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Randomized phase 2 study of valproic acid combined with simvastatin and gemcitabine/nab-paclitaxel-based regimens in untreated metastatic pancreatic adenocarcinoma patients: the VESPA trial study protocol

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Abstract

Background Metastatic pancreatic ductal adenocarcinoma (mPDAC) patients have very poor prognosis highlighting the urgent need of novel treatments. In this regard, repurposing non-oncology already-approved drugs might be an attractive strategy to offer more-effective treatment easily tested in clinical trials. Accumulating evidence suggests that epigenetic deregulation is a hallmark of cancer contributing to treatment resistance in several solid tumors, including PDAC. Histone deacetylase inhibitors (HDACi) are epigenetic drugs we have investigated preclinically and clinically as anticancer agents. Valproic acid (VPA) is a generic low-cost anticonvulsant and mood stabilizer with HDAC inhibitory activity, and anticancer properties also demonstrated in PDAC models. Statins use was reported to be associated with lower mortality risk in patients with pancreatic cancer and statins have been shown to have a direct antitumor effect when used alone or in combination therapy. We recently showed capability of VPA/Simvastatin (SIM) combination to potentiate the antitumor activity of gemcitabine/nab-paclitaxel in vitro and in vivo PDAC preclinical models.

Methods/Design VESPA is a patient-centric open label randomized multicenter phase-II investigator-initiated trial, evaluating the feasibility, safety, and efficacy of VPA/SIM plus first line gemcitabine/nab-paclitaxel-based regimens (AG or PAXG) (experimental arm) versus chemotherapy alone (standard arm) in mPDAC patients. The study involves Italian and Spanish oncology centers and includes an initial 6-patients safety run-in-phase. A sample size of 240 patients (120 for each arm) was calculated under the hypothesis that the addition of VPA/SIM to gemcitabine and nab-paclitaxel-

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based regimens may extend progression free survival from 6 to 9 months in the experimental arm. Secondary endpoints are overall survival, response rate, disease control rate, duration of response, CA 19.9 reduction, toxicity, and quality of life. The study includes a patient engagement plan and complementary biomarkers studies on tumor and blood samples.

Conclusions VESPA is the first trial evaluating efficacy and safety of two repurposed drugs in oncology such as VPA and SIM, in combination with standard chemotherapy, with the aim of improving mPDAC survival. The study is ongoing. Enrollment started in June 2023 and a total of 63 patients have been enrolled as of June 2024.

Trial registration EudraCT number: 2022-004154-63; ClinicalTrials.gov identifier NCT05821556, posted 2023/04/20.

Keywords Pancreatic cancer, Valproic acid, Simvastatin, Drug repurposing, Gemcitabine, nab-paclitaxel

Background

Despite all the recent advances in cancer therapies, pancreatic ductal adenocarcinoma (PDAC) patients have very poor prognosis. Incident rates are increasing, with 5.7 per 100,000 men and 4.1 per 100,000 women in 2020, accounting for 466,000 deaths [1–3]. Notably, diagnosis of PDAC often occurred with advanced disease as metastatic PDAC (mPDAC). For mPDAC, chemotherapy is the only effective treatment option, however resistance as well as adverse effects are major challenges and median progression-free survival (PFS) of standard first-line treatment regimens do not exceed 7 months [4]. Thus, there is an urgent necessity of new treatment options for this disease.

In this regard, repurposing non-oncology already-approved drugs, might be an attractive strategy to offer more-effective treatment options easily translatable in early clinical trials. At the same time, considering the elevated costs estimated for new oncology drugs, not affordable by National Health System (NHS), mechanistic based repurposing in cancer treatment of cheap and safe non-anticancer drugs already in clinical practice, represents an interesting approach with a relevant impact on NHS.

Accumulating evidence suggests that epigenetic deregulation is a hallmark of cancer and has a major contribution to disease development, progression as well as resistance to antitumor treatment in several solid tumors including pancreatic cancer [5, 6]. Histone deacetylase inhibitors (HDACi) are one of the most prominent classes of epigenetic drugs that we have been investigating as anticancer agents for a long time, both preclinically and clinically [6]. Mechanistically, HDACi influence chromatin structure, which in turn regulates gene expression, by inhibiting histone deacetylase. Moreover, by deacetylating non-histone proteins, HDACi can modify cellular processes independent of gene expression. As a result, they may be involved in the control of several aberrant pathways in cancer, such as apoptosis, cell cycle, and DNA repair. A large number of HDACi are currently in clinical development as anticancer agents, with three (vorinostat, romidepsin, and belinostat) approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma [7–9], while panobinostat being the first HDACi approved for the treatment of recurrent multiple myeloma in combination with bortezomib and dexamethasone [10]. Interestingly, preclinical data indicate that RAS-mutant cancer cells, which occur in more than 90% of mPDAC, are more susceptible to DNA damage and apoptosis induced by HDACi [11]. In PDAC patients limited clinical data on the combination of HDACi plus chemotherapy has been reported, demonstrating good tolerability, and, in some cases, despite the small number of patients enrolled, encouraging efficacy [12].

Valproic acid (VPA) is a generic low-cost anticonvulsant and mood stabilizer in the treatment of maniac depression (bipolar affective disorder) being used for over 50 years. VPA has HDAC inhibitory activity and anticancer properties [13], that have been also demonstrated in pancreatic cancer models in monotherapy [13–15] or combined with gemcitabine [16, 17]. Due to its safe use as a chronic therapy for epileptic disorders, it may be a good candidate drug to be combined in novel anticancer regimens. VPA has significantly longer plasma half-life than other HDACi, making its pharmacokinetics more suitable for clinical use (7–9 h in humans) [18]. Several phase I and II studies using VPA to treat hematologic and solid malignancies, demonstrated that VPA administration was well tolerated by the patients, resulting also in some encouraging tumor responses [19–24]. We are currently exploring VPA in combination with conventional chemotherapy regimens in different solid tumors [25–27], overall confirming that this approach is feasible and safe [28].

The mevalonate pathway (MVP) regulates the biosynthesis of cholesterol, an essential component of mammalian cell membranes and a precursor of critical cell signaling components. Cancer cells reprogram cholesterol metabolism to satisfy their increased nutrient demands and support their uncontrolled growth, thereby promoting tumor development and progression [29]. In detail, MVP provides metabolites for post-translational protein prenylation such as farnesylation and

geranyl-geranylation, which are crucial for the downstream signaling activity of small GTPases for instance Ras, Rho, and Rac, which are heavily involved in tumor initiation and progression [30]. As a result, altered cholesterol metabolism pathways suggest exploitable strategies in the context of cancer therapy.

Statins, which were developed as lipid-lowering drugs, inhibit HMG-CoA reductase (HMGCR), the first step of the MVP, preventing cholesterol formation and the protein prenylation branch and have demonstrated a direct antitumor effect in different tumor models [31], including pancreatic cancer preclinical models in monotherapy [32, 33], as well as in combination with gemcitabine [34] or paclitaxel [35].

Recently we have demonstrated for the first time the synergistic antitumor interaction between VPA and simvastatin (SIM) in metastatic prostate cancer (PCa) experimental models, mechanistically related with the capacity of the combined approach to target the cancer stem cells (CSCs) compartment via the inhibition of the oncogene YAP. Based on this evidence we then showed the ability of VPA/SIM combination to sensitize PCa cells to docetaxel, and to revert docetaxel resistance, both in vitro and in vivo [36]. Interestingly, it was reported that statin use, rather than cholesterol level, was associated with lower mortality risk in patients with pancreatic cancer and that statins appear to improve survival through a lipid-independent mechanism [30]. A meta-analysis on a large population demonstrated that, among individual statins used as cholesterol lowering agents, SIM seemed safer and more tolerable than other statins [37]. Gemcitabine/nab-paclitaxel (AG), represents a standard of care for advanced inoperable PDAC with median PFS in first-line treatment of 6 months [38]. More recently, a better efficacy of PAXG regimen (gemcitabine nab-paclitaxel/with capecitabine and cisplatin) compared to AG (median PFS of 8.3 vs. 6.1 months; $p=0{\cdot}01$) was shown [39]. However, the reported overall survival with these regimens, or with the alternative first line option FOLFIRINOX [folinic acid; fluorouracil (5-FU), irinotecan and oxaliplatin], remains less than 1 year.

Notably, more recently VPA/SIM combination was patented by us, since we demonstrated for the first time in pancreatic cancer models that combination treatment has a synergistic antitumor effect and potentiates AG doublet treatment in both in vitro and in vivo PDAC models (patent application PCT/EP2023/058948) [40]. Mechanistically, unpublished findings from our group suggest that the mechanism of the synergistic antitumor interaction is at least in part dependent on the VPA/SIM-mediated reversion of TGF-β-regulated epithelial-to-mesenchymal (EMT) transition. These findings appear essential considering that the EMT program have been reported able to render mPDAC cells more invasive and resistant to therapy-induced apoptosis as well as to promote accumulation of myofibroblasts [33], leading to increased fibrosis [34]. At same time, TGF-β signaling, that has been identified as tumor promoter in mPDAC playing a critical role in both the epithelial and stromal compartments [41], is crucial for the fibrotic process mediate by EMT [42] (Fig. 1). Drawing from these considerations, we designed the randomized phase II VESPA trial to explore the safety and efficacy of VPA/SIM combination added to gemcitabine and nab-paclitaxel-based regimens as initial therapy of mPDAC patients.

Methods/Design

Aim

This study aims to investigate if VPA in combination with SIM may improve the efficacy of first-line gemcitabine and nab-paclitaxel-based regimens and extend PFS as compared with chemotherapy alone, in patients with mPDAC. Safety and quality of life (QoL) will also be assessed. Correlative studies are planned on tumor and blood samples to identify potential biomarkers of toxicity and efficacy helping to define personalized treatment strategy and adding new insight into the antitumor mechanism of the combination approach.

Study design

VESPA is a patient-centric open label randomized proof of concept, multicenter, superiority phase-II trial, evaluating if VPA in combination with SIM plus first line gemcitabine/nab-paclitaxel-based regimens (AG or PAXG), can extend PFS in mPDAC patients compared to chemotherapy alone (Fig. 2). The study involves Italian and Spanish oncology centers and includes an initial 6-patients safety run-in-phase in the experimental arm. Randomization is stratified according to the center, Eastern Cooperative Oncology Group (ECOG) performance status (PS) (0 vs. 1) and chemotherapy backbone regimen (AG vs. PAXG).

Endpoints

The primary endpoint is PFS, defined as the time from randomization to the first documentation of objective disease progression by RECIST 1.1 criteria, or death due to any cause, whichever occurs first, in mPDAC patients treated by experimental combination compared to standard conventional treatment.

Secondary endpoints are objective tumor response rate (ORR), duration control rate (DOR), disease control rate (DCR), overall survival (OS), overall toxicity rate and quality of life (QoL).

Exploratory objectives are the evaluation of the prognostic and predictive significance of DNA mutational status and transcriptomics profiling performed on archived baseline tumor samples or on newly obtained samples

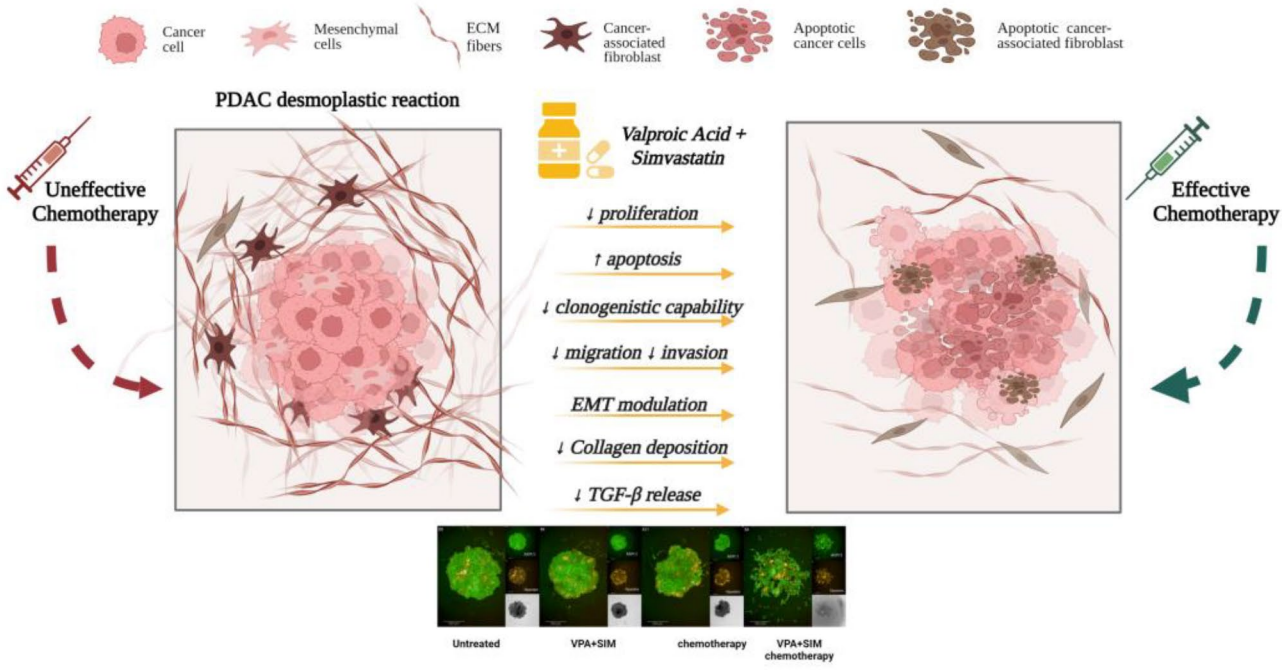

Fig. 1 Antitumoral effects of valproic acid/simvastatin combination treatment in PDAC models

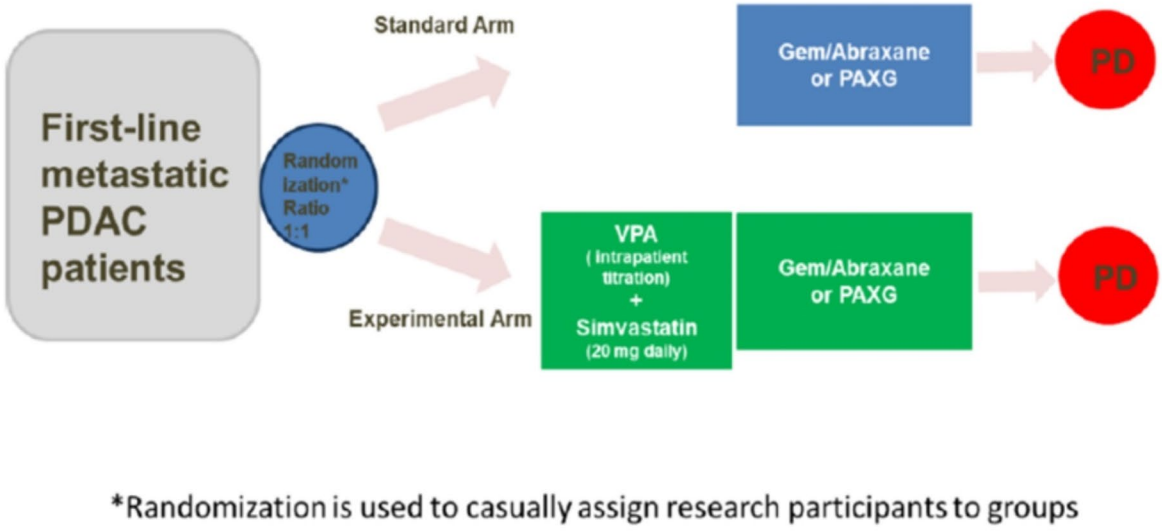

Fig. 2 Treatment plan. Patients will be randomized electronically 1:1 to one of the two arms: (**A**) Standard: Nab-paclitaxel 125 mg/m^2 followed by gemcitabine 1000 mg/m^2 on days 1, 8, and 15 (AG); or nab-paclitaxel 150 mg/m^2, followed by gemcitabine 800 mg/m^2, followed by cisplatin 30 mg/m^2 on days 1 and 15, and oral capecitabine 1250 mg/m^2 on days 1–28 (PAXG). (**B**) Experimental: Chemotherapy (AG or PAXG) + simvastatin oral daily at a fixed dosage of 20 mg in combination with increasing doses of valproic acid administered oral daily from day − 7 with an intra-patient titration for a final target serum level of 50–100 µg/ml (see below)

from primary tumors and/or resected metastases when available. Moreover, on tumor tissue will be also explored the prognostic and predictive value of several biomarkers evaluated by immunohistochemistry (IHC) to characterize the molecular subtype of the tumors and to confirm the preclinical mechanistic insight in the observed synergism between VPA and SIM. Blood samples will be also collected longitudinally during the treatment to examine: (a) metabolomic profiling by NMR Spectrometer; (b) circulating cytokines and chemokines by Luminex technology; (c) a panel of circulating microRNAs evaluated and cftDNA by real-time PCR; (d) drugs pharmacokinetics (PK).

Eligibility criteria

The primary patient inclusion and exclusion criteria are presented in Table 1. Patients with untreated histologically or cytologically confirmed diagnosis of mPDAC are eligible if have an ECOG performance status of 0–1 at study entry. Other inclusion criteria are: patients aged ≥ 18 years, with at least one Imaging-documented measurable lesion, according to RECIST 1.1 criteria, as well as adequate bone marrow, renal and liver function. Subjects are excluded from the trial if they have received previous chemotherapy or any other medical treatment for mPDAC (previous adjuvant chemotherapy is allowed if terminated > 6 months previously), major surgical intervention within 4 weeks prior to enrollment, prior treatment with an HDACi or compounds with HDACi-like activity, such as VPA or if they have used statins, fibrates, or any other hypercholesterolemia treatment in the last three months before the study. Patients with known or suspected brain metastases or with uncontrolled systemic disease are also excluded.

Table 1 Selection of patients

Inclusion Criteria
1. Written informed consent to study procedures and to correlative studies
2. Histologically or cytologically proven metastatic PDAC
3. No prior treatments (chemotherapy, radiation or surgery) for mPDAC
4. Either sex aged ≥ 18 years
5. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 1 at study entry
6. Imaging-documented measurable disease, according to RECIST 1.1 criteria
7. Known dihydropyrimidine dehydrogenase (DPD) activity is mandatory for patients enrolled in PAXG scheme
8. Adequate bone marrow haematological function: absolute neutrophil count (ANC) ≥ 1.5×10^9/L AND platelet count ≥ 100×10^9/L AND haemoglobin ≥ 9 g/dL
9. Adequate liver function: total bilirubin ≤ 1.5 x upper limit of normal (ULN) or ≤ 2 in case of biliary stent) and aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ≤ 5 X ULN
10. Adequate renal function: serum creatinine ≤ 1.5 mg/dL OR creatinine clearance ≥ 60 mL/min in males and ≥ 50 mL/min in females (calculated according to Cockroft-Gault formula)
Exclusion Criteria
1. Prior malignancy within one year. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer
2. Prior chemotherapy or any other medical treatment for metastatic PDAC (previous adjuvant chemotherapy is allowed if terminated > 6 months previously)
3. Patients who have had prior treatment with an HDAC inhibitor and patients who have received compounds with HDAC inhibitor-like activity, such as valproic acid
4. Current use of statins or fibrates or any medication for hypercholesterolemia for any time during the 3 months before the study
5. Proven hypersensitivity to statins and to any component of the other medications used in the trial
6. Major surgical intervention within 4 weeks prior to enrollment
7. Pregnancy and breast-feeding
8. Brain metastasis
9. Hepatitis or any severe liver disorder
10. Evidence of severe or uncontrolled systemic disease or any concurrent condition which in the investigator's opinion makes it undesirable for the patient to participate in the study, or which would jeopardize compliance with the protocol, or would interfere with the results of the study
11. Patients with long QT-syndrome or QTc interval duration > 480 msec or concomitant medication with drugs prolonging QTc
12. History of poor co-operation, non-compliance with medical treatment, unreliability or any condition that may impair the patient's understanding of the Informed consent form
13. Participation in any interventional drug or medical device study within 30 days prior to treatment start
14. Patients who cannot take oral medication, who require intravenous alimentation, have had prior surgical procedures affecting absorption, or have active peptic ulcer disease
15. Sexually active males and females (of childbearing potential) unwilling to practice contraception during the study and until 6 months after the last trial treatment

Treatment plan

Patients will be randomized electronically 1:1 to one of the two arms and the treatment should be started within 3 days from randomization. In standard arm nab-paclitaxel will be administered as a 125 mg/m^2 intravenous infusion in 30 min followed by gemcitabine 1000 mg/m^2 intravenous infusion over 60 min on days 1, 8, and 15 every 4 weeks in the AG combination. In the PAXG combination nab-paclitaxel will be administered as a 150 mg/m^2 intravenous infusion, followed by gemcitabine 800 mg/m^2 intravenous infusion over 60 min, followed by cisplatin 30 mg/m^2 intravenous infusion over 60 min on days 1 and 15, and oral capecitabine 1250 mg/m^2 on days 1–28.

In the experimental arm chemotherapy will be administered as described for standard arm, while VPA + SIM treatment will start 7 days before chemotherapy (Fig. 2). VPA and SIM are used at their non-cancer approved indications, as these dosages have been shown to be preclinically effective in combination treatment with the two drugs plus chemotherapy [36, 40]. Specifically, starting at day −7 simvastatin will be administered oral daily in the evening at a fixed dosage of 20 mg in combination with increasing doses of VPA administered oral daily with an intra-patient titration using slow releasing tablet (Depakin Chrono®). The titration strategy will be applied in each patient to achieve a serum concentration between 50 and 100 µg/ml, as reported in Table 2. Treatment will be administered orally starting at day −7 with 500 mg in the evening to reach full dosages of 1500 mg daily, representing the recommended values for the treatment of epilepsy. In the morning of day 1 and thereafter biweekly, with the goal to keep target serum level indicated, VPA serum concentration will be checked by drawing a blood sample within 2 h after taking the morning dose using a commercially available valproate test. Consequently, the dose of VPA will be adjusted depending on the reached steady level. A diary containing details about the daily home administration of VPA and SIM will be dispensed by the Investigator to the patient.

One cycle consists of 28 days of treatment for both arms. Patients will continue to receive study treatment up to 6 cycles until disease progression, unacceptable toxicity, physician's decision, patient's refusal, or any other discontinuation criteria. Continuation of treatment, over the six cycles, will be allowed only in case of clinical benefit, defined as continuous decrease of CA19-9 concentration or radiological response, without unacceptable toxicity. Surgery can be carried out in case of appropriate tumor shrinkage will be evident at response evaluation and resectability should be evaluated by a multidisciplinary team. All subjects who finish treatment, whichever the reason, will enter in the follow-up and they will be followed until death and data on subsequent treatment will be collected.

Table 2 Valproic acid titration: the dose will be increased according to the following scheme:

Days	Morning dose*(mg)	Midday dose*(mg)	Evening dose*(mg)
-7	0	0	500
-6	300	0	500
-5	500	0	500
-4 & -3	500	300	500
-2 & -1	500	500	500

*The interval between doses will be 12 h on days −7 to -5 and 8 h from day −4

Safety run-in phase: A Safety Monitoring Committee (SMC), comprising of two specialists who are not engaged in the trial's conduct and a patient organization representative, will initially examine safety data when the first six patients in each chemotherapeutic backbone (AG or PAXG) receive at least two rounds of the trial treatment. The SMC will assess whether the study treatment combination for each chemotherapy backbone (AG or PAXG) is judged feasible and no major safety concerns arise compared to standard treatment. (Supplementary Fig. 1). The SMC will examine safety data on a regular basis, usually every 3 months from the date of the first patient-in, including adverse events, major adverse events, adverse events of special interest, and relevant laboratory data. Furthermore, an independent data monitoring committee, comprising SMC members plus a statistician and a medical oncologist not involved in the study, will monitor data on progress and safety to ensure the safety of study participants and preserve clinical trial integrity.

In case of hematologic and non-hematologic toxicities, chemotherapeutic drugs (either AG or PAXG regimen) will be reduced as reported in supplementary Tables 1 and 2. In the event of a grade 2 muscular cramp, the SIM dose will be lowered to 10 mg/day until the cramp is grade 1. If grade 2 muscular cramp continues after dosage decrease, or if grade 3 muscle cramp occurs, SIM will be definitively stopped. In the case of grade 2 somnolence or fatigue, the VPA dose will be reduced by -500 mg/day steps until it reaches grade 1, regardless of the actual serum level. VPA will be permanently suspended if the patient shows grade 3 somnolence or fatigue. VPA must be suspended if asymptomatic QTc prolongation develops (QTc > 500 ms or QT prolongation > 60 ms but not associated with symptoms). After 24 h, the ECG must be redone. If the incident is resolved, VPA can be resumed, but the dose will be reduced by -200 mg/day; if QT prolongation is confirmed, VPA treatment must be stopped [43]. VPA has to be stopped if symptomatic QTc prolongation develops (QTc > 500 ms or QTc > 60 ms and associated with symptoms indicative of ventricular tachyarrhythmia). If the blood VPA concentration is

more than 150, the VPA dosage is reduced by 500 mg/day, even if the patient is asymptomatic.

Study procedures

Assessment and procedures, including those for exploratory objectives (see below) are illustrated in Fig. 3. All screening/baseline assessments must be performed within 28 days from randomization.

Response evaluation is planned every 8 weeks thereafter until disease progression (PD), by CT or MRI scan of the chest, abdomen, and pelvis, CEA, CA19.9, or any test that was positive at baseline. The Investigator will code the response using the RECIST (v 1.1). All patients will be included in the analysis of the response rate.

Patients will be assessed for toxicities before each treatment cycle throughout the study treatment and up to four weeks after last cycle of treatment in accordance with the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE-NCI) version 5.0. To assess adverse events, clinicians will consider clinical examination (including PS and vital parameter evaluation), comprehensive hematology and biochemistry before to any day-treatment of each cycle; ECG (for the experimental arm only) at day 1 of the first cycle and thereafter every 8 weeks. In patients with QT prolongation, the ECG will be repeated after 24 h; any further test considered clinically necessary by the Investigator. Patients who discontinue study drug for any reason (e.g., adverse events (AEs), etc.) other than disease progression will continue to be followed. Recurrence of disease will be documented, as well as initiation of another cancer therapy.

Quality of Life will be assessed by using EORTC QLQ-C30 and QLQ-PAN26 EORTC questionnaires that must be compiled within 2 weeks prior to randomization (baseline) and thereafter every 8 weeks during treatment and after treatment, until 40 weeks after randomization. The EORTC QLQ-C30, version 3.0, explores 5 multi-item functional subscales (physical, role, emotional, social and cognitive functioning); three multi-item symptom scales (fatigue, pain, and emesis); a global health status subscale; and six single items (financial impact and symptoms such as dyspnea, sleep disturbance, appetite, diarrhea, and constipation). The QLQ-PAN26, dedicated to pancreatic cancer patients, includes 26 questions with 4-category response exploring symptoms, effect of treatment, psychological and social aspects including sexual activity, and will be evaluated/validated under an agreement with EORTC.

Biomarkers

In cancer therapy, there is an unmet need for pharmacodynamic and predictive biomarkers. In particular, the identification of predictive biomarkers for toxicity and efficacy, as well as therapeutic drug monitoring that leads to dosage optimization, could improve patients' QoL, treatment costs, and the clinical implementation of the treatment approach evaluated in this trial.

Biomarkers will be evaluated on archival tumor tissues from metastases (always recommended) and/or primary tumors or on newly obtained biopsies at baseline. When available, on the bases of a separate additional, not mandatory for the inclusion in the study, informed consent, biomarkers will be evaluated on metastases tissues during treatment and/or at progression. Blood samples will be collected at baseline, during treatment, and at progression. Biomarkers will be correlated with clinical response, patient outcome and toxicity.

The recent discovery of both genomic and transcriptional PDAC subtypes is yielding biological insights with potential therapeutic implications in mPDAC [44]. Thus, in the current trial, we will evaluate genomics and transcriptomics profiling from primary tumor tissues and/or metastases (when available) at baseline and eventually during treatment in patients undergoing curative surgery, by Next Generation Sequencing (NGS) and RNAseq technology, respectively.

Molecular Subtypes characterization of the tumor will be also performed, particularly basal phenotype identification by specific markers (GATA 6, Keratin 81 and KIF3b). Emerging evidence also suggest that some mPDAC subtypes have more severe epigenetic alterations [45], emphasizing the importance of a detailed molecular characterization of baseline mPDAC tissue samples from trial patients. We will further use IHC and real-time PCR to confirm our preclinical published and preliminary data by comparing normal mucosa with tumor at baseline (possibly within the diagnostic biopsy) and on tumor tissues (when available, either primary tumor and/or resected metastases) during treatment. To confirm our preclinical mechanistic insight into the observed synergism between VPA and SIM, the expression of MVP enzymes (i.e., HMGR) as well as a specific panel of EMT, CSC, and differentiation markers will be evaluated. Moreover, we will test HDAC isoforms and global histone and protein acetylation (H&P-Ac), to be correlated with VPA effect; expression of DNA repair genes (i.e., RAD51, XRCC1) that may affect sensitivity to chemotherapy. Analysis of expression levels at baseline tumor tissue vs. normal mucosa and/or baseline tumor tissues vs. eventually resected tumor/metastases after treatment will be performed when possible.

Circulating biomarkers, which enable real-time monitoring, can help to address additional challenges such as early response prediction and resistance, pharmacokinetics and drug-drug interactions. Peripheral blood samples for exploratory biomarker studies are collected at baseline and at several time points during treatment

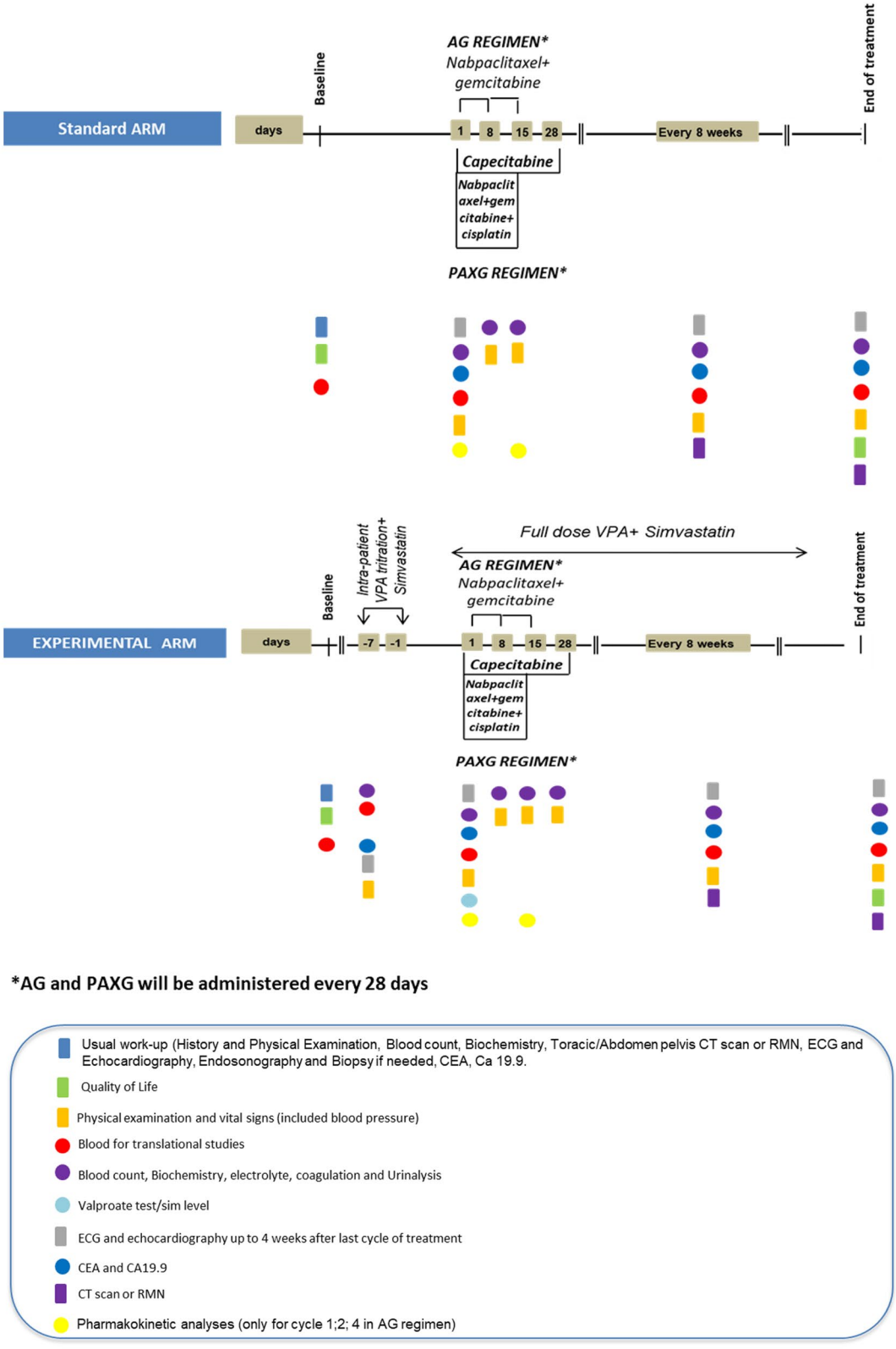

Fig. 3 Study procedures

and, immediately, stored at -80 °C (Fig. 3). Biomarkers in blood will be determined by sequencing and qRT-PCR.

VPA serum level will be measured by commercial test on day 1 of the first cycle (before treatment) and biweekly thereafter (day 1 and day 15 of each cycle) until the end of treatment with VPA. Drugs pharmacokinetics studies will be set up on SIM and on chemotherapeutics employed in AG regimens (Fig. 3).

Aberrant metabolism is an emerging hallmark of cancer [46] and recent observations suggest that cancer cells undergo specific metabolic changes that can be used to stratify patients [47]. In this context, metabolomics analysis in serum is minimally invasive and easily accessible for disease monitoring, and thus has a strong potential for identifying novel biomarkers of treatment benefit in cancer patients. Nuclear Magnetic Resonance (NMR) is a powerful nondestructive and affordable technique for identifying and quantifying complex metabolite mixtures using small sample volumes [47, 48]. Based on our preliminary experience in preclinical models and patient samples from other tumor types [49], we will evaluate in plasma patient the metabolomic profiling at baseline and during treatment by NMR to early distinguish patients for whom the treatment is effectively befitting and beneficial.

Cytokines regulate immune cell trafficking into tumors, have been implicated in tumor development and progression, and their abnormal production is linked to the development of drug resistance in pancreatic cancer [50]. Notably, in pancreatic cancers, a clear interplay between metabolic changes and cytokines has been demonstrated [50]. Furthermore, mounting evidence suggest that HDACi, including VPA [51], and statins [52, 53], have an immunomodulatory effect. On these foundations, we will investigate a large panel of circulating cytokines and chemokines in patient peripheral blood samples using by a multiplex biometric ELISA-based immunoassay in the plasma, as previously described by our group [54].

Histones and proteins acetylation will be also evaluated on peripheral blood lymphocytes (PBL) as described by P. Munster et al. [24].

Deregulation of microRNAs (miRNAs) among epigenetic aberrations contributes to mPDAC carcinogenesis by promoting the expression of proto-oncogenes or inhibiting the expression of tumor suppressor genes [45, 55]. Circulating miRNAs have also been investigated as potential prognostic and predictive biomarkers in the context of mPDAC, including treatment response [45, 56]. Based on our previous published data [57], we will investigate a panel of circulating miRNAs using qRT-PCR as previously reported [58]. Similarly, because cftDNA (Cell-free circulating tumor DNA) levels have been linked to survival and treatment response, we will also estimate cftDNA in serum using qRT-PCR to identify specific mutations.

Biomarker analysis is exploratory by nature, therefore these data will be analyzed in the majority of cases after the primary research analysis is completed. Despite numerous tests are yet planned, the definitive list of analysis has to be determined, because the rapid evolution of novel markers identification connecting with disease activity, treatment efficacy or safety. Furthermore, because the values remain uncertain, the results will not be reported to the patients.

The above cited biomarkers will be correlated to the clinical outcome to define the predictive and/or prognostic role of the alterations detected. A final algorithm including the biomarkers with predictive value will be built to generate more robust tool for patient response and toxicity evaluation.

Statistical analysis

All efficacy analysis will be conducted using the intention-to-treat (ITT) method. Compliance and safety assessments will include all individuals who received at least one dosage of the allocated trial therapy. There will be no 'per-protocol analysis'. Patients lost to follow up or alive at the end of the study will be censored on the date of the last tumor assessment. In as much as expected median PFS for mPDAC is 6 months for AG regimen [38] and 8 months for PAXG regimen (PACT-19 trial) [39] assuming that 20% of the patients will receive PAXG therapy, we can broadly assume a median PFS of 6.3 months in the control arm.

Taken these considerations, our hypothesis is to test an HR=0.70 corresponding to an expected median PFS in the experimental arm of 9 months. Setting power to 80% and significance level to 10%, using log-rank test we need to enroll 114 patients per arm; considering 5% of dropout we plan to accrual a total of 240 patients (120 for each arm) in around 30 months. The date of end of study will be the date of the last visit of the last patient. The choice of this significance level was made according to Rubenstein et al. [59] which consider an alpha value of 0.10 as a standard for phase 2 trials because there is still another phase, namely phase 3, to change clinical standard.

The main analysis will be performed after 196 events (progressions or deaths) are observed.

If the null hypothesis of no difference between arms will be refused, the possible evolution in a phase 3 trial with OS as endpoint and a more stringent significance level (i.e. 5%) will be considered.

Kaplan-Meier product-limit approach will be used to describe PFS and OS curves. For each patient and for each type of toxicity described according to CTCAE, the worst degree ever suffered during treatment will be used for the analysis. EORTC questionnaires will be managed

according to standard rules for their analysis reported in the EORTC manuals [60].

Due to the small sample size, statistical analysis of biomarkers data will be conducted with the aim of hypothesis generation and a complete description of data will be done. For biomarkers that might change over time, as a consequence of treatment, levels before and after treatment will be compared with appropriate univariate or multivariate statistical tests, based on the type of data and accordingly to the number of samples available. Correlation with outcome will be performed with appropriate statistical tests. Serum levels of VPA throughout treatment will be described and compared between different acetylator phenotypes, with appropriate statistical tests. *P* values ≤ 0.05 will be considered significant, and no adjustment is planned for multiple comparisons due to the exploratory nature of the analysis.

Patient engagement plan

The VESPA trial was planned as patient centric with patients' voice contributing to shape and implement research through a patient engagement plan that will be defined in collaboration with patient associations (Eurordis and Beacon). Being part of the Steering and the Safety Monitoring Committees patients will contribute to each step of the study and particularly to early detection of safety and procedures issues and will ensure the implementation of the trial according to the original plan. To ensure patient adhesion and involvement in the study, patients took part in the development and finalization of the informed consent form, as well as in the review procedures, assessments, and schedules including the patient's diary. Patients will also participate in the following practical activities: establishing international and national patients boards to implement patient engagement and to monitor the trial; creating a user-friendly fact sheet on the VESPA Study (in English, Spanish, and Italian) (Supplementary material appendix I); creating a user-friendly and patient-oriented informed consent form, with graphics and in layman's language (in English, Spanish, and Italian); and setting up focus groups to ensure gathering patient feedback as well as multi-stakeholder conference on pancreatic cancer to increase awareness of the disease. As part of patient's engagement plan a patients-friendly language report of the results of the trial will be prepared to be shared with patients and their care-givers. Finally, results will be disseminated by using newsletter, social media implantation, and webinar reports directed to patients, general public and policy makers to attract awareness on PDAC and accelerate the transition to clinic practice. A dedicated email address has been created by coordinator center to receive information regarding the trial itself, including details about eligibility and logistics.

Discussion

Despite advances in therapy, the prognosis for mPDAC remains poor. Combination chemotherapy FOLFIRINOX and AG represents the gold standard first-line treatment in patients with mPDAC, as both regimens were proven superior to gemcitabine alone. The longest mOS observed with first-line FOLFIRINOX and the data of NAPOLI-3 study (NCT04083235) [61], that compared the combination of fluorouracil, leucovorin, liposomial irinotecan and oxaliplatin (NALIRIFOX) with AG, support the use of triplet chemotherapy as first-line therapy in mPDAC with good performance status. However, in a recent randomized phase III trial FOLFIRINOX was found not to be superior to AG, reporting a median OS of 14 months vs. a median OS of 17 months observed with AG. Moreover, FOLFIRINOX was burdened by more frequent severe gastrointestinal toxicities compared to AG [62].

Anyway, the impact of these treatment regimens on OS of patients with mPDAC remain poor. Furthermore, target therapy and immunotherapy, alone or in combination with chemotherapy, have failed to demonstrate a benefit in mPDAC.

As a result, intrinsic cancer biology characteristics suggest the need for new combinatorial medicines.

Our proposal will address these issues by analyzing for the first time in this environment a unique therapeutic strategy based on the use of VPA, a safe and generic medication with HDACi activity, plus SIM in combination with a consolidated therapy.

In particular, by exploring the efficacy and safety of VPA/SIM in combination with gemcitabine/nab-paclitaxel-based regimen in patients with untreated mPDAC we expect to validate a novel and affordable therapy in a very poor prognosis type of cancer that could be easily implemented into the clinical practice.

Moreover, by correlative biomarkers studies we expect to improve dosage optimization and eventually to suggest how to select responsive/resistant patients. Whichever the result, the proposed study will also add new insights into the antitumor effects (and the mechanisms) of HDACi, and in particular of VPA, plus SIM, in combination with gemcitabine/nab-paclitaxel-based chemotherapy in mPDAC. In vitro and in silico drug assessment will support optimal design of the clinical trial, assess combination treatment efficacy and drug-drug interaction prediction.

Finally, through the patient engagement plan, we expect to demonstrate the critical role of patients' involvement to improve outcome of clinical (and translational) research programs representing an example to be followed within our research Institutions and beyond.

National Health Systems, notably the Italian and the Spanish NHS, are unlikely to continue to sustain the cost

explosion of new cancer medications for much longer. Considering this situation, optimizing consolidated anticancer drugs as well as mechanistic-based repurposing in cancer treatment of cheap and safe non-anticancer drugs already in clinical practice, such as VPA and SIM, represent an appealing strategy for providing more effective and affordable cancer treatments to patients.

We do not anticipate problems regarding with patient compliance to treatment because VPA and SIM are a well-known and safe drugs, and our intra-patient treatment approach should ensure good compliance, as confirmed by preliminary data on the absence of adverse events in the Revolution trial [25]. In any case, we have developed a pre-planned dose reduction/treatment interruption programmed in case of adverse events to guarantee optimal treatment compliance. We expected to improve dosage optimization by analyzing potential predictive biomarkers of toxicity and effectiveness, as well as drug pharmacokinetics, using methodologies that might be adopted in clinical practice.

Finally, it is important to mention that this phase II trial could be a pilot experience for performing drug repurposing in high mortality tumors where therapeutic approaches are poor and ineffective.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-024-12936-w.

Supplementary Material 1

Author contributions

Development of the concept and design: AB, AL, DG, AA; Development of methodology: AB, AL, EP, LS, FF, AI, MSR, FB, MLGB, MRG, GT, MM, MR, CF, ER, CK, EDG, DG, AA; Writing, review and/ revision of manuscript, prepared the figures: AB, AL, EP, EDG; AA. All authors reviewed the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Certified independent Ethical Committee of the Istituto Nazionale Tumori IRCCS G. Pascale (Determina Dirigenziale 13/03/2023 N.358) and by the Italian and Spanish regulatory drug bodies AIFA and AEMPS. Approval was confirmed by institutional Review Boards of all participating centers. The procedures set out in this study protocol are designed to ensure that the principles of the Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH) and the Declaration of Helsinki are respected in the conduct, evaluation and documentation of this study. Participants will provide written informed consent prior to participation in this study. The study started in June 2023. It is planned that patient's enrollment will be completed in 30 months with an expected total study duration of approximately 40 months, considering an additional 10 months of follow up from last patient enrolled. At the moment 69 patients were screened and 63 enrolled. The study was registered in EU Clinical Trial Register (EudraCT 2022-004154-63) and in ClinicalTrial.gov (NCT05821556).

Competing interests

The authors declare no competing interests.

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