

Review article

Personalized models of heterogeneous 3D epithelial tumor microenvironments: Ovarian cancer as a model[☆]

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ABSTRACT

Intractable human diseases such as cancers, are context dependent, unique to both the individual patient and to the specific tumor microenvironment. However, conventional cancer treatments are often nonspecific, targeting global similarities rather than unique drivers. This limits treatment efficacy across heterogeneous patient populations and even at different tumor locations within the same patient. Ultimately, this poor efficacy can lead to adverse clinical outcomes and the development of treatment-resistant relapse. To prevent this and improve outcomes, it is necessary to be selective when choosing a patient's optimal adjuvant treatment. In this review, we posit the use of personalized, tumor-specific models (TSM) as tools to achieve this remarkable feat. First, using ovarian cancer as a model disease, we outline the heterogeneity and complexity of both the cellular and extracellular components in the tumor microenvironment. Then we examine the advantages and disadvantages of contemporary cancer models and the rationale for personalized TSM. We discuss how to generate precision 3D models through careful and detailed analysis of patient biopsies. Finally, we provide clinically relevant applications of these versatile personalized cancer models to highlight their potential impact. These models are ideal for a myriad of fundamental cancer biology and translational studies. Importantly, these approaches can be extended to other carcinomas, facilitating the discovery of new therapeutics that more effectively target the unique aspects of each individual patient's TME.

Statement of significance

In this article, we have presented the case for the application of biomaterials in developing personalized models of complex diseases such as cancers. TSM could bring about breakthroughs in the promise of precision medicine. The critical components of the diverse tumor microenvironments, that lead to treatment failures, include cellular- and extracellular matrix- heterogeneity, and biophysical signals to the cells. Therefore, we have described these dynamic components of the tumor microenvironments, and have highlighted how contemporary biomaterials can be utilized to create personalized *in vitro* models of cancers. We have also described the application of the TSM to predict the dynamic patterns of disease progression, and predict effective therapies that can produce durable responses, limit relapses, and treat any minimal residual disease.

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1. Introduction

Precision medicine, wherein a treatment is tailored to a patient's specific tumor characteristics, has become widely popular in the last 10 years [1–3]. In precision medicine, therapies are

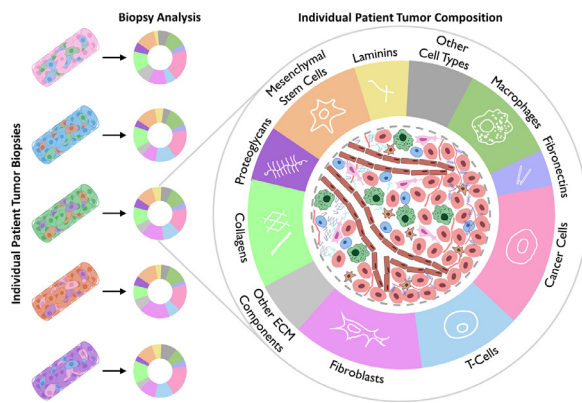


Fig. 1. Cancers are composed of distinct and heterogeneous microenvironments. Human diseases, including cancers, are complex heterogeneous ecosystems. Although cancers can be categorized based on histological subtype, components of the TME can vary drastically between patients. Informed by analysis of these distinct components, biomaterials-based technologies enable the development of tumor specific models. These personalized models represent new paradigms in precision medicine and a systems level approach to improving clinical outcomes.

specifically optimized based on molecular profile, cancer type, stage, and biomarkers for each patient. This process currently involves obtaining the genetic profile of the patient, which is then used to anticipate patient specific drug metabolism, response, and toxicities. Given the high molecular heterogeneity in many cancer types, the concept of personalized or precision medicine is intuitive. This is even more so, when other factors that contribute to clinical outcomes, such as ethnicity, gender, age, socioeconomic status, geographic location, and disability are considered. Precision treatments are particularly effective in increasing the treatment efficacy of cancers with targetable mutations in a subset of patients [4–6]. However, many cancers lack targetable genetic mutations, thereby making precision treatment difficult [7]. In addition, the efficacy of cancer cell-targeting therapies are often compromised by the non-cancerous components of the tumor microenvironment (TME) [8–10]. This includes tumor supporting cells and tumor secreted extracellular matrices (ECM) which form complex multifaceted interactions with cancer cells that act to modulate chemoresistance [11–14]. Unfortunately, high level cell-cell and cell-ECM interactions are often omitted in contemporaneous model systems leading to underrepresented cellular, molecular, and ECM heterogeneity. Moreover, this is thought to have a substantial impact on clinical translation [15–17].

In light of this oversight, we propose the thorough analysis of the genetic, molecular, cellular, and ECM composition of patient biopsies, performed concomitantly with pathologic diagnosis, to guide construction of *in vitro* personalized tumor-specific models (TSM). Generation of 3D microscale TSM based on the comprehensive analysis of each patient's tumor would be followed by high throughput assays. This process would have numerous applications, ranging from personalized drug screening to identification of novel biomarkers. If successfully implemented, these applications have the potential to change the landscape of precision medicine and decrease mortality rates in many cancers. Here we present this approach through the lens of high grade serous ovarian cancer (HGSOC), which is highly heterogeneous and notorious for development of chemoresistance, relapse, and poor outcomes. Despite the focus on HGSOC in this review, we postulate that these same methods can be extended to different epithelial cancer types with their own unique genomic, molecular, and microenvironmental characteristics [18–20] (Fig. 1). Using HGSOC as a model disease allows us to highlight patient-specific differences in the TME, which may not be ubiquitous in all other epithelial tumors, but

serves as a starting point from which pragmatic parallels can be drawn to other tumors.

In the first section of this review, we start by introducing ovarian cancer and its various sources of heterogeneity, including genetic, molecular, cellular, and ECM differences between and within patients. We then briefly outline how these features interact to confer heterogeneous responses to treatment. Subsequently, we review current models that recapitulate each aspect of heterogeneity before describing our proposed TSM model system and potential key applications.

2. Defining heterogeneity in the ovarian cancer microenvironment

2.1. Characteristics of high grade serous ovarian cancer

Ovarian cancer is the most lethal of all gynecological malignancies and 5th leading cause of cancer deaths among women worldwide [21,22]. As the most common type of ovarian cancer, HGSOC accounts for over 60% of ovarian cancer deaths. Patients with HGSOC are often diagnosed at advanced stages when tumors have metastasized to peritoneal organs. They receive standard therapy that combines cytoreductive surgery and platinum-taxane chemotherapy. More than half of the patients become increasingly resistant to the conventional chemotherapies within 6 months of treatment, which eventually leads to tumor relapse and death [23,24].

Characterizing these tumors reveals genomic profiles with frequent copy number variations (CNV) but also a notable deficit in mutational burden. There are only a handful of genetic mutations common between patients and they are not uniformly expressed across all HGSOC which complicates the use of targeted therapies [25–27]. Given that the majority of women with ovarian cancer have recurrent tumors and no common somatic mutations to stratify them, accelerated screening of novel or synergistic therapies are critically needed. On the other hand, large-scale HGSOC datasets have been used to stratify ovarian cancer patients into molecular subtypes based on gene expression [27,28]. This stratification has prognostic and therapeutic relevance [29], however, no clinical use has been validated [30].

Another major contributor to ovarian cancer malignancy is the unique metastatic pattern that differs from those of most other epithelial malignant diseases. Most frequently HGSOC disseminates via the transcoelomic route, with about 70% of patients developing peritoneal metastases [31,32]. Specifically, metastatic multicellular tumor spheroids disseminate with the accumulated ascites (tumor fluid) in the peritoneal cavity and spread to the surrounding abdominal organs (colon, intestine, liver, pancreas, lungs) [32,33]. They are also transported by peritoneal fluid flow to adhere to the mesothelium that covers the peritoneal surfaces where they initiate metastatic colonies [33,34]. This unique spreading pathway creates a microenvironment that enriches cancer stem-like cell (CSC) populations and treatment-resistant cancer cells [12]. Given the different characteristics of the microenvironments along various stages of metastasis, the development of *in vitro* 3D TSM that resemble each HGSOC-specific TME (Fig. 1) is critical for identifying therapeutics that can effectively target drug-resistant cancer cell populations. In order to recreate these microenvironments in TSM, it is necessary to better understand both the cellular and acellular components, and the role that each plays in disease progression and chemoresistance. These components and their complex interactions are described below.

2.2. Cellular heterogeneity in the TME

Cellular heterogeneity can be defined on two levels. It can refer to cell level metabolic, genetic, epigenetic, and phenotypic dif-

ferences between cancer cells, or it can refer to the higher level composition of all cell types in the tumor microenvironment (i.e. a tumor with high versus low immune infiltration) [28,35–38]. Both definitions of cellular heterogeneity can influence disease progression and chemoresistance, stressing the need to incorporate both in models for precision medicine.

2.2.1. Cellular heterogeneity within the cancer cell population in the TME

HGSOC is highly heterogeneous in terms of its histopathological architecture, genetic alterations, and cellular composition, which results in variable responses to chemotherapies. The histopathological architectures of HGSOC are solid masses of cells with papillary, glandular, or cribriform features, which are often accompanied by areas of extensive necrosis [39]. Some HGSOC resemble the appearance of endometrioid or transitional cell carcinoma. The morphology of these tumors is distinct, but they show similar immunoreactivity to typical HGSOC and are diagnosed accordingly [40]. HGSOC tumors share genomic features, including frequent *TP53* mutations and homologous recombination deficiency. Mutations in *NF1*, *BRCA1*, *BRCA2*, *RB1*, and *CDK12* are also frequent [27,41]. Most HGSOC exhibit large genetic gains and losses suggesting genomic instability. Common copy number alterations are gains in *MYC* and *KRAS* genes and losses in *PTEN*, *RB1*, and *NF1* genes. Primary HGSOC can have highly heterogeneous genomic alterations, which are associated with high recurrent risk and poor prognosis [42]. Acquisition of genetic mutations over the course of disease progression is an example of the clonal evolution model of tumor heterogeneity wherein somatic mutations result in a mixture of phenotypes that may respond differently to treatment [43].

In addition to genetic heterogeneity, cancer cells are also subject to epigenetic variation which influences cell phenotypes and treatment response [18,35,44]. Epigenetic changes are heritable modifications to gene expression that do not change the DNA, but rather alter accessibility of genes through various mechanisms including DNA methylation, histone modifications, and microRNA. These epigenetic changes occur more often than genetic mutations and happen early on in tumor progression, highlighting their role in developing diverse mixtures of tumor cells and the potential impact of drugs targeting epigenetic dysregulation [44]. In ovarian cancer, an important example of epigenetic alterations is the hypermethylation of *BRCA1*. This results in decreased *BRCA1* expression which in turn promotes genomic instability in the ovarian cancer cells and leads to heterogeneous phenotypes [45]. While a detailed discussion of epigenetic modifications is beyond the scope of this review, readers are referred to more comprehensive reviews dedicated entirely to the topic [18,44,45]. Importantly, epigenetic alterations are also reported to be involved in the reprogramming of cancer cells into a rare, but clinically relevant population of cancer cells termed cancer stem-like cells (CSC) [46].

CSC are a clinically relevant subpopulation that is responsible for tumor initiation, metastatic disease, recurrence, and resistance to chemo/radio-therapy [47–51]. Like normal stem cells, CSC maintain a level of pluripotency, which enables self-renewal and differentiation [49,52,53]. In addition, CSC are highly plastic cells with a heterogeneous population defined by different epigenetic and metabolic states which they pass on to their progeny. CSC progeny comprise most of the tumor mass and tend to be more responsive to chemotherapy than CSC [53]. This ultimately leads to the development of a heterogeneous tumor cell population generated from distinct CSC [35,50,51,54,55].

Self-renewing tumorigenic CSC have been identified and isolated in many cancers, including, leukemia [56], breast [57], ovarian [58], colon [59,60], prostate [61,62], brain [63–65], pancreatic [66], melanoma [67], myeloma [68], and lung [69]. Importantly, while some CSC markers are shared between different can-

cer types, some cancers also have unique CSC markers [70]. Even among common CSC markers, recent work by Dzobo *et al.* found no consistent pattern of ALDH1A1, CD44, CD24, EPCAM, ICAM1, CD90, CXCR4, NES, CD133, ABCB1, and ABCG2 expression in colon, pancreatic, lung, and esophageal cancers [71]. This cross-cancer heterogeneity in CSC populations indicates that CSC targeting therapies may not share efficacy across cancer types. In ovarian cancer, CSC are identified by an amalgam of biomarkers including, CD133, ALDH, CD44, CD24, and CD117, and their presence is an indicator of reduced progression-free survival and poor patient outcomes [72–84]. Ovarian CSC phenotypes are dictated in part by interactions with the other cells in the TME, which will be discussed in Section 2.2.2.

The resistance of CSC to treatment is attributed to overexpression of ABC transporters, enhanced ALDH activity, response to DNA damage, epithelial to mesenchymal transition (EMT), and dormancy [85,86]. Due to these resistance mechanisms, CSC are capable of surviving primary therapies and repopulating the tumor with a heterogeneous population of cancer cells [87]. In ovarian CSC, it has also been suggested that heterogeneity within the CSC population may increase the odds of the development of spontaneous escape variants [88]. Anoikis resistance is another key feature of ovarian CSC, which allows them to survive in non-adherent conditions like the malignant ascites fluid that accumulates in the peritoneal cavity [51,87].

2.2.2. Cellular heterogeneity in the non-cancer cell composition in the TME

Tumors are a heterogeneous mixture of host cells and tumor cells that interact dynamically to drive tumor progression [89,90]. Specifically, the proportion of epithelial, endothelial, lymphocyte, myeloid, and stromal cell components varies significantly between tumors [91–93]. The high degree of cellular heterogeneity both within and between patients' tumors can affect drug response and prognosis, causing major challenges in the clinical management of many cancers [38,90]. In this section we will review the heterogeneity of cell compositions in various types of HGSOC microenvironments and discuss how these cells contribute to heterogeneity in the ECM in Section 2.3 (Fig. 2).

HGSOC have several unique microenvironments including the primary tumor, the malignant ascites, and secondary metastatic sites. Each of these microenvironments is accompanied by different cellular and acellular characteristics that affect tumor progression [94]. While there is overlap between the cells that are present in each microenvironment, their relative proportions vary and this can influence the signals received by the tumor cells [94]. In primary HGSOC tumors, epithelial cells have been shown to make up as much as ~68% of the population, while in metastatic tumors, epithelial cells may only make up as few as 10% [94]. However, the immune cell composition is known to be highly variable and the stroma makes up anywhere from 10% to 60% of the tumor, indicating that a fair degree of heterogeneity exists even within the primary tumor [91–93].

CSC are enriched through dynamic interactions with cells in the surrounding TME, such as carcinoma-associated mesenchymal stem cells (CA-MSC), carcinoma-associated fibroblasts (CAF), endothelial cells (EC), and macrophages [89,90]. These interactions are exemplified by mesenchymal stem cells (MSC) which reside in the ovary and can be recruited to the primary tumor from distant locations in the body through soluble signaling. These MSC can then be converted into CA-MSC by the cancer cells [95–97]. Once converted, they have been shown to increase the number of CSC, enhance chemo-protection for CSC, and lead to tumor growth, either through paracrine signaling or indirectly by differentiation into CAF [97–105]. Importantly, CA-MSC are distinct from CAF and normal MSC, and can differentiate into several other critical com-

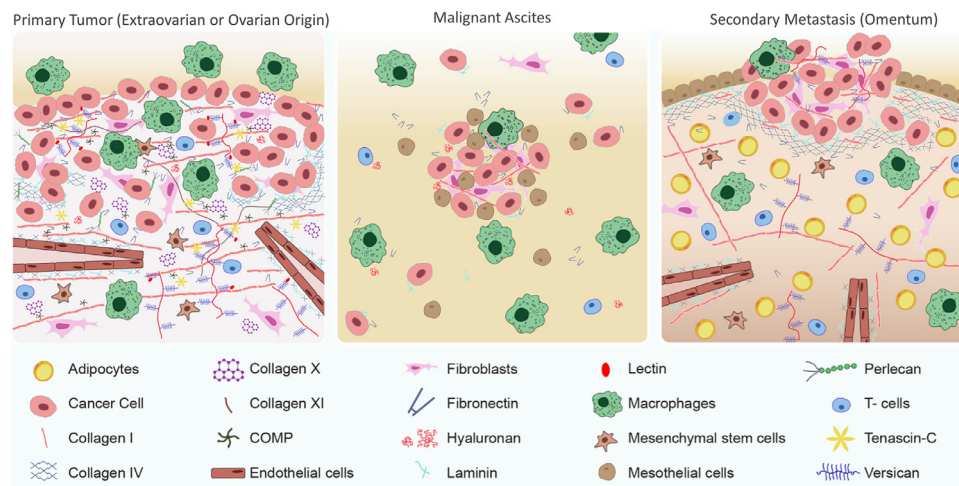


Fig. 2. Distinct and heterogeneous cellular and ECM composition and organization are present in primary and metastatic ovarian cancers. (Left) Production of ECM in the primary ovarian tumor is gradually dysregulated over time, with the deposition of new proteins not normally found in the ovarian germinal epithelium and tunica albuginea. The basement membrane is also degraded in the primary tumor aiding in dissemination. The primary tumor is infiltrated by tumor-associated macrophages, T cells, carcinoma-associated fibroblasts and endothelial cells. (Middle) The malignant ascites contains suspended single cells and cellular aggregates comprised of cancer cells, CSC, carcinoma-associated fibroblasts, mesothelial cells, macrophages and T cells. ECM such as fibronectin, hyaluronan, and collagen type I are found within cellular aggregates, and in and around the fluidic ascites. (Right) Secondary omental metastases consist of colonizing cellular spheroids from the ascites. In these sites, cancers cells begin to produce new basement membrane, collagens, and hyaluronan. In all three TME, the cell and ECM sub-types are indicated with the representative schematic (not drawn to scale).

ponents of the tumor stroma including fibroblasts, osteocytes, and adipocytes [95,105], further contributing to cellular heterogeneity. Additionally, EC in the primary tumor and secondary tumor locations can also support CSC and tumor cells [106,107]. Not only have EC been shown to induce self-renewal pathways in CSC [108], but they also protect them from cisplatin and paclitaxel treatment [109–111]. Similar to CSC recruitment of MSC to the tumor, CSC can recruit EC to the tumor through secretion of angiogenic signals, like VEGF, SDF-1. This occurs when hypoxia develops as a result of the tumor outgrowing its vasculature. Hypoxic microenvironments activate Oct-4, Sox2, Notch, VEGF, and c-MYC expression which stabilizes HIF-1 α and promotes survival capacity of the CSC [70,89,112,113]. EC additionally contribute to disease progression through their role in the formation of the unique malignant ascites TME [32,114].

The malignant ascites is the buildup of fluid in the peritoneal cavity that accompanies various pathologies, including HGSOE [32]. While the exact cellular context of the ascites changes with disease progression [115], the predominant cell populations in the ascitic TME include cancer cells [116,117], CAF [116,117], leukocytes [116,117], and mesothelial cells [118]. Within the ascites, the cancer cell population contains a high proportion of treatment-resistant CSC [11,117,119]. Cells within the ascites often aggregate, to form tumor spheroids that are highly malignant, metastatic, and resistant to chemotherapy [116,120,121]. The non-tumor cells in these spheroids influence cancer cell phenotype and promote malignant characteristics. Among them, CAF are fibroblasts that are reprogrammed into a pro-tumoral phenotype. They promote EMT, tumor cell attachment to the mesothelium, and subsequent displacement of mesothelial cells [91,116,120]. This ultimately allows tumor spheroids to initiate tumor growth at secondary locations. CAF also have immunomodulatory functions, can promote angiogenesis, inhibit cancer cell apoptosis, and produce and remodel ECM [91,116,122]. CAF also have the propensity to promote stemness, chemoresistance, and tumor growth [91,97,122].

Tumor associated macrophages (TAM), another predominant cell type found in the malignant ascites (in addition to the primary tumor), are similar in their ability to drive components of disease progression such as angiogenesis, angiostasis, metastasis,

and the function of other immune cells in the TME [123–125]. Like CAF, ovarian cancer cells also reprogram macrophages that are recruited to the tumor, into a pro-tumoral phenotype termed M2-like alternatively activated macrophages [125]. These macrophages can contribute to immunosuppression by attracting regulatory T cells, inhibiting the anti-tumor response of natural killer (NK) cells and cytotoxic T cells, and inhibiting maturation of dendritic cells (DC). They can also promote drug resistance, decrease apoptosis, and promote stemness in ovarian cancer cells [125,126]. Despite the widespread influence that M2-like macrophages have on tumor progression, their prognostic effect depends on the proportion of anti-tumoral M1-like classically activated macrophages, which are also present in the TME. The M1:M2 macrophage ratio in patients can vary widely, resulting in different immune responses and altered prognosis. Expectedly, higher M1:M2 ratios are associated with better outcomes for the patient [125,127].

In addition to macrophages, a host of other immune cells can be found in the ovarian cancer TME including NK cells, regulatory T cells, DC, CD8+ and CD4+ T cells, B cells, and myeloid derived suppressor cells (MDSC). Each of these cell types play a role in the complex immune response present in a tumor, with some cells like MDSC, regulatory T cells, and DC, serving tumor suppressive functions and others such as CD8+ and CD4+ T cells having antitumor effects [93,128,129].

Within the immune cell category there is remarkable heterogeneity, even within the same tumor site [38,130–132]. The immune cell context of a tumor can change throughout tumor progression and can depend on factors like tumor location and treatment status [38,130,132]. For example, a recent study in HGSOE examined immunogenomic changes before and after neoadjuvant treatment in paired samples. Astoundingly, the authors observed that in treatment-naïve patients some areas of the same tumor contained immune cells while others completely excluded them [38]. Furthermore, they found that variation in immune cell genes, particularly those associated with T cells and NK cells, were responsible for the highest degree of variance between different treatment-naïve patient samples. This suggests the importance of immune heterogeneity in interpatient differences. Thorough characterization of these samples revealed that immune exclusion was

associated with amplification of MYC targets and WNT signaling, which has previously been related to immune evasion [38,133]. The authors also noted increased NK cell infiltration and oligoclonal T cell expansion following treatment neoadjuvant therapy, demonstrating the plasticity of the heterogeneous immune microenvironment when perturbed [38].

Another study of immune heterogeneity in HGSOC compared the immune cell infiltration in the primary tumor and its surrounding stroma to the infiltration of corresponding omental or peritoneal metastases. Interestingly, they found variance in the number of CD45+, CD3+, CD8+ and PD-1+ cells between all primary samples and matched metastatic samples. Furthermore, they found that immune cell infiltration in the stroma of the omentum was significantly greater than in the primary tumor stroma [130]. Post treatment analysis of the same patients/samples, revealed that an increased intratumoral CD3+ infiltration in the primary tumor was associated with platinum sensitivity indicating that high pretherapeutic CD3+ infiltration could be an indicator of favorable platinum response. Similarly, higher intratumoral CD8+ infiltration in peritoneal metastases compared to the primary tumor was also associated with platinum sensitivity [130]. These results are concordant with previous work indicating the CD3+ and CD8+ T cells are prognostic indicators for ovarian cancer [131,134,135]. Contrarily, high PD-1+ expression in peritoneal metastases is linked to poor response to platinum therapy [130]. This finding agrees with reports that PD-1 activation is associated with decreased anti-tumor immunity [136]. Overall, this study highlights the potential utility of immune context characterization in predicting treatment response and disease progression. As a result of these findings, the immune cell composition needs to be taken into account in development of personalized models. More comprehensive discussion of the immune system's role in cancer has been reviewed previously [128,137].

Aside from the prominent cells found directly in the malignant ascites, mesothelial cells and adipocytes can both interact with the tumor at secondary tumor sites in the peritoneal cavity and from afar through soluble signaling in the ascites to exert additional influence on disease progression [120]. Mesothelial cells, for example, can secrete factors like lysophosphatidic acid (LPA) to promote tumor cell adhesion, migration, and invasion [138,139]. They can also produce factors that inhibit drug induced apoptosis in cancer cells, following stimulation by the ascites [139,140]. Additionally, mesothelial cells are capable of transitioning into CAF via TGF- β signaling [141] to advance disease progression. Recently, mesothelial cells have even been found to induce platinum resistance in peritoneal metastasis through cell-to-cell interactions [142].

Adipocytes are prominent in the omentum, which is one of the most common sites of ovarian cancer metastasis, as well as subcutaneous tissues and the mesenteric membrane. From these locations, adipocytes can interact with tumor cells through secretion of adipokines and lipokines. Specifically, adipocytes in the omentum can attract cancer cells through IL-8 secretion and promote their proliferation through transfer of fatty acids. Importantly, adipocytes have recently been shown to confer chemoresistance to cancer cells through activation of the Akt pathway in cancer cells and secretion of a chemo-protective lipid, arachidonic acid [143]. Contrarily, adipocytes may contribute to suppression of ovarian cancer through cross-talk with ovarian cancer cells via secreted protein acidic and rich in cysteine (SPARC) [144]. These studies underscore the fact that the specific phenotype of the adipocytes and cancer cells may determine the result of adipocyte-cancer cell interactions.

As in the primary and ascites TME, ovarian cancer cells that have metastasized to secondary locations are subjected to even more diverse cellular microenvironments that can further alter the phenotype of cancer cells [145]. For example, in the omentum,

adipocytes and mesothelial cells are in closer proximity to cancer cells, which is different from the TME in the ascites, where cancer cells are surrounded by mostly CAF and leukocytes. In the omentum, adipocytes are abundant with localized *milky spots* filled with immune cells, CAF, adipose-derived MSC, and vascular cells [146–149].

2.2.3. Effect of hypoxia on cellular heterogeneity (cancer and non-cancer) within the TME

When discussing cellular heterogeneity, it is also important to note the role of hypoxia in producing heterogeneous cell populations. As tumors develop, they outgrow their vasculature which leads to development of hypoxic pockets of cells [150]. These pockets of cells develop altered metabolism in order to survive the harsh conditions, which influences their proliferation, migration, and invasion [51,151]. HIF-1 α plays a central role in cellular response to hypoxia and its expression is associated with poor outcomes in ovarian cancer [152]. In ovarian cancer hypoxia has been implicated in CSC enrichment through HIF-1 α mediated activation of NF- κ b signaling and consequent upregulation of SIRT1 [112]. Not only has hypoxia been shown to enrich for cancer stem cell phenotypes in ovarian cancer, but it also promotes CSC chemoresistance via HIF-2 α mediated upregulation of *BCRP*, a gene encoding a transporter that can expel drugs [112,153].

Hypoxic conditions also influence the non-cancer TME cells and how they interact with the cancer cells. For example, in hypoxic environments CAF stabilize HIF-1 α leading to a metabolic switch to aerobic glycolysis and corresponding lactate production. This lactate can then be used by cancer cells to promote tumor growth [150,154]. Furthermore, CAF are also known for pro-tumorigenic ECM remodeling, which may be linked to hypoxia given that fibroblasts cultured in hypoxia have been shown to promote fibrosis and elevated ECM transcripts [150,155]. This ultimately can influence the metastatic potential of the cancer cells [150]. Intuitively, as mentioned above, hypoxia also results in the secretion of angiogenesis promoting factors such as VEGF by tumor and stromal cells. This can lead to some new vessels temporarily restoring normoxic conditions until rapidly growing tumor cells once again exceed the reach of the new vessels. This generates further heterogeneity by creating both chronic and acute hypoxic regions as well as promoting the development of disorganized vasculature [150].

Unsurprisingly, immune cells are not exempt from the effects of hypoxia. Tumor cells in hypoxic conditions can recruit macrophages to the tumor through secretion of chemoattractants [156]. Recruited macrophages are then polarized into an M2-like phenotype to promote tumor progression [156]. Interestingly, myeloid-derived suppressor cells (MDSC) have been shown to enhance immuno-suppression in hypoxia although they can also obtain an immuno-stimulatory phenotype [150]. A final example of the role of hypoxia in cellular heterogeneity stems from HIF-1 α stabilization. This causes a metabolic switch to glycolytic dominated metabolism in cancer cells [51,150]. This switch ultimately creates competition for nutrients between the hypoxic cancer cells and T cells, a battle that T cells are unlikely to win. Without sufficient nutrients, the anti-tumor effects of T cells are stymied. T cells in hypoxia may even differentiate into regulatory T cells, which contribute to immunosuppression [150]. Given this evidence it is clear that as hypoxia emerges throughout a tumor, pockets of cells will also emerge with differential, often tumor supporting behavior and thereby contribute to cellular heterogeneity.

Given the effects that each cell type can have on tumor progression and chemoresistance, and the wide ranging TME present in ovarian cancer, it would be unreasonable to assume that a successful therapy for the primary tumor will be similarly effective in secondary locations such as the ascites or the omentum. Consequently, it is critical that these sources of heterogeneity be care-

Table 1
Heterogeneous microenvironmental cells reprogram the extracellular matrix (ECM). The Table illustrates selected examples of the various cell types remodeling the tumor ECM through gene upregulation and increased protein and protease secretion, to promote tumor growth, angiogenesis, and metastasis.

Type of Cell	Impact on extracellular matrix (ECM)	Refs.
Cancer stem-like cells (CSC)	Overexpress several collagens, periostin, mucin 1, and tenascin-C (TN-C). Highly express decorin, lumican, biglycan, versican, aggrecan. Produce high levels of hyaluronic acid.	[171–189,189,190]
Cancer-associated mesenchymal stem cells (CA-MSC)	Differentiate into CAFs and adipocytes. Remodel surrounding collagen matrix.	[103,191–193]
Cancer-associated fibroblasts (CAF)	Increase collagen production through JAK1 activation. Remodel ECM to provide ideal stiffness. Create actomyosin tracks in ECM for cancer cells to follow. Secrete versican and increased amounts of collagens. Upregulate COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, COL5A1, COL5A2, COL6A1, COL6A3, and collagen support genes, secreted protein acidic and rich in Cysteine (SPARC), SERPINH1, and SERPINE1. Increased expression of MMP2, MMP11, and TIMP1.	[94,194–197]
Endothelial cells (EC)	Upregulation of lysyl oxidase homolog 2 (LOXL2). Secrete SPARC related modular calcium binding 2 (SMOC-2), cysteine rich with EGF like domains 2 (CRELD-2), microfibril-associated glycoprotein 2 (MAGP-2), lumican, extracellular matrix protein-1 (ECM-1). Highly express COL4A1, COL4A2, SERPINH1, and SPARC.	[94,198,199]
Tumor-associated macrophages (TAM)	Secrete osteopontin, osteoactivin, collagens, fibronectins, and truncated fibronectin. Secrete matrix metalloproteases, cathepsins, lysosomal and a disintegrin and metalloproteinase (ADAM) proteases, and the urokinase-type plasminogen activator (uPA). Secrete TN-C, fibronectins (FN1).	[200–206]
Adipocytes	Upregulation of tumor necrosis factor (TNF- α), osteopontin, MMP9, versican, and leptin. Secrete and process collagen type VI. Secrete endotropin.	[207–210]
Mesothelial cells	Secretes collagen type I. Secrete fibronectin.	[163,211]
Myeloid derived suppressor cells (MDSC)	Secrete MMPs and cathepsins. Releases TGF- β to induce LOX production. Remodel basement membrane.	[212–214]
Neutrophils	Secrete MMP-9 during angiogenesis.	[215,216]
Dendritic cells	Release indoleamine 2,3-dioxygenase (IDO) which catalyzes tryptophan and prompts tumor angiogenesis and metastasis.	[217]
B cells	Generate interleukin 10 (IL-10) and immunoglobulins (IgG) which form antigen-IgG complexes to recruit immunosuppressive myeloid cells.	[218]

fully considered when developing precision medicine models. The effects of this potential variability between and within patients' tumors are compounded by the ECM in the TME. The various cell types within primary and metastatic tumor sites remodel the ECM in a context-specific manner, which in and of itself impacts drug response (Table 1).

2.3. ECM heterogeneity in the TME

2.3.1. Cellular influence on the ECM composition

Cells within the TME not only affect disease progression via soluble and insoluble signaling, but also exert influence through ECM remodeling (Table 1). CAF in particular, are responsible for a large portion of ECM remodeling in ovarian cancers. Fibroblasts secrete ECM proteins and proteases to develop ECM scaffolding within the ovarian cortex. However, once fibroblasts differentiate to CAF, they remodel the ECM through increased secretion of ECM proteins such as collagens, fibronectins, and tenascin-C. Increased secretion of proteases and ECM modulators including matrix metalloproteinases (MMP), lysyl oxidases (LOX), and transglutaminases (TGM), which degrade and crosslink ECM components, is also observed [91,116,157]. Interestingly, a specific subset of CAF have been shown to produce longer collagen fibers that correlate significantly with poor clinical outcomes in head and neck, esophageal, and colorectal cancers [158]. These data support the role of phenotypic heterogeneity within the tumor, further linking the TME to disease progression and patient outcome. While CAF are one of the most prominent remodelers of the ECM, many other cell

types have been shown to manipulate these microenvironments. In ovarian cancer specifically, TAM are delineated from normal macrophages based on a set of 19 commonly upregulated ECM-related genes including *LOX*, *LUM*, *COL5A1*, *COL5A2*, *COL3A1*, *COL1A1*, *COL1A2*, and *MMP-19* among others [159]. Cancer cells [91,159], adipocytes [160], EC [75], MSC [161], mesothelial cells [162,163], MDSC [164], neutrophils [165], and T cells [166] also aid in ECM remodeling. Conversely, it is well known that the ECM can also influence cell phenotypes [167–170]. These bidirectional relationships highlight the urgent need for precision models that address cellular and ECM heterogeneity together, in order to more accurately reflect *in vivo* disease progression.

2.3.2. Significance of ECM in classification and clinical outcomes in HGSOc

The important relationship between cellular heterogeneity, ECM, and clinical outcome is apparent in the recently defined major subtypes (mesenchymal, immunoreactive, differentiated, proliferative and anti-mesenchymal) of HGSOc determined from RNA-seq profiling [4,27,219]. The mesenchymal subtype is represented by enhanced desmoplastic response and poor overall survival. The defining expression profile for this subtype includes increased ECM production, remodeling, cellular adhesion, signaling, and angiogenesis, which is due to a large fraction of CAF [93]. The second subtype, termed the immunoreactive subtype, is characterized by slow tumor growth, elevated cytokine and immune activation marker expression, as well as high CD3+ T cell infiltration. This subtype also features significantly better overall survival. Similarly, the dif-

ferentiated subtype is associated with a higher overall survival than the mesenchymal subtype and is characterized by a low stromal response, tandem to an elevated epithelial signature and reduced growth rate. The proliferative subtype expresses activated cell cycle and mesenchymal markers, as well as reduced immune infiltration and is associated with worse prognosis [4,27,219,220]. Finally, the anti-mesenchymal subtype is associated with down-regulation of genes in the mesenchymal subtype. As a result, this subtype is also associated with higher overall survival [106], further cementing the relationship between the immune and mesenchymal contents of each of these subtypes and overall survival. Interestingly, recent work by Pires et al. showed that an effective T cell response resulted in lower density ECM [166]. This potentially links the improved prognosis in immunoreactive tumors to differences in ECM density brought on by distinct cell composition. This can also be expanded to the other tumor subtypes, as varying cellular and extracellular compositions might also indicate therapeutic response. However, despite direct correlations of The Cancer Genome Atlas (TCGA) stratification to patient outcomes, they have yet to be utilized clinically to determine personalized treatment strategies. These RNA-seq identified HGSOC subtypes are a reminder of the cellular and extracellular complexity and heterogeneity, which necessitate personalized TSM to screen therapeutic responses. However, the different cellular and ECM compositions of the five subtypes of HGSOC, are a complex and technical challenge to recapitulate *in vitro*. As a result, attempts to produce personalized models of both the cellular and ECM components in ovarian cancer with the RNA-seq identified subtypes have been limited. In order to study the impact of collective gene expression of ECM and their related proteins on the treatment efficacy, new advances in our understanding of matrisome signature, and structure are outlined below.

2.3.3. ECM composition in the TME

The matrisome comprises approximately 300 discrete ECM proteins, each with the capacity to self-assemble into macromolecular superstructures [221]. In cancers, this milieu of macromolecules deviates heavily from the baseline of benign tissue [14,222]. Moreover, as observed in mesenchymal subtype survival statistics, ovarian cancer progression is highly correlated to matrisome dysregulation and reformation [223]. Recent advances in proteomics, imaging, single cell analysis, and bioinformatics have reinvigorated interest in how ECM structure, orientation, and composition change over tumor progression. Similar advances in 3D cell culture have demonstrated feedforward mechanisms relating to protein expression and cellular decision making [14,224]. Since the highest protein transcript expressions in a third of all ovarian cancers relate to ECM and adhesion [225], it is imperative that the personalized TSM are comprised of extracellular components to characterize and investigate the role of ECM in ovarian cancer outcomes.

Categorized by macromolecular organization and chemical structure, the ECM is generally divided into three subgroups, collagens, proteoglycans, and glycoproteins [170,221,226]. In the ovarian stroma collagens subgroup, collagen type I and III specifically, are thought to be the most abundant [227]. In normal ovarian tissue, collagen type I and III can be highly crosslinked, however during tumor progression collagen crosslinking becomes defective [228]. Second harmonic generation (SHG) confocal microscopy has discovered vast degradation and secretion of collagen type I fibers in HGSOC. Wherein the collagen type I of ovarian stroma, consisting of nonspecific fiber orientation, is degraded by tumor cells and replaced with a neo-reactive fiber structure [229]. This new malignant-type collagen fiber network has a higher degree of orientation, noted by a dense and ordered structure with comparative regularity over the network expanse (Fig. 2) [230]. Given that collagen type I fiber morphology and abundance are directly linked to

migration and drug resistance in ovarian cancer cells respectively [185,231], the TSM must incorporate this ECM component.

Immunohistochemistry (IHC) is commonly used to analyze ECM. However, the process of fixing, sectioning, and staining samples can damage the structure of ECM and limits the information that can be obtained from IHC analysis. Recent advances in proteomic approaches, such as peptide extraction, ultra-high pressure liquid chromatography, mass spectrometry, and bioinformatics, have provided new opportunities to obtain insight into the dynamic changes in ECM during tumor progression. A comparative study by Pearce et al., has revealed a complex ECM landscape at varying HGSOC disease stages [92,232]. Interestingly, their findings confirm a contested theory whereby the relative abundance of collagen type I and III actually decreases in late stage and secondary tumors, likely due to a gradual dysregulation of ECM production in the TME. In addition, the authors identify the expression of fibronectin, cartilage oligomeric matrix protein (COMP), versican, collagen XI, collagen X, collagen I, and hyaluron to be highly correlated to disease score and tumor stiffness. As COMP, versican, and collagen XI are normally reserved for stiff tissues such as bone or cartilage, their presence in HGSOC further highlights ECM dysregulation in the TME. Intriguingly, a decrease in key basement membrane genes relating to laminin, collagen type IV, and perlecan were also found in late stage, primary tumors. Decay of basal membrane components have previously been linked to intraperitoneal dissemination [170,228,233]. Similarly, increased expression of glycosaminoglycans (GAG) and proteoglycans such as hyaluron and versican in the metastatic tumor are associated with cancer cell migration by expanding local tissue space, increasing tumor modulus, and improving mesothelial adhesion [233–236]. Beyond migration, hyaluron is also thought to promote chemoresistance through CD44 binding, PI3K/AKT, p53, and MAPK survival pathways [237,238]. Another ECM component, tenascin-C, is found between the epithelia and the stroma of malignant tumors, with its expression levels being closely associated with tumor grade [239]. Tenascin-C also mediates $\alpha v \beta 3$, $\alpha v \beta 5$, $\alpha 6 \beta 4$ integrin interactions in concert with periostin, fibronectin, and type I and IV collagen, which has been shown to promote paclitaxel resistance in ovarian cancer [240,241]. The glycoprotein, fibronectin is also found to be highly expressed in metastatic tumors, and is closely correlated to tumor stage and growth [242–245]. Moreover, fibronectin is thought to play a prominent role in transcoelomic metastasis, with high concentrations found in patient ascites, associated with mesothelial adhesion and metastasis (Fig. 2) [163,246–248].

The ECM at metastatic sites is also remodeled to promote disease progression. For example, the omentum undergoes ECM remodeling during colonization of tumor cells. Fibrillar collagens are the most prevalent macromolecule, with substantial clusters of collagen type I entwined between fibroblasts, below the mesothelium surface, and within the basement membrane. Similar to primary tumors, a general increase in glycoprotein and proteoglycan production is observed in the TME of omental metastasis (Fig. 2) [232]. Interestingly, proteins that define the basement membrane are relatively replete in omental metastasis, theoretically aiding in the establishment of the tumor colony [249]. Specifically, an increased expression of laminin, collagen type IV and fibronectin are observed in the basement membrane of omental metastases [247,250–252].

2.3.4. Targeting the components of ECM to improve patient outcomes

Due to the relationships between ECM, cells, and clinical outcomes, targeting critical ECM components can improve the efficacy of precision treatment [8,253–255]. The promise of targeting ECM components is exemplified by losartan, an FDA approved antihypertensive agent that blocks angiotensin II receptor type 1 (AT1) [256]. In ovarian cancer bearing mice, losartan treatment signifi-

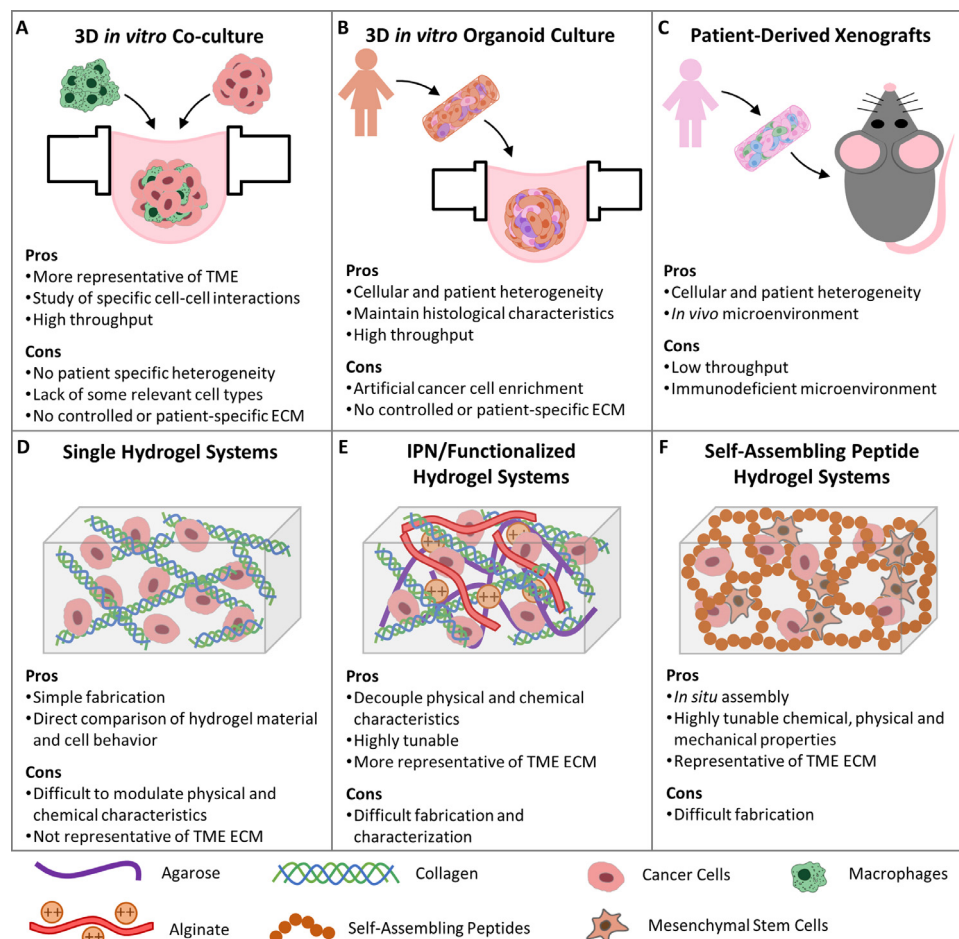


Fig. 3. Selected examples of contemporary 3D platforms that can be applied towards biomaterials-based patient-specific multiscale tumor models. (A) Heterospheroids in 3D suspension co-culture, that comprise of small number of ovarian cancer stem-like cells and M2-like alternatively activated macrophages, as described in [95]. (B) 3D patient-derived *in vitro* ovarian cancer organoids grown in Matrigel, as characterized in [204]. (C) *In vivo* patient derived xenograft (PDX) models in immune compromised animals shown in [207]. (D) 3D hydrogels based on a single ECM component, such as collagen type I, as discussed in [145]. (E) 3D interpenetrating hydrogel created from multiple ECM components, for example, alginate-agarose-collagen type I as presented in [223]. (F) Self-assembling 3D scaffold, for example, peptide amphiphiles and keratin hydrogel, as described in [229]. (All schematics are original works based on previously published methods.)

cantly lowered collagen and hyaluronic acid concentration in addition to decreasing the number of fibroblasts and α -SMA⁺ stromal cells. The decrease of ECM resulted in lowered solid stress and increased intra-tumoral chemotherapy penetration. In breast and pancreatic cancers, losartan has been shown to cause decreases in intratumoral expression of thrombospondin-1 (THBS-1) and activation of TGF- β in CAF. As a result, losartan is now being evaluated in Phase II clinical trials for treatment of pancreatic cancer along with chemotherapy [257]. Given the heterogeneity of ECM components in HGSOc and the promise of precision ECM targeting treatments, it is vital to integrate different components of the ECM into personalized models.

Further, targeting ECM protein stabilizers that contribute to tumor stroma stiffness and crosslinking of collagen and elastin is also a promising lead [258,259]. For example, the mitigation of LOX resulted in decreased tumor burden and collagen remodeling in HGSOc associated omental metastasis [211,260]. Similarly in pancreatic cancers, the combination of LOX inhibition and gemcitabine reduced collagen fibril density, metastasis, and extended disease-free survival in mouse models [261]. In yet another example, LOX inhibition reduced collagen cross-linking and fibronectin assembly, increased drug penetration, induced apoptosis, and re-sensitized triple negative breast cancers to chemotherapy [262,263]. These successes have led to clinical trials where LOX inhibition efficacy is

being investigated in myelofibrosis and pancreatic cancer patients [264]. Given the increased prevalence of collagens and elastin in mesenchymal and other ECM-rich stromal HGSOc subtypes, it is essential to target these components and their modifiers that generate, stabilize, and orient structural proteins.

2.4. Extracellular matrix properties within the TME

In healthy individuals both the cellular and acellular contents of the ovaries are tightly regulated by the peritoneal membrane. However, during tumor progression, components of the TME are gradually dysregulated. In primary tumors, this dysregulation (Sections 2.2 and 2.3) aids in tumor cell dissemination into the peritoneal cavity, often leading to the formation of malignant ascites [265,266]. Retention of ascitic fluid in the peritoneal cavity prompts a unique mechanical environment, further augmenting the mechanical forces present in the ovarian TME, including matrix stiffness, shear stress, compression, and tensile stresses [12,267,268].

As a consequence of HGSOc associated desmoplasia, the ECM in ovarian tumors continuously stiffens [269]. The average Young's modulus for ovarian tumors is cited at 5 kPa but contains discrete regions with moduli ranging from 16 to 35 kPa [269,270]. Elevated ECM stiffness has been shown to influence metastasis, inva-

sion, proliferation, and chemoresistance [16,253,271–275]. Numerous studies have proven stiffer substrates enhance the metastatic phenotypes of cancer cells [276–280]. However within the field of ovarian cancer, studies have resulted in contradictory findings [269,281–284].

Ovarian cancer cells within the peritoneal ascites experience a range of compressive and shear stresses, originating from hydrostatic pressure, growth induced stress, as well as ascitic, vascular, and interstitial fluid flow respectively [12]. In the intraperitoneal space, shear stress is estimated to range between 0.14 and 11 dynes/cm², while compressive stress is thought to range between 4.7 and 18.9 kPa [285–287]. External dynamic stimuli, including shear stress and compression, have been correlated with increased metastasis, CSC enrichment, chemoresistance, and proliferation in a variety of cancers. The effects of compression on ovarian cancer is currently thought to be contradictory and requires further attention [288–293]. Evidently, the full role that mechanical stimuli plays in ovarian tumor progression and treatment response is not well understood. This represents yet another gap in knowledge that could be filled with investigation using the patient-derived TSM.

2.5. Evidence of complex interactions within the TME

As outlined above, heterogeneity in ovarian cancer is made up of cellular, ECM, and mechanical components. Yet, rather than existing in isolated states, TME constituents are inherently coupled, cooperating together to promote events within tumorigenesis. When cells in the tumor remodel the ECM, they invariably change the mechanical properties of their surroundings. This in turn alters cellular phenotypes and consequently, disease progression and chemoresistance. Within breast tumors for instance, a tight correlation exists between immune infiltration, ECM density, and drug response [169,263]. Cohorts of tumors that respond well to treatment display muted ECM density and a higher degree of T cell infiltration [294–296]. Understanding this interconnected system has enabled researchers to predict outcomes such as relapse more accurately [169,297–299]. In ovarian tumors, stiffness correlates strongly with disease stage whereby late stage tumors generally have a higher modulus [92]. The increase in stiffness and tumor stage promotes pliability in ovarian cancer cells, further promoting migration and invasive potential [300,301]. This feed forward mechanism demonstrates how stromal cells change ECM, in turn affecting cancer cell behavior, and finally how this behavior aids in disease progression. Coupling events like these highlight why comprehensive models are crucial in our understanding of ovarian cancer behavior, and why we might need to alter our approach to precision medicine.

3. Making personalized TME models for HGSC

Due to the diversity in origin, architecture, and composition of HGSC [220], conventionally available models need to be further improved to meet the needs of clinical translation [10,11,33,302,303]. In this section, we will review contemporary models used to replicate tumor cellular and acellular heterogeneity and discuss the critical next steps in the development of TSM for precision medicine applications. As evidenced throughout this review, the proposed patient-informed models contain enormous potential to improve patient care and clinical outcomes.

3.1. Models of cellular heterogeneity

Conventional approaches of drug screening include the use of isogenic cell lines in 2D culture or propagated as xenografts, genetically engineered mouse models (GEMM), patient-derived

xenografts (PDX), and patient derived organoids (PDOs) [10,302–304]. While 2D cell line cultures are easy to use and inexpensive, they have long been known to poorly represent *in vivo* conditions, making observed drug responses unreliable [305]. Newer 2D models might use cell lines derived from patient samples, however generation of a new cell line is prone to low success rates and fibroblast contamination. Moreover, the cell lines that are successfully established will have persevered through a strong selection pressure in 2D *in vitro* conditions making them a poor representation of the heterogeneous tumor cell population [19].

GEMM and PDX on the other hand are labor- and time-intensive, making it challenging for them to contribute to drug screening and individualized therapy on a clinical scale [19,305,306]. Furthermore, GEMM are limited in their ability to consistently generate tumors on a reliable timeline as these tumors form with heterogeneous latency periods and growth rates [307]. PDX formed from diverse populations of patient cells maintain cellular heterogeneity, however are susceptible to copy number alterations with passaging and loss of human immune cells in the tumor which may result in unrepresentative drug responses compared to the patient responses [308]. The immunocompromised nature of PDX [307] also prevents evaluation of a functioning immune system in drug response. Overall, mouse models have struggled to translate into the clinic, with only about 5% of the drugs tested in mouse models performing well enough in phase III trials to be licensed for clinical usage [308]. However, a promising new variation of mouse model, called ‘humanized mice’ has been developed with human immune cells, allowing for evaluation of tumor interactions with the immune system [308]. Despite this improvement, the technical challenge of developing these models remains, and hinders use as high throughput screening systems.

On the other hand, 3D *in vitro* co-cultures are ideal for high throughput drug screening and evaluation of interactions between two or more cell types. For example, using recently developed 3D co-culture heterospheroids of CSC and CA-MSC, PDGF, and Hedgehog crosstalk was found to be a key signaling mechanism involved in increasing stemness, metastatic potential, and chemoresistance in CSC [309]. Similar heterospheroid models were utilized to show that ovarian cancer cells reprogram normal ovarian and omental MSC into pro-tumoral CA-MSC, presenting evidence that ovarian cancer cells catalyze the formation of their own pro-tumoral microenvironment [95]. Similarly, to dissect the signaling between immune cells and ovarian tumor cells, recent 3D models have featured co-cultures with immune cells. Since the adaptive and innate immune systems are both responsive and influential to the ovarian TME, they both have roles in the initiation and resolution of inflammatory response [32,33]. Ovarian cancer cells form spheroids in the ascitic fluid due in part to their interactions with macrophages, and these heterospheroids are thought to aid in transcoelomic metastasis [126]. Utilizing a hanging drop non-adherent 3D suspension model, ovarian CSC and activated macrophages can be brought in close association, simulating the physiologic environment of non-adherent malignant ascites. These 3D heterospheroids illustrate that pro-tumoral macrophages promote chemoresistant and invasive phenotypes in CSC, further leading to CSC enrichment. This model was used in the discovery that reciprocal paracrine signaling via WNT/ β -catenin between macrophages and ovarian CSC promoted pro-tumoral environments including polarization of macrophages into M2-like phenotypes, and increased expression of the stem marker ALDH in CSC. This suggested that the WNT/ β -catenin pathway could be an effective target for new therapeutics that specifically eradicate the immunomodulation of macrophages by CSC [167]. These types of models have been made with tumor cells and endothelial cells [310], mesothelial cells [311], and adipocytes [144]. While co-cultures are advantageous in examining interactions between two or three cell

types, they still do not replicate patient-specific cellular and acellular heterogeneity.

PDO have been instrumental in our ability to replicate cell-cell interactions with realistic cell compositions *in vitro* and have had some success in predicting drug response [19,305]. PDO are formed from either a single patient cell or a heterogeneous population of patient-derived cells grown into organoids that are embedded in basement membrane extract with an appropriate growth factor and small molecule cocktail to replicate the *in vivo* TME [19]. A panel of ovarian PDO have been successfully created for long term *in vitro* culture of all subtypes of epithelial ovarian cancers. The panel of organoids was generated with cells from primary tumors, metastatic lesions, ascites, and pleural puncture. Through in-depth analysis, they demonstrated that tumor organoids maintain histological characteristics of the primary source samples including nuclear and cellular atypia and expression of tumor biomarkers like p53 and pax8. This study showed that passaging the organoids did not result in any genomic changes compared to the original tumors. The organoids also show hallmarks of ovarian cancers, including the significant number of copy number variations, recurrent mutations and tumor heterogeneity [36]. However, a drawback of these organoids is their cell composition, as tumor organoids averaged $88 \pm 23\%$ of cancer cell content, while the actual tumors contained only $49 \pm 9\%$ tumor cell content across all samples [19]. HGSOc organoids have also been utilized for screening compounds, while maintaining the intra- and inter-patient tumor heterogeneity and mutation status, and matching the parental tumors genetically and functionally [312–314]. Another drawback to conventional organoid cultures is the use of basement membrane extract, such as Matrigel [19]. As we discussed above, the dynamic interactions between the ECM and the cells in a tumor have a profound effect on tumor progression and clinical outcomes, making the use of extracts with uncontrolled ECM composition problematic. A more thorough review of these PDO models was published previously [10].

3.2. Models of ECM heterogeneity

The ECM constituents of the TME make up a rich mosaic of collagens, proteoglycans, and glycoproteins. Each macromolecule has a unique function, position, and concentration that impart chemical and physical cues to surrounding cells. Changes to the local tumor ECM impact cancer progression and individual patient response to treatment [14,16,315]. Cataloging and modeling the TME and cell-matrix interactions are vital to the long-term improvement of patient outcomes. ECM constituents, similar to TME cell heterogeneity, are disparate and unique to individuals. It is therefore important that 3D biomaterials-based personalized models are improved to better replicate a tumor's protein rich ECM [316]. Although a plethora of 3D model systems have been utilized to study cancers, few capture the complexity of the tumor ECM, and none combine the vast biochemical and biophysical interactions present in a singular model.

The most widely utilized contemporary model for 3D ovarian carcinomas is the murine derived Matrigel, a solubilized basement membrane harvested from mouse sarcoma. However, with undefined constituents, batch to batch variability, and poor biophysical properties, it does not accurately replicate the ovarian tumor stroma [317]. Moreover, Matrigel fails to replicate the physiology of the primary tumor, as the basement membrane is lost during disease progression (Section 2.3.3). Naturally derived protein scaffolds address some of these limitations by providing physiologically relevant chemical cues and magnifying key cell-matrix interactions. For example, the role of $\alpha 5 \beta 1$ -integrin interactions during ovarian metastasis was discovered using various fibronectin coated surfaces [269,318]. Studies have documented an increase in ordered

collagen type I expression in ovarian carcinomas, enhancing the fibrotic microenvironment at primary and secondary sites [319]. 3D collagen matrices have induced MMP-mediated matrix degradation in ovarian cancer cell lines which may support recent proteomics and SHG findings, whereby mature collagen matrices are replaced by ordered fibers [320]. Collagen type I alone has been shown to increase ovarian cancer cell invasion *in vitro* [321,322]. The modulus of collagen gels generally ranges from 1 to 4 kPa, with a scaffold of 3.6 mg/mL providing a modulus of 1.7 kPa (Poisson's ratio, 0.3) [321,323]. Notwithstanding, collagen fiber density has been shown to impact cellular behavior independent of the material's bulk stiffness [323]. This decoupling of stiffness and collagen associated adhesion motif (GFOGER) further contextualizes the complexity of the TME. Therefore, in order to create personalized TSM, it is vital to control both material stiffness and collagen concentration within collagen containing biomimetic scaffolds. Although GFOGER is the relevant binding site in the collagen triple helices, RGD cues have also been widely investigated in cancer. Shear thinning and partially crosslinked gelatin scaffolds (which have exposed RGD epitopes) achieve a higher stiffness than collagen scaffolds, and can be 3D printed to fine tune pore size and permeability [324].

Hydrogels have also been created using interpenetrating networks (IPN) to decouple physical and chemical characteristics within TME biomimetics. Thus, IPN hydrogels can be designed to contain relevant bioactive substrates while maintaining physiologically relevant mechanical signaling, including modulus, viscoelasticity, pore size, and permeability. For example, combining collagen type I and collagen type III together has shown increased invasion of transformed fallopian tube epithelial cells compared to collagen type I alone [325]. IPN hydrogels may also allow for the manipulation of discrete components in a spatio-temporal manner. A recent study has shown that collagen type I morphology can be manipulated using multiphoton excited photochemistry to align fibers within a solubilized gelatin methacrylate scaffolding [229]. Porosity can also be fine-tuned in IPN hydrogels, for example varying collagen concentration in agarose + alginate IPN achieved average pore sizes of 66.6 to 62.8 μm within ovarian cancer cultures [326]. Polysaccharide hydrogels such as alginate and agarose are bioinert, and can replicate the highest regions of stiffness present in ovarian tumors [12,327]. Even when exposed to long durations of physical perturbations, such as those present in studies involving mechanotransduction, agarose maintains form and structure [12]. Ovarian cancer cells, encapsulated in 3% (w/v) agarose and 0.5 mg/mL collagen type I IPN hydrogels exposed to elevated compressive stress, have shown increased invasiveness and chemoresistance [328]. The capacity to decouple physical and chemical signaling is mirrored by 4 and 8 arm PEG, with each arm capable of functional modification to include RGD or MMP motifs [282,329]. Using a PEG-MMP-RGD functionalized hydrogel system (1.5/2.0/2.5% (w/v)), ovarian cancer cells have been shown to express variable proliferative rates and spheroid formation faculty in response to increased stiffness ($G' = 241/637/1201 \text{ Pa}$) [282].

Self-assembling peptide-based bioinks can be programmed to assemble into well-defined structures *in situ* [330]. With the capability to form fine-tuned alignments, attain a range of mechanical properties (0.6 to 205 kPa), and incorporate protein-mimetic epitopes that encourage angiogenesis and migration, they make an appealing prospect for the future of personalized TSM [331,332]. In a recently published report, ovarian cancer cells, MSC, and EC were encapsulated within peptide amphiphiles/keratin (PA/K) bioinks specialized with organized ECM proteins. These bioinks included HHL, RGD, and GHK epitope sequences, to enhance ECM co-assembly, cell adhesion, and proliferation respectively [333]. The PA/K peptide hydrogels reached a stiffness of 7 kPa, and were shown to maintain stability for up to 28 days under cell culture

conditions. Another self-assembling peptide, RADA16-I (1% (w/v)), recapitulated the effects of collagen type I models of ovarian cancer. RADA16-I also mimics the porosity of tumor ECM (with a pore size of 5–200 nm and nano fibers approximately 1000 nm in length) [334]. While the methods outlined above have advanced our understanding of cancer cell-ECM interactions, they lack patient and disease-specific cell and ECM compositions.

3.3. Proposed personalized tumor-specific precision medicine platform

Given the indisputable and coupled relationship of cellular and ECM heterogeneity with therapy response, we propose that the next step in the development of personalized tumor models for precision medicine will need to be grounded in evidence from functional and descriptive -omics data. Specifically, we propose a thorough characterization of patient biopsies using existing and emergent technologies to determine molecular, cellular, and ECM composition. In light of innovative work from the Hynes group, leveraging proteomics has been shown to faithfully enumerate the matrisome of complex diseases [335–338]. Despite the elucidation of breast and pancreatic cancer matrisome, limited investigation has been done to define the matrisome of ovarian cancer (Section 2.3). Next generation imaging and informatics modalities, including Multiplex FISH [339], multiplexed ion beam imaging time of flight (MIBI-TOF) [340], and tissue based cyclic immunofluorescence (t-CyCIF) [341] high throughput histology will also provide crucial spatial and quantitative snapshots of tumor heterogeneity (including protein and cellular content). The utilization of peptide extraction, ultra-high pressure liquid chromatography, mass spectrometry, mass cytometry [342], single cell sequencing [93], and bioinformatics can further inform cellular and acellular composition.

Information gleaned from comprehensive characterization of a patient's heterogeneous tumor composition using these tools allows for personalized re-engineering of the tumor with the same composition *in vitro* for drug screening or other investigations. However, due to the limited size of patient samples it will be important to select only the most effective characterization techniques. Generating these TSM within a high throughput culture system (e.g. a 384-well hanging drop plate) enables drug screening to determine which TME compositions, or more generally, which patients might respond to certain treatments or combination treatments (Fig. 4). By incorporating both cellular heterogeneity and ECM complexity into personalized disease platforms, patient-specific cell-cell and cell-ECM signaling will be more complete. As a result, we expect that treatment response predictions will be more accurate, thereby improving personalized clinical decisions and outcomes for cancer patients [238,333].

4. Applications of personalized tumor-specific models

Personalized tumor-specific models have the potential to improve patient care and clinical outcomes. In this section, we will identify and discuss the potential high-impact applications that could be realized with these individualized models. TSM can be utilized in high throughput assays whereby drug response data, corresponding to individualized tumor compositions, is collected over time and analyzed *in silico* to determine the appropriate course of action (Fig. 4). This workflow could be used for numerous critical applications.

4.1. Personalized screening for drug compounds and combinations

Using the data obtained from characterizing patient biopsies, personalized TSM can be generated and used for screening. Based

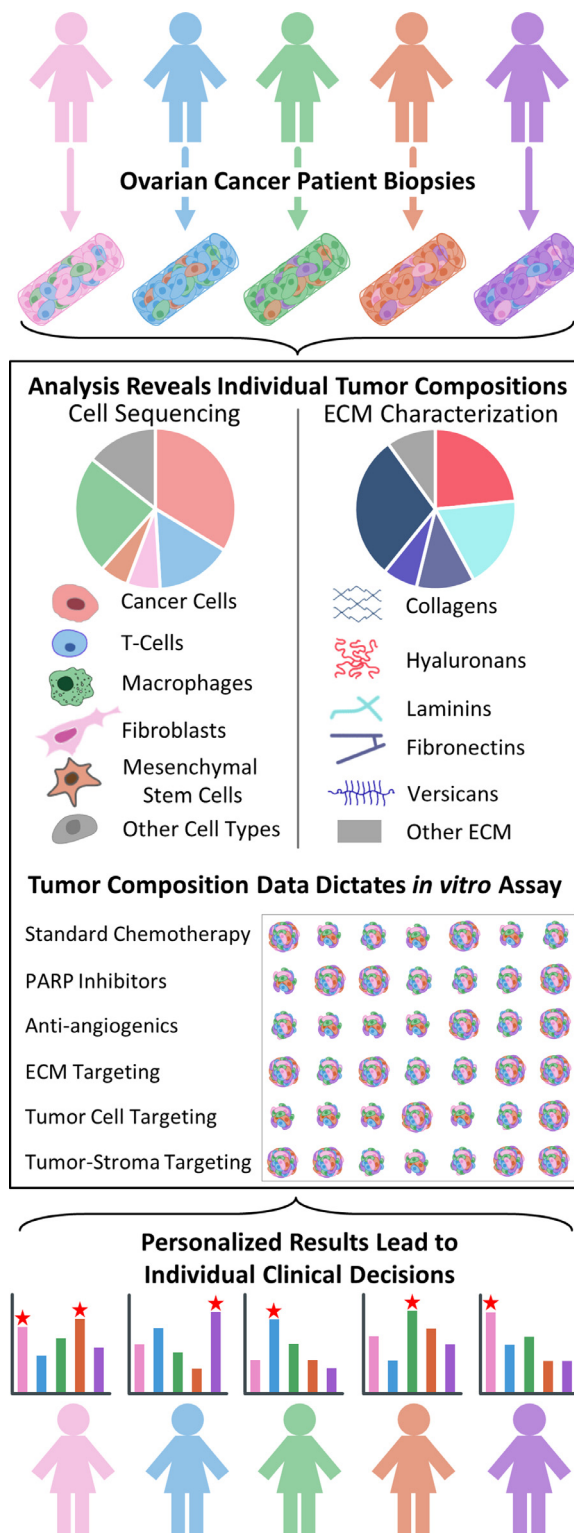


Fig. 4. Proposed clinical workflow for patient-derived tumor-specific 3D models which can predict therapy response and identify the most effective, yet non-toxic therapies or combinations, leading to sustained and durable responses.

on the results of the screening, a drug or a combination of drugs will be selected. Next, the features that are most important in determining a patient's response to a wide variety of treatments could be discerned *in silico*. This could expedite the discovery of effective adjuvant combination treatments to reduce disease burden and improve outcomes.

4.2. Identify biomarkers of chemosensitivity

Identifying novel biomarkers of chemosensitivity is another possible function of TSM. After generating TSM from a large cohort of patients over time, patterns and tumor characteristics associated with responses to specific therapies could be identified. In the long term, complex combinations of biomarkers indicating sensitivity to certain therapies may be recognized using *in silico* models. This would enable rapid clinical treatment decisions following tumor characterization, potentially without the need for TSM. With more precise sets of biomarkers and decreased time to treatment, clinical outcomes could drastically improve.

4.3. Predict and prevent relapses and treat minimal residual disease

Most HGSOc relapse within the first 2 years of diagnosis, even after optimal de-bulking, chemo-, and targeted-therapies [344–346]. As the tumor cells evolve post-therapy and develop resistance, the proposed TSM model could be applied to identify features responsible for minimal residual disease. Accordingly, optimal cocktails of compounds could be designed to prevent relapse. Along with TSM, predictive models would calculate appropriate sequential treatments thereby extending remission period.

TSM could also be utilized to study quiescent tumor cells, which are conventionally difficult to study *in vitro*. As quiescent cancer cells that have escaped previous therapies resurface again in the TSM, their evolution can be studied and monitored to understand how different residual clonal populations take hold. These results can then be used to identify the druggable targets in the clonal chemoresistant populations that emerge following various primary treatments.

4.4. Predict the characteristics of future relapses

By utilizing TSM, tumor recurrence could be modeled through serial passaging, a previously established process [343] wherein TSM would be created, dispersed after a period of culture, and then re-generated from the same cell population. This process would facilitate analysis of the development of chemoresistance and the enhancement of CSC populations simultaneously.

5. Conclusions

In this article, we have laid out why personalized tumor-specific models (TSM) are crucial for improving precision medicine outcomes. The tumor microenvironment (TME) is made up of an amalgam of cellular and acellular components that all interact dynamically and drive disease progression. However, it is difficult to capture the complex cellular and acellular features of the TME in conventional animal models and *in vitro* platforms. By omitting many of these features, biochemical and biophysical cues as well as epigenetic changes are lost in many contemporary models. This loss of biomarkers leads to flawed drug screening results which fail to select the optimal drug for a patient. For this reason, we propose taking elements of the TME, obtained through thorough biopsy characterization, and integrating them into personalized TSM in high throughput culture systems. In theory, this will recapitulate the patient's heterogeneity, and thus could produce a representative chemoresistance profile. Once this process is repeated with a significant number of patients, TSM may facilitate identification of biomarkers for chemosensitivity and relapse. The identified biomarkers can then be probed in the initial characterization of another patient's tumor (without the need to generate the TSM and perform the drug screening) to identify the expected effectiveness of various treatment options and the likelihood of recurrence. This would minimize the lag time between diagnosis and

the start of treatment, which could further improve outcomes. Importantly, although we presented this proposal through the example of ovarian cancer, we postulate that the same approaches may be useful for other types of cancers, and in general for many human diseases.

Analogous to our advances in genomic, proteomic, matrisome, and other emergent platforms, we will soon be able to 'sequence' both the cellular and ECM constituents of each individual tumor. This will enable the recreation of biomimetic 3D TME for each patient, including cell and ECM constituents in appropriate abundance and orientation. Each TSM can be multiplexed on high throughput arrays and integrated with mathematical modeling to determine the best course of treatment. This system will facilitate the discovery of new therapeutics that target patient-specific features. Ultimately, TSM that integrate both biomaterials and personalized data could be a significant next step towards the goal of personalized medicine.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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