T.C.
DOKUZ EYLÜL UNIVERSITY
IZMIR INTERNATIONAL
BIOMEDICINE AND GENOME
INSTITUTE

PATIENT-DERIVED TUMOR ORGANOIDS FOR PREDICTION OF DRUG RESPONSE

EMİNE BERNA BIÇAK

MOLECULAR BIOLOGY AND GENETICS
MASTER'S PROGRAM

MASTER OF SCIENCE THESIS

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Dokuz Eylül Üniversitesi İzmir Uluslararası Biyotıp ve Genom Enstitüsü Genom Bilimleri ve Moleküler Biyoteknoloji Anabilim Dalı, Moleküler Biyoloji ve Genetik ~~Doktora~~ /Yüksek Lisans programı öğrencisi Emine Berna Bıçak "İlaç Yanıtının Tahmini İçin Hasta Kaynaklı Tümör Organoidleri" konulu Yüksek Lisans tezini 16/06/2022 tarihinde başarılı olarak tamamlamıştır.

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Emine Berna Bıçak, who is a student at the Department of Molecular Biology and Genetic Master's program of the Dokuz Eylul University's Izmir International Biomedicine and Genome Institute, with the student ID 2018850021, succeeded her Master's thesis titled "Patient Derived Tumor Organoids for Prediction of Drug Response" as a result of her thesis defense on 16/06/2022.

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ABBREVIATIONS

3D	Three Dimensional
5-FU	5 Fluorouracil
ASCs	Adult Stem Cells
CAPIRI	Capecitabine and Irınotecan
CAPOX	Capecitabine and Oxaliplatin
CRC	Colorectal Cancer
ECM	Extracellular Matrix
EGF	Epidermal Growth Factor
EM	Expansion Medium
ESCs	Embriyonic Stem Cells
FAP	Familial Adenomatous Polyposis
FDA	Food and Drug Administration
FOLFIRI	5-FU and Irınotecan
FOLFOX	5-FU and Oxaliplatin
H&E	Hematoxylin and Eosin Stain
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
IC_{50}	Half Maximal Inhibitory Concentration
iPSCs	Induced Pluripotent Stem Cells
LARC	Locally Advanced Rectal Cancer
PDCO	Patient Derived Cancer Organoids
PDO	Patient Derived Organoids
PDX	Patient Derived Xenografts
TRG	Tumor Regression Grade

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PATIENT-DERIVED TUMOR ORGANOIDS FOR PREDICTION OF DRUG RESPONSE

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ABSTRACT

A key limitation in cancer treatment is the lack of valid predictive biomarkers, which reduces the efficacy of treatments. Personalized medicine strategies include protein-, RNA-based and genome-based stratification, though in oncology, precision medicine has been largely based on genomic biomarkers. However, less than half of patients are eligible for genetically matched treatment and for the majority of anticancer agents no genetic markers are available. A promising predictive biomarker is individualized tumor response testing using patient-derived organoids (PDOs), in which anticancer agents are screened ex vivo on PDOs to predict clinical response. PDOs have been developed for a variety of tumors and are stem-cell derived, three-dimensional self- organizing structures comprised of epithelial cells, mimicking its corresponding tumor. This study aims to establish the organoids of colorectal cancer (CRC) patients by using in-house protocol and to predict drug response. The patient cohort of our study consists of CRC patients over the age of 18. Fresh surgical material taken from the patients reached our laboratory within 24 hours at +4^{o}C. After dissociating the tumor tissue mechanically and chemically, cells were counted and embedded in matrigel. After 3-4 week culture, organoids were tyrpsinized into single cell and passaged into matrigel containing 96 well plate as 6000 cells/5ul dome. Whenever organoids reformed, drug (5-FU, FOLFOX, FOLFIRI, CAPOX,..) were added in different concentrations. After 7 day incubation, viability analyses were performed by CellTiter-Glo 3D Viability Assay kit. All experiments were replicated four times and statistical analyses were done by one-way ANOVA. Finally, IC_{50} of drugs were calculated by GraphPad Prism 8 program. We were able to establish organoids from tumor tissues with a success rate of 90%. By performing drug screening for various anti-cancer agents on these organoids, we determined that each patient's response to treatments is different. We have seen that targeted antibodies increase the efficacy of treatment in one patient. We have successfully predict drug responses for 5 patients as a proof of concept study.

Key words: Patient-derived organoid (PDO), colorectal cancer (CRC), drug screening

İLAÇ YANITININ TAHMİNİ İÇİN HASTA KAYNAKLI TÜMÖR ORGANOİDLERİ

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ÖZET

Kanser tedavisinde önemli bir sınırlama, tedavilerin etkinliğini azaltan geçerli öngörücü biyobelirteçlerin olmamasıdır. Kişiselleştirilmiş tıp stratejileri, protein, RNA bazlı ve genom bazlı tabakalaşmayı içerir, ancak onkolojide hassas tıp büyük ölçüde genomik biyobelirteçlere dayanmaktadır. Bununla birlikte, hastaların yarısından azı genetik olarak uyumlu tedavi için uygundur ve antikanser ajanların çoğu için hiçbir genetik belirteç mevcut değildir. Umut verici bir öngörücü biyobelirteç, klinik yanıtı tahmin etmek için antikanser ajanların PDO'larda ex vivo olarak tarandığı, hastadan türetilmiş organoidler (PDO) kullanılarak bireyselleştirilmiş tümör yanıtı testidir. PDO'lar çeşitli tümörler için geliştirilmiştir ve kök hücreden türetilmiş, epitel hücrelerinden oluşan ve karşılık gelen tümörü taklit eden üç boyutlu kendi kendini organize eden yapılardır. Bu çalışma, kurum içi protokol kullanarak kolorektal kanser (CRC) hastalarının organoidlerini oluşturmayı ve ilaç yanıtını tahmin etmeyi amaçlamaktadır. Çalışmamızın hasta grubunu 18 yaş üstü CRC hastaları oluşturmaktadır. Hastalardan alınan taze cerrahi materyal +4ºC'de 24 saat içerisinde laboratuvarımıza ulaştırıldı. Tümör dokusu mekanik ve kimyasal olarak ayrıştırıldıktan sonra hücreler sayıldı ve matrigel içine gömüldü. 3-4 haftalık kültürden sonra, organoidler tek hücreye dönüştürüldü ve 6000 hücre/5ul dot olarak 96 oyuklu plaka içeren matrigel içine gömüldü. Organoidler tekrar oluştuğunda, ilaç (5-FU, FOLFOX, FOLFIRI, CAPOX,..) farklı konsantrasyonlarda eklendi. 7 günlük inkübasyondan sonra, CellTiter-Glo 3D Viability Assay kiti ile canlılık analizleri yapıldı. Tüm deneyler dört tekrarlı gerçekleştirildi ve istatistiksel analizler One Way ANOVA ile yapıldı. Son olarak GraphPad Prism 8 programı ile ilaçların IC_{50} değerleri hesaplandı. Tümör dokularından %90 başarı oranıyla organoidler oluşturabildik. Bu organoidler üzerinde çeşitli anti-kanser ajanları için ilaç taraması yaparak her hastanın tedaviye verdiği yanıtın farklı olduğunu belirledik. Hedeflenen antikorların bir hastada tedavinin etkinliğini artırdığını gördük. Kavram çalışmasının bir kanıtı olarak 5 hasta için ilaç yanıtlarını başarılı bir şekilde tahmin ettik.

Anahtar Kelimeler: Hasta kaynaklı organoid, kolorektal kanser, ilaç taraması

1. INTRODUCTION AND PURPOSE

1.1 Statement and Importance of the Problem

Colorectal cancer is the third most common type of cancer in the world. And it ranks second in cancer-related deaths. Although there have been important developments in cancer treatment strategies with the development of technology, there are still limitations. A major limitation in cancer therapy is the lack of valid predictive biomarkers that reduce the efficacy of treatments. Personalized medicine strategies include protein, RNA-based and genome-based stratification, but precision medicine in oncology relies heavily on genomic biomarkers. However, less than half of patients are eligible for genetically matched therapy, and no genetic markers are available for most anticancer agents. For this reason, a modeling tool is required to predict clinical response and save the patient from the severe side effects and loss of time of ineffective treatments. PDCOs, in which anticancer agents can be screened ex vivo, are a promising predictive biomarker.

1.2 Aim of the Study

In this study, our aim is to establish organoid culture from tissues taken from patients diagnosed with CRC by using in-house protocol and to predict drug response by performing drug screenings.

1.3 The hypothesis of the Study

We hypothesize that patient-derived cancer organoids can be used for prediction of drug response. Live biobanks can be created from PDCOs that are subsequently expanded and cryopreserved.

GENERAL INFORMATION

2.1 Organoid Based Personalized Medicine

Organoids are 3D structures made up of self-organizing cells that can mimic the functional and structural properties of an organ. These features are that it contains more than one cell type, has an organization similar to the cell organization of the organ, and has the ability to perform organ-specific functions. In addition, it mimics the typical organization that the organ performs during its embryonic development. Organoids have become an in vitro modeling tool that preserves the genetic, phenotypic and behavioral characteristics of organs in recent years (Lancaster & Knoblich, 2014). Signaling pathways are important in organ development and these pathways direct organoid formation. Therefore, there is a need for organ-specific growth factors and inhibitors in the organoid growth medium.

Organoids can be produced from both pluripotent stem cells (embryonic stem cells and induced pluripotent stem cells) and adult stem cells. Using these starting materials, organoids of many organs such as retina, stomach, lung, small intestine, colon and liver were formed. (Clevers, 2016) Organoid culture can be established from healthy tissues as well as from diseased tissues. Disease modeling can be done with tissues taken from patients or with organoids genetically manipulated with CRISPR-Cas9 technology (Li et al., 2020).

Firstly, Yoshiki Sasai et al. tested the mimicry of development in living things with organoid culture. As a result, they cultured the brain, retina, and pituitary gland. Later, methods were developed and organoids of intestine, stomach, liver, lung and kidney were obtained from iPSC. There are 3 different germ layers where each of these organs develops. iPSCs are differentiated into these germ layers in the presence of cocktail media and various growth factors (Figure 2.1). Since the embryonic development of organs is summarized during this differentiation protocol, it makes it possible to model developmental diseases, genetic diseases and infectious diseases (Li et al., 2020).

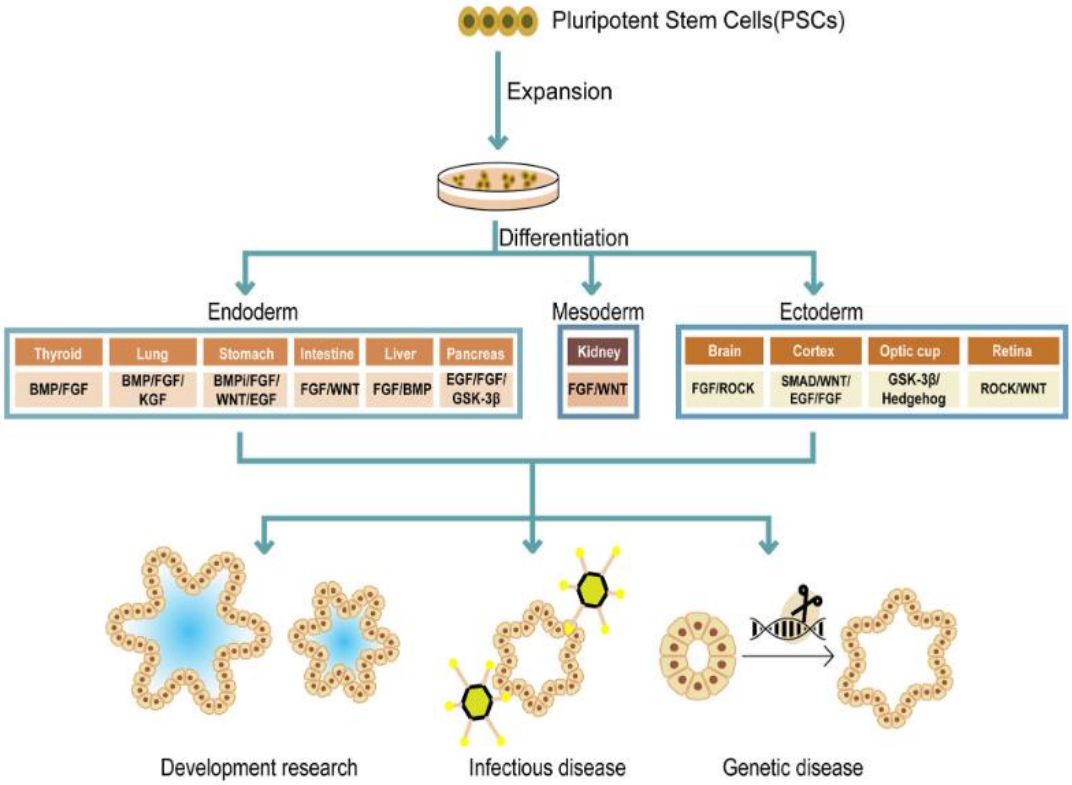

Figure 2.1: Culture strategy and applications of plurtotent stem cell derived organoids. (Li et al., 2020)

Organoids produced from ASCs, on the other hand, brought a new perspective to modeling. First, Sato et al obtained intestinal organoids by isolating LGR5+ stem cells that can differentiate into all intestinal cells. Stem cells embedded in ECM were cultured with Wnt agonist R-spondin, epidermal growth factor (EGF), bone morphogenetic protein inhibitor Noggin and showed that these cells self-organized, proliferated and differentiated. Since then, patient-derived organoids have been made from tissue obtained through biopsy or surgical resection by modifying growth factor cocktails. Organoids formed after an average of 2 weeks can be expanded by passage and frozen to form a biobank. They also maintain their genetic stabilization throughout the passages. These advantages put organoids a step ahead for disease modeling. In addition, patient-derived organoid cultures are obtained from both healthy and patient cells of the patient, allowing them to grow in parallel. Thus, living tumor organoid biobanks can be created and these biobanks enable personalized therapy applications (Li et al., 2020).

2.1.1 Importance of Personalized Medicine in Cancer

Cancer was the cause of nearly 10 million deaths in 2020. The most common types of cancer are breast, lung, colon and prostate cancers. About 70% of cancer deaths are caused by tobacco use, alcohol consumption, low fruit and vegetable consumption, and lack of physical activity. The most important factors that increase survival are early diagnosis and effective treatment. With the development of new generation sequencing technology, the genetic structure of many cancer types has been clarified. In the light of this genetic information, research and development studies have been carried out for molecular targeted drugs. Despite these advances, treatment options for some types of cancer are limited. Each patient's response to therapeutics is different due to their genetic makeup. This has a great impact on the course of treatment. For this reason, the selection of therapeutic agents should be based on genetic changes rather than cancer localization (Figure 2.2). With personalized treatment, severe side effects and time loss caused by useless treatments given to the patient can be prevented (Shiihara & Furukawa, 2022).

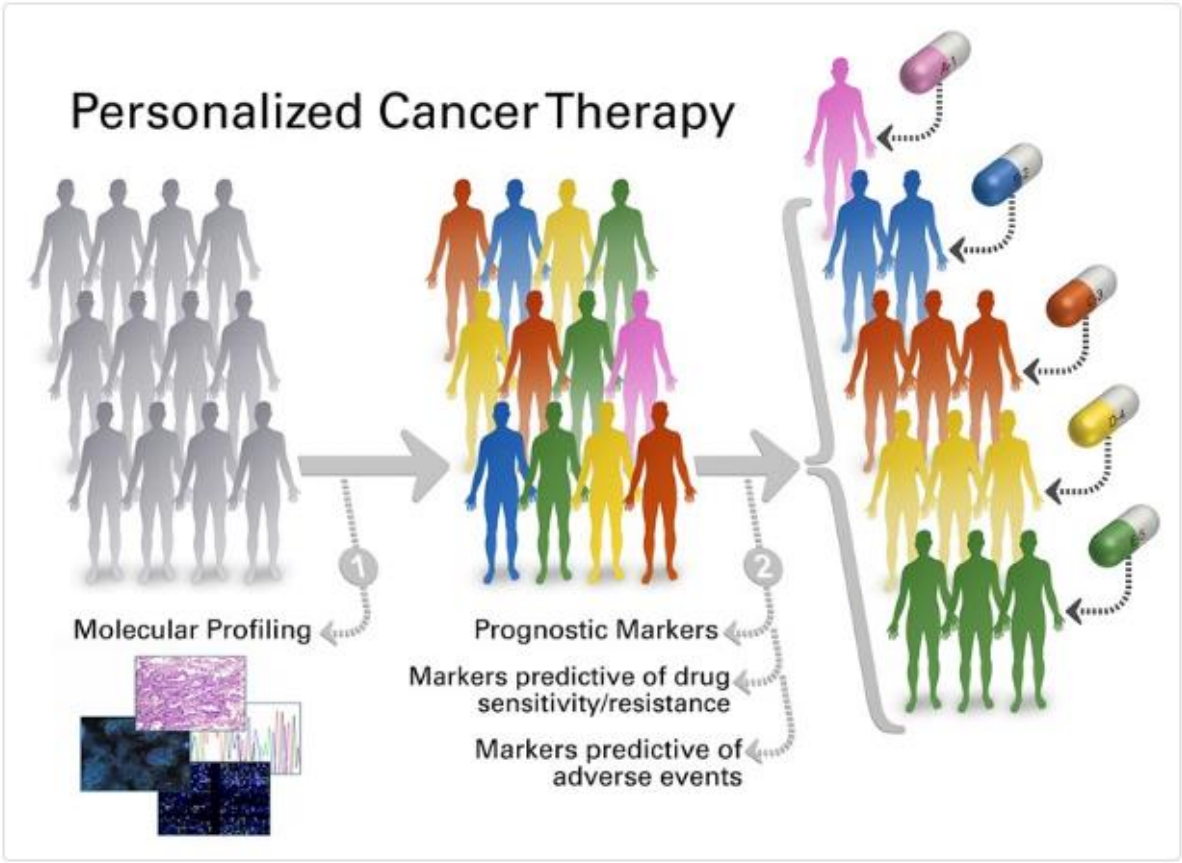

Figure 2.2: Personalized therapy steps. (pct.mdanderson.org, 2022)

Personalized medicine is an approach to create a treatment strategy by considering the genomic profile of the patient, lifestyle, and environmental conditions. In order to find the most accurate treatment strategy in cancer treatment, the genomic profile of the tumor should be looked at. Anticancer agents selected according to the genomic profile increase the efficiency of the treatment. To confirm these selections, drug screening should be performed on tumor-derived cell lines. Susceptibility testing of patient-derived cell lines to anticancer agents is a viable tool to investigate molecular targeted therapies. However, cell lines do not always accurately recapitulate the primary tumor tissue, so the results of drug scans with 2D cell lines are not accurate. Organoids are similar to the original tumor tissue from which they originated, both in terms of molecular and physical structure and function. According to studies, the genomic profile remains stable even in long-term organoid cultures. Because of these properties, patient-derived cancer organoids give more reliable results for cancer research and personalized treatments than 2D cell culture and patient-derived xenograft models, and are suitable for use as a cancer model for translational research.

2.1.2 Patient Derived Cancer Organoids

Patient-derived organoids are 3-dimensional models in which many diseases can be modeled, drug screenings and preclinical studies can be done. Briefly, PDO culture is initiated by obtaining cells by mechanically and chemically lysis of patient tissue and embedding these cells, usually in a matrigel or basement membrane extract (BME). Growth medium is added and allowed to grow so that the cells can receive the necessary nutrients and small molecules. Genetic diseases, infectious diseases, many types of cancer can be modeled with PDOs. (Li et al., 2020) PDO biobanks that can be created for many diseases are of great importance for personalized treatment (Li et al., 2020). Today, patient-derived cancer organoids can be made for many cancer types. Currently, patient-derived organoid cultures of many cancer types such as prostate, breast, colon, liver, brain and pancreas have been successfully obtained (Liu et al., 2021).

It has been proven by many studies that patient-derived cancer organoids reflect the genetic and pathological features of the primary tumor. Various immunohistochemical stains were performed and they were shown to share a common morphology with the primary tumor.

In addition, it was determined that the markers used in the diagnosis were preserved. Genome sequencing and RNAseq showed that patterns at the molecular level were conserved. These genomic patterns are maintained across passages. Patient-derived cancer organoids provide great advantages over 2D culture models in terms of cell polarization, drug response, and mutation status. Considering all the features and advantages mentioned, it is clear that PDOs have a wide range of uses in medicine (Yang et al., 2018).

2.1.3 Patient Derived Cancer Organoids vs Other Modeling Systems

Two-dimensional cultured cell lines have been used as an in vitro research tool over the past 10 years. It is easy to handle, cheaper compared to organoid culture. But the biggest shortcoming of 2D culture to cancer modeling is heterogeneity. Cell lines are homogeneous and genetic and phenotypic traits are not stable. In contrast, the original tumor tissue consists of different cell types and exhibits high heterogeneity. These shortcomings prevent cell lines from being used in personalized medicine.

Preclinical in vitro models reflecting tumor heterogeneity are needed in cancer research. Patient-derived xenograft models (PDX) were first obtained in 1953. In this modeling, primary tumor tissue is transplanted into immunodeficient mice. Tumor structure and relative proportion of tumor cells and stromal cells are largely preserved. PDXs are better than cell lines as they can reflect tumor heterogeneity. However, its establishment is difficult and the rate of expansion is slow. It is also not amenable to genetic manipulation and high-throughput analysis is very expensive. In the last 10 years, 3D cell culture technology has developed rapidly. Organoid culture is a less costly and faster modeling tool than PDXs. It can allow for genetic modifications. They can be rapidly expanded and cryopreserved and used in the formation of biobanks. They are suitable for use in high-throughput analysis (Figure 2.3) (Li et al., 2020). These advantages in organoid technology have filled many shortcomings in cell lines and PDXs, making them a promising tool in disease modeling and personalized medicine applications.

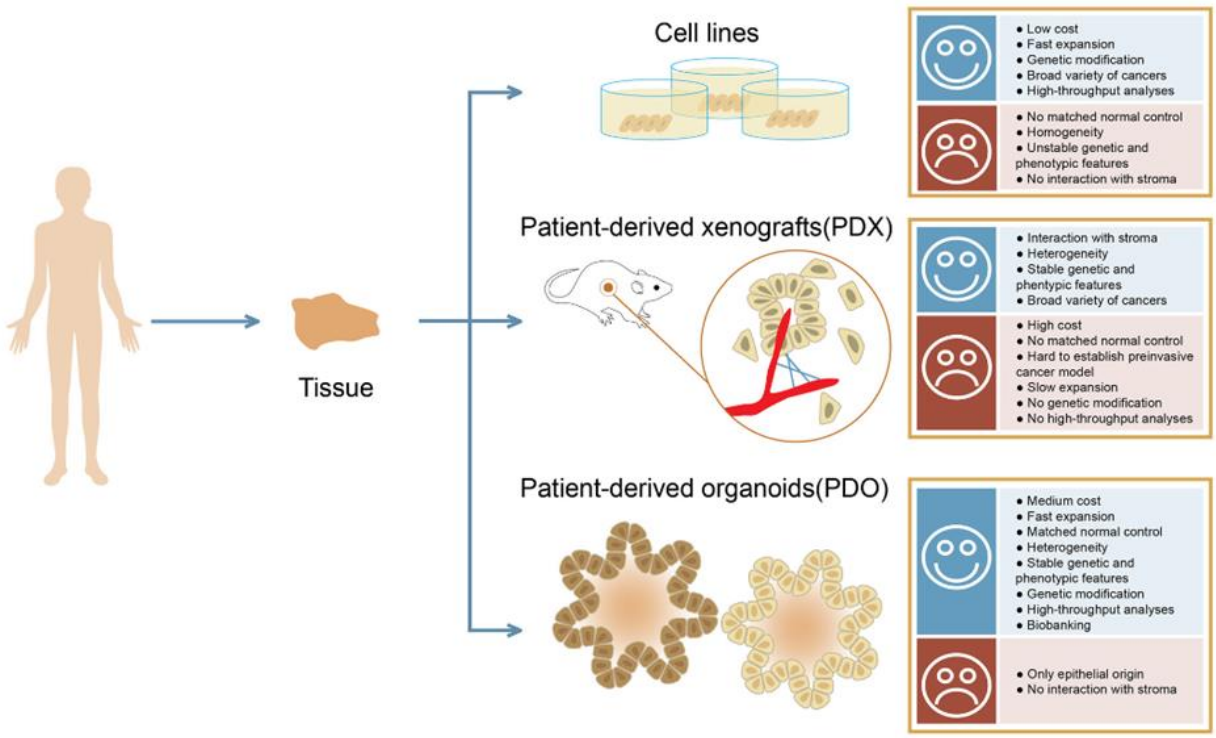

Figure 2. 3 Comparison of cell lines, patient-derived xenografts and organoids. (Li et al., 2020)

2.2 Colorectal Cancer

Colorectal cancer is the 3rd most common cancer type in the world. Despite the technological advances in diagnosis and treatment, its prevalence and fatality rate is quite high. In the last 5 years, a survival rate of less than 10% has been reported for patients with advanced stages due to failure of treatment regimens (Schmitt & Greten, 2021).

Colonoscopy is often used for the diagnosis of colorectal cancer. In addition, abdominal ultrasound and chest radiography are also performed. Good imaging of the tumor is very important for treatment planning. Endorectal ultrasound, CT, or MRI can be used for local staging. Positron emission tomography (PET) is not preferred for imaging primary cancer, but is used to detect recurrent colorectal cancer. There is no definitive method for the detection of colorectal cancer metastasis. Therefore, MRI and CT are routinely used for diagnosis (Weitz et al., 2005).

When we look at the risk factors of colorectal cancer, we can divide it into 3 classes. Sporadic cases make up the majority of 88-94%. The rate of cases caused by inflammatory

bowel disease is 1-2%. Colorectal cancer causes 70% of deaths due to ulcerative colitis. The risk of inflammatory bowel disease causing colorectal cancer varies depending on the duration of the disease and the extent of inflammation. Hereditary colorectal cancer cases occur in 5-10% of cases. It has two main forms, hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP). FAP is an autosomal dominant disease. 80% of patients have adenomatous polyposis coli (APC) gene mutation. Untreated FAP generally causes colorectal adenoma by age 40 years. HNPPC, on the other hand, is inherited in an autosomal dominant manner. HNPPC-based tumors often show a molecular feature called microsatellite instability (MSS). This molecular feature is mutations in short repetitive DNA sequences and are used in diagnosis. Tumors from HNPPC generally develop until the patient reaches 50 years of age and are usually on the right side. Diagnosis is difficult as they do not exhibit typical phenotype features (Weitz et al., 2005).

2.3 Intestinal Cancer Organoids and Colorectal Cancer Organoids

Intestinal organoids are structurally composed of highly polarized epithelial layers with a central lumen. Their crypt-like structures protrude outward from the center. While enterocytes form the lumen surface, secretion by goblet cells occurs towards the lumen (Figure 2.4).

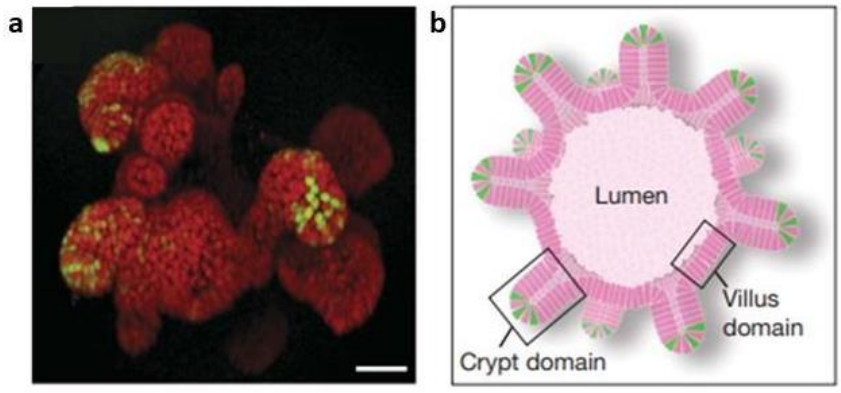

Figure 2.4: 3D reconstructed confocal image after 3 weeks in culture. (Sato et al., 2009)

(a) Lgr5–GFP+ stem cells (green) are localized at the tip of crypt-like domains.

(b) Schematic representation of a crypt organoid, consisting of a central lumen lined by villus-like epithelium and several surrounding crypt-like domains.

Intestinal cancer organoids have been established and characterized in many studies. LGR5+ stem cells located in the crypt region are stem cells that can differentiate into all intestinal cells in vivo. This suggested that intestinal organoids could be established from a single LGR5+ stem cell. Sato et al isolated crypts at the ends of intestinal villi and cultured them by embedding in matrigel, a mimic of an extracellular matrix. They characterized these organoids by performing various immunohistochemical and immunofluorescent stainings on these organoids. They are cultured in growth medium containing 3 recombinant proteins: R-spondin-1 is the LGR5 ligand and Wnt signal enhancer. Noggin is an EGF and BMP inhibitor. In addition, Wnt3a is needed because the formed colonic epithelial tissue produces very little WNT3a (Clevers, 2016; Sato et al., 2009).

Sato et al. reported that colorectal cancer organoids respond differently to Wnt3A, Rspondin-1, SB202190 and oxygen concentration. Some tumors require Wnt pathway activators, while others require a hypoxic environment (Fujii et al., 2016). The resulting CRC organoids were highly similar to primary tumors in terms of mutation and transcriptomic profile, histological subtypes. Proteomic analyzes on organoids showed that each patient's organoid has a different profile (Clevers, 2016; Xu et al., 2018).

In conclusion, the key components for establishing an intestinal organoid culture are: a potent source of WNT, a potent activator of tyrosine kinase receptor signaling like EGF, inhibition of BMP/Tgfb signals, and Matrigel. Organoid culture can be created not only from isolated LGR5+ stem cells but also from primary tissue pieces (Clevers, 2016).

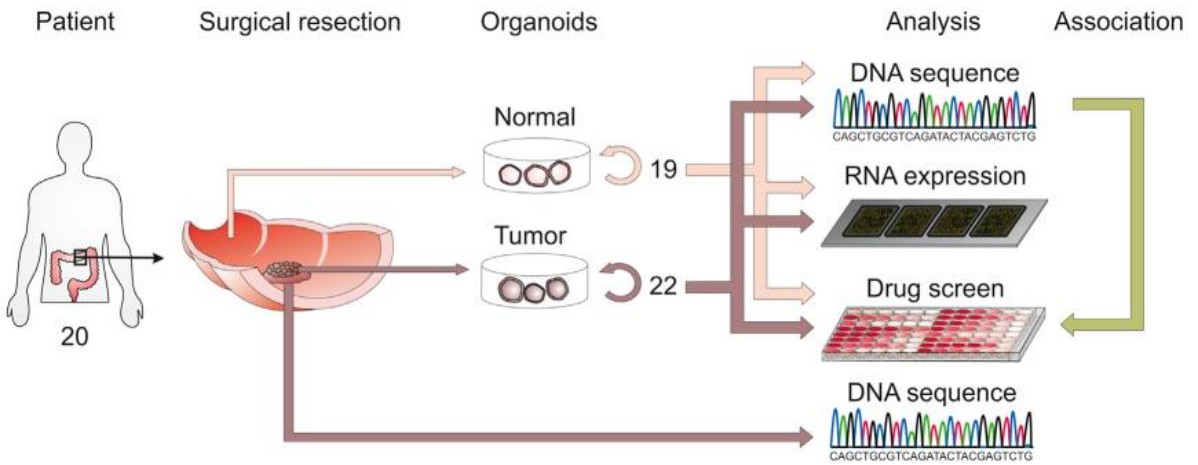

Figure 2.5: Summary of the procedure for genomic analyzes and drug screening of organoids in a CRC biobank. (van de Wetering et al., 2015)

A CRC organoid biobank was created with surgical materials containing both healthy and tumor tissue from previously untreated CRC patients. RNA sequencing analysis and DNA sequencing were performed in the created organoids and original tissues. It has been reported that the genomic alteration between CRC organoids and primary tissue is similar (Figure 2.5). When organoid sections of many patients were compared with primary tissue sections, they were shown to have similar morphology (Figure 2.6) (van de Wetering et al., 2015).

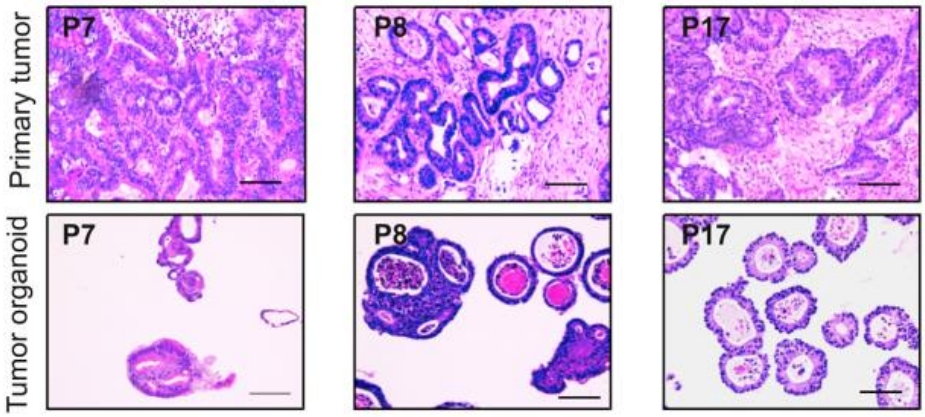

Figure 2.6: H&E staining images of primary tissue and tumor organoids derived therefrom. (van de Wetering et al., 2015)

2.4 Drug Response Prediction with Patient Derived Cancer Organoids

Despite advances in cancer treatment, 9.5 million cancer-related deaths were reported worldwide in 2018. The most important limitation in cancer treatment is the unpredictability of treatment response in individual patients. For this reason, patients both receive a costly inefficient treatment and are exposed to toxic side effects. Personalized medicine practices are important in terms of increasing the effect of treatment and patient survival rate. Currently, there are protein, RNA, and genome-based applications, but less than half of cancer patients are eligible for genetically compatible treatments. In addition, there are no genetic markers for most of the drugs used in cancer treatment (Wensink et al., 2021).

We can talk about patient-derived organoids as a promising technology for the prediction of drug response. Many anticancer agents are screened ex vivo to predict patient clinical response in these PDOs. For the first time in 2018, Vlachogiannis G et al published that drug response prediction in cancer patients could be made through PDOs (Vlachogiannis et al., n.d.).

G. Emerens Wensink et al published a review comparing 17 studies using PDO-based drug screening in 2021 (Table 2.1) (Wensink et al., 2021). In this review article, different drug screening methods used by researchers are examined. The clinical validity of the pdo-based drug screening method was evaluated by comparing these drug screening results with clinical studies.

Table 2.1: Characteristics of drug screening studies performed in PDCOs of many cancer types. (Wensink et al., 2021)

Table 1. Study characteristics.

Publication (author, year, study name)	Study design	Tumour type & stage	Drug screen cohort		Clinical response association cohort		Treatment
			Patients	PDOs	Patients	PDOs	
Ooft, 2019[21] TUMOROID	Prospective cohort, observational	mCRC	29	35	29	35	FOLFIRI (>1st line); Irinotecan (>1st line); FOLFOX (mixed lines)
Chalabi, 2020[22] NICHE	Prospective cohort (within phase II trial), observational	CRC (Stage III)	11	12	11	12	Nivolumab + ipilimumab (neoadjuvant)
Ganesh, 2019[29]	Observational cohort	RC (non-metastatic & metastatic)	14	23	9	17	5-FU & FOLFOX; Radiation
Yao, 2020[26] CinClare	Prospective, observational (within phase III trial)	LARC	80	80	80	80	Chemoradiation (capecitabine versus CAPIRI; neoadjuvant)
Narasimhan, 2020[28] APOLLO	Prospective, offers assay-guided treatment to treatment refractory patients	mCRC (peritoneal)	15	17	9	9	FOLFOX, FOLFIRI, regorafenib, vandetanib, gemcitabine
Vlachogiannis, 2018[11]	Prospective, observational, using PDOs from 4 prospective phase I/II trials	mCRC, mGC, mGOC	15	19	15	19[a]	TAS-102, Cetuximab, Regorafenib (CRC); Paclitaxel (GC); 5-FU + cisplatin (GOC)
Steele, 2019[31]	Observational cohort	GC (non-metastatic & metastatic)	6	6	2	2	EOX
Tiriac, 2018[23]	Observational cohort	Pancreatic cancer (Stage II–IV)	57	66	9	12	5-FU; Gemcitabine + nab-paclitaxel; 5-FU + SN-38 + gemcitabine; 5-FU + SN-38 + oxaliplatin; 5-FU + oxaliplatin; 5-FU + gemcitabine
Sharick, 2020[17]	Observational cohort	Pancreatic cancer (non-metastatic); Breast cancer (not specified)	24	24	10	10	Gemcitabine + 5-FU, oxaliplatin + 5-FU, 5-FU or FOLFIRINOX (pancreatic cancer). AC-T (breast cancer)
Li, 2018[27]	Observational cohort	Oesophageal cancer (non-metastatic)	8	8	5	5	ECX, ECF, CF
Driehuis, 2019[34]	Observational cohort	HNSCC (non-metastatic)	14	14	7	7	Radiation (postoperative with curative intent, primary and adjuvant)
Sachs, 2018[14]	Observational cohort	Breast cancer (metastatic)	NR	12	2	2	Tamoxifen
Phan, 2019[18]	Observational cohort	Ovarian carcinoma (Stage IV)	4	4	2	2	Carboplatin
De Witte, 2020[32]	Observational cohort	Ovarian carcinoma (non-metastatic & metastatic)[b]	23	36	5	7	Carboplatin + paclitaxel.
Votanopoulos, 2019[19]	Observational cohort	Melanoma (Stage III–IV)	7	9	5	7	Pembrolizumab, nivolumab, ipilimumab, dabrefinib/trametinib
Mazzocchi, 2018[20]	Observational cohort	Mesothelioma (metastatic)	2	2	2	2	Cisplatin + pemetrexed.
Jacob, 2020[30]	Observational cohort	Glioblastoma (WHO grade IV)	7	8	5	6	Radiation + temozolomide.

In the study performed by Ooft et al, the patient cohort consisted of metastatic colorectal cancer patients. In studies conducted by taking 67 biopsy materials from a total of 61 patients, the success rate of organoid establishment was reported as 63%. Some of the samples failed due to bacterial contamination, some due to insufficient cell number. Initially, irinotecan was tested in 10 patients. Growth rate inhibition metrics (GR) of each condition were calculated 6 days after exposure to SN-38, the active metabolite of irinotecan, and dose-response curves (DRCs) were generated. They developed a GR-score-based classifier that accurately identifies nonresponders to irinotecan and evaluated prediction performance using leave-one-out-cross-validation (LOOCV). They then tried a combination of 5-FU and irinotecan (FOLFIRI) in 12 patients. They stated that a reliable result was obtained when they evaluated the prediction performance of this combination drug. They also reported that 50% of PDOs most susceptible to FOLFIRI treatment had significantly higher progression-free survival, suggesting that PDO susceptibility parallels clinical conditions (Ooft et al., 2019).

In the study published by Ganesh et al., drug screening was performed by creating rectal cancer tumoroids. In this context, 21 RC tumoroids were created and screening was performed for the combination of 5-FU and 5-FU Oxaliplatin alone in these tumoroids. Area under curve (AUC) graphs were created. We investigated whether differential ex vivo 5-FU or FOLFOX susceptibility was associated with clinical outcome and progression-free survival (PFS). They reported that clinical response and drug screening response were consistent with each other in 7 patients. Radiation therapy was also applied to 19 tumoroids in this study. While resistant tumoroids are the organoids of patients who relapse after radiation and surgery, it has been reported that the organoids of patients whose clinical response is greater than 50% of tumor size reduction are more sensitive. In summary, it has been shown that results correlated with clinical response in both radiation therapy and chemotherapy (Ganesh et al., 2019).

Yao et al investigated the chemotherapy response of locally advanced rectal cancer organoids (LARC). A total of 80 LARC organoids were screened for 5-FU and irinotecan drugs and radiation (Figure 2.7).

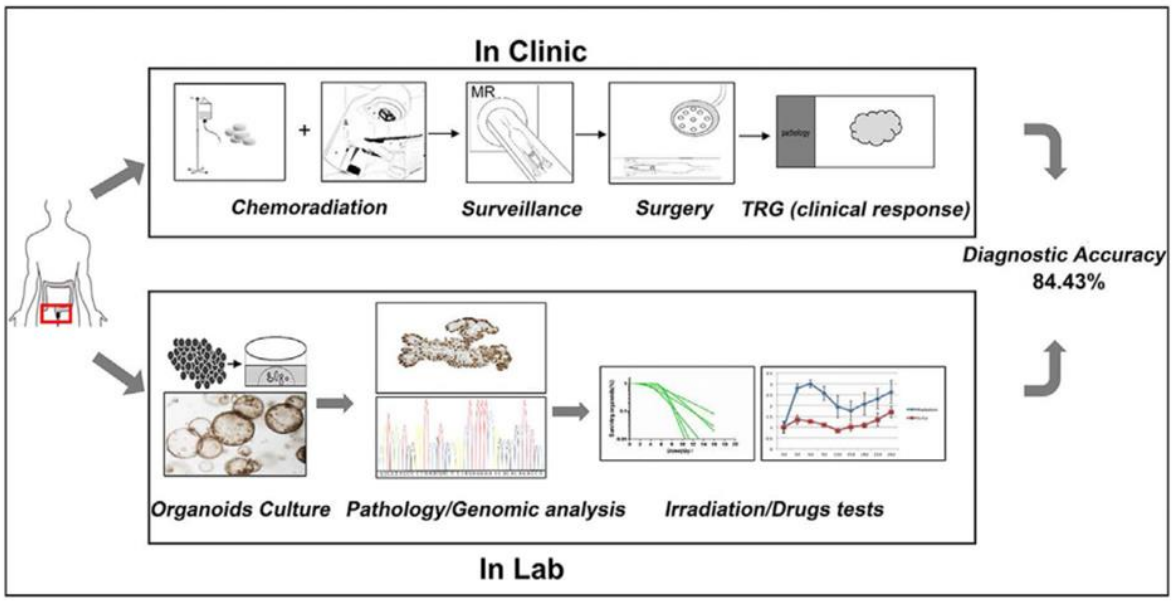

Figure 2.7: Workflow of clinical applications and drug screenings on organoids (Yao et al., 2020).

A total of 74 patients underwent surgery and tumor regression grade (TRG) scores are available. The postoperative TRG score is an important criterion for evaluating the patient's response to therapy. Patients have TRG scores to be compared with the responses of their PDO.

There are 31 patients with TRG scores of 0 and 1, and these patients have been reported to be susceptible to neoadjuvant chemoradiation. The remaining 43 patients were reported to have TRG scores of 2 and 3 and were resistant to neoadjuvant chemoradiation. As a result, when the comparison data were collected in this article, it was reported that the chemoradiation responses of the patients and the responses of their PDOs were compatible with 84.43% accuracy, 78.01% sensitivity and 91.97% specificity (Figure 2.8) (Yao et al., 2020).

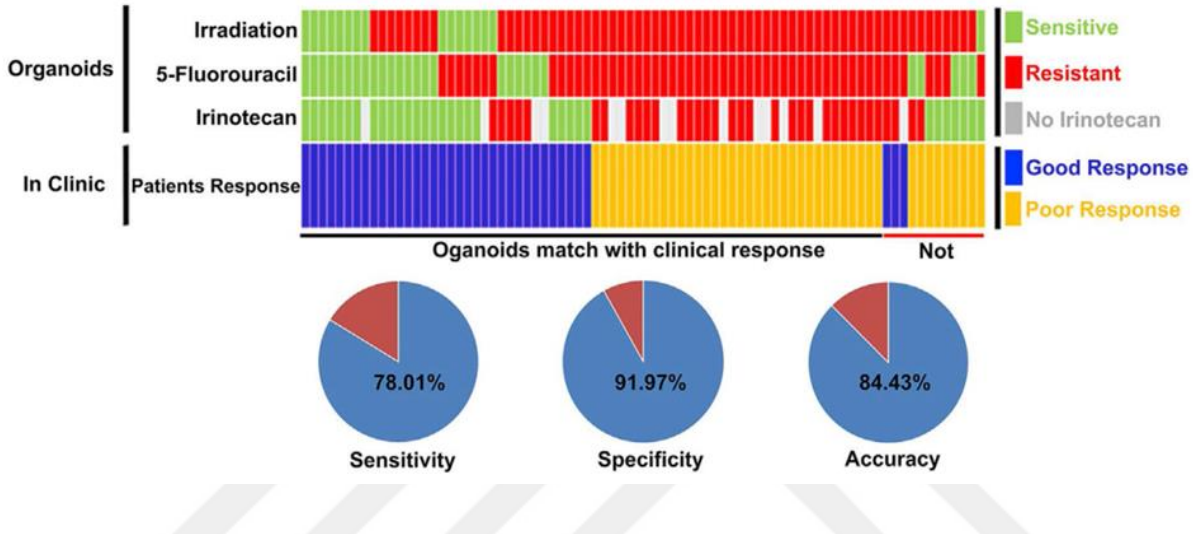

Figure 2.8: Organoid response data match patients' clinical outcomes (Yao et al., 2020)

Vlachogiannis et al published a paper examining the response to treatment of PDOs in patients with metastatic gastrointestinal and colorectal cancer in 2018. A total of 110 samples from 71 patients enrolled in four prospective phase I/II clinical trials were used in the study. A drug library consisting of a total of 55 drugs was prepared and these drugs were screened in patients. They reported that PDOs were successful in predicting the targeted agent and chemotherapeutic response, with 100% sensitivity, 93% specificity, and 88% positive predictive value (Vlachogiannis et al., n.d.).

2. MATERIALS AND METHODS

3.1. Type of Research

This study used an experimental research method.

3.2. Time and Place of the Research

It was found ethically appropriate by the Dokuz Eylul University Ethics Committee with the decision no 2020-040 on 25/09/2020. This project was supported by TÜBİTAK-TEYDEB with the number 7201148 within the scope of 1507 projects. Experimental procedures were carried out at ORGANO-ID Biotech Company Laboratory between March 2021 and June 2022.

3.3. Research Population, Sampling, and Experimental Groups

The study material of this research is the surgical materials obtained from colorectal cancer patients over the age of 18, together with their consent form.

3.4. Research Materials

3.4.1. Human Tissue Samples

Surgical resection of tumor tissue of approximately 20 mm x 20 mm x 10 mm was obtained from the patients. The tissues taken were transferred to the transfer medium (Table 3.1) by the pathology specialist under a fume hood, using sterile equipment, and examined macroscopically. All samples taken were delivered to the laboratory at +2-8^{o}C conditions within 24 hours.

2.4.2. Organoid culture buffers/mediums

Table 3.1: Organoid culture buffers and mediums

Buffer/Medium	**Components**
Basal medium (BM)/adDMEM+++, 500 ml	485 ml Advanced DMEM/F12 (Invitrogen), 5 ml HEPES (Gibco), 5 ml Penicillin/Streptomycin (Gibco), 5 ml Glutamax (Gibco)
Expansion medium (EM)	Advanced DMEM/F12 +++ (Invitrogen) supplemented with 1% B27 (Gibco), 1% N2 (Invitrogen), N-acetyl-L-cysteine (Sigma Aldrich), Gastrin (Sigma Aldrich), R-SPO1 conditioned medium (in-house), WNT3a Surrogate-Fc Fusion Protein (ImmunoPrecise), Human Recombinant Noggin Protein (Peprotech), Prostaglandin E2 (Tocris), hEGF (Peprotech), SB202190 (Sigma Aldrich), Y-27632 (Tocris), Nicotinamide (Sigma Aldrich), A83-01 (Tocris)
Transfer Medium	100 ml adDMEM+++ , Y-2763 (Tocris), Normocin (Invivogen), Amphotericin B (Gibco), Primocin (Invivogen)
Dissociation medium	96 ml adDMEM+++ , Collagenase XI (Sigma), Dispase II (Sigma), Hyaluronidase (StemCell), Y-2763 (Tocris)

Wash Buffer	97 ml 1X PBS (Gibco), %1 Penicillin/Streptomycin (Gibco), HEPES (Gibco), Normocin (Invivogen), Primocin (Invivogen), Amphotericin B (Gibco)
1X PBS	50ml 10X PBS (Gibco), 450 ml autoclaved ddH2O

2.4.3. Enzymes and Kits

Table 3.2: Enzymes and Kits

Kit	Vendor	Catalogue#	Purpose of Use
Trypsin	Thermo Fisher Scientific	25200056	It is used in organoid passage and drug screening when making single cells.
CellTiter-Glo 3D Cell Viability Assay	Promega	G9682	It is used to analyze the viability of organoids depending on the amount of ATP.

Dual-Luciferase Reporter Assay System	Promega	E1910	It is used to determine the activity of in-house RSPO-1 and WNT3a conditioned media.

2.4.4. Laboratory Equipments

Table 3.3: Laboratory Equipments

Device Name	Vendor	Catalogue#
Centrifuge	Nuve	NF 800R
EVOS™ XL Core Imaging System	Thermo Fisher Scientific	AMEX1000
Centro LB 963 Microplate Luminometer	Berthold Technologies	70325
Multiskan™ GO Microplate Spectrophotometer	Thermo Fisher Scientific	51119300

2.4.5. Software and databases

Table 3.4: Software and databases

Software	**Purpose of Use**	**Company/Web page**
ImageJ	It is used to edit microscope images of organoids.	https://imagej.nih.gov/ij/index.html
GraphPad Prism 8	It is used for analyzing CellTiterGloassay data and plotting graphs.	https://www.graphpad.com/scientific-software/prism/

3.5. Research Variables

Patient-derived organoid culture is the dependent variable. The anticancer agents used in drug screening in patient-derived organoids are the independent variable.

3.6. Data Collection Tools/Methods

3.6.1 Establishment of Patient Derived CRC Organoids

3.6.1.1 Tissue processing before dissociation

Tumor tissue was washed 2-3 times with washing buffer containing antibiotics. It was transferred to sterile petri dish and necrotic yellowish tissues were removed using forceps and lancet. Viable pink tissue was minced to increase surface area and digestion efficiency.

3.6.1.2 Dissociation of patient tumor tissue and plating of tumor cells

The minced tissue pieces were transferred to the enzyme-containing digestion medium and incubated at 37°C in a shaker for approximately 40 minutes. When the mixture became cloudy, AdDMEM+++ was added and centrifuged at 4°C to stop the reaction. The supernatant was removed and the pellet was resuspended with adDMEM+++. The mixture was passed through cell strainers. The filtered cells were centrifuged again and the supernatant aspirated. The cell pellet was mixed with matrigel and the matrigel dots were seeded into a preheated 6 well plate. The expansion medium required for the growth of colorectal cancer cells was prepared by adding colorectal cancer-specific small molecules to the rich growth factor cocktail. When the matrigel solidified, EM containing primocin was added to cover the dots and removed to the incubator (Figure 3.1) (Driehuis et al., 2020; Engel et al., 2020; Sato et al., 2011; van de Wetering et al., 2015).

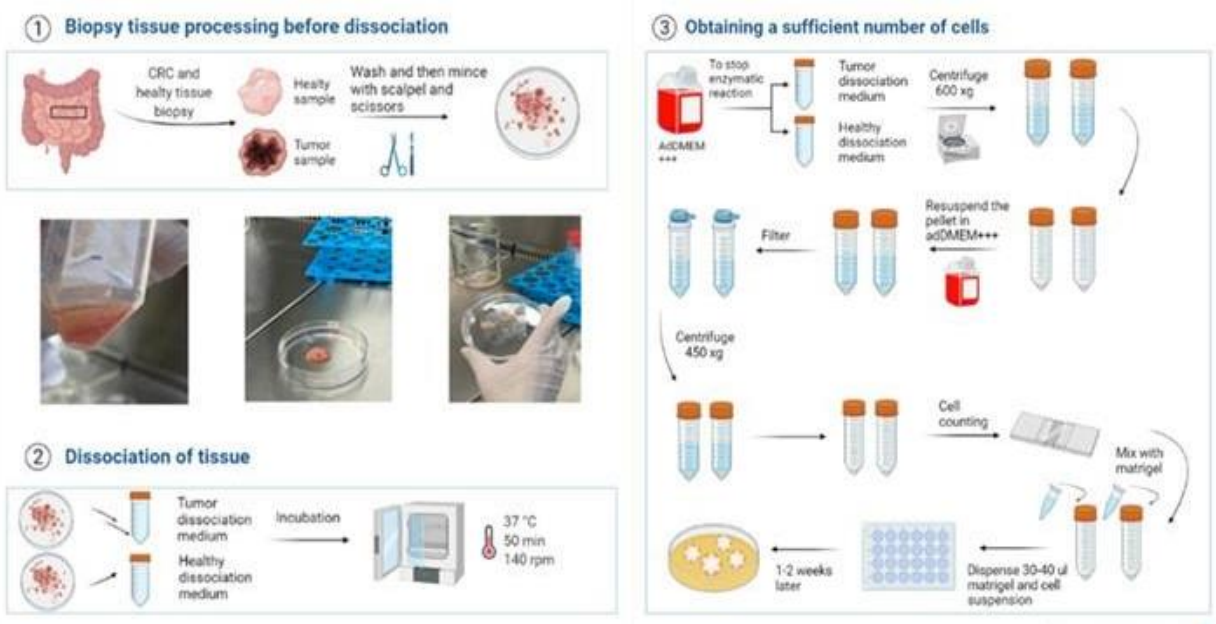

Figure 3.1: PDCO establishment chart. (in-house protocol)

3.6.2 Passaging of Colorectal Cancer Organoids

AdDMEM+++ was added to 15 ml sterile tubes coated with FBS and put on ice. EM in the well was discarded. Matrigel dots were mechanically disrupted using P1000 pipette with 1

ml of ice cold AdDMEM+++. Organoids were collected to 15 ml sterile tube and washed the well with an additional 1 ml of basal medium. The tube was centrifuged at 400 xg at 4ºC for 5 minutes and supernatant was removed until 500 ul remained. Organoids were mechanically fragmented by pipetting with a P200 pipette. Trypsin was used for enzymatic digestion as needed in this step depending on the size of the organoids. 1 ml of trypsin was added to the organoids and incubated at 37ºC for 5-10 minutes. It was completed to 10 ml with AdDMEM+++ and centrifuged at 400 xg 4ºC for 5 minutes. The supernatant was removed and the pellet was resuspended with matrigel. The mixture was seeded in dots on a preheated 6 well plate. The plate was inverted and incubated at 37°C for 10 minutes to polymerize the matrigel. The solidified dots were covered with EM and the plate was removed to the incubator. (Driehuis et al., 2020)

3.6.3 3D cell culture of HCT-116

HCT-116 cells grown in 10% FBS DMEM medium were collected into 15 ml sterile tube by trypsinization. It was centrifuged for 5 minutes at 300 xg. The supernatant was aspirated and the pellet was resuspended in 1 ml of basal medium. Cells mixed with trypan blue at a ratio of 1:1 were counted by placing them in the neubauer counting chamber. Matrigel dots of 3000 cells/5 µl dots were seeded into each well of the 96 well plate for 3D culture.Wells were covered with 100 µl of basal medium.

The experiment was set up with 4 replicates for each drug combination. Concentrations in table 3.5 were determined for the combination of FOLFOX and FOLFIRI. Cell titer glo viability assay for 3D culture was performed on the 7th day.

Table 3.5: Drug concentrations used in 3D HCT-116 culture drug screening

FOLFOX		FOLFIRI	
5-FU (uM)	Oxaliplatin (uM)	5-FU (uM)	SN-38 (nM)
200	5	200	160
100	2.5	100	80
50	1.25	50	40

25	0.625	25	20
12.5	0.312	12.5	10

3.6.4 CellTiter-Glo 3D Viability Assay

The reagent of the CellTiter Glo 3D Cell Viability Assay kit was removed from -20ºC to room temperature. Before starting the assay, the reagent and the plate were allowed to come to room temperature. The growth medium content in the wells was aspirated and 25 µl of CellTiter-Glo® 3D Reagent was added to the top of the matrigel domes. Domes were disrupted by incubating for 30 minutes on a horizontal shaker at room temperature, protected from light. Approximately 30 µl of the contents in the wells were transferred to a black 96 well plate. Luminometric measurements were made at 1 second/well.

3.6.5 Determination of Drug response in Patient Derived CRC organoids

3.6.5.1 Micro dome organoid setup for Drug Screening

Organoids collected in a sterile 15 ml tube as at the beginning of the passage protocol were transformed into single cells by trypsinization. It was centrifuged at 400 xg at 4C for 5 minutes and the supernatant was aspirated until 1 ml remained. The cells were resuspended and mixed with trypan blue and the cells were counted in the counting chamber. The tube was centrifuged again and the supernatant was aspirated. The pellet was mixed with matrigel. Cells were seeded into wells of 96 well plate with 6000 cells/5u matrigel dome (Figure 3.2) The experiment was set up with 4 replicates for each drug combination. Cell titer glo assay was performed on day 7 (Engel et al., 2020; Ooft et al., 2019).

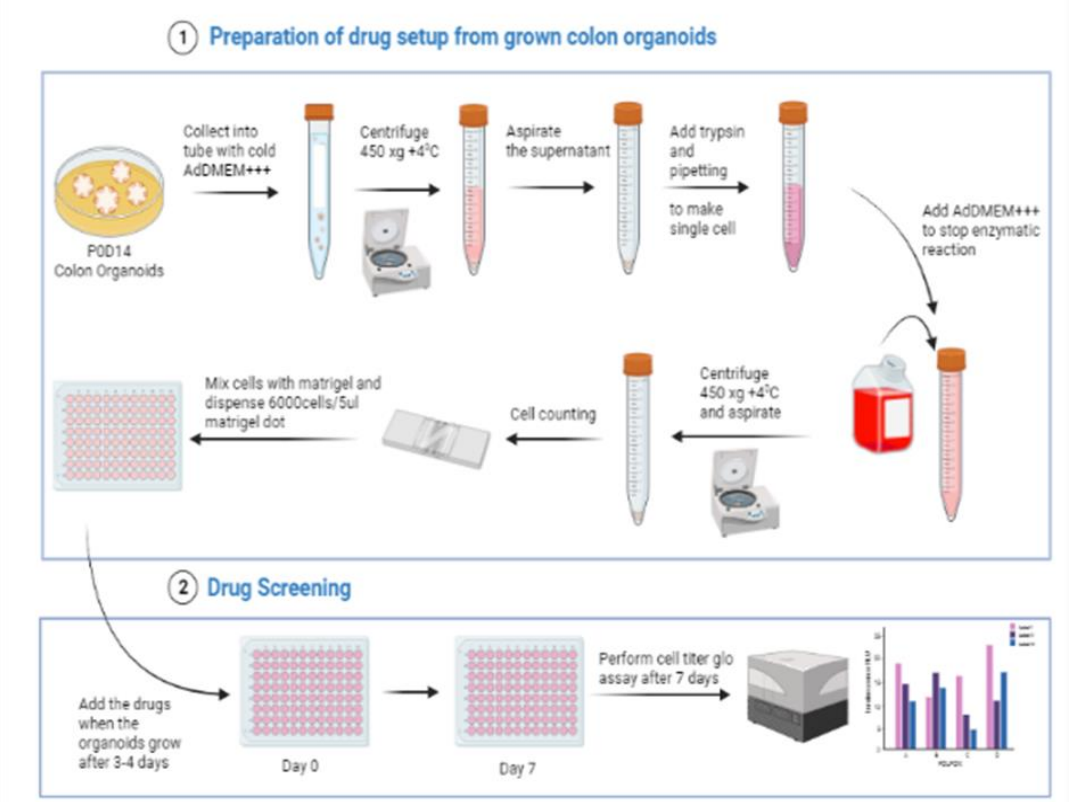

Figure 3.2: Preparation of drug screening experimental setup from grown PDCOs. (in-house protocol)

3.7. Research plan and timeline

Table 3.6: Research timeline

Work Package (WP)	**MONTHS**														
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Patient-derived CRC Organoid Culture	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**	**x**				
Optimization experiments with 2D culture					**x**	**x**									

Drug screening in patient-derived organoids							**x**	**x**	**x**	**x**	**x**	**x**	**x**		
Writing Thesis													**x**	**x**	**x**

3.8. Data Analysis

Cell titer glo 3D cell viability assay data were analyzed using graphpad prism program. One way anova method was used for analysis. All doses were compared with the control group and it was determined which dose had the lowest effect on organoid viability.

3.9. Limitations of the Research

The limitations of this study are the insufficient number of cells from the patient's surgical material, the low efficiency of organoid formation from the cells obtained, and the expensive matrigel chemical used in organoid culture.

3.10. Ethical committee approval

The ethics committee reviewed our study with protocol number 2020-040 on September 25, 2020, and found it to be ethically suitable.

3. RESULTS

The workflow of this study is summarized below (Figure 4..1). Within the scope of the project, the tissues collected with the consent of the patients with CRC were brought to the laboratory, and then they were dissociated and tumor cells were obtained. Organoid culture establishment was performed from the obtained tumor cells. Within the scope of the master's thesis of Melis Kanık, another graduate student in our laboratory, WES analysis and histopathological analyzes were performed on primary tumor tissue and PDCOs obtained from them, and the characterization of the organoids produced was performed. Before performing drug screenings with PDCOs, optimization trials were carried out in 3D culture in the HCT-116 cell line, which is the CRC cell line, to determine the dose ranges of the drugs to be screened. After that, an experiment was set up for drug screening by transforming PDCOs into single cells. In order to see the effect of the drugs, the organoids were exposed to the determined drugs for seven days. At the end of seven days, the vitality assay was performed and the data were analyzed with the GraphPad Prism 8 program.

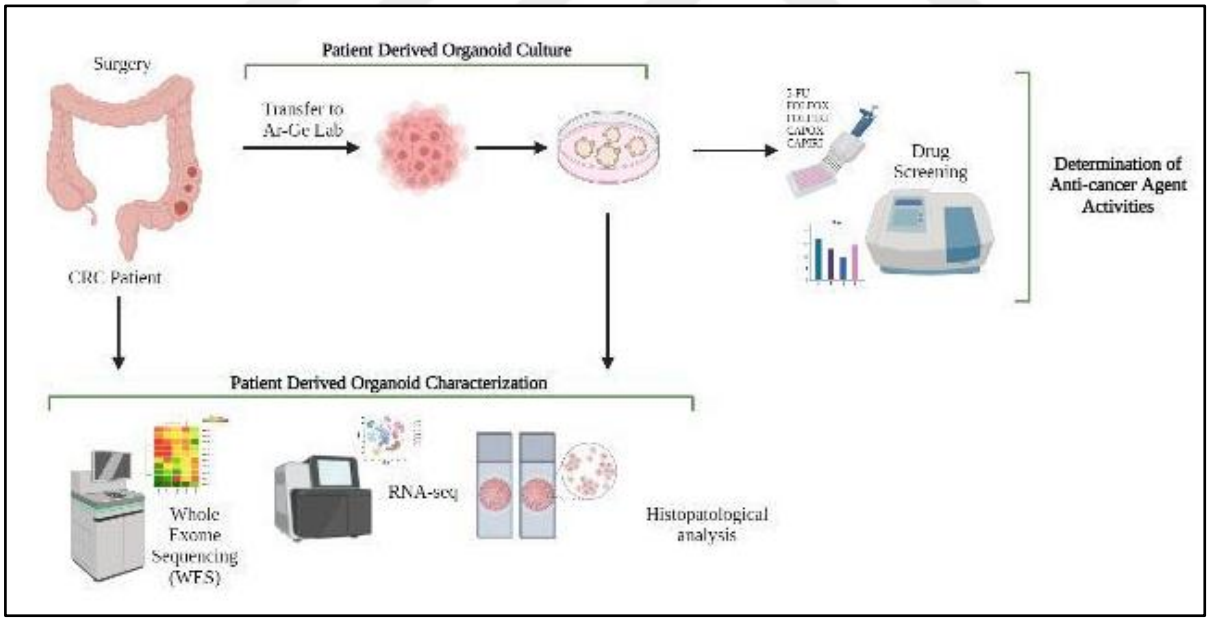

Figure 4.1: Shematic work-flow of this study.

4.1 Patient Derived Cancer Organoids

4.1.1 Characteristics of Patients

Within the scope of this research study, surgical resection material of a patient with a diagnosis of colorectal cancer was obtained. Tissues were taken from a total of 15 patients between May 2021 and May 2022. During the protocol optimization, organoid setup could not be performed from the samples of 5 patients. Samples of 1 patient were lost due to bacterial contamination. Organoid setup of the remaining 9 patients was successfully performed. In this study, drug screening was performed from the organoids of 5 patients. The diagnosis of all these patients is adenocarcinoma. Tumors of three patients were located in the sigmoid, and tumors of two patients were located in the right colon (Table 4.1)

Table 4.1: Clinical data from the patients diagnosed as colonic adenocarcinoma

Patient	**Age**	**Sex**	**Tumor Location**	**Lymph Node etastases**	**Pathologic Tumor Stage**	**Screened Drugs**
P1 (KH-FĞ-091221)	73	Female	Right colon	No	T3N0	5-FU FOLFOX FOLFIRI FOLFIRI+Cetuximab CAPIRI CAPOX
P2 (KH-ŞR-140122)	58	Female	Sigmoid colon	Yes (11/15)	T4bN2b	FOLFOX CAPIRI Capecitabine SN-38
P3 (KH-AM-211221)	63	Male	Sigmoid colon	Yes (3/11)	T3N1b	5-FU FOLFOX FOLFIRI CAPIRI CAPOX
P4 (KH-MAK-240222)	60	Male	Sigmoid colon	Yes (22/27)	T3N2b	FOLFOX FOLFIRI
P5 (KH-TZ-110322)	72	Male	Right colon	No	T3N0	FOLFOX FOLFIRI

4.1.2 Establishment and Passaging of PDOs

For PDO establishment, the surgical material was first dissociated and tumor cells were obtained. Then the cells were embedded in matrigel and covered with expansion medium. The EM content is similar for many types of cancer. However, the signaling pathways that need to be active or suppressed specific to the cancer type are different. WNT-3a and WNT promoter R-spondin1 molecules are essential for organoid establishment from normal epithelial cells. When we look at CRC cases, 90% have mutations that abnormally activate the Wnt signaling pathway. Therefore, we used the Wnt dependence of cells to selectively establish and expand CRC tumoroids.

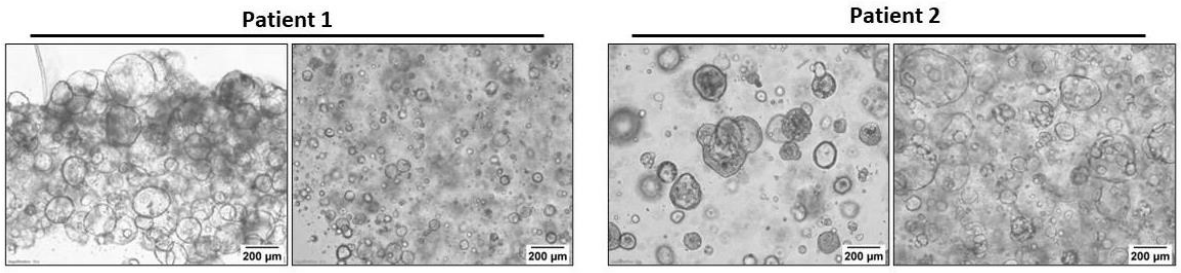

Figure 4.2: PDCO images of different patients

The number of organoids composed of primary cells varied between patients. Some patients had thousands of organoids, while others only had 100-200 organoids. This difference in expansion capacity is due to the heterogeneity of tumor cells. Likewise, some patients had increased growth rates after the first passage, while others had limited growth rates and proliferation capacity. Because of these limitations, some patients could not be included in the drug studies.

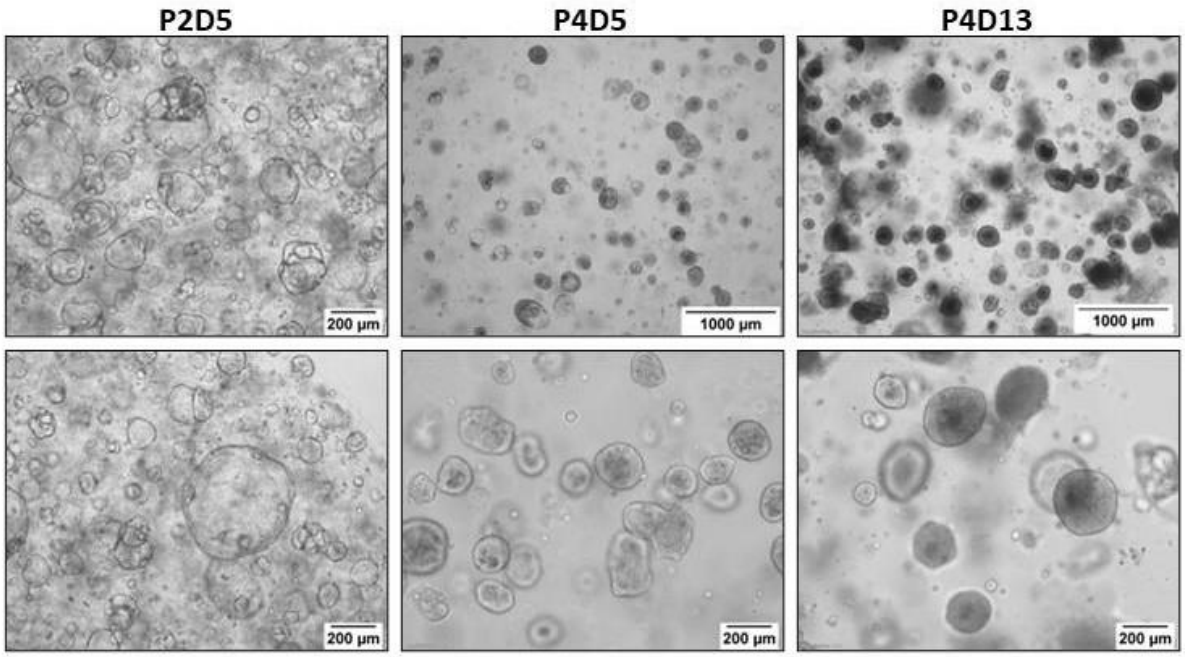

Figure 4.3: Light microscope images of tumor organoids of the Patients 2.

PDOs can exhibit different morphological structures. Due to tumor heterogeneity, some patients' organoids are filled, dark and dense, while others grow in the form of empty rings. This variation can be between patients or even in organoids of the same patient in the same matrigel dome (Figure 4.2). Morphological differentiations may occur in PDOs as the number of passages progresses. Organoids, which appear as hollow in passage 2, may become solid, denser and darker when they reach passage 4 (Figure 4.3). Cells from the tissue bring with them the ECM. It may appear as pollution when viewed under a microscope. However, this ECM content has a positive effect on organoid formation. As the number of passages progresses, it decreases and disappears (Figure 4.4).

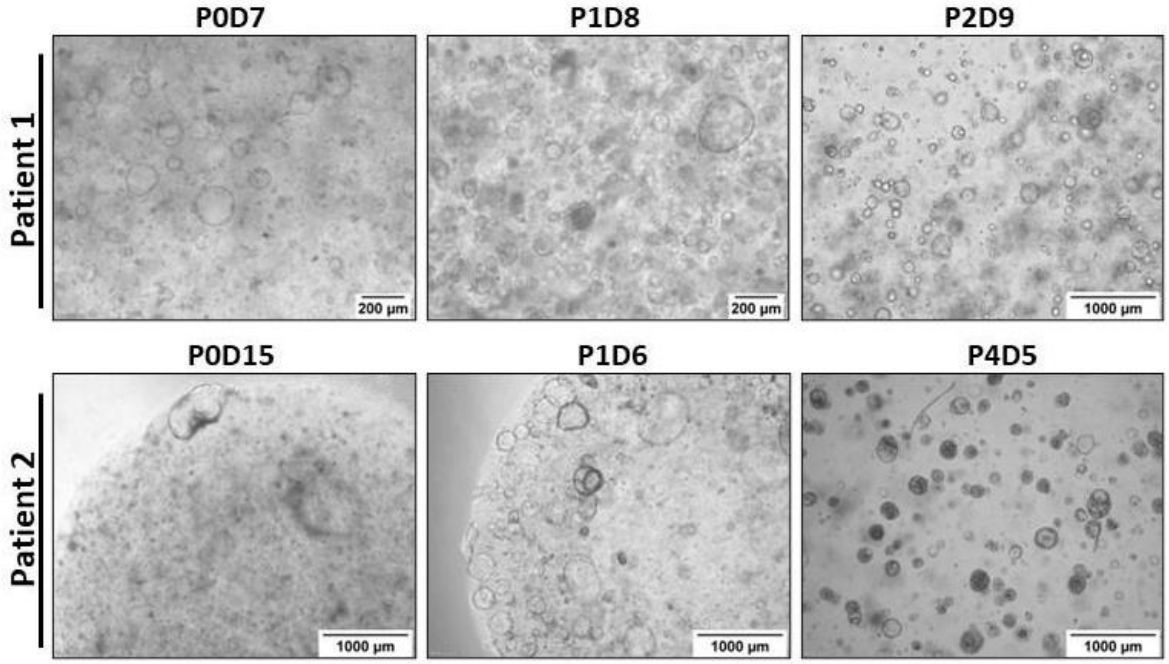

Figure 4.4: Light microscope images of organoids through passages.

PDOs are passed on average once every 2 weeks. When structures become too large they sometimes tend to protrude from the matrigel dome. Or the structures may become too large and the inside of the dome may become denser (Figure 4.5). These views show they need the passage. If the organoid growth rate is slow in some patients, the matrigel structure begins to deteriorate before the structures grow sufficiently. Ripples can be seen in the boundary lines of the dome. In this case, a passage can be made just to refresh the matrigel. If the size of the structures is less than 200um, passage can only be made by mechanical disruption. As the organoids increase in size, they become more difficult to fragment by pipetting. In this case, the enzymatic digestion method should also be used. It was observed that organoids treated with trypsin enzyme during passage had an increase in growth rate compared to those that were mechanically lysed. As a result of all these efforts, we were able to establish organoids from tumor tissues with a success rate of 90%.

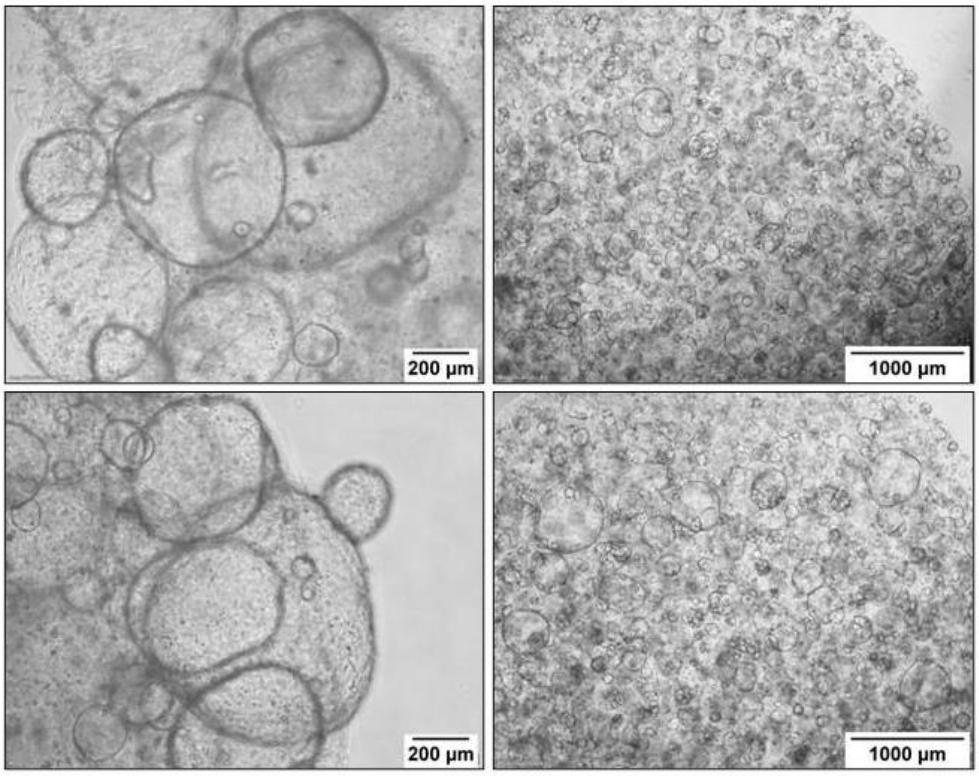

Figure 4.5: PDCO culture images that need the passage.

4.2. Understanding of Drug Response in 3D CRC Cell Line Models

Before starting drug screening experiments in PDOs, dose optimization experiments were performed for FOLFOX and FOLFIRI drug combinations in the HCT 116 which is CRC cell line. Experiments were performed in 3D culture and IC_{50} values were calculated.

4.2.1 3D Culture of HCT-116 Cell Line

HCT116 cells were first seeded in a T75 flask. Enough proliferating cells were transformed into single cells. For 3D setup, cells suspended with matrigel were seeded as a 5 µl dome in a 96-well plate. The domes were covered with basal medium.

4.2.2 Drug Response in HCT-116 Cell Line

In drug screening experiments, the aim was to determine the dose range and IC50 values of drugs. FOLFOX and FOLFIRI were used for this screening. According to the viability analysis obtained from the 3D culture, FOLFOX had a 2-fold significant effect even at the lowest dose. The IC_{50} dose of the drug was calculated as 49.63 µM 5-FU and 1.24 µM Oxaliplatin. FOLFIRI had a 4-fold significant effect at the lowest dose. The IC50 value was calculated as 35.5 µM 5-FU and 28.40 nM SN-38. The reason for the IC50 dose to be so high in the HCT-116 cell line is that it may have shown resistance throughout the passage. After these experiments, drug screening experiments were started in PDOs.

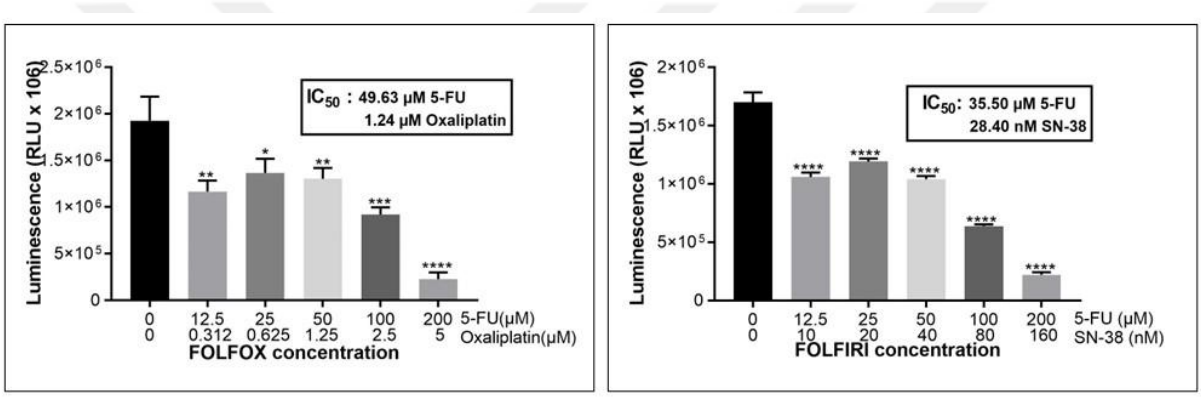

Figure 4.6: Graphs of drug screening in 3D culture of HCT-116.

4.3 Personalized Therapy Studies in PDOs

5-FU, Capecitabine, SN-38, FOLFOX, FOLFIRI, CAPOX and CAPIRI were screened in PDCOs. In one patient, a targeted therapy agent was screened in addition to cytotoxic agents. For drug screening in PDOs, organoids were made into single cells by enzymatic fragmentation. Cells were suspended with matrigel and seeded as 5 µl domes in a 96-well plate. Any concentration higher than 10 µM is toxic to the human body. When calculating the IC50, doses higher than 10 µM were used, since the lethal dose should also be completely.

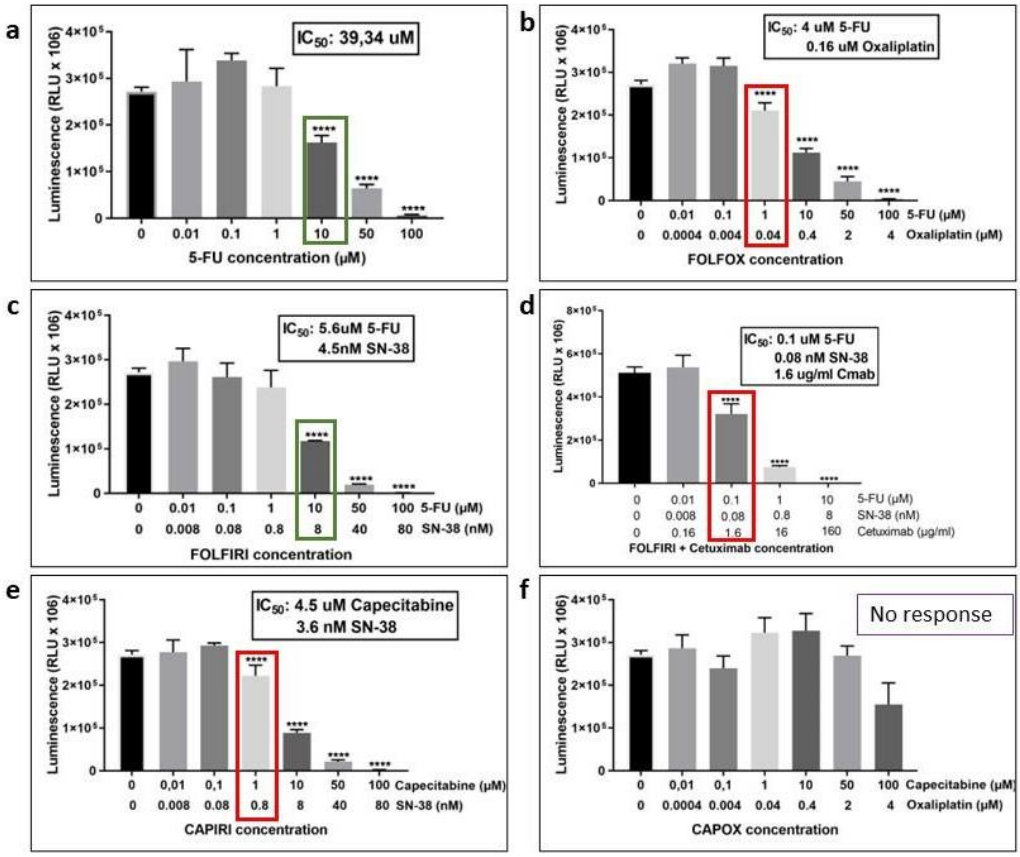

Figure 4.7: Drug screening results of Patient 1.

Organoids of patient 1 responded at low dose to FOLFOX and CAPIRI combined treatments (b,e). Moreover, the lowest dose response is observed when FOLFIRI is combined with the anti-EGFR antibody Cetuximab, which is the targeted agent (d). However, it responded to 5-FU and FOLFIRI at very high doses (a,c). The patient didn't respond to CAPOX (f).

In patient 1, the 5-FU agent is lethal at a toxic dose (green frame). For this reason, it is not possible to apply it in the clinic. On the other hand, we can see that the IC_{50} value decreased in the FOLFOX combination. As a result, it can be said that the patient is more sensitive to the combination of 5-FU and oxaliplatin rather than 5-FU alone. When we look at the FOLFIRI combination, we see that it inhibits the growth of organoids at doses of 5.6 µM 5-FU and 4.5 nM SN-38. In this patient, when we added the targeted agent, the anti-EGFR antibody Cetiuximab, to FOLFIRI, we observed that the IC_{50} value decreased significantly. We can say that this patient is sensitive to FOLFIRI and Cetuximab (Figure 4.7).

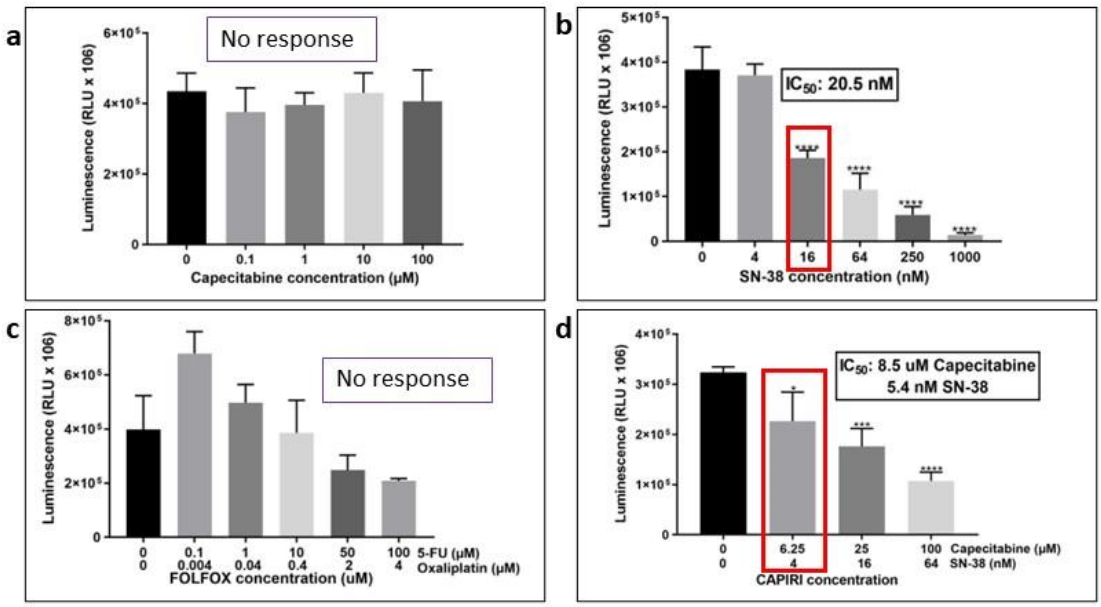

Figure 4.8: Drug screening results of Patient 2.
Although a low dose response was obtained when the SN-38 agent was used alone, the lowest dose response was obtained in the CAPIRI treatment when it was combined with Capecitabine (b,d) The patient didn't respond to FOLFOX and Capecitabine (a,c).

In patient 2, we can see that the FOLFOX combined therapy was not effective. It is also resistant to the agent Capecitabine alone. We can say that the patient's tumor responded to CAPIRI combined therapy at low doses and was resistant to SN-38 alone (red frames) (Figure 4.8).

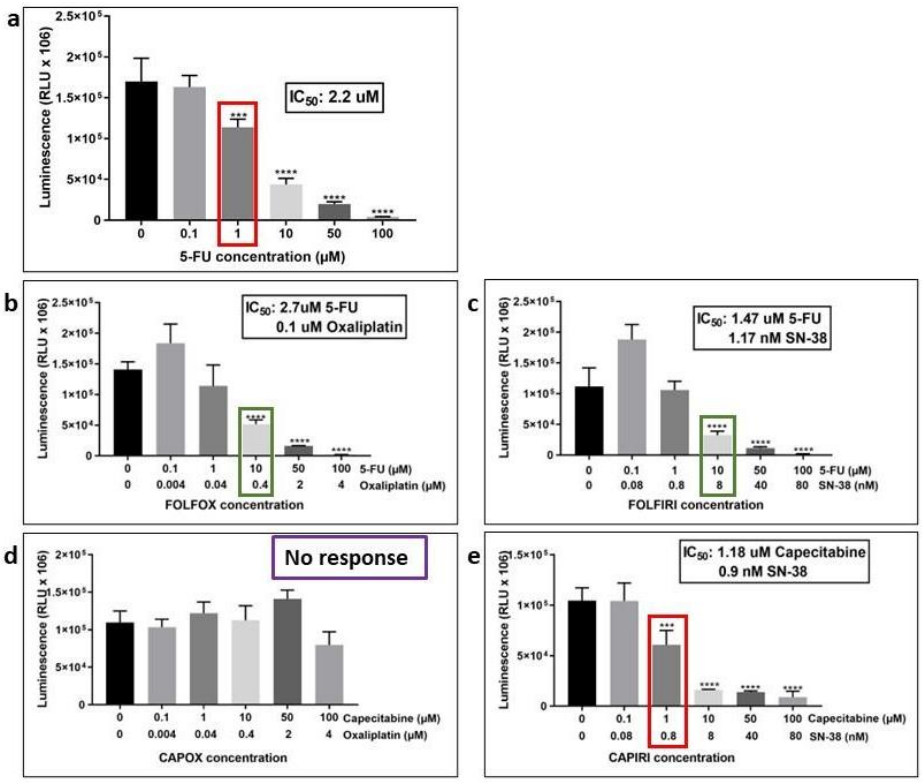

Figure 4.9: Drug screening results of Patient 3.

Organoids responded to 5-FU and CAPIRI treatments at low dose (a,e). Compared to these, it responded to FOLFOX and FOLFIRI treatments at higher doses (b,c). It didn't respond to CAPOX therapy (d).

In patient 3, unlike the first patient, there was no significant difference between the IC_{50} values of the combined therapy of 5-FU and FOLFOX alone. In this patient, organoid growth was inhibited by combinations except CAPOX. We can say that the therapy to which the patient is most sensitive is CAPIRI combined therapy (Figure 4.9).

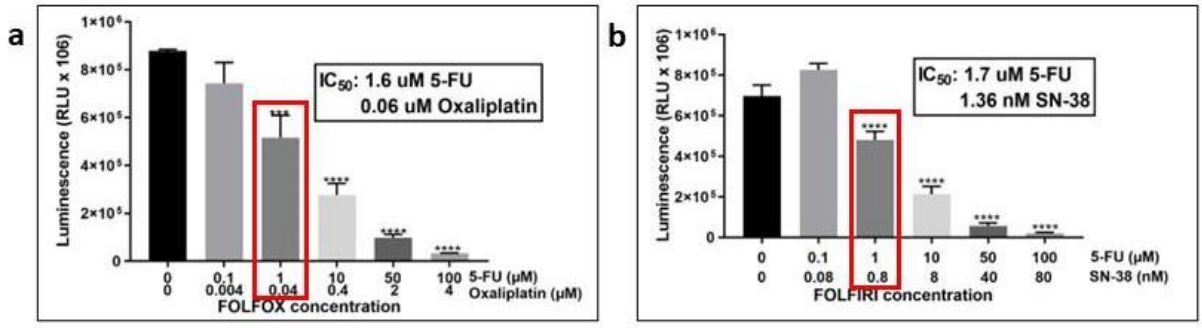

Figure 4.10: Drug screening results of Patient 4.

The patient's organoids responded to both FOLFOX and FOLFIRI treatments at a low dose (a,b).

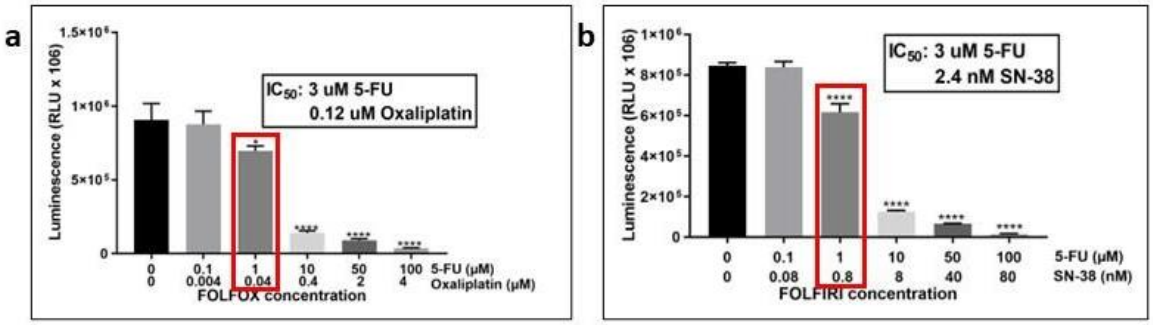

Figure 4.11: Drug screening results of Patient 5.

The patient's organoids responded to both FOLFOX and FOLFIRI treatments at a low dose (a,b).

We found that the FOLFOX and FOLFIRI combined therapies screened in patients 4 and 5 had close IC_{50} values (Figure 4.10, Figure 4.11).

As a result, we determined that each patient's response to treatment was different by screening drugs for various anti-cancer agents on organoids we obtained from patients. We show that targeted antibodies increased the effectiveness of the treatment in one patient. As a proof of concept study, we successfully predicted drug responses for 5 patients.

4. DISCUSSION

In recent years, there have been important developments in the treatment of cancer with the development of technology. However, cancer remains the second leading cause of death worldwide. The major limitation in cancer treatment is the unpredictability of treatment response for individual patients. Patients are exposed to severe side effects by applying standard treatment. Personalized medicine applications are mostly made with genomic biomarkers. However, only a minority of cancer patients are suitable for genetically compatible therapy. One of the reasons for this is the absence of genetic markers for most anticancer agents. For this reason, it becomes very important to develop a modeling system in which drug response can be predicted in order to use personalized medicine effectively and to increase survival.

Organoids are the cell culture system that allows the most realistic observation to study human biology and disease development in healthy and diseased cells (Tuveson & Clevers, n.d.). Patient-derived cancer organoids are a modeling system in which anticancer agents can be tested ex vivo and clinical response can be predicted. Modeling has been done with PDCOs for many cancer types. Many articles have shown that drug response can be predicted with PDCOs (Ooft et al., 2019). PDCOs are superior to other preclinical model systems in that they reflect tumor heterogeneity, maintain their stability over time, and can be used in high-throughput drug screening.

The overall aim of this project is to generate PDCOs from patient tumor tissues, to test the ability of these PDCOs to predict patients' drug responses, and to highlight the importance of personalized therapy in cancer treatment by showing that patient responses may differ. The tested drugs are cytotoxic anticancer agents called standard-ofcare chemotherapy (5-FU, FOLFOX, FOLFIRI, CAPOX, CAPIRI). As can be seen in the results, the responses and IC_{50} values of the tested PDCOs to drugs vary from person to person, as we would like to underline. Among the reasons for this change are tumor heterogeneity, microenvironment, and mutation profile of the patient. Cetuximab with the anti-EGFR receptor was also tried as a targeted therapy for one patient. And organoids exposed to Cetuximab with FOLFIRI were found to be more sensitive than FOLFIRI. However, we are aware that the patient mutation profile should be known in order to see the effect of targeted therapies more accurately.

There are studies with drug screening with PDCOs for many types of cancer. Oof et al. showed the effect of irinotecan monotherapy in two patient groups (stable disease, and progressive disease, patients) diagnosed with metastatic colorectal cancer. Scans using SN-38, the active metabolite of irinotecan, showed that the widest effect window was obtained at 3.2 nM SN-38. And they showed that patients with stable disease are more sensitive to this treatment regimen than patients with progressive disease. They then compared PDCO drug response with clinical response in the combined treatment of 5-FU and irinotecan. They found that 50% of PDCOs sensitive to FOLFIRI treatment had a high progression-free survival rate, so PDCO responses significantly reflected the clinical response. In our study, we tried FOLFIRI in 4 of 5 PDCOs. One patient was resistant to 5-FU alone, while responding to FOLFIRI combination therapy at a significantly lower concentration. Another patient was sensitive to both treatments. But the FOLFIRI combination still had a lower IC50. For 5-FU treatment alone, one of our patients' organoids was sensitive and had an IC_{50} value of 2.2 µM, while in another patient the IC_{50} value was 39 µM, which is a toxic dose. These results emphasize the importance of personalized therapy in cancer.

In summary, we created patient-derived cancer organoids with a 90% success rate. As a result of the drug screenings we performed with these organoids, we observed that the patient's response changed individually. However, there are some limitations to using this system as a tool for predicting drug response. The first and most important limitation is that surgical resection cannot always be obtained from patients. In our study, all of our samples are surgical material and much larger than a skin biopsy. Thus, despite the losses experienced during the protocol, we were able to reach the required number of cells for the establishment of organoig culture. However, when we tried to perform organoid establishment with skin biopsy, the cells we obtained were not enough. For this reason, an efficient organoid formation did not occur. We will continue to work on culturing from the biopsy sample by improving our protocol. As a second limitation, we can say that organoid culture is very expensive. This will increase the cost of the service to be given to the patient. The matrigel we use to mimic ECM in our experiments is quite expensive. By producing a similar material locally, the cost of organoid culture can be reduced. Therefore, it can be facilitated for patients to benefit from personalized therapy during the treatment process.

5. CONCLUSION AND FUTURE ASPECTS

In this study, we questioned the feasibility of organoid-based personalized therapy in our country. In order to use not only standard of care chemotherapy, but also targeted therapies, which are agents that give more effective results in treatment, studies in which the mutation profile of the patient is known can be carried out, and the effectiveness of the treatment can be further increased. And when the organoid culture is successfully established from the biopsy material, more patients can be provided with personalized treatment.

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İZMİR BİYOTIP VE GENOM MERKEZİ
GİRİŞİMSEL OLMAYAN ARAŞTIRMALAR ETİK KURULU (İBG-GOEK)
KARARI

Toplantı Tarihi	: 25/09/2020	**Toplantı Günü**	: Cuma
Toplantı Sayısı	: 12	**Toplantı Saati**	: 10:30

Sayın Şerife Esra ERDAL BAĞRIYANIK,

2020-040 Protokol No'lu; sorumlusu olduğunuz "Kolorektal Karsinoma Tümöroidlerinde Kişiye Özel Kemoterapi Etkinlik Testinin Geliştirilmesi" başlıklı araştırmanın uygulanmasında etik açıdan sakınca olmadığına oy birliği ile karar verilmiştir.

Bilgilerinizi ve gereğini rica ederiz.

Dr. Serap ERKEK
Başkan V.

Pandemi süresince online ortamda gerçekleştirilen toplantımızda alınan kararlar tek imzalı olarak düzenlenmektedir. Pandemi sona erdikten sonra ıslak imzalı karar belgesi teslim edilecektir.