Repurposing of Valproic Acid and Simvastatin to potentiate first line chemotherapy regimen in metastatic pancreatic cancer patients: from preclinical evidence to clinical testing in VESPA trial

Alfredo Budillon[1], Maria Serena Roca[2], Federica Iannelli[2], Veronica Barile[2], Rossella Migliorino[2], Eugenia Passaro[2], Cristina Testa[2], Laura Addi[2], Laura Grumetti[2], Tania Moccia[2], Carlo Vitagliano[2], Lucrezia Silvestro[3], Francesca Foschini[3], Carmine Carbone[4,5], Lorenzo Priori[5], Maddalena Fratelli[6], Laura Fiorenza[6], Maria Laura Garcia Bermejo[7], Bruno Sainz Jr[8], Francesca Bruzzese[9], Diana Giannarelli[10], Mercedes Rodriguez Garrote[11], Giampaolo Tortora[4,5], Michele Milella[12], Michele Reni[13], Elena Di Gennaro[2], Christine Kubiak[14], Eve Scott[15], Claudia Fuchs[16], Alessandra Leone[2], Antonio Avallone[3] .

1 Scientific Directorate, Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale" – IRCCS, Naples; Italy; 2 Experimental Pharmacology Unit-Laboratory of Naples and Mercogliano (AV), Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale" – IRCCS, Naples; Italy; 3 Experimental Clinical Abdominal Oncology; Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale" – IRCCS, Naples; Italy; 4 Medical Oncology, Department of Medical and Surgical Sciences, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; 5 Medical Oncology, Department of Translational Medicine, Catholic University of the Sacred Heart, Rome, Italy; 6 Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy; 7 Biomarkers and Therapeutic Targets Group, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; 8 Biomarkers and Personalized Approach to Cancer group (BIOPAC), Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; 9 Animal Facility Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori "Fondazione G. Pascale" – IRCCS, Naples, Italy; 10 Facility of Epidemiology and Biostatistics, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy; 11 Molecular Epidemiology and Predictive Tumor Markers group, Area 3, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain; 12 Section of Innvovation Biomedicines-Oncology Area, Department of Engineering for Innovation Medicine (DIMI), University of Verona and University and Hospital Trust (AOUI) of Verona, Italy; 13 Department of Medical Oncology, University "Vita-Salute San Raffaele", IRCCS- Ospedale San Raffaele, Milan, Italy; 14 ECRIN-ERIC (European Clinical Research Infrastructure Network-European Research Infrastructure Consortium, Paris, France.; 15 Beacon: for rare diseases, Cambridge, United Kingdom; 16 EURORDIS - Rare Disease Europe.

BACKGROUND

- Patients with metastatic pancreatic ductal adenocarcinoma (PDAC) have a very poor prognosis, despite all the improvements in cancer therapy [Lambert et al. Semin Oncol, 2021], indicating the urgent need for new treatments. Repurposing already-approved non-oncology medications may be a desirable approach in terms of helping to provide efficacious therapeutic alternatives that are easily transferred to early clinical trials.
- In tumor models including PDAC, valproic acid (VPA), a generic low-cost anticonvulsant with histone deacetylase (HDAC) inhibitory activity, has been shown to have anticancer characteristics when used alone [Luo D et al. Carcinogenesis, 2020] or in combination with gemcitabine [Lin T et al. JECCR, 2019].
- As we recently shown [Roca MS et al. JECCR, 2022], HDAC inhibitors have the ability to sensitize PDAC cells to gemcitabine/abraxane doublet. VPA in combination with conventional chemotherapy is under investigation in different solid tumors, and the results generally support the viability and safety of this strategy [Avallone A et al. BMC cancer, 2016; Budillon A et al. Ann Onc, 2018].
- Originally designed to decrease cholesterol by blocking HMG-CoA reductase, statins have also shown a direct antitumor impact when used alone or in combination with chemotherapy and target treatment in a wide range of tumor models, including pancreatic cancer [Gupta V et al, Cancer Lett 2018].
- We have just demonstrated that VPA and the cholesterol-lowering drug simvastatin have a preclinical synergistic anticancer interaction in metastatic prostate cancer models. Additionally, the combination therapy has the potential to both sensitize prostate cancer cells to docetaxel and reverse docetaxel resistance. This impact has a mechanistic connection to the combined approach's ability to suppress the oncogene YAP and target the cancer stem cells compartment [Iannelli F et al. JECCR, 2020].

KEY FINDINGS

- **In a panel of human and murine pancreatic ductal adenocarcinoma cells, we demonstrated a strong synergistic antiproliferative and pro-apoptotic effect of valproic acid (VPA) and simvastatin (SIM) combination, either alone or plus chemotherapy. This effect was strengthened by a technical cross-validation carried out in the framework of the EU-funded REMEDI4ALL project.**
- **Synergistic antitumor interaction was further observed as impairment of clonogenic capability as well as growth inhibition in 3D models, such as fibroblast/tumor cell microtissues and patient derived-organoids.**
- **The antitumor efficacy has been confirmed in vivo in orthotopic and heterotopic xenograft pancreatic ductal adenocarcinoma models in nude mice.**
- **Mechanistically, we also provided evidences that VPA/SIM combination regulate several protumorigenic pathways through TGF-β and YAP signaling modulation, thus potentiating chemotherapy.**
- **These findings represent the rationale for the ongoing VESPA trial (EudraCT: 2022-004154-63-NCT: 05821556), a multicentric, patient-centric, open-label, proof-of-concept, "randomized phase 2 study of Valproic acid combinEd with Simvastatin and gemcitabine/nab-paclitaxel-based regimens in untreated metastatic Pancreatic Adenocarcinoma patients," that has already enrolled 32 patients.**
- **Overall, we proposed a novel and affordable combination therapy, based on two orally safe and generic drugs, to sensitize a widely employed first-line treatment in poor prognosis mPDAC patients.**

Preclinical evidences of synergistic antitumor effect of VPA/SIM alone or in combination with gemcitabine/taxol

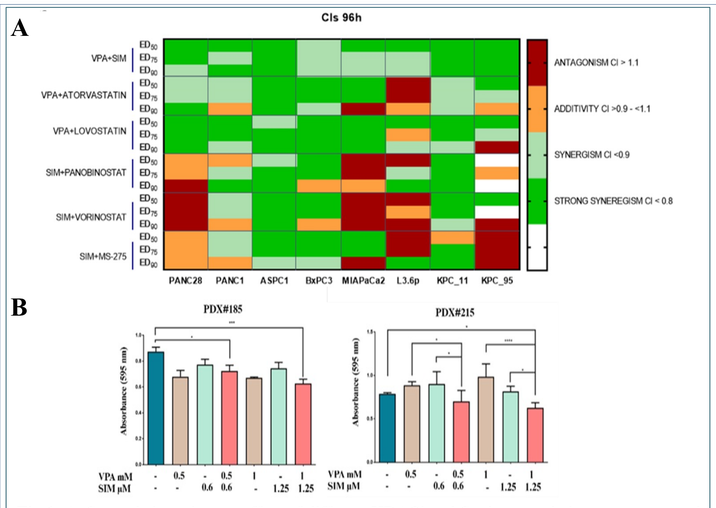


Fig 1. **A**. Synergistic antitumor effect of different HDACi and Statins association were measured as combination indexes (CIs) in a panel of PDAC cell lines. Interestingly, the best results were obtained when VPA was associated with SIM, compared to other HDACi/Statins combinations. **B**. Two PDX-derived cell lines (PDX 185 and PDX 215) showed a sensitivity to VPA, SIM and their combination at two different concentrations (0,5 and 1 mM VPA; 0,625 and 1,25 µM SIM).

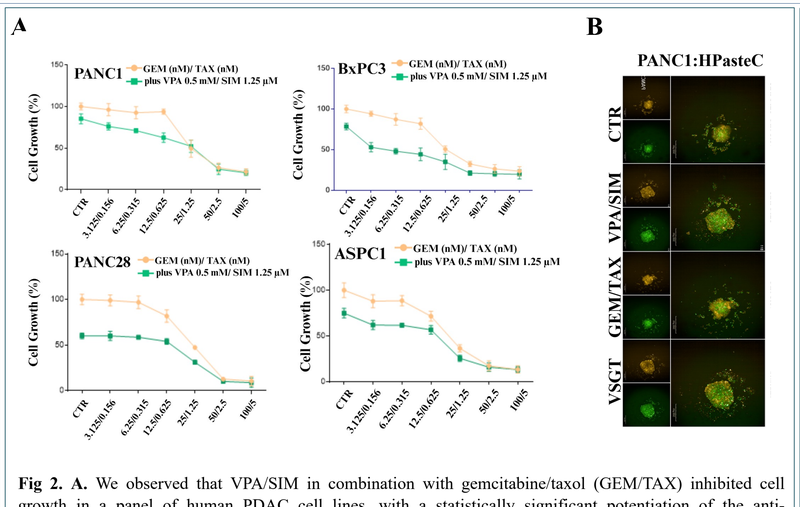


Fig 2. A. We observed that VPA/SIM in combination with gemcitabine/taxol (GEM/TAX) inhibited cell growth in a panel of human PDAC cell lines, with a statistically significant potentiation of the anti-proliferative effect of the drugs. **B.** The impact of combination on PDAC microenvironment was assessed on 3D PANC1 spheroids cocultured with HPasteC fibroblasts. VPA/SIM either alone or in combination with GEM/TAX (VSGT) lead to a disruption and disintegration of microtissues**.**

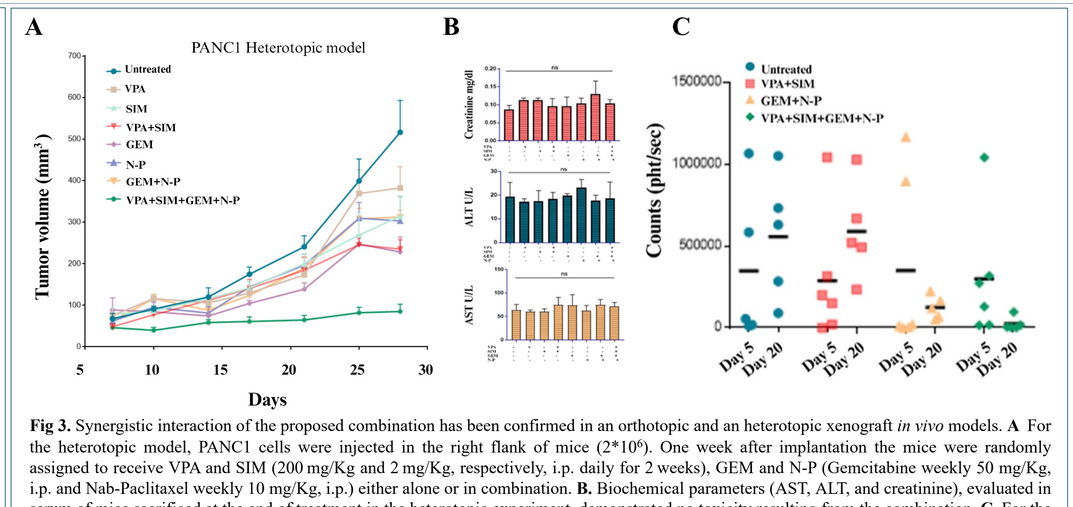
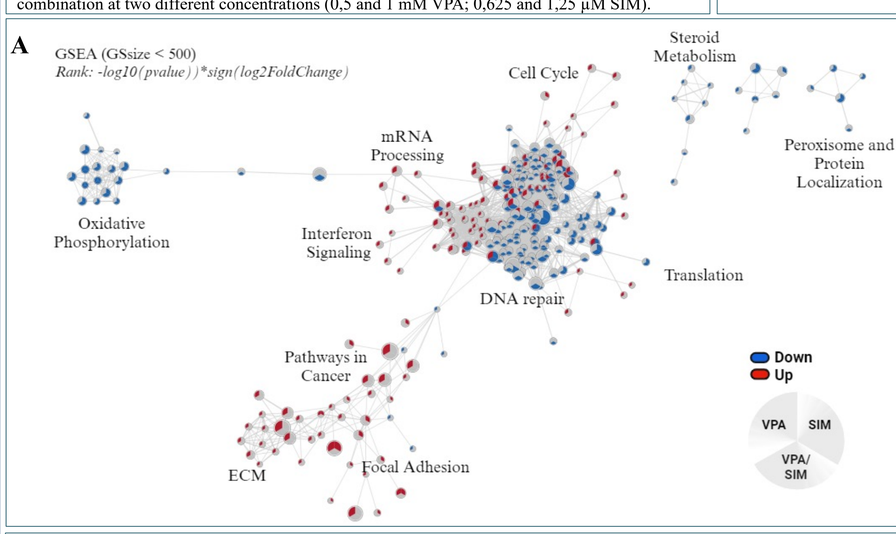
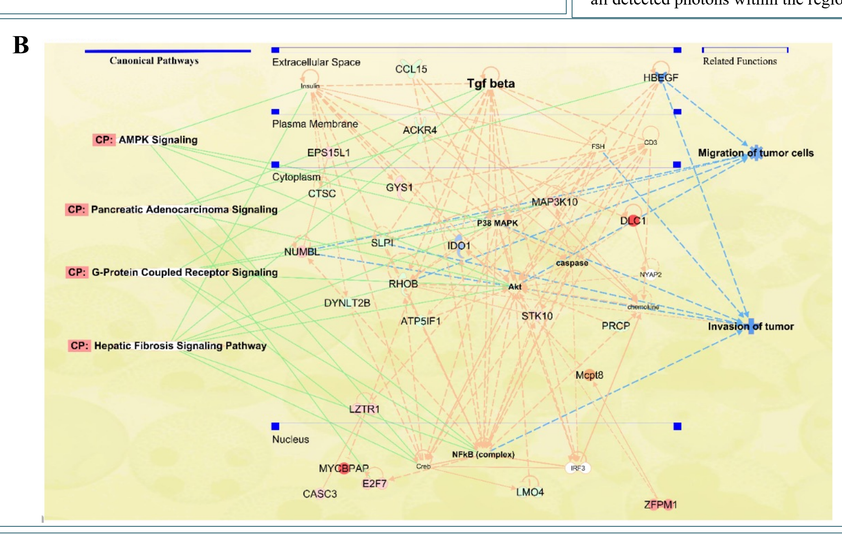


Fig 3. Synergistic interaction of the proposed combination has been confirmed in an orthotopic and an heterotopic xenograft *in vivo* models. **A** For the heterotopic model, PANC1 cells were injected in the right flank of mice ($2*10^6$). One week after implantation the mice were randomly assigned to receive VPA and SIM (200 mg/Kg and 2 mg/Kg, respectively, i.p. daily for 2 weeks), GEM and N-P (Gemcitabine weekly 50 mg/Kg, i.p. and Nab-Paclitaxel weekly 10 mg/Kg, i.p.) either alone or in combination. **B.** Biochemical parameters (AST, ALT, and creatinine), evaluated in serum of mice sacrificed at the end of treatment in the heterotopic experiment, demonstrated no toxicity resulting from the combination. **C.** For the orthotopic model, PANC1_LUC cells were injected into the pancreatic gland of mice ($5*10^4$). One week after implantation the mice were randomly assigned to receive subtherapeutic doses of VPA/SIM combination (200 mg/Kg and 2 mg/Kg, respectively, i.p. daily for 2 weeks), and GEM/N-P (Gemcitabine weekly 25mg/Kg, i.p. and nab-Paclitaxel weekly 20 mg/Kg, i.p.). Tumor volume was quantified as the mean of the sum of all detected photons within the region of the tumor/s, at the indicated time point, and was compared with the mean at day 1 (T0).





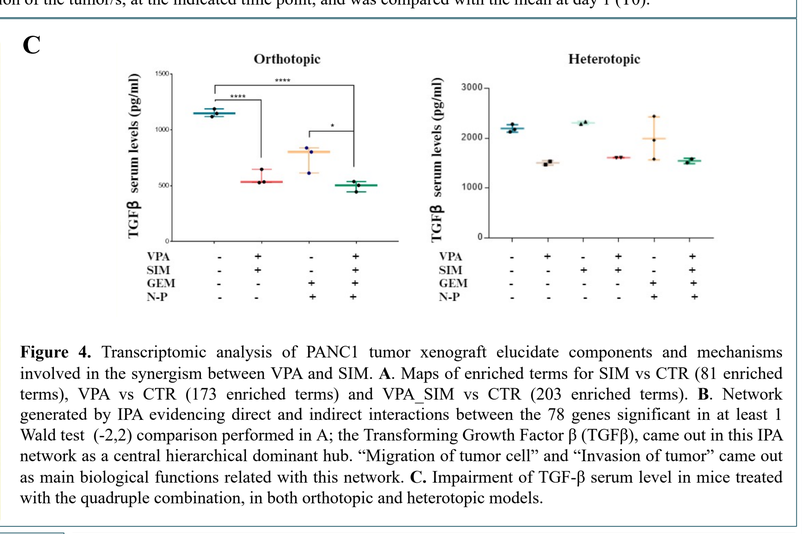
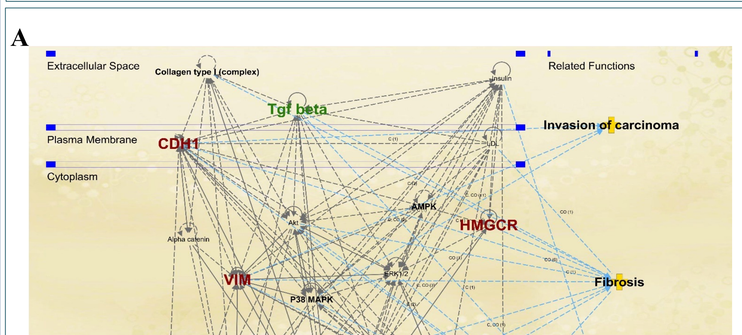


Figure 4. Transcriptomic analysis of PANC1 tumor xenograft elucidate components and mechanisms involved in the synergism between VPA and SIM. **A**. Maps of enriched terms for SIM vs CTR (81 enriched terms), VPA vs CTR (173 enriched terms) and VPA_SIM vs CTR (203 enriched terms). **B**. Network generated by IPA evidencing direct and indirect interactions between the 78 genes significant in at least 1 Wald test (-2,2) comparison performed in A; the Transforming Growth Factor β (TGFβ), came out in this IPA network as a central hierarchical dominant hub. "Migration of tumor cell" and "Invasion of tumor" came out as main biological functions related with this network. **C.** Impairment of TGF-β serum level in mice treated with the quadruple combination, in both orthotopic and heterotopic models.



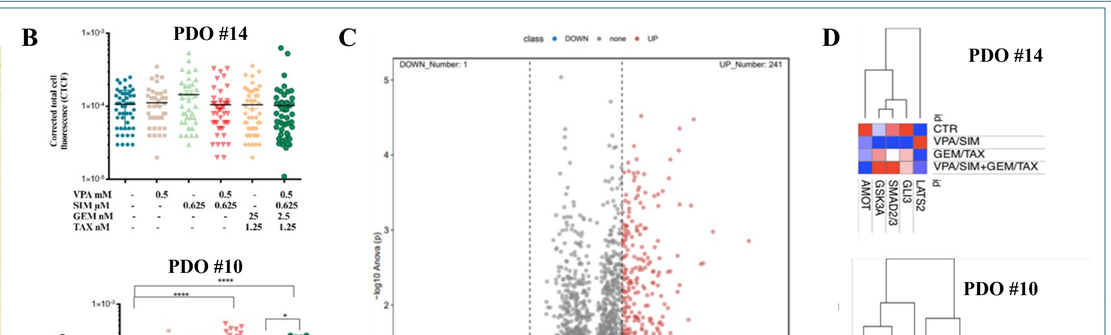
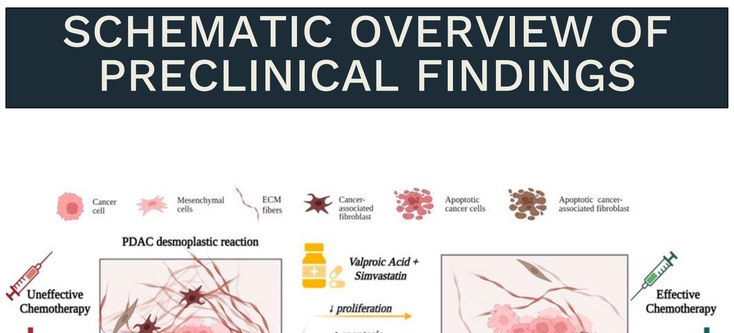


Figure 5. A. Network generated by IPA searching for known target of VPA and SIM (HDAC, HMGCR and YAP) and EMT markers (VIM and CDH1) evidencing indirect and direct interactions between them. Migration and invasion were confirmed as biological function associated with this network and "Fibrosis" came out as new important function. **B.** Drug-induced apoptosis in patient-derived organoids measured as Caspase 3 cleavage on PDO #14 (resistant to quadruple combination) and on PDO #10 (sensitive to quadruple combination). **C**. Volcano Plot of differentially expressed protein between PDO #14 and #10 at basal level. Proteins upregulated are highlighted in red (241 proteins) and downregulated protein is highlighted in light blue (1 protein). **D**. Heatmap of Hippo pathway proteins modulated by treatment in resistant PDO #14 and sensitive PDO #10. PDO #14 resulted less enriched in proteins belonging to Hippo pathway, and less modulated by treatment.

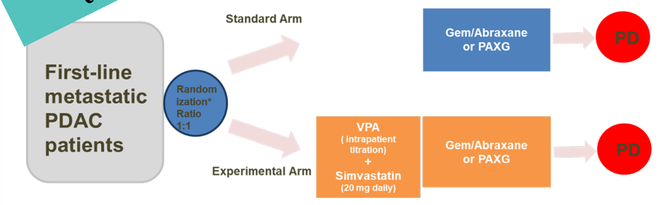
SCHEMATIC OVERVIEW OF PRECLINICAL FINDINGS



Multicentric, proof-of-concept, open label "Randomized phase 2 study of Valproic acid combinEd with Simvastatin and gemcitabine/nab-paclitaxel-based regimens in untreated mPDAC patients"-The VESPA trial

32 Patients enrolled

DESIGN OF THE STUDY

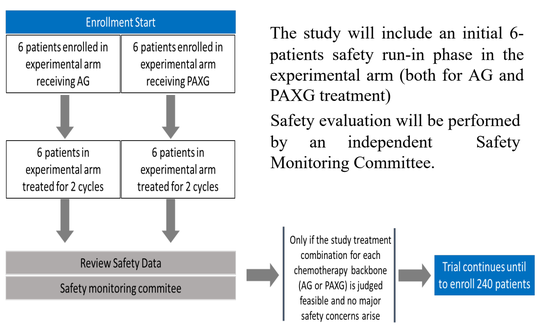


STANDARD: Nab-paclitaxel 125 mg/m^2 followed by gemcitabine 1000 mg/m^2 on days 1, 8, and 15 (AG); or nab-paclitaxel 150 mg/m^2, followed by gemcitabine 800 mg/m^2, followed by cisplatin 30 mg/m^2 on days 1 and 15, and oral capecitabine 1250 mg/m^2 on days 1–28 (PAXG).

EXPERIMENTAL: Chemotherapy (AG or PAXG) + simvastatin oral daily at a fixed dosage of 20 mg in combination with increasing doses of VPA administered oral daily from day -7 with an intra-patient titration for a final target serum level of 50-100 µg/ml.

A total of 240 patients, 120 for arm, will be enrolled

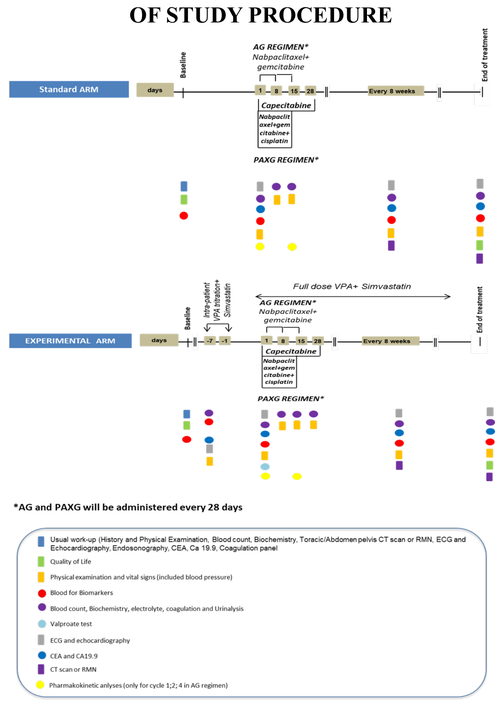
SAFETY RUN-IN PHASE



The study will include an initial 6-patients safety run-in phase in the experimental arm (both for AG and PAXG treatment)

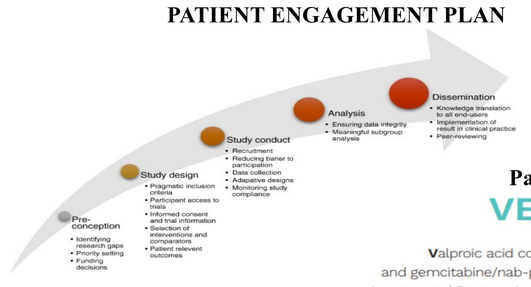
Safety evaluation will be performed by an independent Safety Monitoring Committee.

SCHEMATIC TIMELINE OF STUDY PROCEDURE



*AG and PAXG will be administered every 28 days

PATIENT ENGAGEMENT PLAN



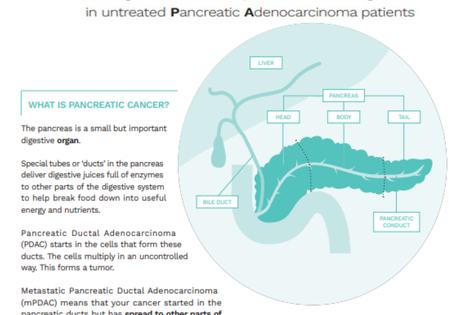
Patient Fact-sheet

VESPA Trial

Valproic acid combin**E**d with **S**imvastatin and gemcitabine/nab-paclitaxel based regimens in untreated **P**ancreatic **A**denocarcinoma patients

The VESPA trial was planned as patient centric since the research design was defined in collaboration with the patient association (Eurordis, Beacon, CPE) as well as with European Clinical Research Infrastructure Network (ECRIN).

Patients contribute to each step of the study, being an active member of the study Steering Committee and Safety Monitoring Committee, to be sure to include patients' needs, as patient-oriented outcomes, design interventions, moral and ethical concerns and safety assessments.



STUDY OBJECTIVES

PRIMARY ENDPOINT	SECONDARY ENDPOINTS
PROGRESSION FREE SURVIVAL	OBJECTIVE TUMOR RESPONSE RATE (ORR)
	DURATION OF OBJECTIVE RESPONSE (DOR)
	DISEASE CONTROL RATE (DCR)
	OVERALL SURVIVAL (OS)
	OVERALL TOXICITY RATE
	QUALITY OF LIFE (QOL)
EXPLORATORY OBJECTIVES	
DRUGS PHARMACOKINETICS	
METABOLOMIC PROFILING	
CIRCULATING CYTOKINES AND CHEMOKINES	
CIRCULATING MICRORNAS EVALUATED AND CFTDNA	
PROGNOSTIC AND PREDICTIVE VALUE OF TUMOR DNA MUTATIONAL PROFILING	
TRANSCRIPTOMICS PROFILING	
PROGNOSTIC AND PREDICTIVE VALUE OF SEVERAL BIOMARKERS EVALUATED BY IMMUNOHISTOCHEMISTRY	

CENTERS

- Istituto Nazionale dei Tumori, IRCCS Fondazione G. Pascale- UOC Oncologia Clinica Sperimentale Addome, Naples, Italy.
- University of Verona Hospital Trust, Department of Oncology, Pancreas Institute, Verona, Italy.
- Università Vita e Salute, IRCCS San Raffaele, Department of Oncology, Pancreas Center, Milan, Italy.
- Università Cattolica Del Sacro Cuore, IRCCS Fondazione Policlinico Universitario Gemelli- Medical Oncology, Rome, Italy.
- Ramon y Cajal Hospital and Health Research Institute (IRYCIS), Madrid, Spain.

METHODS

Cell Culture The pancreatic cancer cell lines PANC1_ATCC, ASPC1, MiaPaca2, PANC28, l3.6pl e COLO_357, and the mouse pancreatic cancer cell lines KPC_11 e KPC_95 were maintained as monolayer cultures and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose, glutamine, and nonessential amino acids and supplemented with 10% heat-inactivated fetal bovine serum and penicillin (100 IU/mL)–streptomycin (100 µg/mL). BxPC3 cell line were grown in RPMI (Roswell Park Memorial Institute) supplemented with 10% fetal bovine serum (FBS, Cambrex, Belgium) heat-inactivated, 50 units/ml penicillin (Cambrex, Belgium), 500 g/ml streptomycin (Cambrex, Belgium), and glutamine 4 mM. ***Proliferation assay***. Cell proliferation was measured in 96-well plates in cells untreated and treated with described drugs as single agent or in combination. Cell proliferation was measured using a spectrophotometric dye incorporation assay (Sulforhodamine B). Synergism, additivity, and antagonism were quantified after an evaluation of the CI, which was calculated by the Chou-Talalay equation with CalcuSyn software (Biosoft,Cambridge, UK). Cell proliferation of PDX-derived cell lines, 185 and 215, was measured using using Cristal Violet colorimetric assay and expressed as absorbance at 590 nm. ***Microtissues.*** PANC1 cells were marked using a fluorescent probe-cell tracker and cultured as microtissues by the ULA System (PerkinElmer). Cancer cells were co-cultured with human pancreatic stellate cells in a ratio of 3:1 as described in literature (Brancato V. et al. 2020) and untreated or treated with drugs for 96h. 3D microtissues were maintained in the incubator and photographed by Opera Phenix microscope (PerkinHelmer) air objective magnification 5X. **In vivo**. All studies have been performed in accordance with "Directive 2010/63/EU on the protection of Animals used for scientific purposes" and made effective in Italy by the Legislative Decree DLGS 26/2014. Female athymic nude mice (NCI-nu), which were 6- to 8-weeks old, were purchased from Envigo Laboratories (Huntingdon, UK). The mice were acclimatized in the Animal Care Facility of CROM–Centro Ricerche Oncologiche di Mercogliano. To produce pancreatic tumors, PANC1-LUC cells were harvested from subconfluent cultures and resuspended in PBS solution. **RNA-Seq** protocol was performed as differential gene expression analysis using Illumina sequencing. Computational analysis including quality control, quantification of gene expression, and differential expression was performing at Mario Negri Institute, Milan. **Patient- Derived Organoids (PDO)** . PDOs were generated from tumor surgical procedures of primitive pancreatic cancer spicemnet. Tumor tissue was processed incubating them in digestion media and plated in matrigel and treated as indicated in the figure. To monitor the sensitivity to different treatments PDO were photographed with air objective magnification 10X and scored by CellEvent Caspase 3/7 Green (Invitrogen, Thermo Fisher Scientific). **Proteomic analysis**. Differentially expressed proteins by Patient-derived organoids at basal level as well as upon treatment was performed by mass spectrometry (LC-MS/MS) and quantified by Progenesis QI for proteomics v. 4.2. Data were filtered using a global FDR < 5% and only proteins with at least one unique identical peptide sequence (p-value < 0.05) were considered identified.

CORRESPOND TO

Alfredo Budillon
a.budillon@istitutotumori.na.it
Alessandra Leone
a.leone@istitutotumori.na.it
Antonio Avallone
a.avallone@istitutotumori.na.it
For Info on VESPA trial
infopazienti@studiovespa.it