



DERIVING PRIMARY CANCER CELL CULTURES FOR PERSONALIZED THERAPY

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ABSTRACT

Cancer is the second-leading cause of death in the world, accounting for one out of six deaths. Consequently, there is an urgent need for new and more effective therapeutic options as well as drug screening methods. Immortal, "stable" cancer cell lines have been employed since the past century to assess drug response but face several disadvantages. They often accumulate new genetic aberrations due to long-term culture and lack the indisputable heterogeneity of solid tumors, therefore, compromising the recapitulation of molecular features from parental tumors. Primary cancer cells have emerged as an attractive alternative to commercial cell lines since they can preserve such properties more closely. Here, we provide an overview of the basic concepts underlying generation and characterization of primary cell cultures from tumor samples. We emphasize the advantages and disadvantages of using these types of cancer cell cultures, and we make a comparison with other types of cultures used for personalized therapy. Finally, we consider the use of primary cancer cell cultures in personalized therapy as a means to improve drug response prediction and therapeutic outcomes. (REV INVEST CLIN. 2019;71:369-80)

Key words: Cancer. Primary cancer cells. Personalized therapy. Cell culture.

INTRODUCTION

Tumor cell lines have been widely used during decades to understand several aspects of tumor biology, including tumor initiation, progression, metastasis, and therapy response. Although easily available, immortalized commercial cancer cell lines face several

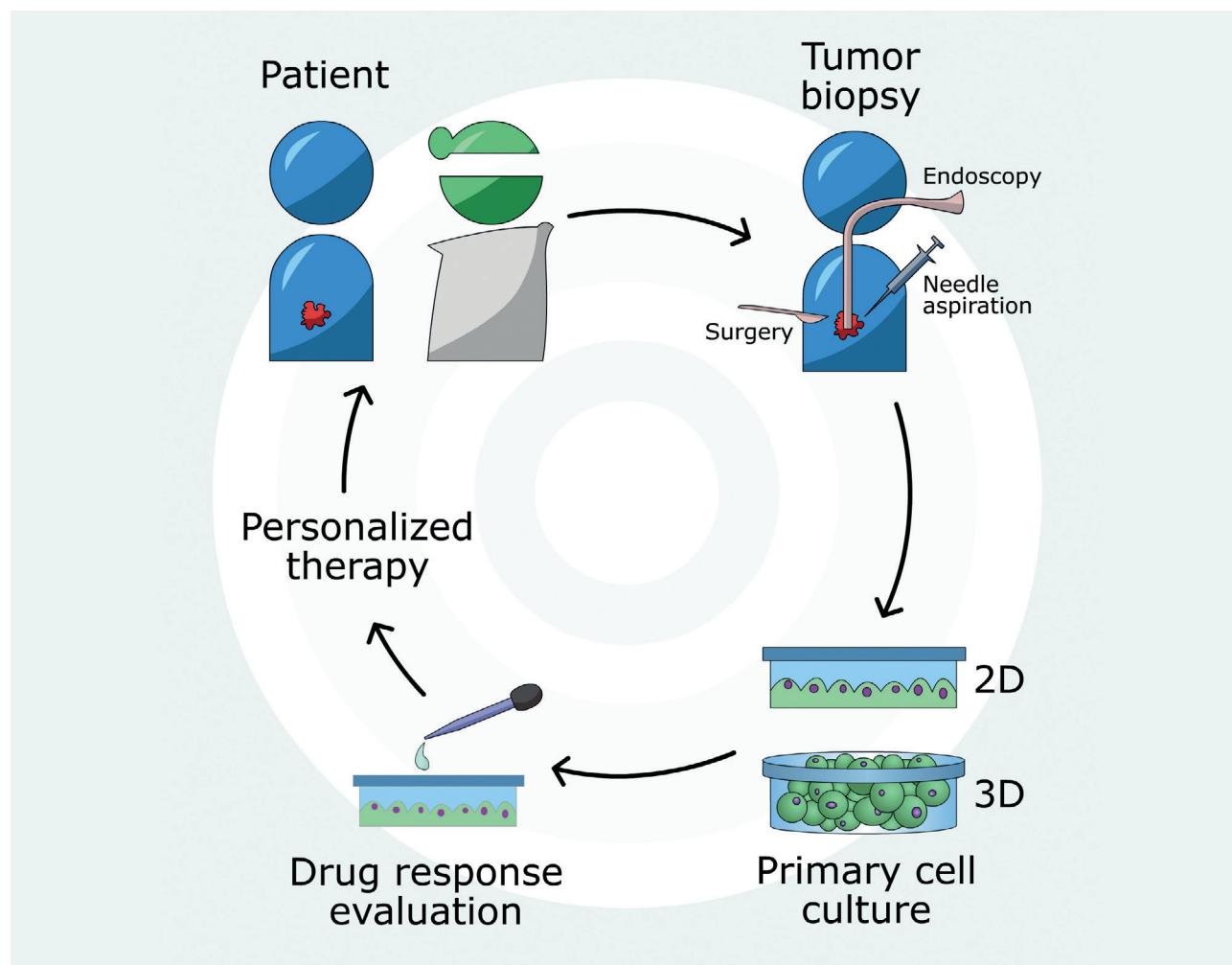
disadvantages for individualized therapy since often they do not fully represent the molecular background of a patient's tumor, and their long-term culture may lead to new genetic aberrations, thus limiting putative clinical correlations. These drawbacks underscore the need for patient-derived cancer cell models that better reflect the molecular characteristics of the original

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Figure 1. Primary cancer cell culture for personalized therapy. Tumor tissue from cancer patients can be obtained by biopsy procedures such as endoscopy, needle aspiration, or surgery. Primary cell cultures can be developed using two-dimensional or three-dimensional culture systems, which are then employed to evaluate the response to specific anticancer drugs to improve prediction of patient's therapy outcome. The approach harnesses drug testing on cancer cells with the same or highly similar molecular background as tumor cells *in situ* to help design a customized therapeutic strategy.



tumors and help design personalized, more efficient anti-cancer therapies. Figure 1 shows the general approach to use primary cancer cell cultures for personalized therapy. Generation of primary cancer cell cultures requires at least five stages that include ethical approval, biopsy acquisition, disaggregation, culture, and authentication.

BIOPSY ACQUISITION AND SOURCES

Biopsies may be obtained by several methods. Endoscopic biopsy, for example, is done using a flexible

tube (the endoscope) carrying attached tools for tissue collection as well as a light and a camera at the end to perform biopsies of bladder (cystoscopy), lung (bronchoscopy), and colon (colonoscopy) tumors, among others. Needle biopsy is used to obtain tumor cells from a suspicious lump. There are several variants of this procedure: (a) fine-needle aspiration, where a thin and long needle is inserted to reach the suspicious area (e.g., for thyroid nodules)¹; (b) core-needle aspiration, which employs a large needle with a sharp tip at the end to extract a column of tissue; (c) vacuum-assisted biopsy, using a suction device to increase the amount of fluid or tissue obtained (e.g.,



Table 1. Methods of tissue disaggregation

Types of tissue disaggregation	Advantages	Disadvantages
Mechanical	High yield Faster than enzymatic disaggregation	Causes mechanical damage
Enzymatic	Yields large numbers of cells with good viability through optimization Faster than explants	May affect cell membrane integrity if it is not well optimized
Chemical	Maintains membrane integrity	Needs to be combined with another method
Automatic dissociator	Easy disaggregation Safe and sterile handling High reproducibility	Expensive

for lung cancer)²; and (d) image-guided biopsy, aided by X-ray, computerized tomography, and ultrasound or magnetic resonance imaging to guide the needle acquiring the biopsy. If the sample cannot be taken by one of the previous methods, surgical biopsy is applied (e.g., breast lump, lymph node, and ovarian cancer)³. This method can be used to remove only part of the abnormal tissue (incisional biopsy) or the entire tumor (excisional biopsy). The sample should be procured under sterile conditions, placed into a tube containing media supplemented with fetal bovine serum (FBS) and antibiotics, and immediately delivered for processing.

Pleural or peritoneal effusions, ascites, or solid tumor tissues are the most common biopsy sources for primary cancer cell culture. Lung, breast cancer, lymphomas, and leukemia among other types of cancer are known to generate pleural effusions when tumor cells invade the pleura⁴. Ascites is common in ovarian, uterine, and cervical cancers⁵. Isolation of tumor cells from effusions or ascites is less complicated than from solid tumors coming from surgery. Usually, effusions are centrifuged; the supernatant is discarded and the cell pellet is resuspended in appropriate media supplemented with FBS, and the growth factors required according to the cancer type. The neoplastic origin can be characterized right after isolation. Pleural effusions are known to provide better efficiency at the establishment of primary cancer cell cultures⁶.

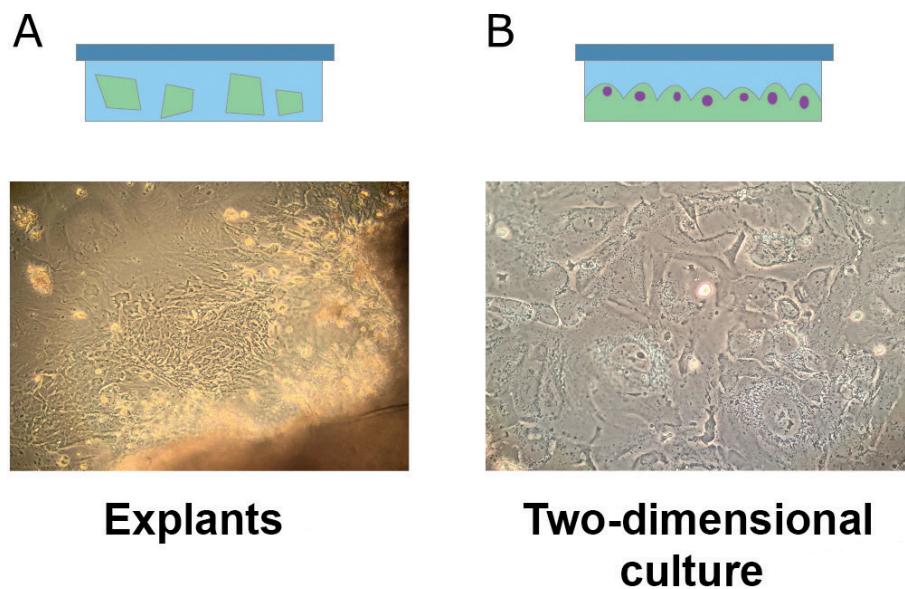
TUMOR DISAGGREGATION

Once the tumor biopsy has been acquired, the next step is to disaggregate tumor cells from the rest of

non-malignant cells that include tumor-associated fibroblasts, immune cells, and blood vessels, among other extracellular matrix components⁷. Separation of tumor cells from the tumor microenvironment may be one of the main difficulties when establishing primary cancer cell cultures. Certainly, the right selection of the disruption method is needed to break down the extracellular matrix and the connective tissue architecture that surrounds cancer cells. Tumor tissue disruption into a single-cell suspension varies from manual homogenization to the use of automated dissociators. Mechanical, enzymatic, and chemical methods or their combinations are used to disaggregate tumor samples. Mechanical dissociation applies physical force to the tissue using a sharp blade, scissors, or simply scraping it on a plate surface. The tissue can also be passed through a steel or nylon mesh and aspirated repeatedly with a pipette or needle gauge⁸. Enzymatic dissociation uses a wide variety of enzymes such as proteases and collagenases that disaggregate the extracellular matrix^{9,10}. Trypsin, elastase, pronase, hyaluronidases, and collagenases are the most common enzymes used for tissue disaggregation. Chemical dissociation disrupts Ca^{+2} and Mg^{+2} -dependent cell junctions using chelators such as ethylenediaminetetraacetic acid or ethylene glycol tetraacetic acid that sequester cations to loosen intracellular junctions¹¹. These three disaggregation methods have their own advantages and disadvantages (Table 1). There is also a semi-automatic tissue dissociator commercially available under the name "gentleMACS" (www.miltenyibiotec.com), featuring preloaded protocols to generate viable single-cell suspensions from tumor tissues¹². Here, right calibration of concentration, time, and temperature is important to obtain good yield and cell viability.



Figure 2. Schematic representation of growing primary tumor cells by explants and two-dimensional (2D) culture. (A) Tumor mass is cut into small pieces and placed on a sticky surface where tumor cells will outgrow from the explant. (B) Primary cancer cells grown in the 2D system.



Depending on the tumor type, dissociation methods should be combined to improve the yield of primary cancer cells.

CULTURE AND MAINTENANCE OF PRIMARY CANCER CELLS

Primary cancer cells are becoming the gold standard as *in vitro* models in cancer research; therefore, their culture conditions should preserve the original tumor characteristics. Culture and maintenance of primary cancer cells are based on methods developed to grow immortalized cancer cell lines. Primary cancer cells can be grown by explants, two-dimensional (2D) or three-dimensional (3D) cultures, which will be described in the following paragraphs.

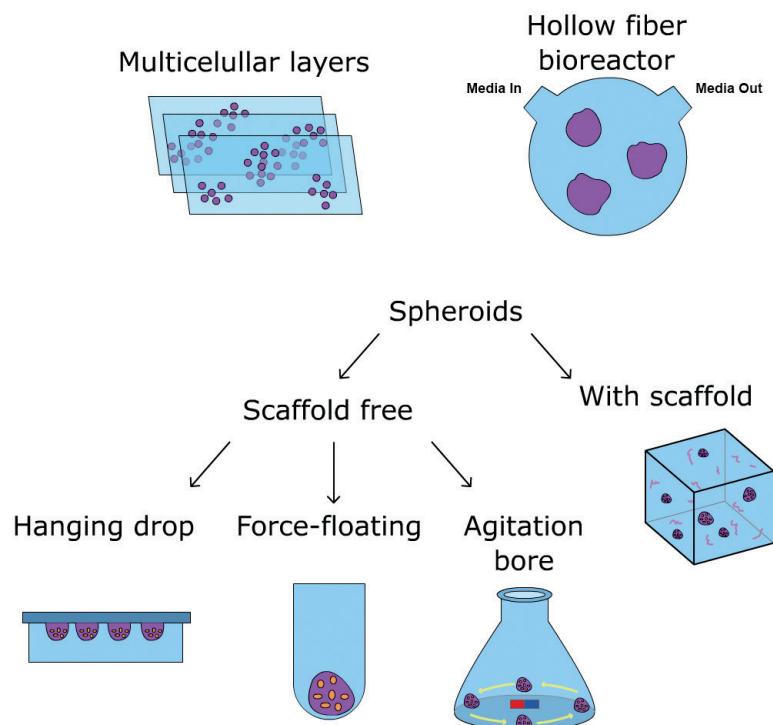
Culturing primary cancer cell cultures by explants has the advantage of retaining the tissue architecture and microenvironment, thus replicating, to some extent, the *in vivo* interactions. In this method, small tumor pieces are placed over a sticky surface that allows tumor to remain attached (Fig. 2a). Cells that outgrow the explant can be lifted off the plate and grown on 2D culture, and the explant can continue generating primary cancer cells¹³. Cancer cell cultures

generated by explants are heterogeneous and might retain genetic variability for several passages. Explant cell cultures have been established from breast, prostate, and lung cancer¹⁴⁻¹⁶.

Growing primary cancer cells by 2D cell cultures (2D) is based on the method used to grow immortalized cancer cell lines. This method was established more than 100 years ago, and at present, the culture conditions are well established. Primary cancer cell lines that have been disaggregated are grown on a flat surface using materials such as plastic or glass; sometimes, the surface is pre-coated with fibronectin, collagen, or synthetic extracellular matrix to improve adherence and spreading (Fig. 2b)¹⁷. The 2D cell culture system has the advantages of easy observation of the monolayer and manipulation of cells while being less expensive than 3D cultures. It has enabled countless breakthroughs in tumor cell biology research and has provided a vast amount of information useful for comparisons. However, the behavior of cells growing as a monolayer is different from that of the tissue *in situ* or even in 3D culture. Flat monolayers cannot reproduce the complex environment of *in vivo* tissue because interactions of surrounding cells are lost in three dimensions. Drug sensitivity may be increased since the surface in contact with the drug is



Figure 3. Schematic diagrams of three-dimensional (3D) primary cell cultures. Multicellular layers: primary cancer cells are embedded within membrane multilayers. Hollow fiber bioreactor is a continuous perfusion system that uses polyvinylidene fluoride fibers within a tube with an inlet where fresh medium flows, and an outlet where cellular products are excreted. Spheroids can be formed using a scaffold or scaffold-free system. Scaffolds are semi-solid matrices from synthetic or natural materials, for example, agarose, collagen, poly (ethylene glycol), and polyesters. The scaffold-free system does not require a solid support, and thus, spheroids are formed by placing cells in suspension (hanging drop), in a low adherent surface or continuous stirring to avoid cell attachment.



larger on a spread cell¹⁸. For these reasons, results obtained with 2D cultures may not be entirely predictive for *in vivo* applications.

The third method for growing primary cancer cells is the 3D culture system, which in recent years has become a popular attempt to recapitulate *in vivo* conditions, including cellular interactions, specific environmental conditions (e.g., hypoxia, angiogenesis, necrosis, and metastasis), or presence of immune cells. In this sense, 3D cultures have proven to be more useful than 2D cultures for translational cancer research. There are different ways to culture cells as a 3D system, and they will be discussed below (Fig. 3).

Multicellular layers

This method uses porous collagen-coated membranes where cells are placed. Then, several membrane

sheets are put on top of each other. It is used with cells that do not aggregate, and its applications include measurement of invasion, drug transport, and resistance¹⁹.

Hollow-fiber bioreactor

3D culture by this method requires cells to be grown into polyvinylidene fluoride (PVDF) capillaries. It has been used to study cancer metabolism and drug resistance. Its main disadvantage comes from the barrier to cell growth that the PVDF fiber implies.

Spheroids

There are two types of spheroid culture systems: scaffold and scaffold free. In the latter, cell aggregates (spheroids) are generated without a solid support by one of the following methods: (a) the hanging



drop, consisting of placing a drop of cell suspension on the underside of a cell culture plate lid to allow cell growth on the inverted lid^{20,21}. Special hanging drop plates are available from companies such as NUNC and 3D Biometrix. (b) The force-floating method, where spheroids are generated by addition of a cell suspension to a low-adhesion plate after centrifugation; the plate is coated with hydrophilic or neutral charge polymer to avoid cell attachment^{22,23}. (c) The agitation-based approaches, where spheroids are spontaneously formed using non-stop stirring. Cells in suspension are placed into a spinner flask with continuous high-speed stirring that prevents cells from attaching. Some advantages of this method are that spheroids are formed uniformly, throughput is high, and distribution of nutrients is homogeneous due to continuous agitation²⁴.

The scaffold-based system for spheroid culture requires the use of extracellular matrix or a semi-solid organic or inorganic material where cells will survive, grow, and proliferate. This scaffold can be natural (agarose, collagen, fibronectin, laminin, or vitronectin) or synthetic (poly[ethylene glycol], poly[N-isopropylacrylamide] polyesters), and cells can just be put on the surface of the scaffold or embedded into it. Collagen type I is the most-used natural scaffold because of its low cost and pore size flexibility that accommodates different levels of matrix stiffness. Each type of cancer cell culture will require specific conditions; hence, concentration and scaffold type should be chosen carefully for optimal tumor cell growth. The scaffold system, however, presents some limitations regarding reproducibility and calibration of biochemical properties of the natural matrices; synthetic matrices can be more homogenous but present other constraints related to their opacity and limited capacity of penetration that hinders diffusion of testing compounds and further analyses.

Mimicking tumor formation *in vitro* through spheroid culture is widely used because it helps integrate various features of the tumor microenvironment. Coculture of tumor cells with fibroblasts, endothelial, or other type of cells can resemble the interactions occurring *in vivo*^{25,26}. Spheroids can reach up to 1 mm in diameter; at this size, they have three regions. The center is a necrotic area due to lack of oxygen and nutrients; the middle area contains

quiescent cells, and the outer layer has highly proliferating cells due to a great exposure to nutrients and oxygen. This structure is similar to *in vivo* conditions. Furthermore, cells may exhibit slower proliferation rates and increased survival when compared to 2D cultures. Molecular changes can also be found; for example, the ovarian cancer cell line SKOV3 grown in spheroids promotes expression of mesenchymal markers (N-cadherin, vimentin, and fibronectin) while diminishing expression of the epithelial marker CD326^{27,28}. When malignant breast cancer cells are grown in laminin-rich extracellular matrix, apoptosis is increased and cell proliferation drops²⁹. 3D cultures have also shown differences in drug response. Breast cancer cell lines grown in a laminin-rich extracellular matrix present resistance to anti-HER2 target drugs when compared to their 2D counterparts³⁰.

PRIMARY CELL CULTURE CHARACTERIZATION AND AUTHENTICATION

Characterization and authentication are important steps after derivation of primary cancer cells. Characterization may include (a) demonstration of absence of cross-contamination; (b) confirmation of the species of origin; (c) connection with the tissue of origin, identification of the lineage to which the cell belongs and the differentiation state within the lineage; (d) determination of malignancy-associated characteristics; (e) predisposition of the new culture to genetic instability and phenotypic alteration; (f) identification of special features unique to the primary cancer cell culture; and (e) evaluation of growing parameters to determine optimal growth conditions.

Specific characterization techniques to be employed can fit into the type of work being carried out in the laboratory; for example, if molecular technology is easily available, then DNA fingerprinting, DNA profiling, or gene expression analysis is likely to be of most use, whereas a cytology laboratory may choose to use chromosome analysis coupled with fluorescence *in situ* hybridization and chromosome painting; a laboratory with immunological expertise may prefer to use major histocompatibility complex



analysis linked with lineage-specific markers. These procedures in combination with functional assays should provide sufficient data to authenticate a primary cancer cell culture. Regardless of the intrinsic laboratory capacities, DNA fingerprinting or DNA profiling and multiple isoenzyme analyses have now become the major standard procedures for cell authentication. Either short tandem repeats³¹ or single-nucleotide polymorphism analyses best characterize human cell lines and can also be employed for primary cancer cells. Changes in morphology are frequently an early sign of deterioration and often indicate that cells are differentiating, contaminated, or experiencing a crisis. Nevertheless, there is the chance that a primary cancer culture could become cross-contaminated with an existing continuous cell line or misidentified by mislabeling or handling mistakes. Literature reports show that most cell lines in the United States in the late 1960s were contaminated with HeLa, a cell line derived from an invasive cervical carcinoma³²⁻³⁴. Characterization studies have become necessary for cell authentication and validation of the derived data.

Some valuable parameters of characterization for lineage and sublineage discrimination are (a) cell-surface antigens, which are particularly useful for hematopoietic cell sorting³⁵. (b) Intermediate filament proteins - they are not involved in cell migration but appear to play a structural role and serve as lineage or tissue markers³⁶⁻⁴¹. (c) Markers of differentiation and specialized function - these markers are unique and specific to the cell type; however, they depend on full expression of the differentiated phenotype. (d) Enzymes - constitutive enzyme levels (in the absence of inducers or repressors), induced or adaptive level (the response to inducers and repressors), and existence of isoenzyme polymorphism are useful parameters⁴². (e) Regulation - tumor cell microenvironment (matrix, adjacent cells) regulates the expression level of many differentiated products. Hence, determination of specific lineage markers may require pre-incubation of the cells in a suitable environment where hormones, growth factors, or extracellular matrix is present at the correct amount and concentration. (f) Lineage fidelity - lineage markers are frequently used as tissue or cell type markers; they are characteristic of cell function rather than its embryonic origin^{43,44}.

BIOLOGICAL STUDIES USING PRIMARY CANCER CELL CULTURES

The use of primary cancer cell cultures for personalized therapy commonly resorts to assay cellular proliferation and migration as key biological properties to evaluate drug response.

Direct measure of DNA synthesis represents the most accurate way to assess cell proliferation. For example, 5-bromo-2-deoxyuridine (BrdU) is a reagent that incorporates into newly synthesized DNA during the S-phase of the cell cycle⁴⁵. After denaturation, specific antibodies can detect the DNA-incorporated BrdU. A variation of this assay employs 5-ethynyl-2'-deoxyuridine⁴⁶. Cellular DNA content and metabolic activity represent indirect parameters to measure cell proliferation and viability. Fluorescent nucleic acid stains are commercially available for high-throughput screening in add-incubate-read workflow^{47,48}. On the other hand, proliferation/viability assays based on cellular metabolism have the advantage of being more economical as compared with the previous methods⁴⁹.

One common metabolic proliferation assay involves intracellular reduction of the tetrazolium salt 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide by NAD(P)H-oxidoreductases of viable cells to form the formazan counterpart⁴⁹⁻⁵¹. [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] (MTS) can be employed in a similar fashion⁵². XTT [2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] and WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium] are converted to soluble formazans in culture medium, but their reduction is thought to occur at the cell surface⁵¹. The soluble compound resazurin can be reduced into the fluorescent product resorufin and generally provides higher sensitivity than tetrazolium compounds^{49,53,54}. Cell staining with crystal violet is a non-metabolic tool that can also be used to examine cellular proliferation^{55,56}.

Cell migration and invasion are important traits that define primary cell phenotypes. Cell migration is of particular interest in cancer given that metastatic



spread commonly represents an advanced stage in tumor progression and is strongly correlated with poor prognosis. One basic method to investigate cellular migration ability is known as the “wound closure assay,” which consists in the creation of a gap on a confluent cell monolayer to let cells on the edges move and close the wound⁵⁷⁻⁵⁹. The transwell migration assay, also known as the Boyden chamber assay, allows studying cellular migration through a physical barrier such as a porous membrane. Coating of the transwell membrane with extracellular matrix proteins turns the experiment into an invasion assay⁵⁹.

PRIMARY CELL CULTURES FOR PERSONALIZED THERAPY

In the era of cutting-edge technology, enormous advances in cancer biology knowledge have led to a vast investment in drug discovery. However, these new target drugs have had limited clinical success due to high toxicity or ineffective response. For these reasons, the development of novel high-throughput screening models for testing new drugs in preclinical studies is vital to translate the results from bench to bedside. The methodology for anticancer drug screening has been developed since 1970s, and both *in vitro* and *in vivo* models have been used. For many years, tumor cell lines were the main models for drug screening; however, they present several caveats and poor clinical prediction power (Table 2). Other methods that better resemble a patient’s tumor have become more attractive and effective, such as patient-derived xenografts (PDXs), circulating tumor cells (CTCs), and primary cancer cell cultures. PDX is an *in vivo* model that retains most of the complexity of the original tumor, including its heterogeneity^{60,61}. However, engraftment efficiency depends on the aggressiveness of tumor and can be influenced by the specific mouse model. The long time to develop the engraftment and cost-efficiency issues are some limitations of this model for drug screening and personalized therapy⁶²⁻⁶⁴ (Table 2). CTCs represent a metastatic stage and are suitable to study molecular changes developing from the primary tumor to the metastasis. At the moment, poor yield and purity are the main disadvantages of using CTCs for personalized therapy⁶⁵ (Table 2). Primary cell cultures represent a very attractive *ex vivo* model for drug

response prediction and informed clinical decision-making to improve therapeutic outcomes (Fig. 1). In recent years, their use has been expanding in pre-clinical studies with interesting results on intra-tumor heterogeneity and drug response from different tumor types. For example, Esparza-López et al. reported an association between cell and receptor tyrosine kinase expression patterns in subpopulations isolated from a primary breast cancer cell culture. Authors reported that platelet-derived growth factor receptor expression in breast cancer subpopulations drives cell proliferation, migration, anchorage-independent cell growth, and high sensitivity to paclitaxel and imatinib¹⁴. Kar et al. established several primary ovarian cancer cell cultures from ascitic fluid of corresponding patients⁶⁶. After four to six passages, ovarian cancer cells were free of fibroblasts and were used to investigate the response to chemotherapeutic agents such as paclitaxel, carboplatin, and curcumin. Each primary culture showed a different percentage of apoptotic cells upon treatment with the single drugs; however, combination experiments demonstrated higher effectiveness. For example, the combination of carboplatin with paclitaxel increased the average percent apoptosis from 14% (individual treatments) to 22% and addition of curcumin increased apoptosis to 45%. Primary cultures uncovered the wide response variation to specific drugs, probably coming from distinct tumor’s molecular profiles. This highlights the potential of primary ovarian cell cultures for individualized therapy.

Another example of drug-response evaluation with primary cell cultures can be found in the work of Karekla et al.¹⁶. They developed a method for explant culture of non-small cell lung cancer (NSCLC) tumor samples retaining both tumor and stromal cells. Non-necrotic sections from fresh NSCLC tumors were identified by hematoxylin and eosin staining, and small tissue fragments were placed in microporous membrane inserts hanging in a well of a 6-well plate with culture medium. After 16-20 h, the inserts were transferred to new wells with fresh medium, and the explants were exposed to cisplatin and the targeted agent tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) for 24 h. The explants were then fixed with paraformaldehyde and embedded into paraffin blocks to be analyzed by immunohistochemistry with cleaved poly-ADP-ribose polymerase (cPARP),



Table 2. Comparison of immortal cancer cell lines, primary cancer cells, PDXs, and CTCs for their use in personalized therapy

Cancer model	Advantages	Disadvantages
Immortal cancer cell lines	Standard culture media and techniques available Bypass ethical considerations on animal experimentation Provide large number of cells useful for comparative studies Suitable for genetic manipulation and molecular studies specific to cancer cells Lower cost	Prone to genetic and phenotypic changes over time Lack representation of tumor heterogeneity Lack relevance to personalized therapy
Primary cancer cells	Reflect the molecular background of patient's tumor cells Clinically relevant cancer cell models, useful for personalized therapy Bypass ethical considerations on animal experimentation Suitable for genetic manipulation and molecular studies specific to cancer cells	Cells may require complex culture medium to grow and retain phenotype Stromal components may outgrow cancer cells in the early culture steps Tumor heterogeneity may be lost, depending on culture technique Generation efficiency depends on type and source of sample
Patient-derived xenografts (PDXs)	Resemble the parental tumor <i>in vivo</i> Reflect the molecular background of a patient's tumor including tumor heterogeneity Clinically relevant cancer cell models, useful for personalized therapy Orthotopic xenografts resemble the growth and metastatic dissemination of a patient's tumor	Deficiency of host immune system Engraftment efficiency may be biased to undifferentiated and more aggressive tumor subtypes Mouse stroma outcompetes the human stromal component Development time may be too long for drug screening and personalized therapy Grafting techniques and mouse strains lack standardization Higher associated costs
Circulating tumor cells (CTCs)	Suitable to investigate the molecular profile of metastatic cancer, which could be different from the primary tumor Collected as liquid biopsy	Current CTCs enrichment methods need improvement to attain better recovery and specificity rates Comprehensive molecular profiling is hindered by CTC yield and purity Drug screening is challenged by low yield of CTC enrichment

Ki-67 and p53 antibodies. Previous experiments with a sample subset allowed the observation that significant drop in cell proliferation (Ki-67) and increase of cell death (cPARP) occurred with culture time beyond 24 h after the initial 16-20 h, whereas serum concentration in the growth medium had no significant effect.

The experiments exhibited the heterogeneous sensitivity of these preclinical models to the drugs and

pointed out an enhanced prediction of chemotherapeutic benefit on a case-by-case basis. Only around 50% of explants responded to cisplatin treatment and most of them at high concentration (50 μ mol/L). Notably, there was a significant correlation between patient survival and explant sensitivity to cisplatin in all tumor stages. In addition, percentage of cPARP staining showed dependency on histotype and was diminished in later tumor stages. Induction of p53 protein expression by cisplatin was associated with



lower levels of cell death and suppression of cell proliferation. Treatment with TRAIL proved successful in one out of twelve samples. This work provides an advantageous approach in terms of time and costs over other methods such as patient-derived xenografts to gain information about NSCLC patient tumor response to anticancer drugs, enabling prediction of treatment response in a culture system that maintains the tumor microenvironment and takes advantage of immunohistochemistry for the specific analysis of tumor cells.

Recently, Kodack et al. reported the success rate of developing primary cancer cells from 568 patient tissues corresponding to a variety of tumor types and sources⁶. They found an average success rate of 26%, most of them being lung tumors. Pleural effusions had a higher rate (42%) compared to core biopsies (23%). The main cause for the lack of cell culture initiation was a low content of cancer cells in the patients' specimens, followed by stromal fibroblast outgrowth. It was suggested that these difficulties could be alleviated by microscopic observation and differential trypsinization since fibroblasts tend to respond faster to cell detachment. Authors also found that a culture system with irradiated fibroblast feeder cells and medium with a defined composition (Dulbecco's Modified Eagle's Medium, F-12, FBS, endothelial growth factor (EGF), insulin, adenine, hydrocortisone, cholera toxin, and Rho-associated protein kinase inhibitor) was more effective than standard growth media to generate primary cell lines (77% vs. 54% from needle biopsies). They went on to develop an immunofluorescence assay for cell viability applicable to biopsy cultures in the presence of stromal cells and found that cancer cells of epithelial origin can be identified by their expression of cytokeratin 8 (CK8) and cytokeratin 18 (CK18), including cells with epithelial to mesenchymal transition. CK8/CK18 could recognize NSCLC cancer cells, squamous cell lung cancer, breast, bladder, colorectal, and pancreatic cancer cells. The antibody pair did not stain human fibroblasts and could reproduce the dose-response curves of pure cancer cells developed with an MTS-metabolic viability assay. Same results were found when cancer cells were cocultured with irradiated feeder fibroblasts. However, removal of EGF and insulin from the medium composition was needed to preserve the response to EGFR and ALK tyrosine kinase inhibitors, and this could slow down

the establishment of the primary cultures. Sensitivity of the NSCLC biopsy cultures to tyrosine kinase inhibitors was found to be consistent with the patients' response.

CONCLUSIONS

The fight against cancer has been moving toward personalized therapy in recent years. As we have discussed in this review, primary cancer cells represent an attractive model to study several aspects of cancer biology since they reflect the molecular and cellular characteristics of the original tumor cells. Culture of primary cancer cells involves a series of steps from ethics approval to sample collection, processing, maintenance, characterization, and authentication. Primary cancer cells are an excellent model for anti-cancer drug testing, overcoming deficiencies of cancer cell lines, or the costs associated with using animal models. As a valuable tool in personalized therapy, their use will likely keep expanding to improve prediction of cancer therapy benefit.

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REFERENCES

1. Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Barani L, et al. Primary cell cultures from anaplastic thyroid cancer obtained by fine-needle aspiration used for chemosensitivity tests. *Clin Endocrinol (Oxf)*. 2008;69:148-52.
2. Carney DN, Bunn PA Jr, Gazdar AF, Pagan JA, Minna JD. Selective growth in serum-free hormone-supplemented medium of tumor cells obtained by biopsy from patients with small cell carcinoma of the lung. *Proc Natl Acad Sci U S A*. 1981;78:3185-9.
3. Hovatta O, Silye R, Abir R, Krausz T, Winston RM. Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. *Hum Reprod*. 1997;12:1032-6.
4. Bezdieniezhnykh N, Lykhova A, Semesiuk N, Okhrimenko R, Kudryavets Y. Establishment and characterization of new breast and ovarian cancer cell lines as a model for studying cellular plasticity in vitro. *Exp Oncol*. 2016;38:94-100.
5. Kar R, Chawla D, Gupta B, Mehendiratta M, Wadhwa N, Agarwal R, et al. Establishment of primary cell culture from ascitic fluid



and solid tumor obtained from epithelial ovarian carcinoma patients. *Int J Gynecol Cancer*. 2017;27:2000-5.

- 6. Kodack DP, Farago AF, Dastur A, Held MA, Dardaei L, Friboulet L, et al. Primary patient-derived cancer cells and their potential for personalized cancer patient care. *Cell Rep*. 2017; 21:3298-309.
- 7. Tsai MJ, Chang WA, Huang MS, Kuo PL. Tumor microenvironment: a new treatment target for cancer. *ISRN Biochem*. 2014; 2014:351959.
- 8. Hayashida Y, Partida GJ, Ishida AT. Dissociation of retinal ganglion cells without enzymes. *J Neurosci Methods*. 2004;137:25-35.
- 9. Cunningham RE. Tissue disaggregation. *Methods Mol Biol*. 2010;588:327-30.
- 10. Quatromoni JG, Singhal S, Bhojnagarwala P, Hancock WW, Albelda SM, Eruslanov E, et al. An optimized disaggregation method for human lung tumors that preserves the phenotype and function of the immune cells. *J Leukoc Biol*. 2015;97: 201-9.
- 11. Li WC, Ralphs KL, Tosh D. Isolation and culture of adult mouse hepatocytes. *Methods Mol Biol*. 2010;633:185-96.
- 12. gentleMACS Dissociator. Miltenyi Biotec. Available from: <https://www.miltenyibiotec.com/US-en/products/mac-s-sample-preparation/tissue-dissociators-and-tubes/gentlemacs-dissociator/gentlemacstm-dissociator.html>.
- 13. Pei XF, Noble MS, Davoli MA, Rosfjord E, Tilli MT, Furth PA, et al. Explant-cell culture of primary mammary tumors from MMTV-c-myc transgenic mice. *In Vitro Cell Dev Biol Anim*. 2004;40:14-21.
- 14. Esparza-López J, Ramos-Elías PA, Castro-Sánchez A, Rocha-Zavaleta L, Escobar-Arriaga E, Zentella-Dehesa A, et al. Primary breast cancer cell culture yields intra-tumor heterogeneous subpopulations expressing exclusive patterns of receptor tyrosine kinases. *BMC Cancer*. 2016;16:740.
- 15. Nguyen EV, Centenera MM, Moldovan M, Das R, Irani S, Vincent AD, et al. Identification of novel response and predictive biomarkers to hsp90 inhibitors through proteomic profiling of patient-derived prostate tumor explants. *Mol Cell Proteomics*. 2018;17:1470-86.
- 16. Karelka E, Liao WJ, Sharp B, Pugh J, Reid H, Quesne JL, et al. Ex vivo explant cultures of non-small cell lung carcinoma enable evaluation of primary tumor responses to anticancer therapy. *Cancer Res*. 2017;77:2029-39.
- 17. Singhvi R, Kumar A, Lopez GP, Stephanopoulos GN, Wang DL, Whitesides GM, et al. Engineering cell shape and function. *Science*. 1994;264:696-8.
- 18. Huh D, Hamilton GA, Ingber DE. From 3D cell culture to organs-on-chips. *Trends Cell Biol*. 2011;21:745-54.
- 19. Kunz-Schughart LA, Freyer JP, Hofstaedter F, Ebner R. The use of 3-D cultures for high-throughput screening: the multicellular spheroid model. *J Biomol Screen*. 2004;9:273-85.
- 20. Kelm JM, Timmins NE, Brown CJ, Fussenegger M, Nielsen LK. Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types. *Biotechnol Bioeng*. 2003;83:173-80.
- 21. Napolitano AP, Dean DM, Man AJ, Youssef J, Ho DN, Rago AP, et al. Scaffold-free three-dimensional cell culture utilizing micro-molded nonadhesive hydrogels. *Biotechniques*. 2007;43:494, 496-500.
- 22. Vinci M, Gowan S, Boxall F, Patterson L, Zimmermann M, Court W, et al. Advances in establishment and analysis of three-dimensional tumor spheroid-based functional assays for target validation and drug evaluation. *BMC Biol*. 2012;10:29.
- 23. Gupta N, Liu JR, Patel B, Solomon DE, Vaidya B, Gupta V, et al. Microfluidics-based 3D cell culture models: utility in novel drug discovery and delivery research. *Bioeng Transl Med*. 2016; 1:63-81.
- 24. Benien P, Swami A 3D tumor models: history, advances and future perspectives. *Future Oncol*. 2014;10:1311-27.
- 25. Kim JB. Three-dimensional tissue culture models in cancer biology. *Semin Cancer Biol*. 2005;15:365-77.
- 26. Burdett E, Kasper FK, Mikos AG, Ludwig JA. Engineering tumors: a tissue engineering perspective in cancer biology. *Tissue Eng Part B Rev*. 2010;16:351-9.
- 27. Kuo CT, Wang JY, Lin YF, Wo AM, Chen BP, Lee H. Three-dimensional spheroid culture targeting versatile tissue bioassays using a PDMS-based hanging drop array. *Sci Rep*. 2017;7:4363.
- 28. Fischbach C, Chen R, Matsumoto T, Schmelzle T, Brugge JS, Polverini PJ, et al. Engineering tumors with 3D scaffolds. *Nat Methods*. 2007;4:855-60.
- 29. Park CC, Zhang H, Pallavicini M, Gray JW, Baehner F, Park CJ, et al. Beta1 integrin inhibitory antibody induces apoptosis of breast cancer cells, inhibits growth, and distinguishes malignant from normal phenotype in three dimensional cultures and *in vivo*. *Cancer Res*. 2006;66:1526-35.
- 30. Weigelt B, Lo AT, Park CC, Gray JW, Bissell MJ. HER2 signaling pathway activation and response of breast cancer cells to HER2-targeting agents is dependent strongly on the 3D microenvironment. *Breast Cancer Res Treat*. 2010;122:35-43.
- 31. Gilbert DA, Reid YA, Gail MH, Pee D, White C, Hay RJ, et al. Application of DNA fingerprints for cell-line individualization. *Am J Hum Genet*. 1990;47:499-514.
- 32. Lavappa KS. Survey of ATCC stocks of human cell lines for HeLa contamination. *In Vitro*. 1978;14:469-75.
- 33. Nelson-Rees WA, Flandermeyer RR. Inter and intraspecies contamination of human breast tumor cell lines HBC and brCa5 and other cell cultures. *Science*. 1977;195:1343-4.
- 34. Gartler SM. Apparent HeLa cell contamination of human heteroploid cell lines. *Nature*. 1968;217:750-1.
- 35. Pereira FG, Metze K, Costa FP, Lima CS, Lorand-Metze I. Phenotypic quantitative features of patients with acute myeloid leukemia. *Neoplasma*. 2006;53:155-60.
- 36. Sudo T, Iwaya T, Nishida N, Sawada G, Takahashi Y, Ishibashi M, et al. Expression of mesenchymal markers vimentin and fibronectin: the clinical significance in esophageal squamous cell carcinoma. *Ann Surg Oncol*. 2013;20 Suppl 3:S324-35.
- 37. Wu DK, de Vellis J. The expression of the intermediate filament-associated protein (NAPA-73) is associated with the stage of terminal differentiation of chick brain neurons. *Brain Res*. 1987; 421:186-93.
- 38. Brouty-Boyce D, Kolonias D, Savaraj N, Lampidis TJ. Alpha-smooth muscle actin expression in cultured cardiac fibroblasts of newborn rat. *In Vitro Cell Dev Biol*. 1992;28A:293-6.
- 39. Bochaton-Piallat ML, Gabbiani F, Ropraz P, Gabbiani G. Cultured aortic smooth muscle cells from newborn and adult rats show distinct cytoskeletal features. *Differentiation*. 1992; 49:175-85.
- 40. Lane EB, Klymkowsky MW. Epithelial tonofilaments: investigating their form and function using monoclonal antibodies. *Cold Spring Harb Symp Quant Biol*. 1982;46 Pt 1:387-402.
- 41. Bignami A, Dahl D, Seiler MW. Neurofilaments in the chick embryo during early development. I. Immunofluorescent study with antisera to neurofilament protein. *Dev Neurosci*. 1980; 3:151-61.
- 42. Grant AG, Duke D, Hermon-Taylor J. Establishment and characterization of primary human pancreatic carcinoma in continuous cell culture and in nude mice. *Br J Cancer*. 1979;39:143-51.
- 43. Binétruy B, Heasley L, Bost F, Caron L, Aouadi M. Concise review: regulation of embryonic stem cell lineage commitment by mitogen-activated protein kinases. *Stem Cells*. 2007;25:1090-5.
- 44. Buckleton JS, Krawczak M, Weir BS. The interpretation of lineage markers in forensic DNA testing. *Forensic Sci Int Genet*. 2011;5:78-83.
- 45. Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science*. 1982;218:474-5.
- 46. Salic A, Mitchison TJ. A chemical method for fast and sensitive detection of DNA synthesis *in vivo*. *Proc Natl Acad Sci U S A*. 2008;105:2415-20.
- 47. Jones LJ, Gray M, Yue ST, Haugland RP, Singer VL. Sensitive determination of cell number using the CyQUANT cell proliferation assay. *J Immunol Methods*. 2001;254:85-98.
- 48. CyQUANT Direct Cell Proliferation Assay. Available from: <http://www.thermofisher.com/order/catalog/product/C35011>.
- 49. Moravec RA, Niles AL, Duellman S, Benink HA, Worzella TJ, et al. Cell Viability Assays. 2013 May 1 [Updated 2016 Jul 1]. In: Sittampalam GS, Coussens NP, Brimacombe K, Grossman A, Arkin M, Auld D, et al. Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences. 2004. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK144065/>
- 50. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55-63.
- 51. Berridge MV, Herst PM, Tan AS. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. *Biotechnol Annu Rev*. 2005;11:127-52.
- 52. Cory AH, Owen TC, Barltrop JA, Cory JG. Use of an aqueous soluble tetrazolium/formazan assay for cell growth assays in culture. *Cancer Commun*. 1991;3:207-12.
- 53. Ahmed SA, Gogal RM Jr, Walsh JE. A new rapid and simple non-radioactive assay to monitor and determine the proliferation of



lymphocytes: an alternative to [³H]thymidine incorporation assay. *J Immunol Methods*. 1994;170:211-24.

54. Chen JL, Steele TWJ, Stuckey DC. Metabolic reduction of resazurin; location within the cell for cytotoxicity assays. *Biotechnol Bioeng*. 2018;115:351-8.

55. Feoktistova M, Geserick P, Leverkus M. Crystal violet assay for determining viability of cultured cells. *Cold Spring Harb Protoc*. 2016;2016:pdb.prot87379.

56. Śliwka L, Wiktorska K, Suchocki P, Milczarek M, Mielczarek S, Lubelska K, et al. The comparison of MTT and CVS assays for the assessment of anticancer agent interactions. *PLoS One*. 2016;11:e155772.

57. Liang CC, Park AY, Guan JL. *In vitro* scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc*. 2007;2:329-33.

58. Jonkman JE, Cathcart JA, Xu F, Bartolini ME, Amon JE, Stevens KM, et al. An introduction to the wound healing assay using live-cell microscopy. *Cell Adh Migr*. 2014;8:440-51.

59. Justus CR, Leffler N, Ruiz-Echevarria M, Yang LV. *In vitro* cell migration and invasion assays. *J Vis Exp*. 2014;88:51046.

60. Nguyen LV, Cox CL, Eirew P, Knapp DJ, Pellacani D, Kannan N, et al. DNA barcoding reveals diverse growth kinetics of human breast tumour subclones in serially passaged xenografts. *Nat Commun*. 2014;5:5871.

61. Cheung PF, Yip CW, Ng LW, Lo KW, Chow C, Chan KF, et al. Comprehensive characterization of the patient-derived xenograft and the paralleled primary hepatocellular carcinoma cell line. *Cancer Cell Int*. 2016;16:41.

62. Zhang L, Liu Y, Wang X, Tang Z, Li S, Hu Y, et al. The extent of inflammatory infiltration in primary cancer tissues is associated with lymphomagenesis in immunodeficient mice. *Sci Rep*. 2015; 5:9447.

63. Hidalgo M, Amant F, Biankin AV, Budinská E, Byrne AT, Caldas C, et al. Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov*. 2014; 4:998-1013.

64. Sereti E, Karagianellou T, Kotsoni I, Magouliotis D, Kamposioras K, Ulukaya E, et al. Patient Derived Xenografts (PDX) for personalized treatment of pancreatic cancer: emerging allies in the war on a devastating cancer? *J Proteomics*. 2018; 188:107-18.

65. Tellez-Gabriel M, Cochonneau D, Cadé M, Jubellin C, Heymann MF, Heymann D, et al. Circulating tumor cell-derived pre-clinical models for personalized medicine. *Cancers (Basel)*. 2018;11:e19.

66. Kar R, Sharma C, Sen S, Jain SK, Gupta SD, Singh N, et al. Response of primary culture of human ovarian cancer cells to chemotherapy: *in vitro* individualized therapy. *J Cancer Res Ther*. 2016;12:1050-5.