

ESMO Translational Research Fellowship

(09/2018 – 09/2020)

Dr Federica Papaccio

FINAL REPORT

Host Institute: **INCLIVA Biomedical Research Institute, Valencia, Spain**

Mentor: **Prof Dr Andres Cervantes, MD PhD**

Project title: **Development of a “living organoid biobank” derived from colorectal cancer patients. Towards a personalized medicine in cancer.**

Home Institute: **Department of Medical Oncology, Università della Campania “L. Vanvitelli”, Naples, Italy**

Rationale and Aim

3D organoids models are based on the capacity of tissue stem cells to reconstitute the diversity of the tissue cell populations. Tissue stem cells require some specific niche factors that are present in their microenvironment. Once embedded in a 3D matrix and in the presence of these factors, human epithelial tissues from different organs, like stomach, small intestine, pancreas duct, liver, bladder and colon can efficiently form organoids (Baker et al. 2010; Boj et al., 2015, Huch et al 2013, Sato et al., 2011; Varley CL et al., 2011). These 3D organoids models are of great interest for both basic and translational research (Fatehullah et al., 2016).

This “mini-organ” culture system can be applied to healthy as well as cancer tissues although the niche factors requirements change during tumorigenesis and some of these factors became dispensable leading to a less stringent culture condition for cancer organoids as compared to healthy normal organoids (Fujii et al., 2016). In a recent publication, it was described that the patient-derived organoids (PDOs) from metastatic, heavily pretreated colorectal (CRC) and gastroesophageal cancer patients show a phenotypic and genotypic profile very similar to those of the original tumor (Vlachogiannis et al., 2018). It is for that reason that PDOs could be used to test alternative treatments, in order to improve the response of the patient, emerging as robust preclinical models.

Therefore, the principal aim of the project is to generate a biobank of PDOs (from both healthy and tumor tissue), that could be used to test different treatments as well as to study the underlying molecular causes of cancer and treatment resistance.

Experimental design

- 1.- To set up an organoids' biobank derived from colorectal cancer tissues with different grades and belonging to different subtypes collected together with the corresponding healthy tissues.
 - 2.- To characterize each organoid by gene expression analysis evaluating if it could reflect all genetic changes and gene expression patterns detected among the original patient tissue.
 - 3.- To study if tumor organoids faithfully recapitulate clinical phenotypes of patient's tumors.
 - 4.- To perform *in vitro* drug testing to predict treatment response in the patient.
- In fig. 1 an overview of the project.

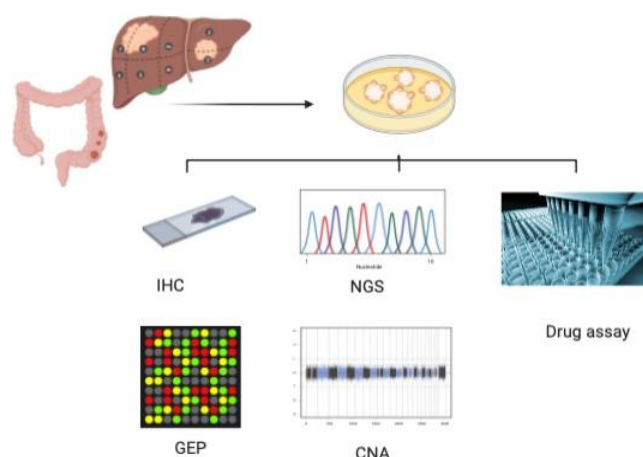


Fig. 1 Overview of the project. IHC: immunohistochemistry; NGS: next generation sequencing; GEP: gene expression profile; CAN: copy number alteration.

Results, Conclusions and Future Perspectives

During the first six months of the project an efficient protocol for the isolation of PDO's has been developed from both surgical pieces and biopsies from consenting colorectal cancer patients from stage I to IV, with a success rate of about 80%. A database has been developed to match patient's characteristics with that of PDO's.

The biobank of CRC living organoids has been established and increased in number. A protocol for expansion and cryopreservation in liquid nitrogen has been developed. Clear growth is detectable along days and passages.

From october 2018 to october 2020 a total number of 34 patients with locally advanced or metastatic colorectal cancer have been included. A total of 50 samples has been collected and processed, obtaining 29 PDOs lines out of 23 patients for whom organoid growth have been observed. This biobank includes PDOs from both first, second and more advanced lines of treatment, going from never-treated patients, to more than 6-months chemo-free, to heavily pretreated samples.

Once established and amplified PDOs are morphologically and molecularly characterized. As regards morphological characterization hematoxylin and eosin staining of paraffin embedded organoids is performed. Immunohistochemistry analysis is also done using markers currently used for CRC diagnosis: alcyan blue, MUC2/5, CDX2, CK20 and Ki67. Their morphology is comparable with that of corresponding patients (fig. 2).

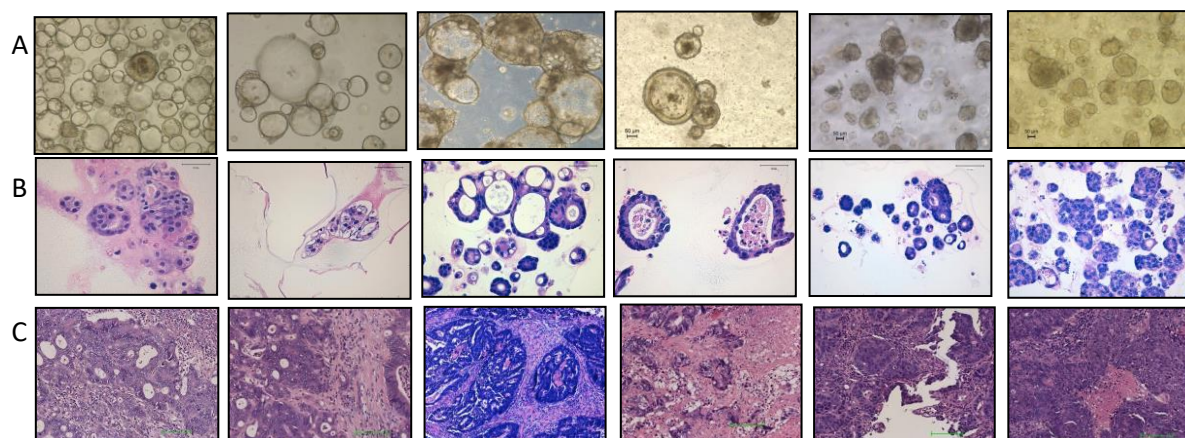


Fig. 2 A: in vitro imaging of selected PDOs with their matched hematoxylin and eosin (H&E) staining (B) and their matched H&E staining of original tumour tissue (C).

In order to perform genomic characterization of PDO's, DNA has been extracted, quantified with Bioanalyzer. Libraries have been prepared and sequenced with an in-house panel (Illumina) and matched with patient's original tissue. In the table 1 are shown some examples of mutations detected. A good concordance has been observed.

Patient	Gene	Mutation	PDOs VAF	Tissue VAF
Pt #2	PIK3CA	p.E545K	0.464	0.2827
	KRAS	p.G12V	0.5106	0.3149
	FBXW7	p.S582P	0.4626	0.2964
Pt #7	TP53	p.C275Y	1	0,2904
	APC	p.Y935*	0,4842	0,1005
Pt #18	BRAF	p.D594G	0.5118	0.3675
Pt #24	PIK3CA	p.H1047R	0.4852	0.1529
	KRAS	p.G12D	0.4978	0.1935
	APC	p.Y935*	0.9965	0.3412
Pt #38	TP53	p.R248W	0.9967	0.5272
	APC	p.Q1367*	0.994	0.4723
	FBXW7	p.R222*	0.6828	0.2438
Pt #50	TP53	p.G245S	0,4893	0,2895
	PIK3R1	p.R348*	0,4993	0,1592
Pt #66	TP53	p.M237I	0,988	0,8701
	KRAS	p.G12V	0,4407	0,4611
	APC	p.E1379*	0,998	0,9554

Table 1. Main driver mutations are shown for a selection of PDOs. VAF: variant allelic fraction.

In order to obtain a more accurate genomic characterization copy number variation (CNV) analysis has been performed with Cytoscan HD (Affymetrix) from PDOs and matched fresh tumour tissue. We were able to detect both gain and losses in copy numbers in both PDOs and matched fresh tumour tissue and to identify genes of interest included in these CNA regions.

CNV of selected genes has been validated with droplet-digital PCR (ddPCR). CNV detection by ddPCR confirms that detected by Cytoscan HD.

Total RNA has been extracted, quantified with Bioanalyzer, libraries have been sequenced with MySeq Illumina. Raw data have been processed according to a bioinformatic pipeline. Normalized reads have been mapped and annotated. The comparison between tissue and PDO expression shows high correlation, indicating that our models preserve in vitro gene expression pattern of original tissue (fig. 3).

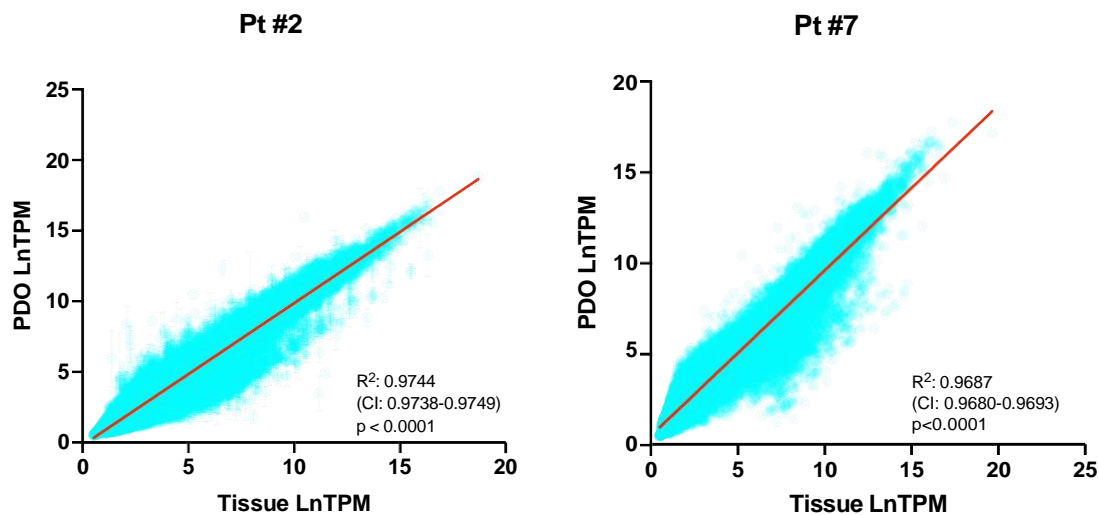


Fig. 3 Comparison of gene expression profile of selected PDOs with their matched tissue.

We developed a protocol for drug assay testing. Briefly, once established PDOs were trypsinized till single cells and plated in a 96-w-plate at a density of 3000 cells/well. After 48h drug treatment with common chemotherapeutic or targeted drugs has been performed at different doses. After 120h CellTiterGlo luminescence test has been performed in order to assess viability. Dose-response curves are built in GraphPad v.8.1 software. Experiments have been conducted with three technical replicates and with at least two biological independent experiments.

In figure 4 there is an example of a RAS wt PDO (Pt #7) treated with erlotinib (used as in vitro anti-EGFR inhibitor). Results are in line with the response of the patient in clinic to cetuximab.

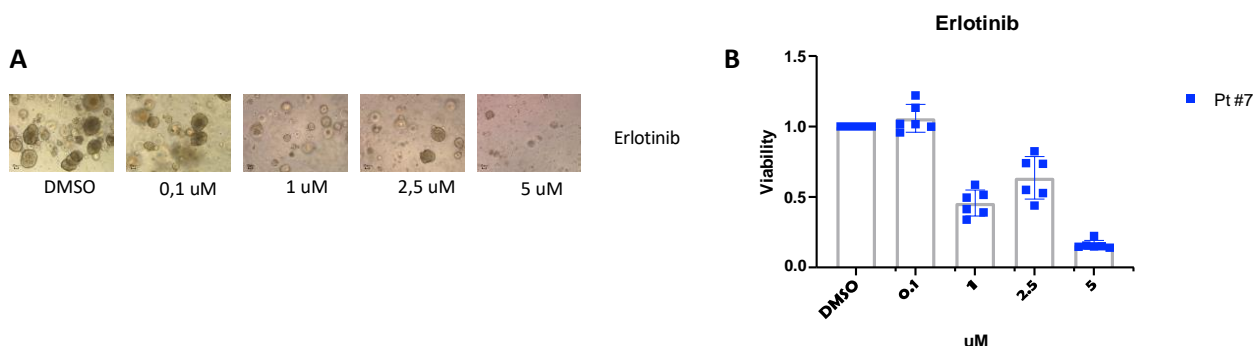


Fig. 4 A: In vitro response of pt#7 PDOs to increasing doses of erlotinib. B: dose-response graph.

In conclusion, we developed a robust efficient protocol for PDOs generation from CRC patients. Our PDOs faithfully recapitulate genomic and phenotypic characteristics of the original tissue. We developed a platform for drug assay with tremendous potential applications as a tool for precision medicine and drug discovery.

List of Publications and Presentations Resulting from the Translational Research Project

- 1) "Development of a living organoid biobank derived from colorectal cancer patients: towards personalized medicine" **Papaccio F**; Gutierrez-Bravo MF, Cabeza-Segura M, Gambardella V, Huerta MS, Martinez-Ciarpaglini C, Rosello S, and others. Annals of Oncology, Vol. 30, v240–v241. (poster ESMO Congress 2019)
- 2) "CNAs concordance between CRC-PDOs and the original tissue reveals relevant oncogenic pathways alterations" **F. Papaccio**, F.G. Valiente, M.F. Gutierrez-Bravo, M. Cabeza-Segura, V. Gambardella, M.D.S. Huerta, C. Martinez-Ciarpaglini, and others Annals of Oncology, Vol. 30, vii9 (poster MAP Congress 2019)
- 3) "Metastatic colorectal cancer derived organoids recapitulate genomic profile and treatment response of the original tumor" **F. Papaccio**, M.F. Gutierrez Bravo, M. Cabeza-Segura, F. Gimeno-Valiente, V. Gambardella, Z. Garzón-Lloría, B. García-Micó, M. Huerta, and others. Annals of Oncology, Vol. 31, S449 (poster ESMO Congress 2020)

At least one research article is expected to be published in the next months.

List of Publications and Presentations resulting from other projects during the fellowship period (if applicable)

- 1) "ZNF518B as a transcriptional factor involved in colorectal cancer progression through the epithelial to mesenchymal transition" F.G. Valiente, N. Tarazona, V. Gambardella, Á Riffo-Campos, **F. Papaccio**, M. Cabeza-Segura, G. López-Rodas, A. Cervantes, and others. Annals of Oncology, Vol. 30, vii16 (poster MAP Congress 2019)
- 2) "An integrated analysis of gene expression profile and chromosomal aberrations highlighted the oxidative stress role in anti-HER2 drug-resistant gastric cancer models" V. Gambardella, F. Gimeno-Valiente, M. Cabeza, **F. Papaccio**, S. Zúñiga-Trejos, J.A. Carbonell-Asins, P. Rentero-Garrido, M. Huerta, and others. Annals of Oncology, Vol. 31, S927 (poster ESMO Congress 2020)
- 3) "3D patient-derived gastric cancer organoids as a tool for functional precision medicine for gastric cancer patients" M. Cabeza, **F. Papaccio**, M.F. Gutierrez Bravo, V. Gambardella, C. Alfaro-Cervello, C. Martínez-Ciarpaglini, Z. Garzón-Lloría, P. Rentero-Garrido, and others. Annals of Oncology, Vol. 31, S919. (poster ESMO Congress 2020)
- 4) "Patient-derived organoids as a tool for modelling localized colorectal cancer" M.F. Gutierrez Bravo, **F. Papaccio**, M. Cabeza, C. Martínez-Ciarpaglini, C. Alfaro-Cervello, Z. Garzón-Lloría, A. Ferrer-Martínez, B. García-Micó, and others Annals of Oncology, Vol. 31, S448. (poster ESMO Congress 2020)
- 5) "Neoadjuvant Chemotherapy in Locally Advanced Rectal Cancer" **Papaccio F**, Roselló S, Huerta M, Gambardella V, Tarazona N, Fleitas T, Roda D, Cervantes A. Cancers (Basel). 2020 Dec 3;12(12):E3611. doi: 10.3390/cancers12123611.
- 6) Precision Medicine to Treat Advanced Gastroesophageal Adenocarcinoma: A Work in Progress. Gambardella V, Fleitas T, Tarazona N, **Papaccio F**, Huerta M, Roselló S, Gimeno-Valiente F, Roda D, Cervantes A. J Clin Med. 2020 Sep 22;9(9):3049. doi: 10.3390/jcm9093049.
- 7) Detection of postoperative plasma circulating tumour DNA and lack of CDX2 expression as markers of recurrence in patients with localised colon cancer. Tarazona N, Gimeno-Valiente F, Gambardella V, Huerta M, Roselló S, Zuniga S, Calon A, Carbonell-Asins JA, Fontana E, Martinez-Ciarpaglini C, Eason K, Rentero-Garrido P, Fleitas T, **Papaccio F**, Moro-Valdezate D, Nyamundanda G, Castillo J, Espí A, Sadanandam A, Roda D, Cervantes A. ESMO Open. 2020 Sep;5(5):e000847. doi: 10.1136/esmoopen-2020-000847.

Selection of Courses and Workshops Attended During the Fellowship

- 1) International Symposium on Advanced Ovarian Cancer – Valencia 2019
- 2) International Gastric Cancer Conference – Prague 2019
- 3) Europea Symposium on Organoids – Milan 2019
- 4) ESMO Congress – Barcelona 2019
- 5) MAP Congress – London 2019
- 6) ESMO Congress – 2020
- 7) MAP Congress - 2020

Valencia, 10.12.2020

Fellow

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