Kan *et al. Molecular Cancer* (2025) 24:125
https://doi.org/10.1186/s12943-025-02328-4

Molecular Cancer

REVIEW Open Access

The application of organoids in investigating immune evasion in the microenvironment of gastric cancer and screening novel drug candidates

Liuyue Kan[1†], Ying Yu[1†], Yaxue Wang[1†], Lei Shi[2†], Tingyuan Fan[3], Hui Chen[4,6*] and Chuanli Ren[3,5,7*]

Abstract
Gastric cancer (GC) is a prevalent digestive system tumor, the fifth most diagnosed cancer worldwide, and a leading cause of cancer deaths. GC is distinguished by its pronounced heterogeneity and a dynamically evolving tumor microenvironment (TME). The lack of accurate disease models complicates the understanding of its mechanisms and impedes the discovery of novel drugs. A growing body of evidence suggests that GC organoids, developed using organoid culture technology, preserve the genetic, phenotypic, and behavioral characteristics. GC organoids hold significant potential for predicting treatment responses in individual patients. This review provides a comprehensive overview of the current clinical treatment strategies for GC, as well as the history, construction and clinical applications of organoids. The focus is on the role of organoids in simulating the TME to explore mechanisms of immune evasion and intratumoral microbiota in GC, as well as their applications in guiding clinical drug therapy and facilitating novel drug screening. Furthermore, we summarize the limitations of GC organoid models and underscore the need for continued technological advancements to benefit both basic and translational oncological research.

Keywords Gastric cancer (GC), Patient derived organoids (PDOs), Immune evasion, Drug candidates

[†]Liuyue Kan, Ying Yu, Yaxue Wang and Lei Shi contributed equally to this work.

*Correspondence:
Hui Chen
chenhuimd@foxmail.com
Chuanli Ren
18051061089@yzu.edu.cn
[1]Department of Laboratory Medicine, Medical College, Yangzhou University, Yangzhou, China
[2]Department of General Surgery, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, No. 98 Western Nantong Road, Yangzhou 225001, China
[3]Department of Laboratory Medicine, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, Yangzhou, China
[4]Department of Geriatrics, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, No. 98 Western Nantong Road, Yangzhou 225001, China
[5]Department of Laboratory Medicine, The Yangzhou Clinical Medical College of Xuzhou Medical University, Yangzhou, China
[6]Northern Jiangsu People's Hospital Affiliated to Yangzhou University, No. 98, Western Nantong Road, Yangzhou 225001, China
[7]The Yangzhou Clinical Medical College of Xuzhou Medical University, No. 98, Western Nantong Road, Yangzhou 225001, China

Introduction

Gastric cancer (GC) is a common malignant tumor of the digestive system, ranking as the fifth most frequently diagnosed cancer worldwide and one of the leading causes of cancer-related deaths [1]. According to 2022 data, new cases of GC exceeded 960,000, with nearly 660,000 deaths, ranking it fifth worldwide for both incidence and mortality [2]. While treatment advancements have prolonged disease-free survival for advanced GC patients, overall survival (OS) improvements are still limited. Chemotherapy remains the standard treatment for advanced GC, with commonly used drugs such as fluoropyrimidines (e.g., 5-fluorouracil (5-FU), capecitabine, and S-1), platinum compounds (e.g., cisplatin and oxaliplatin), docetaxel, and epirubicin [3–5]. Conventional chemotherapy regimens for GC frequently adopt a "one-size-fits-all" strategy, neglecting the variability in individual responses to treatment [6, 7]. With the advent of more targeted therapies, such as the monoclonal antibody trastuzumab targeting HER2, ramucirumab targeting VEGF, zolbetuximab targeting Claudin 18.2 and immune checkpoint inhibitors (ICIs) like PD-1 monoclonal antibodies, the heterogeneity in individual drug responses has become more evident, limiting OS outcomes for patients with advanced GC [8, 9]. Consequently, tailoring treatment strategies to the molecular characteristics of tumors and the individual drug responses of patients is essential for enhancing their prognosis. While high-throughput sequencing data offers mutation details from tumor tissues for precision medicine, it falls short in replicating the complex in vivo microenvironment, hindering accurate predictions of patient treatment benefits. Therefore, there is an urgent need for a preclinical model that reflects the primary tumor's genetics and evaluates drug responses for precision therapy.

Organoids are a three-dimensional (3D) cell culture system derived from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) or adult stem cells (AdSCs) or progenitor or differentiated cells from normal or tumor tissues [10–14]. In vitro, they form cell clusters capable of self-assembly and self-renewal, maintaining the genomic stability and tumor heterogeneity characteristics of the original organ [15]. Organoids provide valuable insights into organ development, disease modeling, cancer progression, drug response, and personalized treatments [16–18]. Compared to xenograft models, organoid cultures require less time and tissue, are easier to handle, and crucially tumor organoids reliably retain the essential features of the primary tumor, even after prolonged passaging [19, 20]. This review explores the forefront of GC organoid research and its clinical implications, highlighting the transformative potential of organoid technology in simulating the TME to forecast immunotherapy outcomes and tailor personalized treatment approaches.

Gastric cancer: etiology, diagnosis and treatment

GC develops due to multifactorial influences, with key risk factors including chronic gastritis caused by prolonged *Helicobacter pylori* infection and untreated gastric ulcers, dietary factors (e.g., high salt intake, consumption of smoked or pickled foods, and low intake of fresh fruits and vegetables), life-style factors (smoking, heavy alcohol consumption), as well as family history and genetic predisposition [21]. GC symptoms are often subtle in the early stages, leading many patients to initially present with upper abdominal discomfort or pain, indigestion, hematemesis, melena, or anemia. Consequently, diagnosis frequently occurs at advanced stages, significantly reducing the likelihood of successful treatment. Current treatment modalities for GC include surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy, with the choice of treatment being primarily determined by cancer stage and the patient's overall health status. Surgical resection remains the standard treatment for early-stage GC, typically involving partial or total gastrectomy. Perioperative chemotherapy is mainly suitable for locally advanced GC that can undergo radical resection based on preoperative evaluation. The basic mode of neoadjuvant chemotherapy, radical surgery and postoperative adjuvant chemotherapy is of great significance in improving the surgical cure rate and long-term survival rate [22]. In advanced-stage GC, palliative chemotherapy is commonly employed to reduce tumor size or alleviate symptoms. The primary agents used are platinum-based drugs (e.g., oxaliplatin, cisplatin) and fluoropyrimidine based drugs (e.g., fluorouracil, capecitabine) [23].

Additionally, targeted therapies for various cancer markers are available, including several key drugs. HER2, as the first and most significant target for GC, has gained global consensus for treatment. The Chinese Society of Clinical Oncology (CSCO) guidelines recommend HER2 testing in GC patients, and if the result is HER2-positive, trastuzumab in combination with chemotherapy is the first-line treatment [23, 24]. VEGF, a crucial factor promoting tumor angiogenesis, is often upregulated in GC cells to enhance tumor growth and metastasis. VEGF-targeted drugs, such as apatinib and ramucirumab, work by limiting tumor blood supply, thereby exerting antitumor effects [25]. Numerous studies have shown their efficacy either as primary drugs or adjuncts in the treatment of various GC [26]. Overexpression of EGFR in GC occurs in 50-63% of cases, with its expression level positively correlating with tumor invasiveness and negatively correlating with differentiation and survival time, suggesting EGFR as a potential therapeutic target. However, clinical trials for anti-EGFR targeted drugs have not

yielded satisfactory results [27, 28]. Recent studies have highlighted Claudin 18.2 as a highly expressed marker in several solid tumors, with up to 60% of patients showing significant expression levels, far exceeding HER2 positivity rates [29, 30]. This has made it a promising target, particularly for HER2-negative GC patients. A wealth of clinical data supports the efficacy of related drugs, and in October 2024, the Food and Drug Administration (FDA) approved zolbetuximab for use in combination with fluoropyrimidine and platinum-based chemotherapy as a first-line treatment for Claudin 18.2-positive, unresectable, advanced, or recurrent gastric or gastroesophageal junction adenocarcinoma, offering new hope for GC patients [9, 31].

However, targeted therapy is not a panacea. For patients with advanced GC, drug resistance in targeted therapy is an important clinical challenge, treatment options become severely limited, resulting in a generally poor prognosis. The five-year survival rate often falls below 10% [32]. According to recent studies, several new treatment strategies are being explored to address this issue. Trastuzumab, the standard first-line targeted therapy for HER2-positive GC, is currently facing significant challenges due to acquired resistance in clinical practice. Approximately 50% of patients develop acquired resistance within one year of treatment, leading to a substantial decline in therapeutic efficacy [33]. Researchers primarily address this challenge by discovering new third-line therapeutic drugs for HER2-positive advanced GC and exploring the molecular mechanisms of trastuzumab resistance. Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate composed of a HER2-targeting antibody and a topoisomerase I inhibitor [34]. It efficiently delivers the cytotoxic payload to HER2-expressing tumor cells, enabling precision therapy. The Phase II clinical trial DESTINY-Gastric06 demonstrated that T-DXd exhibited clinically meaningful benefits in Chinese patients with HER2-positive advanced GC who had received at least two prior lines of anti-tumor therapy, achieving a median OS of 12.4 months, thereby generating evidence to justify T-DXd's use in this population [35]. Supported by evidence from the DESTINY-Gastric01-06 trial series, T-DXd was granted marketing authorization by China National Medical Products Administration on August 5, 2024, indicated for adult patients with HER2-positive locally advanced or metastatic gastric/gastroesophageal junction adenocarcinoma following ≥ 2 prior treatment regimens [36–38]. This approval provides a new third-line treatment option for HER2-positive advanced GC patients in China. Meanwhile, numerous novel therapeutics are under active development including bispecific antibodies and next-generation antibody-drug conjugates which are expected to further enhance treatment efficacy for HER2-positive GC [39]. Nevertheless, the molecular mechanisms underlying trastuzumab resistance remain poorly understood. Elucidating these mechanisms and developing targeted strategies to overcome resistance are crucial for improving clinical outcomes in HER2-positive GC patients. Recent studies have revealed that prolonged trastuzumab exposure induces chromatin remodeling, thereby activating transcriptional expression of the YAP gene. This gene mediates adaptive drug resistance in tumor cells through concurrent regulation of both AKT/mTOR and ERK/mTOR signaling pathways. Notably, combined administration of a YAP inhibitor significantly restores tumor cell sensitivity to trastuzumab, providing novel therapeutic insights for overcoming clinical resistance [40]. Another study revealed that glycolysis in trastuzumab-resistant HER2-positive GC exhibits circadian oscillations synchronized with the BMAL1-CLOCK-PER1-HK2 axis. Chronotherapy combining the HK2-targeted glycolytic inhibitor metformin with trastuzumab effectively reversed trastuzumab resistance. These findings introduce circadian clock regulation into trastuzumab treatment and propose a potentially effective chronotherapeutic strategy to overcome trastuzumab resistance in GC [41]. In addition to cell-autonomous mechanisms, remodeling of the TME also plays a critical role in the development of drug resistance. Fu's team elucidated that HER2-positive GC cells activate the CCR2-ZC3H12A-TRAF6/3 signaling cascade through CCL2 secretion, thereby suppressing M1 macrophage polarization and ultimately promoting drug resistance development. To target this pathway, they developed an anti-CD40-scFv-linked anti-HER2 bispecific antibody that not only specifically binds HER2 but also locally activates M1-polarization of tumor-associated macrophages, effectively overcoming resistance while avoiding systemic immunotoxicity [42]. From a metabolic microenvironment perspective, Shi Min team demonstrated that drug-resistant cells exhibit hyperactive glutamate metabolism concomitant with significant upregulation of GLS1 expression. Tumor-derived GLS1 was demonstrated to exacerbate drug resistance through dual mechanisms: promoting M2-like macrophage polarization and stimulating pro-angiogenic activity. The study proposes a novel combinatorial therapeutic strategy simultaneously targeting glutamine metabolism, angiogenesis inhibition, and M1 macrophage polarization, which demonstrates synergistic effects in reversing drug resistance [43]. These findings not only expand our understanding of targeted therapy resistance mechanisms but also highlight the crucial role of TME regulation in developing innovative treatment approaches. Consequently, research is increasingly shifting focus from the tumor cells themselves to the TME, particularly the role of immune cells. By modulating the TME, it may be possible to enhance the

immune system's ability to recognize and attack cancer cells, thereby improving the effectiveness of treatment. Immunotherapy has become a focal point of research in this area. Key types of immunotherapies include ICIs, cell-based immunotherapy, and tumor vaccines. ICIs are the most extensively studied form of immunotherapy, targeting the programmed cell death receptor 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA4) to block negative regulatory pathways in T cells and enhance their anti-tumor responses [44–46]. Additionally, new immune checkpoints, such as lymphocyte activation gene-3 (LAG-3), have emerged as the third ICI checkpoint to receive approval, following PD-1 and CTLA4 [47, 48]. In GC treatment, early-phase clinical trials have evaluated the efficacy of relatlimab in combination with other ICIs, such as nivolumab (NCT03610711, NCT03662659, NCT03704077), with promising results. Moreover, immune checkpoints like T cell immunoglobulin and ITIM domain (TIGIT) and T cell immunoglobulin and mucin-domain containing-3 (TIM-3) are expected to become the next approved ICIs [49]. Thus, ICIs have become a critical therapeutic tool for many cancers, particularly in refractory tumors like melanoma, non-small cell lung cancer, renal cell carcinoma, and head and neck squamous cell carcinoma, where they have shown significant efficacy either alone or in combination [50–52]. However, in advanced GC, only a subset of patients responds to ICIs. This discrepancy may be attributed to intrinsic molecular changes that create distinct immune microenvironments in different patients. GC molecular subtypes are primarily classified into the Cancer Genome Atlas (TCGA) and the Asian Cancer Research Group (ACRG) subtypes. The TCGA classification includes Epstein-Barr Virus (EBV), microsatellite instability (MSI), genomically stable (GS), and chromosomal instability (CIN) subtypes, while the ACRG classification includes MSI, microsatellite stability (MSS)/TP53$^-$, MSS/TP53$^+$, and MSS/epithelial-mesenchymal transition (EMT) subtypes [53, 54] (as shown in Fig. 1). Some EBV and MSI subtypes are more sensitive to immunotherapy, but such basic classifications alone are insufficient to identify all patients suitable for immunotherapy [55]. Therefore, identifying the appropriate candidates for immunotherapy remains a significant challenge in GC research. Currently, reliable molecular biomarkers to predict immunotherapy efficacy are limited. Biomarkers such as the combined positive score (CPS) score of PD-L1, MSI

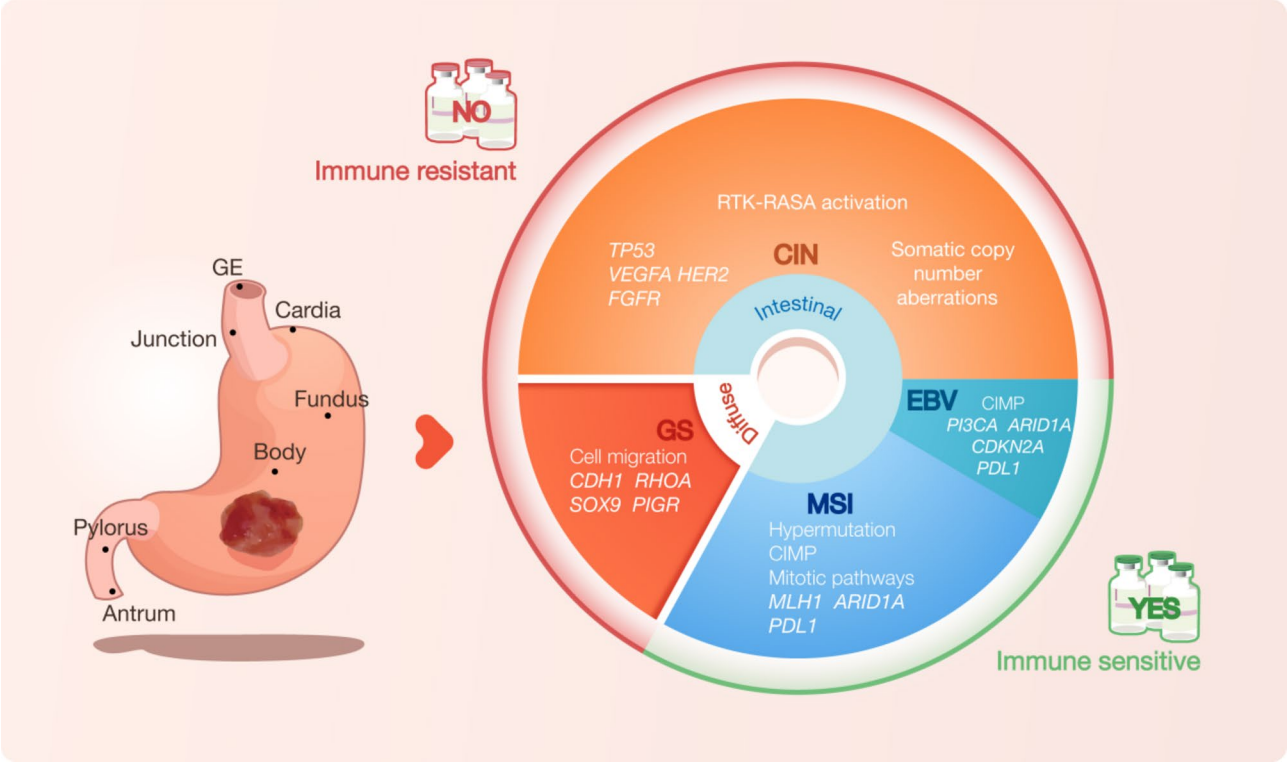

Fig. 1 The molecular subtypes and histological classification of gastric cancer. Histologically, it is categorized into two main types: intestinal and diffuse. Molecular subtyping has revealed four primary subtypes: Epstein-Barr Virus (EBV), Microsatellite Instability (MSI), Chromosomal Instability (CIN), and Genomically Stable (GS). Among these, patients with EBV, MSI, and CIN subtypes predominantly display an intestinal-type profile, while those within the GS subtype exhibit a diffuse-type predominance. Each molecular subtype is associated with distinct driver genes, with altered genes indicated in italics. EBV and MSI subtypes exhibit greater sensitivity to immunotherapy [67, 68]

or dMMR status, and tumor mutational burden (TMB) are among the most widely recognized indicators [56–59]. In the Chinese subgroup of the CheckMate 649 study, the nivolumab-plus-chemotherapy group demonstrated a 3-fold improvement in the 5-year OS rate and a 43% reduction in mortality risk compared to chemotherapy alone in HER2-negative advanced GC patients with PD-L1 CPS ≥ 5. Improvements in progression-free survival (PFS) and OS were also observed in patients with CPS ≥ 1 and in all randomly assigned patients. Regardless of PD-L1 CPS expression levels, nivolumab combined with chemotherapy showed significant benefits in both OS and PFS [60, 61]. The KEYNOTE-062 trial evaluated pembrolizumab, chemotherapy, or a combination of pembrolizumab and chemotherapy in patients with advanced unresectable or metastatic GC and CPS ≥ 1. Compared to chemotherapy alone, the combination therapy did not improve PFS or OS. In patients with CPS ≥ 10, the combination therapy also failed to demonstrate superior OS outcomes [62]. The prediction of immune therapy response based on PD-L1 expression remains inconsistent, and CPS cannot account for all scenarios. Microsatellite highly unstable (MSI-H), as a pan cancer biomarker for ICI response, demonstrates excellent performance in predicting ICI efficacy across various cancer types [59]. However, there is controversy regarding whether and how to use neoadjuvant or adjuvant chemotherapy for locally advanced dMMR/MSI-H gastric or gastroesophageal junction (GEJ) adenocarcinoma patients. dMMR/MSI-H tumors exhibit a better prognosis compared to MSS tumors, and the pathological complete response (pCR) rate of platinum-based and fluoropyrimidine-based neoadjuvant chemotherapy is relatively low, ranging from 3 to 11% [63, 64]. A phase II clinical study of NEONIPIGA investigated the sequential use of nivolumab and ipilimumab as neoadjuvant therapy for resectable dMMR/MSI-H gastric or GEJ adenocarcinoma. The study reported a pCR rate of 59% (17/32), which represents an encouraging outcome [65]. Another phase II trial investigating neoadjuvant therapy for resectable gastric or gastroesophageal junction adenocarcinoma with MSI-H found that the pCR rate was 60%, and the major pathological response rate was 80% following neoadjuvant therapy with tremelimumab and durvalumab. Notably, PD-L1 CPS was not associated with treatment outcomes, while TMB showed no significant correlation with pCR [66]. This provides a promising therapeutic regimen for gastric or gastroesophageal junction adenocarcinoma patients with MSI/dMMR. However, studies have shown that up to 50% of dMMR colorectal cancer patients do not respond to ICIs treatment, underscoring the importance of additional biomarkers or features in predicting treatment response [55]. Thus, there is an urgent need for personalized preclinical models capable of predicting immune therapy responses. These models would better simulate the growing diversity of emerging immune therapies, thereby maximizing efficacy and minimizing unnecessary toxicity. Current research models for GC primarily include tumor cell lines, animal models, and patient-derived xenograft models (PDTX); however, each has limitations (see Fig. 2). The absence of accurate models for GC initiation and progression limits understanding of the disease mechanisms and hinders drug research. In contrast, patient-derived organoids (PDOs) are distinguished by their exceptional ability to preserve the key characteristics of the primary tumor and reconstruct its tumor immune microenvironment (TME). This distinctive advantage renders PDOs an essential model for advancing our understanding of tumor biology and evaluating therapeutic strategies.

Organoids

Timeline of organoid development

In 2009, Clever's team pioneered the successful development of intestinal organoids by culturing $Lgr5^+$ stem cells embedded in a basement membrane extract (BME) rich in laminin and supplemented with growth factors such as R-spondin, Noggin, and epidermal growth factor (EGF) [72]. This resulted in the development of self-organizing crypt-villus-like epithelial structures, which established the groundwork for organoid technology. Using this approach, labs have improved cell isolation and growth factor methods to create organoids from various normal and tumor tissues, successfully forming brain organoids [73, 74], stomach [75–80], esophagus [81], lung [82], liver [83], pancreas [84], kidney [85], salivary gland [86], ovary [87], fallopian tube [87], breast [88], colon [89], and prostate [90]. However, this method constructs only epithelial organoids, excluding stromal components [91]. In parallel, the air-liquid interface (ALI) method preserves the natural architecture of the source tissue, including epithelial cells, endogenous stromal cells, and immune cells, maintaining the original microenvironment. This technique is therefore utilized in cancer oncogene modeling and TME studies [84, 88]. With technological advancements, novel methods like microfluidic culture [92] and organ-on-chip have emerged [93], although the scaffold-based Matrigel method remains more widely used for now. In recent years, the integration of emerging technologies with organoid technology (Fig. 3) has led to notable advancements. This includes hydrogel-based organoids mimicking lymphatic tissue, spatiotemporal omics for detailed organoid analysis, and organoid models for autoimmune diseases like celiac disease.

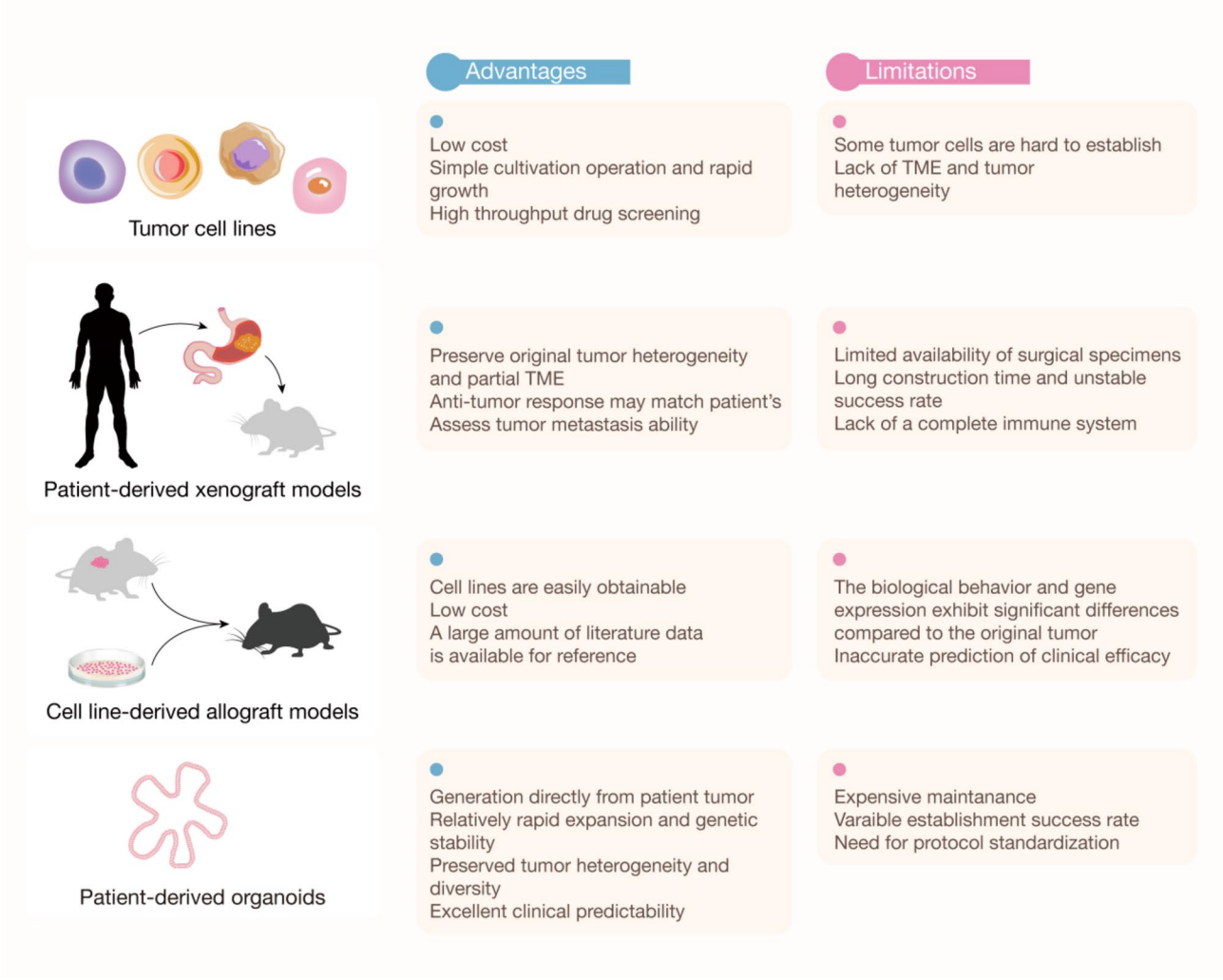

Fig. 2 Summary for the main characteristics of GC preclinical models [69–71]

Organoid construction

The main methods currently used for constructing organoids include the scaffold-based Matrigel method [106], ALI culture [107], microfluidic chips [92], and organoids-on-chip (OoC) [93]. The scaffold-based Matrigel involves enzymatically digesting biopsy specimens to gather gastric gland or cancer cells. The isolated cells are mixed with Matrigel, and then supplemented with a medium containing various growth factors. The ALI method allows co-culturing of organoids with both epithelial and stromal cells [108]. The ALI method uses Boyden chambers (cell culture inserts), which contain a porous membrane layer at the bottom. Tissue fragments mixed with collagen Matrigel are placed into the chamber, and then a medium enriched with nutrients and growth factors is added to the surrounding culture dish. Cells in the ALI method are directly exposed to air, increasing oxygen supply compared to the scaffold-based Matrigel method [109]. A key advantage of the ALI method is its inclusion of stromal cells and its capacity to extend the duration of the TME [110, 111].

Clinical applications of organoids

PDOs accurately mimic the structure, function, histopathology, biomarker expression, and genetic traits of their original tissues [112]. Therefore, organoids are an ideal in vitro human model system for studying organ development, disease modeling, cancer pathogenesis, and drug screening [113, 114] (Fig. 4). However, clinical evidence supporting their use has been limited. The Jaulin team embarked on a feasibility study focused on Functional Precision Medicine (FPM) using organoids cultivated from colorectal cancer (CRC) patients. Researchers successfully developed 25 PDOs from core needle biopsy samples and conducted pharmacological testing with 25 FDA-approved anticancer agents to evaluate their clinical utility. They devised a scoring system, named "chemoscape," to identify responders in vitro. This

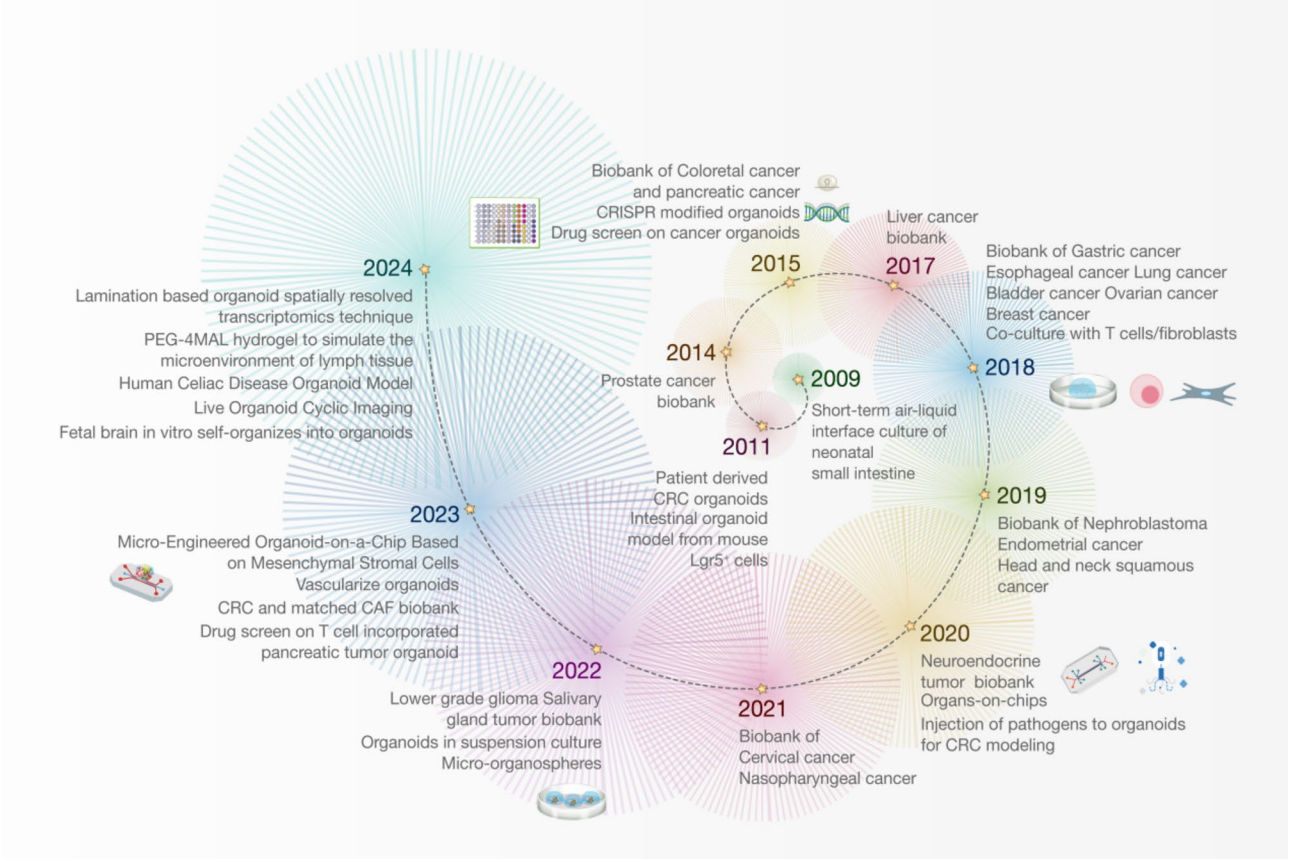

Fig. 3 A brief timeline of organoid development [94–105]

system calculates the mean response of each drug across a population, facilitating the proactive identification of significant sensitivities in specific PDOs. Consequently, it enables clinicians to implement highly personalized treatment strategies for individual patients [115]. Additionally, Boilève's team conducted the largest prospective study to date, involving 87 patients, to gather clinical evidence on applying PDO-based FPM in advanced pancreatic cancer. The researchers utilized their proprietary chemogram drug sensitivity profiling to identify PDOs' sensitivity or resistance to specific drugs. The average sample turnaround time was 6.8 weeks, enabling the identification of at least one potentially effective drug for over 90% of patients, with a sensitivity of 83.3% and specificity of 92.9% [116]. This finding offers significant insights for the development of novel combination therapy clinical trials. Recently, the teams of Agudo and Yilmaz used an orthotopic transplantation method with CRC organoids to identify SOX17 as a key factor in promoting tumor immune evasion. Their research showed that in CRC, SOX17 drives the transition of $LGR5^+$ tumor cells into immune-evasive $LGR5^-$ cells through epigenetic reprogramming. It also diminishes the sensitivity of tumor cells to IFN-γ, inhibits the infiltration of effector $CD8^+$ T cells, creating an immunosuppressive TME that fosters CRC progression [117]. The research highlights the significant value of employing organoid models in cancer research, providing insights for developing therapeutic strategies for targeting early-stage cancers. While further validation in interventional precision oncology trials is needed, PDOs already offer significant opportunities for developing new drugs and combination therapies.

Organoids and artificial intelligence

Despite its wide use, organoid technology still faces challenges and limitations in its construction and evaluation. The initial organoid construction process is highly dependent on manual operations, which restricts reproducibility and reliability. Moreover, organoid cultures produce extensive data in traceability assessments, and efficiently converting this data into insights on disease mechanisms and drug responses is a major research challenge. Integrating Artificial intelligence (AI) into GC organoid research offers new opportunities by streamlining construction, enhancing data analysis, and providing insights into complex biology, thereby advancing precision medicine and accelerating disease modeling and therapeutic discoveries.

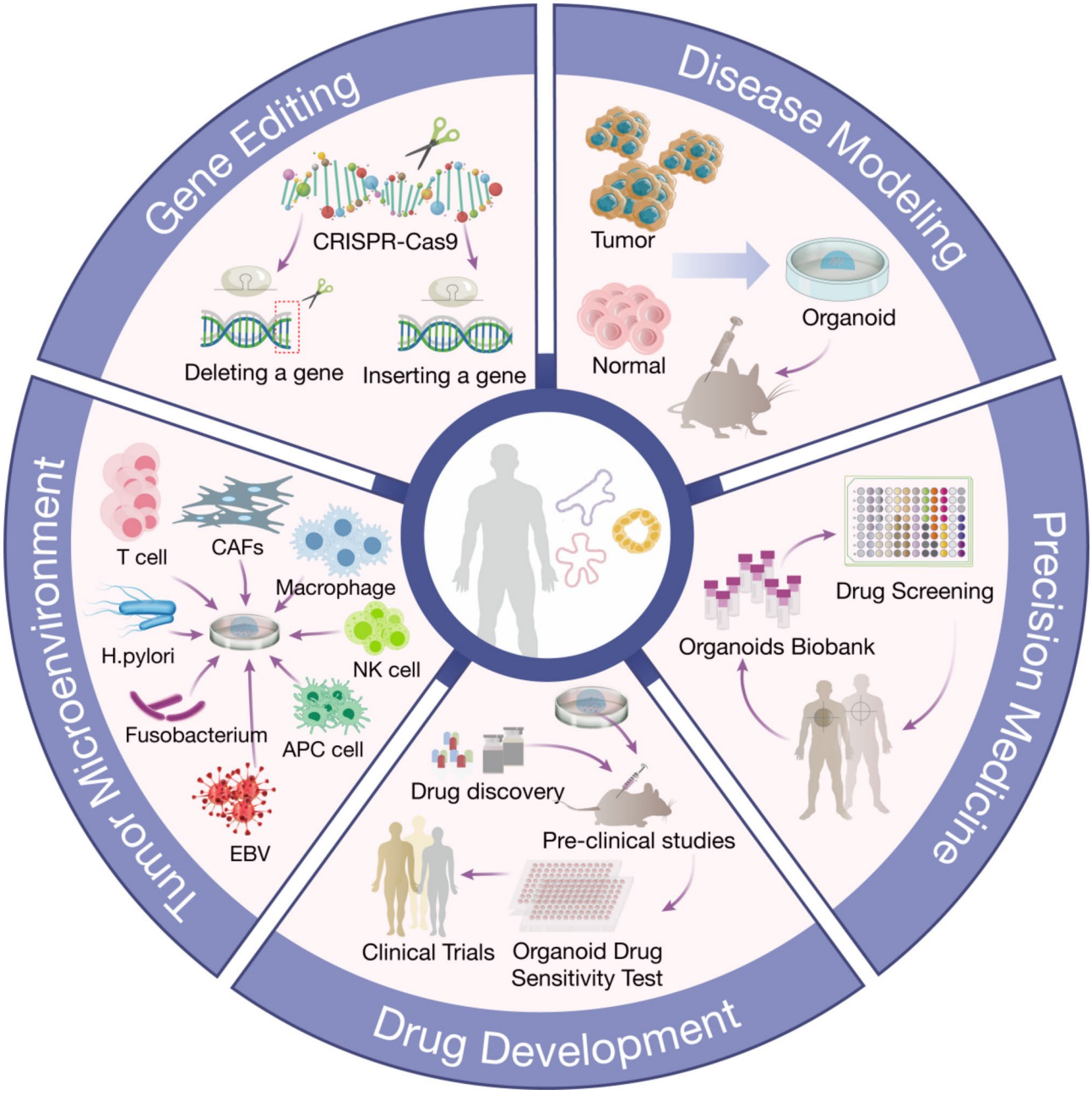

Fig. 4 Overview of possibilities for tumor organoids in clinical applications. Patient derived organoids can be used for disease modeling, precision medicine, simulating tumor microenvironment in vivo, drug development and gene editing, etc. Tumor organoids represent a powerful bridge between preclinical research and clinical application, offering unprecedented opportunities for personalized medicine, drug discovery, and improving patient outcomes in cancer treatment

AI, originating from computer science, aims to mimic human cognitive functions, such as visual perception and decision-making [118]. Machine learning is a subset of AI that uses algorithms to iteratively learn from data, thereby automating decision-making and prediction [119]. AI technologies, particularly machine learning algorithms, present vast potential for the clinical application of organoids. Firstly, AI can optimize Matrigel design for enhanced performance, automate quality control through image analysis, and dynamically monitor culture conditions [120]. AI can analyze high-throughput omics data to offer insights into functional and structural parameters, aiding in the development of more efficient and higher-quality organoids [121]. This accelerates the transition from laboratory research to clinical application. AI can streamline in vitro biobank construction by automatically classifying and labeling samples, improving management and access efficiency through pattern

recognition and data mining. AI-driven predictive models can accelerate the drug screening process by analyzing the responses of organoids to different drugs and identifying potential candidate drugs [122, 123]. Compared to traditional drug screening, this approach significantly reduces the cost of time and money, while improving the success rate of drug development. AI can analyze cellular interactions and changes in organoids using in vitro disease models, simulating disease progression and enhancing understanding of disease mechanisms [124, 125]. In the future, ongoing advancements in artificial intelligence technology are anticipated to enhance the efficiency of GC organoid research significantly, thereby establishing a robust foundation for precision medicine and the development of novel therapeutics.

Organoids and the immune microenvironment

Immunotherapy and immune model organoids

Immunotherapy is a therapeutic approach that leverages the body's innate defense mechanisms to combat diseases, particularly cancer. Alongside surgery, radiation therapy, chemotherapy, and targeted therapies, immunotherapy has become a cornerstone in cancer treatment [126, 127]. Unlike traditional treatment methods, tumor immunotherapy does not directly attack cancer cells, but specifically clears tumor lesions by activating or enhancing the immune system. By stimulating the activity of T cells, B cells, NK cells, and other immune components, tumor immune escape is overcome, and immune cells are awakened again [128]. In addition, immunotherapy also intervenes in the TME, inhibits the function of immunosuppressive molecules, relieves the inhibitory state of the immune system, and restores its killing ability against tumor cells. Therefore, the focus of immunotherapy is not limited to cancer cells, but rather the systemic immune system and TME [67, 129–131]. The TME is a complex milieu composed of various cytokines, chemokines, other factors secreted by tumor cells and the cellular components predominantly includes tumor stromal cells, fibroblasts, endothelial cells, as well as innate and adaptive immune cells [132–135]. These components are pivotal in driving tumor progression. During tumorigenesis, tumor cells interact with and continuously adapt to their surrounding stromal elements, collectively forming the tumor mass [136–139] (Fig. 5). Thus, understanding immune infiltration within the TME is essential for enhancing response rates and developing novel immunotherapeutic strategies for cancer treatment. It is crucial to emphasize that immune cells within the TME regulate tumor growth through their coordinated interactions, and the efficacy of immunotherapy is contingent upon the effective collaboration between innate and adaptive immune cells [140]. Currently, clinical immunotherapy modalities encompass ICIs, adoptive T cell therapy (ATCT), oncolytic virotherapy, cancer vaccines and cytokine therapies [141–145]. Some ICIs, represented by PD-L1/PD-1 and CTLA-4, have been widely used in clinical trials [146–149]. Among them, nivolumab and pembrolizumab have been approved by the US Food and Drug Administration (FDA), and combination chemotherapy is recommended as the first-line treatment for GC [23]. In contrast, adoptive T cell therapy is still in the exploratory stage of early clinical trials in the treatment of GC. The self-developed autologous CAR-T therapy targeting Claudin18.2 by China research team, Ltd. - Sutri cel injection (CT041) has achieved significant breakthroughs in the phase II clinical trial CT041-ST-01 (NCT04581473) for the treatment of GC, and compared with the control group, PFS significantly improved [150, 151]. CT041 has been officially included in the list of breakthrough therapeutic drugs by the China National Medical Products Administration, with the proposed indication being advanced gastric/esophagogastric junction adenocarcinoma with positive Claudin18.2 expression that has undergone at least second-line treatment failure in the past. This marks an important step forward in the field of solid tumor CAR-T therapy. So far, only four oncolytic viruses have been approved for market worldwide, with indications including nasopharyngeal carcinoma, melanoma, and glioblastoma [152]. Multiple oncolytic viruses have entered the clinical trial stage, but the results of oncolytic viruses targeting GC have not been reported yet. In addition, there are literature reports that advanced GC can achieve complete remission for up to 25 months after receiving the new antigen dendritic cell vaccine, but other experimental data is still lacking [153, 154]. Multiple clinical trials of cytokine therapy for solid tumors are currently underway, and no results have been disclosed for GC experiments [155, 156]. Therefore, ICIs are the most widely used immunotherapy method in clinical practice. However, in patients with solid tumors, the outcomes of single-agent immunotherapies are often suboptimal. This can be attributed to the distinctive characteristics of the cancer-immunity cycle (CIC) in solid malignancies. The CIC describes a cyclical process in which the local TME and the host's systemic immune system interact [157]. When immunotherapy aims to overcome immune dysfunction and enhance the CIC, any disruption at any step of the cycle may limit the immune system's ability to control tumor growth effectively [158]. A thorough understanding of the CIC is integral to clarifying the principles of tumor immunotherapy and guiding the development of innovative treatment strategies. Consequently, considerable attention has been directed toward developing accurate tumor models in immunology research, particularly for testing anti-tumor immunotherapies. In tumor immunology, the reliance on 2D cell lines and humanized animal models has often

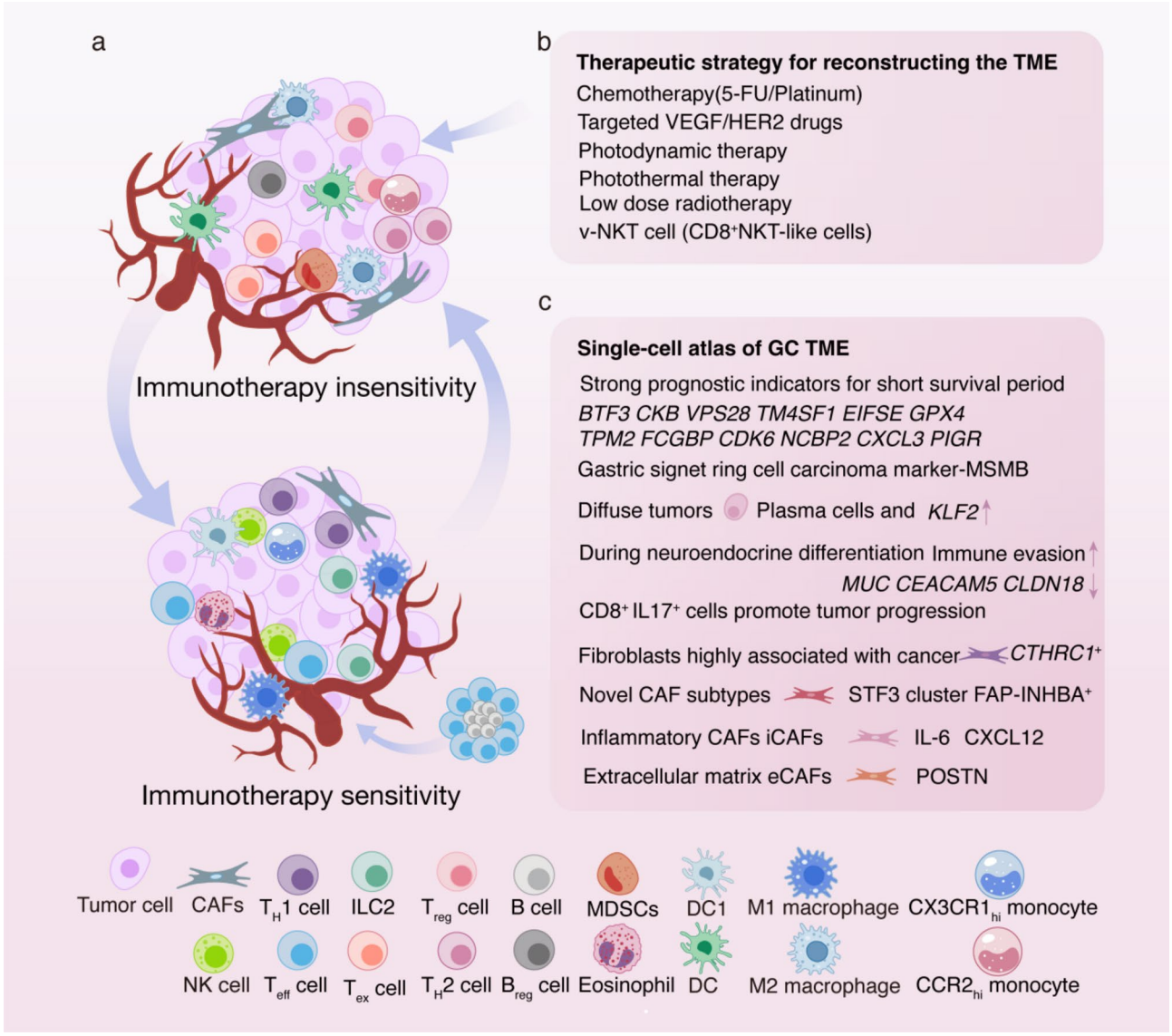

Fig. 5 The dynamically evolving tumor immune microenvironment and the comprehensive landscape single-cell atlas of GC. (**a**) The TME is composed of CAFs, tumor associated macrophages (TAMs), MDSCs, tumor associated neutrophils (TANs), DCs and various types of lymphocytes. According to the degree of response to immunotherapy, it is divided into two categories: "cold tumors" and "hot tumors". The characteristics of "cold" tumors are rich in M2 macrophages, MDSCs, T_H2 cells, Treg cells, terminal exhausted $CD8^+$T (Tex) cells, regulatory B (Breg) cells, $CCR2_{hi}$ monocytes, and mature DC. On the contrary, the cell profile of "hot" tumors includes $CD8^+$T (Teff) cells, T_H1 cells, NK cells, group 2 innate lymphocytes (ILC2s), M1 macrophages, eosinophils, $CX3CR1_{hi}$ monocytes, type 1 dendritic cells (DC1), and tertiary lymphoid structures (TLSs). (**b**) Some therapeutic strategy can reconstructe the TME that transform "cold" tumors into "hot" tumors and this is beneficial for immunotherapy [160, 161]. (**c**) Single-cell sequencing enables a detailed characterization of each cell's state, revealing distinct immune responses and identifying potential therapeutic targets [162–170]

yielded suboptimal results. The advent of in vitro 3D models, however, has helped to address this limitation. The following discussion will focus on the development and clinical application of commonly used immune-functional organoid models. These models include co-culture systems, air-liquid interface techniques, microfluidic organ-on-chip platforms, and micro-organoid spheres [139, 159] each with their respective advantages and disadvantages, as illustrated in Fig. 6. These immune model organoids enable simulation of tumor-immune interactions to a certain extent, improving drug testing and mechanistic studies, and advancing cancer research and therapy development [110, 139].

Co-culture of organoids with immune cells

The extracellular matrix (ECM) can influence adaptive immune responses by either facilitating pathways for T cell infiltration into tissues or directly inhibiting T cell proliferation [171]. Additionally, stromal cells, represented by cancer-associated fibroblasts (CAFs), interact

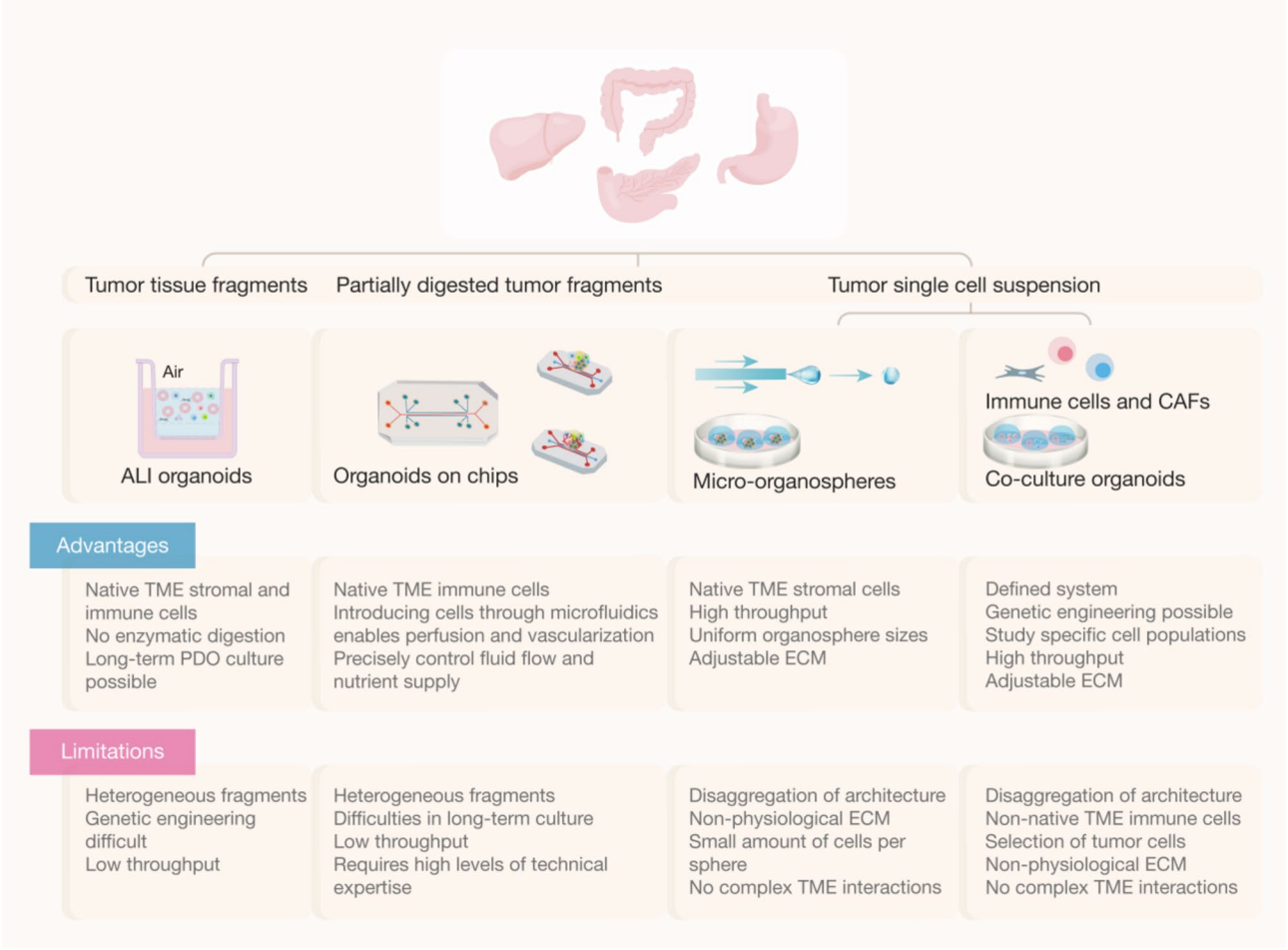

Fig. 6 Advantages and limitations of common tumor immune organoid models. From the perspective of the construction process, ALI organoids directly use tissue fragments, Organoids on chips use partially digested tissue fragments, while Micro-organospheres and co-culture organoids use single-cell suspensions, resulting in a gradually increasing degree of digestion. This means that the difference from the native immune environment of the source tissue gradually increases, ALI organoids is excellent. What's more, the emergence of vascularized organoid on chips has brought us one step closer to highly simulated in vivo tumor microenvironments. The figure is adapted from the work of Calvin J. Kuo's team, with some modifications [139]

with immune cells to significantly impact tumor progression. CAFs are fibroblasts observed in the TME near cancer cells, displaying functional heterogeneity with dual roles in cancer development [172]. While CAFs predominantly promote tumor growth, in certain contexts, they may exhibit anti-tumor functions [172, 173]. CAFs drive cancer progression by enhancing cell growth, invasion, migration, angiogenesis, and therapy resistance. They secrete cytokines, chemokines, Extracellular vesicles, and ECM to alter immune and metabolic responses, fostering tumor development [172, 174, 175].

Research has shown that CAFs secrete immunosuppressive cytokines that can polarize macrophages to an M2 phenotype, leading to exhaustion and loss of $CD8^+$ T cells, which facilitates malignant tumor progression [176]. In 2022, the team led by Strobel established a pancreatic ductal adenocarcinoma (PDAC) organoid model and co-cultured it with primary CAFs isolated from tumor specimens, creating a tumor-CAF co-culture organoid model. Through fluorescence staining, they demonstrated direct contact between CAFs and organoids and observed that CAFs promote tumor cell proliferation. Additionally, researchers have found that CAFs promote inflammation and EMT in this model through using single-cell RNA sequencing and fluorescence staining, suggesting that CAFs may lead to enhanced chemotherapy resistance in tumor cells [177]. Zhao and colleagues established a co-culture system of GC organoids with matched CAFs to investigate their interplay under the influence of 5-FU and oxaliplatin. The study revealed that co-cultured organoids exhibited significantly enhanced drug resistance compared to organoids cultured independently, highlighting the role of CAFs in mediating tumor resilience to chemotherapy [178]. This tumor-CAF co-culture organoid model accurately simulates the human TME, serving as a realistic platform to

evaluate drug responses and cell interactions, and promises better insights into patient-specific drug reactions and TME complexities.

T cells are the primary agents in immunotherapy for cancer patients. However, it is quite difficult to use the patient's own tumor reactive T cells to study their interaction mechanism with tumor cells. Cattaneo and colleagues introduced an innovative technique for cancer immunotherapy research, which involves co-culturing tumor organoids with the patient's own peripheral blood lymphocytes (PBLs) to generate tumor-reactive T cells. The yield of tumor-reactive $CD8^+$ T cell populations can reach about 33–50% in patients with non-small cell lung cancer (NSCLC) and microsatellite instability-high CRC [159]. Researchers co-cultured three GC organoids with autologous T cells to examine the dynamic interplay between organoids and immune cells. In one case, the introduction of autologous T cells led to increased structural damage and apoptosis within the GC organoids. This finding underscores the potential of co-culturing PBMCs with autologous tumor organoids to induce specific tumor-reactive T cell responses, offering a valuable platform for studying the interactions between TME components and tumor cells [178]. This technique marks a milestone in precision medicine and tumor immune response research. It enables the evaluation of novel immunotherapy drugs, facilitates research into patient-specific tumor immune response mechanisms, and allows exploration of immune evasion mechanisms. Additionally, it provides a platform for assessing the efficacy of ICIs, offering a powerful tool for advancing personalized cancer immunotherapy.

CAR-T cells are genetically engineered T cells designed to express a chimeric receptor targeting specific antigens, allowing them to precisely identify and eliminate cancer cells through direct cytotoxicity [179]. However, CAR-T therapy for solid tumors is still in the exploratory stage, and it has been challenging to predict patient responses before treatment. The co-culture of organoids and CAR-T cells presents a novel solution for predicting the efficacy and toxicity assessment in CAR-T therapy. In 2021, Chen and colleagues successfully established a bladder cancer organoid-CAR-T cell co-culture system to evaluate CAR-T cell-mediated cytotoxicity against MUC1-expressing bladder cancer organoids, creating a preclinical ex vivo testing platform. This model holds promise as an effective supplement for personalized CAR-T therapy [180]. Moreover, the broader application of organoid-CAR-T cell co-culture models remains to be explored, with potential applications in drug sensitivity testing, high-throughput screening, and gene editing studies. This advancement could accelerate the development of precision CAR-T therapies, offering a more accurate approach for predicting therapeutic outcomes in cancer treatment.

Air-liquid interface organoids

In 2018, Kuo's team successfully developed PDOs using the ALI method. The ALI-PDOs model retains the endogenous innate immune cell components, fibrous elements, and even cellular matrix components from the original tumor tissue, maintaining a high-fidelity preservation of the original tumor T cell receptor repertoire at both genetic and immune levels [110]. Remarkably, this model enables the simulation of ICIs (anti-PD-1 and anti-PD-L1) activating tumor antigen-specific tumor-infiltrating lymphocytes (TILs) and inducing tumor cell death in vitro. This breakthrough allows for immuno-oncology research on primary tumor cells and their native immune components within TME organoids and promotes personalized immunotherapy. The ALI-PDOs model offers a promising platform for understanding immune responses and assessing personalized treatment strategies, leading to more precise and effective immunotherapy.

Microfluidic organoids on chips

Although traditional organoids hold significant advantages in personalized treatment selection and improving patient outcomes, they face limitations in PDAC. Most PDAC patients are diagnosed at an advanced stage when tumors are unresectable. The source of patient tissue is often from fine-needle aspiration biopsies, which provide limited sample volume and pose challenges for cultivation. To address this, Revzin's team developed an innovative microfluidic organoid culture platform. This platform can sustain cancer organoid growth similarly to Matrigel cultures. Organoids can be created from small tissue samples via needle biopsy, retaining the primary tumor's genetic traits and drug responses, including immune elements [181]. Furthermore, Cherne and colleagues, through an optimized organoid flow chip and a polysaccharide-based synthetic hydrogel, VitroGel®ORGANOID-3, developed an advanced microphysiological immune-cell-epithelial co-culture microfluidic device model. This model was designed to investigate the intricate interactions between dendritic cells, gastric epithelium, and the microbiota, thereby expanding our understanding of the immune surveillance role of mononuclear phagocytes and their involvement in gastritis and related diseases [182]. This makes them ideal for identifying personalized treatment plans, facilitating efficient drug testing and combination therapy selection for advanced cancer patients, and potentially improving the effectiveness of targeted treatments for solid tumors.

Micro-organospheres

In clinical practice, treatment decisions typically need to be made within 14 days after diagnosis, but current PDO cultures often cannot complete drug sensitivity testing within this timeframe, leading to potential treatment delays. Ding and colleagues addressed this issue by employing droplet emulsification microfluidic technology with temperature control and minimized dead volume. They transformed original tumor samples from patients into single-cell suspensions, combined them with 3D Matrigel, and mixed them with biphasic liquids (oil) to rapidly generate thousands of micro-organospheres (MOS) from a small amount of patient tissue. In this study, the time from sampling metastatic CRC tissue to obtaining a reliable drug sensitivity report was only 14 days, making it ideal for guiding clinical treatment decisions. MOS not only captures original tumor cells but also allows for T cell participation, enabling evaluation of immunotherapy efficacy, such as PD-1 inhibitors [183]. With further research and application, this technology holds promise for achieving higher efficacy and lower side effects in future cancer treatments, paving the way for precise, targeted therapy in clinical oncology.

Gastric cancer organoids and immune evasion

Traditionally, malignant cell precursors and the host immune system are believed to exist in a dynamic equilibrium, where the host immune system can eliminate malignant cell precursors and control tumor growth until the tumor cells acquire genetic or epigenetic changes that enable immune escape [184, 185]. For example, NK cells and $CD8^+$ T cells are crucial in restricting the growth of microscopic tumors [186–189]. However, clinically progressive tumors can be observed only after malignant tumor cells have successfully evaded immune recognition and elimination [185].

Malignant tumor cells employ a variety of mechanisms to facilitate immune evasion, which form the basis for local invasiveness and metastatic lesions. These evasion strategies also underlie the challenges and recurrences in established tumors that resist treatment [190, 191]. Understanding these immune evasion mechanisms through GC organoid models provides insight into the TME and immune-tumor interactions, allowing researchers to better design therapies that address these challenges in immunotherapy and precision oncology.

Based on this, L. Galluzzi's team proposed a new framework to categorize cancer immune evasion, identifying that most mechanisms of immune escape by malignant cells involve the "three Cs": Camouflage, Coercion, and Cytorotection (Fig. 7). Camouflage: This refers to malignant cells avoiding detection or recognition by immune effector cells. This can occur due to defects in antigen processing and presentation, limited secretion of chemokines—whether associated with immunogenic cell death (ICD) or not, or the formation of stromal barriers that prevent immune cell infiltration. Coercion: For malignant cells that fail to camouflage, coercion enables immune escape by inhibiting immune effector cell activity. This involves altered expression of immunomodulatory ligands on cancer cells, defects in damage-associated molecular pattern (DAMP) signaling or pro-inflammatory cytokine signaling, and/or the release of immunosuppressive metabolites in the TME. This process suppresses the activity of immune effector cells, including dendritic cells (DCs), NK cells, T_H1-polarized $CD4^+$ T cells, and $CD8^+$ cytotoxic T lymphocytes (CTLs), while promoting the activity of immunosuppressive cells such as regulatory T cells (Treg cells), specific TAM subsets, and myeloid-derived suppressor cells (MDSCs). Cytoprotection: This involves mechanisms that protect malignant cells from immune cytotoxicity. It includes defects in immune synapse formation, activation of downstream cell death pathways, or initiation of compensatory responses such as autophagy, allowing malignant cells to evade immune destruction [192]. These categories provide a structured understanding of the strategies that tumors use to evade immune recognition and elimination, laying the groundwork for developing more effective immunotherapies targeting these specific evasion mechanisms.

Therefore, successfully blocking tumor cells from evading the host immune system is essential for enhancing the effectiveness of both contemporary immunotherapies and conventional treatment approaches. In recent years, researchers have increasingly concentrated on the link between immune evasion and GC. Ji's team, utilizing CRISPR-Cas9 technology, identified TRIM28 as a critical regulatory factor of PD-L1 expression in GC cells. TRIM28 stabilizes PD-L1 by inhibiting its ubiquitination and promoting its SUMOylation, and activates the TBK1-IRF1 signaling pathway to enhance PD-L1 transcription. This leads to immune suppression and promotes GC progression, revealing a novel role of TRIM28 in regulating PD-L1 protein stability [193]. This discovery may serve as a potential therapeutic strategy to improve immunotherapy for GC in the future. Due to the off-target effects and mechanisms of immune evasion, targeted therapies have not been highly effective in significantly extending the survival of GC patients. Research has shown that circular RNAs (circRNAs) might play a physiological role in GC progression [194, 195]. CircRNAs can interact with microRNAs (miRNAs), competitively binding with miRNA response elements to regulate the expression of miRNA-targeted genes [196]. Miao and colleagues discovered that hsa_circ_0136666 is highly expressed in GC and acts as a sponge for miR-375. Mechanistic investigations revealed that hsa_circ_0136666 promotes PRKDC

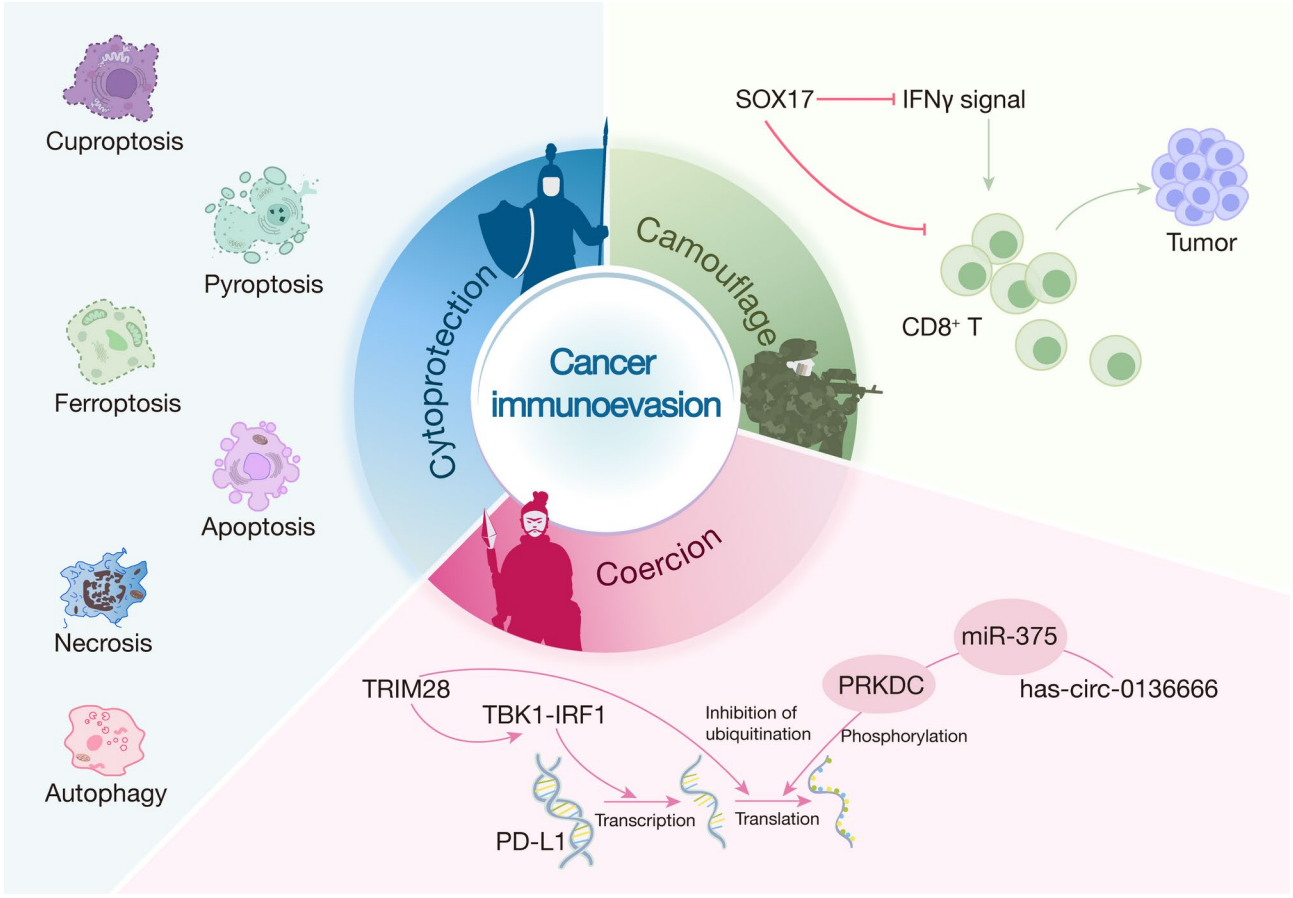

Fig. 7 A brief introduction to immune evasion in tumors. Tumor cells employ various mechanisms to avoid immune responses, which can be summarized as the "Camouflage" mechanism that hides cancer cells from immune recognition, the "Coercion" that directly or indirectly interferes with immune effector cells and the "Cytoprotection" that protects malignant cells from immune cell toxicity. In the process of tumor progression, multiple parts mentioned above are often involved simultaneously. Therefore, understanding immune evasion is crucial for developing immunotherapies, as these treatments aim to overcome these evasive strategies and empower the immune system to recognize and eliminate cancer cells more effectively. This figure is adapted from the work of L. Galluzzi's team with modifications [192]

expression through miR-375, leading to the phosphorylation and stabilization of PD-L1, thereby promoting GC progression and immune evasion [197]. This finding highlights the role of hsa_circ_0136666 as an immune target and provides a theoretical basis for enhancing the efficacy of anti-PD-L1 therapies in GC.

The rise of immunotherapy has brought new hope to cancer patients. With the development of immune organoid technology, researchers now have a platform that can authentically replicate the diversity and physical structure of the TME, providing insights into the interactions between immune cells and tumor cells. Chakrabarti and colleagues explored the effects of combining anti-HER2 targeted therapy with anti-PD1 ICIs by co-culturing human GC organoids with CTLs and myeloid-derived suppressor cells. Their findings revealed that HER2-targeted drugs could inhibit CTL effector functions and PD-L1 expression through the PI3K-AKT-mTOR pathway. It has been demonstrated that HER2-induced PD-L1 may drive immune evasion in tumor cells [198]. In summary, these studies not only provide new insights into the mechanisms of immune evasion in gastric and colorectal cancers but also may aid in developing new immunotherapy strategies and prognostic biomarkers.

Gastric cancer organoids and intracellular bacteria

The microbiome is an integral part of the human body, significantly impacting cancer risk, clinical pathology, treatment responses, and tumor prognosis [199–202]. Studies have demonstrated a strong correlation between the microbiome and the progression of various cancers, including colorectal [203, 204], pancreatic [205], lung [206], breast [207], and GC [208]. Within tumors, hypoxia and necrosis often create a chronic immunosuppressive microenvironment conducive to bacterial growth [209]. As a result, researchers have increasingly detected bacteria within solid tumors, shifting focus towards understanding the link between intracellular

bacteria and tumor progression. It has been found that intracellular bacteria can promote cancer development and progression through various mechanisms, including DNA damage [210–214], epigenetic modifications [215–217], inflammatory responses [218, 219], and modulation of the host immune system [220–223]. These findings suggest that bacteria within the TME may play an active role in shaping cancer behavior and therapy outcomes, making the microbiome a potential target for innovative cancer treatments and a promising field for further investigation in GC organoid studies.

Helicobacter pylori infection is the most common bacterial infection in GC. It stimulates gastric acid secretion which can result in peptic ulcers and a subset of patients may gradually progress to GC [224]. Neutrophil alkaline phosphatase (NAP) is a key virulence factor of *H. pylori*, which promotes gastric mucosal damage by activating neutrophils to produce reactive oxygen species (ROS) and by enhancing neutrophil adhesion to gastric epithelial cells. NAP also stimulates the release of various pro-inflammatory chemokines, increasing the secretion of gastrin and pepsinogen, contributing to gastric mucosal injury [225]. The progression to GC is a prolonged and dynamic process. *H. pylori* has a well-established role in the initiation and progression of GC, with factors such as inflammatory mediators, epithelial cell apoptosis, and genetic mutations contributing to its pathogenesis. However, the exact mechanisms by which these factors operate are still not fully understood. Further research into how *H. pylori* infection leads to GC is crucial for advancing prevention and treatment strategies [226]. Traditionally, it was believed that the highly acidic environment of the stomach prevented most bacteria from surviving, with *H. pylori* being the only microorganism able to colonize. However, recent research has revealed microbial dysbiosis in the stomachs of GC patients, with distinct bacterial profiles and dominant bacterial populations. Studies have shown an abundance of *Fusobacterium* in GC tissues compared to normal tissues, particularly associated with poorer prognosis and shorter OS in patients with diffuse-type GC. *Fusobacterium* infection appears to influence the phenotypic characteristics, microecology, and metabolic functions of GC cells, suggesting a link between *Fusobacterium* and the carcinogenesis, progression, and prognosis of GC [227]. Additionally, research by Zhong and colleagues identified an increased presence of *Candida albicans* in GC. *C. albicans* may contribute to GC development by reducing fungal diversity and abundance within the stomach [228]. When *C. albicans* invades the mucosal epithelium, it triggers apoptosis and necrosis, disrupting the immune barrier of the epithelium and leading to structural alterations [229]. This suggests a potential role for *C. albicans* in GC progression by weakening epithelial defenses and creating an environment conducive to cancer development. Furthermore, Yu Jun's team discovered that *S. anginosus* is enriched in the gastric mucosa of patients with GC. Studies conducted in both conventional and germ-free mice revealed that *S. anginosus* infection spontaneously induces gastric inflammation, atrophy, mucinous metaplasia, and lesions. Further mechanistic investigations revealed that the surface protein TMPC of *S. anginosus* interacts with the Annexin A2 (ANXA2) receptor on gastric epithelial cells, thereby facilitating bacterial adhesion and colonization. angin of the gastric mucosa. This interaction activates the mitogen-activated protein kinase (MAPK) signaling pathway, promoting cell proliferation and inhibiting apoptosis, thereby contributing to tumorigenesis [230]. However, the researchers have yet to explore the interactions between *S. anginosus* and other cells within the gastric TME, as well as its interactions with other microbial communities in GC. This will be a key focus for future studies on the mechanisms of GC. Building on this, we can further investigate the role of *S. anginosus* within the GC immune microenvironment using GC organoid models. These insights underscore the significance of the gastric microbiome beyond *H. pylori*, suggesting that other microbial species may also contribute to GC initiation, progression, and patient outcomes. Further research into these associations may reveal novel targets for diagnostics, treatment, and prevention of GC.

Choosing an appropriate preclinical model to assess the impact of intracellular bacteria on tumor progression is crucial for advancing cancer research. Organoids closely resemble their tissues of origin in both structure and function, making them suitable for modeling the impact of microbes on tumorigenesis, tumor progression, and therapeutic efficacy [112]. Organoids provide a reliable platform to model how microbial interactions influence tumor behavior. For instance, Puschhof and colleagues have used organoids and organ-on-chip systems to evaluate the effects of microbiota on intestinal and colonic epithelium [231]. In 2022, a study led by Cai's team first demonstrated that intracellular bacteria in breast cancer tissue can promote tumor colonization and metastasis. The researchers detected intracellular bacteria in breast cancer tissues using an optimized Taqman qPCR method. Through a co-culture system of intracellular bacteria and organoids, they found that different intracellular bacteria exhibit varying levels of cell invasion and colonization capabilities [232]. Research indicates that *H. pylori* secretes vacuolating cytotoxin A (VacA), colonizes in the stomach, initiating a series of detrimental effects. This toxin induces vacuolation, inflammation, and apoptosis within gastric epithelial cells, while also compromising epithelial integrity. Furthermore, VacA disrupts mitochondrial function by targeting lysosomal

calcium channels or binding directly to mitochondria, ultimately leading to cellular energy deficiencies. These pathological changes contribute to the death of gastric mucosal cells, which accelerates the progression of peptic ulcers and fosters conditions conducive to GC development [233–236]. Despite the availability of various antibiotic treatments for *H. pylori*, the escalating issue of antibiotic resistance has rendered traditional therapeutic approaches increasingly challenging. Therefore, uncovering novel therapeutic targets and strategies, particularly those addressing the mechanisms of VacA, has emerged as a critical area of research. In a study led by Son, the team employed human antrum gastric organoid models (hAGOs) derived from human pluripotent stem cells (hPSCs) to mimic the *H. pylori* infection process. They focused on the mitochondrial damage induced by VacA in antral cells, investigating its underlying molecular mechanisms. Their findings revealed that VacA compromises the gastric mucosal barrier by impairing mitochondrial function in antral mucous cells, leading to energy metabolism disturbances and decreased mucus secretion [237].These insights are pivotal in advancing the understanding of how *H. pylori* infection contributes to gastric mucosal damage. A series of experiments confirmed that intracellular bacteria can indeed enhance tumor colonization and metastasis. The above findings emphasize the utility of organoid models for studying the role of bacteria in cancer biology and highlight the potential of organoid-based systems as preclinical models for evaluating how tumor-resident microbes influence cancer progression and metastasis.

Application of gastric cancer organoids in tumor drug development

PDOs have demonstrated remarkable potential in predicting drug sensitivity and clinical responses in GC. Their ability to reliably assess reactions to chemotherapy and targeted therapies, combined with their rapid establishment and turnaround time, making them ideal for clinical applications [238]. Recently, the U.S. FDA formally recognized organoids' utility in drug screening and sensitivity testing, signaling a shift toward replacing traditional animal models with organoid-based experiments [239]. This recognition underscores the efficacy and reliability of organoid models in preclinical research. The summary of retrievable clinical trial information related to GC organoids is shown in Table 1.

Clinical guidance for gastric cancer treatment using organoids

Emerging evidence supports the use of PDOs derived from metastatic gastrointestinal cancer patients to guide clinical treatment. By comparing drugs response of PDOs in vitro, orthotopic xenograft models in mice and patient outcomes in clinical trials, researchers have demonstrated a sensitivity of 100%, specificity of 93%, positive predictive value of 88%, and negative predictive value of 100% for predicting responses to targeted and chemotherapy drugs [240]. These findings highlight the utility of PDOs in functional genomics research, enabling in vitro modeling of cancer behaviors and incorporating molecular pathology insights into early clinical trial decision-making [240]. For instance, Yu's team successfully developed stable organoid lines from primary gastric tumors and lymph node metastases. These organoids displayed consistent drug response profiles with their source tissues, accurately predicting tumor cell sensitivity to specific chemotherapy drugs and providing robust experimental support for clinical decision-making [241]. For patients with locally advanced GC, neoadjuvant chemotherapy is a standard recommendation to reduce tumor size, eliminate micrometastases, and optimize conditions for subsequent surgery or radiotherapy. PDOs offer a valuable tool for refining drug selection in these cases, tailoring neoadjuvant regimens to individual patients and improving therapeutic outcomes. While neoadjuvant chemotherapy significantly improves median survival in patients with locally advanced esophagogastric adenocarcinoma (EGC), approximately 63% of patients show minimal pathological responses, emphasizing the need for personalized approaches. To address this, researchers cultured PDOs from endoscopic biopsy samples of 120 patients with locally advanced EGC. Drug responses were evaluated for single and combination agents in the FLOT regimen (5-fluorouracil, leucovorin, oxaliplatin, and docetaxel), revealing significant variation in drug sensitivity among organoids. By analyzing these responses, researchers established a threshold for distinguishing responders from non-responders, achieving a sensitivity of 90%, specificity of 100%, and accuracy of 92% [242]. This approach represents a groundbreaking method for individualizing chemotherapy in EGC, improving patient outcomes and enhancing quality of life. With broader application, PDO-based strategies could revolutionize precision oncology by offering a transformative tool for optimizing treatments in esophagogastric cancers.

Gastric cancer organoids for drug screening and new drug development

Antitumor drug screening has relied on conventional tumor cell cultures and PDTX models traditionally. However, these models have significant limitations in replicating in vivo conditions, emphasizing the urgent need for more human-relevant systems to reduce the high costs of drug development. PDOs, which retain the genetic and pathological characteristics of their original tissues, represent a promising alternative [243]. PDOs enable patient-specific drug testing in vitro, potentially

Table 1 Clinical trials related to gastric cancer organoids

Status	ClinicalTrials.ID	Study Type	Enrollment	Registration Dates	Subject	Location
Recruiting	NCT06519500	Observational	40	2024-07-19	Generation of Organoids of Neuroendocrine Neoplasms of the Gastro-Entero-Pancreatic Tract Obtained From Patients Undergoing Surgery	Rome, Italy
Unknown status	NCT05351398	Observational	54	2022-04-01	The Clinical Efficacy of Drug Sensitive Neoadjuvant Chemotherapy Based on Organoid Versus Traditional Neoadjuvant Chemotherapy in Advanced Gastric Cancer	Sahnghai, China
Recruiting	NCT05842187	Interventional	50	2023-04-10	In Vitro Organoid Drug Sensitivity-Guided Treatment for Metastatic Pancreatic and Gastric Cancer (ODYSSEY)	Hangzhou, China
Recruiting	NCT06196554	Observational	40	2023-12-01	Gastric Cancer Organoids in the Screening of Neoadjuvant Drugs	Beijing, China
Unknown status	NCT05203549	Observational	250	2022-01-10	Consistency Between Treatment Responses in PDO Models and Clinical Outcomes in Gastric Cancer	Shanghai, China
Recruiting	NCT05652348	Observational	48	2022-12-07	Response Prediction of Hyperthermic Intraperitoneal Chemotherapy in Gastro- Intestinal Cancer (Hi-STEP1)	Dresden, Germany
Unknown status	NCT05442138	Observational	54	2022-06-28	A Study on the Potential Benefit of Neoadjuvant Therapy for AGC Patients	Not provided
Recruiting	NCT06100003	Observational	104	2023-10-07	A Clinical Study Aims to Assess the Consistency of Clinical Efficacy in Gastric Cancer Treatment and Drug Susceptibility Outcomes Using a Novel Drug Susceptibility Testing Method	Shenyang, China
Active, not recruiting	NCT03429816	Interventional	40	2018-02-05	OPPOSITE: Outcome Prediction Of Systemic Treatment in Esophagogastric Carcinoma (OPPOSITE)	Dresden, Germany Heidelberg, Germany
Recruiting	NCT05508399	Observational	28	2022-08-17	Biomarker Analysis of Tislelizumab Combined With Chemotherapy for Perioperative Treatment of G/GEJ Adenocarcinoma	Xi'an, China
Recruiting	NCT02495337	Observational	100	2015-05-27	Tissue Collection Protocol for Gastroesophageal Cancers	Toronto, Canada

Notes: Data from https://clinicaltrials. American Clinical Trial Registry

increasing success rates, shortening development timelines and reducing overall research costs. In 2018, Yan and colleagues established a GC organoid biobank, incorporating primary tumors, lymph node metastases and non-tumor organoids from 34 GC patients. This biobank encompasses nearly all known molecular subtypes of GC across different disease stages. Validation studies confirmed that the morphology, transcriptomic profiles, and genomic characteristics of long-term cultured organoids closely mirrored those of in vivo tumors. Researchers conducted large-scale drug screening, testing both FDA-approved therapies and drugs in clinical trials using this biobank. Notably, a stemness STAT3-target inhibitor-Napabucasin, mTOR inhibitor-Vistusertib and the ATR inhibitor-VE-822 showed significant sensitivity in organoid models, demonstrating the utility of organoid biobanks for identifying targeted therapies, guiding clinical drug selection, and accelerating anticancer drug development [17]. Expanding on this work, Wang and colleagues developed a gastric tumor organoid (GTO) biobank to conduct high-throughput drug screening while incorporating clinical prognosis insights. Their study revealed that drug sensitivity results in GTOs correlated strongly with the long-term clinical outcomes of corresponding patients. Additionally, they generated gastric normal organoids (GNOs) from the normal gastric epithelial tissues of the same patients. Drug sensitivity testing using GNOs provided valuable information on potential adverse effects, enabling a comprehensive strategy to select therapies that balance efficacy with minimal toxicity. By integrating data from GTOs and GNOs, this approach facilitates personalized treatment plans, optimizing clinical outcomes while minimizing side effects [244].

Similarly, Zhao and colleagues established a biobank of 57 GC organoids from 73 patients and screened six conventional chemotherapy drugs (5-fluorouracil, oxaliplatin, cisplatin, paclitaxel, doxorubicin, and SN-38). RNA sequencing identified distinct gene expression patterns in chemotherapy-sensitive and chemotherapy-resistant organoids. Tumor suppressor genes and pathways were upregulated in organoids sensitive to 5-FU or oxaliplatin,

whereas proliferation and invasion-related pathways were enriched in resistant organoids. Follow-up studies on 12 primary GC patients demonstrated a strong correlation between organoid drug responses and clinical outcomes in 11 cases, providing robust evidence for the clinical utility of GC organoids in drug screening and personalized therapy [178]. Research into diverse drug screening strategies continues to grow. Yang and colleagues used PDOs from different GC subtypes to evaluate 11 small-molecule kinase inhibitors. They observed that intestinal-type PDOs (IPDOs) were broadly sensitive to kinase inhibitors, while diffuse-type PDOs (DPDOs) exhibited limited sensitivity. However, treatment of DPDOs with Aurora kinase inhibitors (AURKi) such as Barasertib and Danusertib induced a senescent phenotype characterized by increased cell size, multinucleated giant cells, and strong senescence-associated β-galactosidase (SA-β-GAL) activity. These senescent cells secreted large quantities of MCP-1/CCL2, recruiting macrophages and polarizing them toward an immunosuppressive M2 phenotype via CCR2 receptor interactions. This immunosuppressive microenvironment inhibited the innate immune response, potentially promoting tumor progression. Based on these findings, a sequential therapy combining AURKi with senolytic agents to clear senescent cells could mitigate the pro-tumorigenic effects of therapy-induced senescence while enhancing therapeutic efficacy in diffuse-type GC [245]. Finally, the development of novel organoid models has enabled deeper investigations into GC pathogenesis.

Mechanistic studies of gastric cancer using organoids

Organoids are widely employed in fundamental research as models for identifying potential therapeutic targets beyond their crucial role in drug screening and new drug development. For instance, Ukai and colleagues developed a series of 5-fluorouracil (5-FU) resistant GC organoids (GCOs) to investigate resistance mechanisms. Through morphological and gene expression analyses of these resistant GCOs, they identified KHDRBS3 as a pivotal factor mediating 5-FU resistance in GC. Further studies demonstrated that knocking out KHDRBS3 reduced chemoresistance, impaired organoid formation, and inhibited tumor growth and metastasis, whereas KHDRBS3 overexpression produced opposite effects. These findings suggest that KHDRBS3 is a critical player in GC and represents a promising therapeutic target [246]. In another study, Ouyang and colleagues established a GC patient-derived xenograft (PDX) models in nude mice, subsequently developing GC organoids derived from these PDXs. Their research revealed that the STAT3 inhibitor W1131 effectively suppressed GC organoid growth by inhibiting the STAT3 signaling pathway and inducing ferroptosis, a regulated form of cell death. Moreover, W1131 significantly enhanced the sensitivity of GC organoids to 5-FU. These results underscore the versatility of GC organoids as models for elucidating drug resistance mechanisms and identifying novel therapeutic targets. The significance of STAT3-related inhibitors in tumor progression suggests that such inhibitors, either alone or in combination with traditional chemotherapeutic agents, hold potential as novel therapeutic strategies for GC [247]. Advancements in gene editing have enabled researchers to engineer organoids with tailored genetic modifications, enhancing their applicability in cancer research. For example, Tan and colleagues employed single-cell sequencing to investigate tumor heterogeneity in GC. They discovered that $CCKBR^+$ gastric adenocarcinoma cells within the TME exhibit stem cell-like properties closely linked to tumor invasiveness and poor prognosis. FOXO was identified as a key regulator of the stemness of $CCKBR^+$ cells. By developing $CCKBR^+$ and $CCKBR^-$ organoids and treating them with a FOXO inhibitor in combination with standard chemotherapy drugs, the researchers demonstrated that FOXO inhibition selectively suppressed the growth of $CCKBR^+$ stem cell-like tumor cells, reduced organoid formation, and inhibited tumor progression. This highlights FOXO inhibition as a potential therapeutic strategy for gastric adenocarcinoma [248]. Similarly, Cai's team used genetically modified organoids to investigate tumor behavior and drug responses, further demonstrating the utility of such models in cancer research. Lastly, studies on circRNAs have identified circ-0008315 as a promising therapeutic target for cisplatin-resistant GC. High-throughput sequencing revealed the upregulation of circ-0008315 in both GC tissues and cisplatin-resistant GC cells, a finding corroborated in cisplatin-resistant GC organoids. Downregulation of circ-0008315 significantly inhibited GC cell proliferation, migration, and EMT in vitro and in vivo. In cisplatin-resistant GC organoid models, suppression of circ-0008315 successfully reversed cisplatin resistance. To translate these findings into a therapeutic application, researchers developed PLGA-PEG nanoparticles targeting circ_0008315, which effectively inhibited GC cell proliferation and overcame cisplatin resistance. Circ-0008315 also holds potential as a prognostic biomarker for GC. This study underscores the promise of circ-0008315 as a therapeutic target in nanomedicine, offering a novel approach to addressing cisplatin resistance and improving patient outcomes in GC [249].

Conclusions

The treatment of GC faces significant challenges, including low early detection rates, pronounced biological heterogeneity, high treatment costs, limitations of current biomarkers, and the lack of ideal preclinical models. An ideal preclinical model should accurately mirror a GC

patient's tumor genome, phenotype, drug response, and maintain the original TME. Addressing this challenge, cancer researchers globally are shifting from a "one-size-fits-all" approach to personalized GC treatment strategies for individual patients. The advent of organoid models has proven transformative in this effort, as they fulfill many criteria for an ideal preclinical model. Organoids retain the morphological characteristics, mutational landscape, and gene profile of the original tumor, encompassing the genetic, epigenetic, and pharmacological heterogeneity. The rapid advancements in precision medicine, immunotherapy, and organoid technology are paving the way for more personalized and effective approaches to diagnosis and treatment of GC. Harnessing these innovations to their fullest potential promises to enhance survival outcomes and improve the quality of life for patients. Moreover, GC organoids can simulate the native TME when cultured within optimized systems. Extensive evidence underscores the indispensable role of organoids in predicting drug sensitivity, facilitating high-throughput screening, advancing novel drug development, and unraveling mechanisms underlying tumorigenesis and progression. These qualities position organoids as invaluable tools for driving individualized cancer treatments, improving therapeutic outcomes, and tailoring interventions to the unique cancer profiles of each patient.

While organoids hold considerable promise as preclinical models, their broad application faces significant barriers. High Cost and Labor-Intensive Culture: The cultivation and maintenance of organoids are expensive and require specialized techniques and materials. Variable Success Rates: The efficiency of GC organoid culture remains inconsistent and is influenced by sample quality, operator expertise, and environmental factors, particularly bacterial or fungal contamination caused by inadequate cleaning of tumor tissue. Lack of Standardized Protocols: The absence of universally accepted protocols for culturing organoids from diverse sample types—such as resected tumors, fine-needle biopsies, lymph node metastases, and GC cells from pleural or peritoneal effusions—compromises reproducibility. Unclear Drug Sensitivity Standards: A lack of defined benchmarks for predicting drug sensitivity complicates the interpretation of experimental data. This makes it challenging for GC patients to achieve significant benefits within a short period. Limitations in Microenvironment Simulation: Organoids can only replicate a simplistic immune microenvironment while lack of vascular structures and influx of new cells from full organism, hindering their ability to fully simulate in vivo conditions. Additionally, evidence supporting the use of PDOs for functional precision medicine in GC remains limited. Overcoming these challenges requires sustained research and refinement to make organoid-based applications more feasible and impactful in the treatment of GC.

Finally, the following concepts are envisioned for the future development of organoids. Optimization of Biological Scaffolds and Matrix Components: Developing scaffolds and culture media that closely mimic the human internal environment is essential for advancing organoid research. Moreover, reducing the cost of culturing GC organoids is equally important. Interdisciplinary Collaboration: Leveraging artificial intelligence (AI) and big data analytics can enable real-time imaging of organoids and the integration of metabolic and genetic data, generating predictive models for disease GC behavior and therapeutic responses. Furthermore, combining organoid technology with 3D bioprinting can expedite GC organoid development and enable efficient high-throughput drug screening. Functional Research In Vivo: Organoids offer promising potential in regenerative medicine, such as in skin or liver transplantation, addressing the global shortage of organ donors [250]. Additionally, organoids incorporating immune microenvironments could be used to study autoimmune disease mechanisms and evaluate drug efficacy [100]. Expansion to Multi-Tissue Symbiotic Systems: Investigating the interactions among multiple organ systems, such as the gut-liver axis and the liver-brain axis, presents a compelling opportunity to deepen our understanding of systemic biology and the mechanisms underlying complex diseases. Collectively, these advancements hold the potential to revolutionize GC organoid research, thereby transforming precision oncology and extending to broader biomedical applications.

Abbreviations

ACRG	Asian Cancer Research Group
AdSCs	Adult stem cells
AI	Artificial intelligence
ALI	Air-liquid interface
ATCT	Adoptive T cell therapy
AURKi	Aurora kinase inhibitors
BME	Basement membrane extract
CAFs	Cancer-associated fibroblasts
CICL	Cancer-immunity cycle
CIN	Chromosomal instability
circRNAs	Circular RNAs
CPS	Combined positive score
CRC	Colorectal cancer
CSCO	Chinese Society of Clinical Oncology
CTLA4	Cytotoxic T lymphocyte-associated antigen 4
CTLs	Cytotoxic T lymphocytes
DAMP	Damage-associated molecular pattern
DCs	Dendritic cells; dMMR: deficient mismatch repair
DPDOs	Diffuse-type gastric cancer patient derived organoids
EBV	Epstein-Barr Virus
ECM	Extracellular matrix
EGC	Esophagogastric adenocarcinoma
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
ESCs	Embryonic stem cells
FDA	Food and Drug Administration
FPM	Functional Precision Medicine
GC	Gastric cancer

GNOs	Gastric normal organoids
GS	Genomically stable
GTO	Gastric tumor organoid
hAGOs	Human antrum gastric organoid models
hPSCs	Human pluripotent stem cells
ICD	Immunogenic cell death
ICIs	Immune checkpoint inhibitors
IPDOs	Intestinal-type gastric cancer patient derived organoids
iPSCs	Induced pluripotent stem cells
LAG-3	Lymphocyte activation gene-3
MAPK	Mitogen-activated protein kinase
MDSCs	Myeloid-derived suppressor cells
miRNAs	microRNAs
MOS	Micro-organospheres
MSCs	Mesenchymal stem cells
MSI	Microsatellite instability
MSS	Microsatellite stability
NAP	Neutrophil alkaline phosphatase
NSCLC	Non-small cell lung cancer
OoC	Organoids-on-chip
OS	Overall survival
PBLs	Peripheral blood lymphocytes
pCR	pathological complete response
PDAC	Pancreatic ductal adenocarcinoma
PDOs	Patient derived organoids
PDTX	Patient-derived tumor xenograft
PFS	Progression-free survival
ROS	Reactive oxygen species
SA-β-GAL	Senescence-associated β-galactosidase
TCGA	The Cancer Genome Atlas
T-DXd	Trastuzumab deruxtecan
TIGIT	T cell immunoglobulin and ITIM domain
TILs	Tumor-infiltrating lymphocytes
TIM-3	T cell immunoglobulin and mucin-domain containing-3
TMB	Tumor mutational burden
TME	Tumor microenvironment
TReg cells	Regulatory T cells
VacA	Vacuolating cytotoxin A
3D	Three-dimensional

Author contributions
LK wrote the original manuscript, LK, YY, YW, WF, HC, LS and CR designed the figures and tables, LK, YY, YW and CR contributed to final editing of the work. All authors contributed to the original preparation of respective sections and revisions of the whole manuscript. All authors read and approved the final manuscript.

Funding
This work was supported by grants from the National Natural Science Foundation of China (82172345, 81573220), Natural Science Foundation of Jiangsu Province (BK20221281) and Crosscooperation project of Subei Peoples' Hospital of Jiangsu Province (SBJC22003), all awarded to Dr. Chuanli Ren.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 9 December 2024 / Accepted: 10 April 2025
Published online: 26 April 2025

References

1. The global. regional, and National burden of stomach cancer in 195 countries, 1990–2017: a systematic analysis for the global burden of disease study 2017. Lancet Gastroenterol Hepatol. 2020;5:42–54.
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74:229–63.
3. Ychou M, Boige V, Pignon JP, Conroy T, Bouché O, Lebreton G, et al. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. J Clin Oncol. 2011;29:1715–21.
4. Cunningham D, Allum WH, Stenning SP, Thompson JN, Van de Velde CJ, Nicolson M, et al. Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med. 2006;355:11–20.
5. Al-Batran SE, Hofheinz RD, Pauligk C, Kopp HG, Haag GM, Luley KB, et al. Histopathological regression after neoadjuvant docetaxel, oxaliplatin, fluorouracil, and leucovorin versus epirubicin, cisplatin, and fluorouracil or capecitabine in patients with resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4-AIO): results from the phase 2 part of a multicentre, open-label, randomised phase 2/3 trial. Lancet Oncol. 2016;17:1697–708.
6. Kang YK, Chin K, Chung HC, Kadowaki S, Oh SC, Nakayama N, et al. S-1 plus leucovorin and oxaliplatin versus S-1 plus cisplatin as first-line therapy in patients with advanced gastric cancer (SOLAR): a randomised, open-label, phase 3 trial. Lancet Oncol. 2020;21:1045–56.
7. Al-Batran SE, Homann N, Pauligk C, Goetze TO, Meiler J, Kasper S, et al. Perioperative chemotherapy with fluorouracil plus leucovorin, oxaliplatin, and docetaxel versus fluorouracil or capecitabine plus cisplatin and epirubicin for locally advanced, resectable gastric or gastro-oesophageal junction adenocarcinoma (FLOT4): a randomised, phase 2/3 trial. Lancet. 2019;393:1948–57.
8. Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet. 2014;383:31–9.
9. Shitara K, Lordick F, Bang YJ, Enzinger P, Ilson D, Shah MA, et al. Zolbetuximab plus mFOLFOX6 in patients with CLDN18.2-positive, HER2-negative, untreated, locally advanced unresectable or metastatic gastric or gastro-oesophageal junction adenocarcinoma (SPOTLIGHT): a multicentre, randomised, double-blind, phase 3 trial. Lancet. 2023;401:1655–68.
10. Huch M, Koo BK. Modeling mouse and human development using organoid cultures. Development. 2015;142:3113–25.
11. Shamir ER, Ewald AJ. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nat Rev Mol Cell Biol. 2014;15:647–64.
12. Xu H, Lyu X, Yi M, Zhao W, Song Y, Wu K. Organoid technology and applications in cancer research. J Hematol Oncol. 2018;11:116.
13. Yang Y, Kong Y, Cui J, Hou Y, Gu Z, Ma C. Advances and applications of Cancer organoids in drug screening and personalized medicine. Stem Cell Rev Rep. 2024;20:1213–26.
14. Zhao Z, Chen X, Dowbaj AM, Sljukic A, Bratlie K, Lin L, et al. Organoids Nat Rev Methods Primers. 2022;2:94.
15. Pang MJ, Burclaff JR, Jin R, Adkins-Threats M, Osaki LH, Han Y, et al. Gastric organoids: progress and remaining challenges. Cell Mol Gastroenterol Hepatol. 2022;13:19–33.
16. Koh V, Chakrabarti J, Torvund M, Steele N, Hawkins JA, Ito Y, et al. Hedgehog transcriptional effector GLI mediates mTOR-Induced PD-L1 expression in gastric cancer organoids. Cancer Lett. 2021;518:59–71.
17. Yan HHN, Siu HC, Law S, Ho SL, Yue SSK, Tsui WY, et al. A comprehensive human gastric Cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening. Cell Stem Cell. 2018;23:882–97.
18. Harada K, Sakamoto N, Ukai S, Yamamoto Y, Pham QT, Taniyama D, et al. Establishment of oxaliplatin-resistant gastric cancer organoids: importance of Myoferlin in the acquisition of oxaliplatin resistance. Gastric Cancer. 2021;24:1264–77.
19. Andersson-Rolf A, Mustata RC, Merenda A, Kim J, Perera S, Grego T, et al. One-step generation of conditional and reversible gene knockouts. Nat Methods. 2017;14:287–9.
20. Koo BK, Stange DE, Sato T, Karthaus W, Farin HF, Huch M, et al. Controlled gene expression in primary Lgr5 organoid cultures. Nat Methods. 2011;9:81–3.
21. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. Lancet. 2020;396:635–48.

22. Lordick F, Carneiro F, Cascinu S, Fleitas T, Haustermans K, Piessen G, et al. Gastric cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. Ann Oncol. 2022;33:1005–20.
23. Wang FH, Zhang XT, Tang L, Wu Q, Cai MY, Li YF, et al. The Chinese society of clinical oncology (CSCO): clinical guidelines for the diagnosis and treatment of gastric cancer, 2023. Cancer Commun (Lond). 2024;44:127–72.
24. Bang YJ, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet. 2010;376:687–97.
25. Xu Z, Hu C, Chen S, Zhang C, Yu J, Wang X, et al. Apatinib enhances chemosensitivity of gastric cancer to Paclitaxel and 5-fluorouracil. Cancer Manag Res. 2019;11:4905–15.
26. Yang Y, Wu X, Li F, Wang N, Zhang M, Sun T, et al. Evaluation of efficacy and safety of apatinib treatment in advanced gastric cancer. J Cancer Res Ther. 2019;15:365–9.
27. Takada H, Sasagawa Y, Yoshimura M, Tanaka K, Iwayama Y, Hayashi T, et al. Single-cell transcriptomics uncovers EGFR signaling-mediated gastric progenitor cell differentiation in stomach homeostasis. Nat Commun. 2023;14:3750.
28. Lei ZN, Teng QX, Tian Q, Chen W, Xie Y, Wu K, et al. Signaling pathways and therapeutic interventions in gastric cancer. Signal Transduct Target Ther. 2022;7:358.
29. Qi C, Chong X, Zhou T, Ma M, Gong J, Zhang M, et al. Clinicopathological significance and immunotherapeutic outcome of Claudin 18.2 expression in advanced gastric cancer: A retrospective study. Chin J Cancer Res. 2024;36:78–89.
30. Antibody Improves Survival in Gastric Cancer. Cancer Discov. 2016;6:Of8.
31. Shah MA, Shitara K, Ajani JA, Bang YJ, Enzinger P, Ilson D, et al. Zolbetuximab plus CAPOX in CLDN18.2-positive gastric or gastroesophageal junction adenocarcinoma: the randomized, phase 3 GLOW trial. Nat Med. 2023;29:2133–41.
32. Zeng D, Wu J, Luo H, Li Y, Xiao J, Peng J, et al. Tumor microenvironment evaluation promotes precise checkpoint immunotherapy of advanced gastric cancer. J Immunother Cancer. 2021;9:e002467.
33. Piro G, Carbone C, Cataldo I, Di Nicolantonio F, Giacopuzzi S, Aprile G, et al. An FGFR3 autocrine loop sustains acquired resistance to trastuzumab in gastric Cancer patients. Clin Cancer Res. 2016;22:6164–75.
34. Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, et al. Trastuzumab Deruxtecan in previously treated HER2-Positive gastric Cancer. N Engl J Med. 2020;382:2419–30.
35. Shen L, Chen P, Lu J, Wan Y, Zheng Y, Ye F, et al. Trastuzumab Deruxtecan (T-DXd) in Chinese patients (pts) with previously treated HER2-positive ($HER2^+$) advanced gastric or gastroesophageal junction adenocarcinoma (GEJA): DESTINY-Gastric06 (DG-06) final analysis. Ann Oncol. 2024;35:S1454–5.
36. Van Cutsem E, di Bartolomeo M, Smyth E, Chau I, Park H, Siena S, et al. Trastuzumab Deruxtecan in patients in the USA and Europe with HER2-positive advanced gastric or gastroesophageal junction cancer with disease progression on or after a trastuzumab-containing regimen (DESTINY-Gastric02): primary and updated analyses from a single-arm, phase 2 study. Lancet Oncol. 2023;24:744–56.
37. Janjigian YY, van Laarhoven HWM, Rha SY, Kozlov V, Oh DY, Gravina A, et al. Trastuzumab Deruxtecan (T-DXd) monotherapy and combinations in patients (pts) with advanced/metastatic HER2-positive ($HER2^+$) esophageal, gastric or gastroesophageal junction adenocarcinoma (GEJA): DESTINY-Gastric03 (DG-03). Ann Oncol. 2024;35:S878.
38. Shen L, Chen P, Lu J, Wan Y, Zheng Y, Ye F, et al. Trastuzumab Deruxtecan (T-DXd) in Chinese patients (pts) with previously treated HER2-positive locally advanced/metastatic gastric cancer (GC) or gastroesophageal junction adenocarcinoma (GEJA): primary efficacy and safety from the phase II single-arm DESTINY-Gastric06 (DG06) trial. Ann Oncol. 2023;34:S1542–3.
39. Gu Y, Wang Z, Wang Y. Bispecific antibody drug conjugates: making 1 + 1 > 2. Acta Pharm Sin B. 2024;14:1965–86.
40. Qiao J, Feng M, Zhou W, Tan Y, Yang S, Liu Q, et al. YAP Inhibition overcomes adaptive resistance in HER2-positive gastric cancer treated with trastuzumab via the AKT/mTOR and ERK/mTOR axis. Gastric Cancer. 2024;27:785–801.
41. Wang J, Huang Q, Hu X, Zhang S, Jiang Y, Yao G, et al. Disrupting circadian rhythm via the PER1-HK2 Axis reverses trastuzumab resistance in gastric Cancer. Cancer Res. 2022;82:1503–17.
42. Sun W, Wang X, Wang D, Lu L, Lin H, Zhang Z, et al. CD40×HER2 bispecific antibody overcomes the CCL2-induced trastuzumab resistance in HER2-positive gastric cancer. J Immunother Cancer. 2022;10:e005063.
43. Hu X, Ma Z, Xu B, Li S, Yao Z, Liang B, et al. Glutamine metabolic microenvironment drives M2 macrophage polarization to mediate trastuzumab resistance in HER2-positive gastric cancer. Cancer Commun (Lond). 2023;43:909–37.
44. Kim ST, Cristescu R, Bass AJ, Kim KM, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 Inhibition in metastatic gastric cancer. Nat Med. 2018;24:1449–58.
45. Xu X, Dennett P, Zhang J, Sherrard A, Zhao Y, Masubuchi T et al. CTLA4 depletes T cell endogenous and trogocytosed B7 ligands via cis-endocytosis. J Exp Med. 2023;220.
46. Rowshanravan B, Halliday N, Sansom DM. CTLA-4: a moving target in immunotherapy. Blood. 2018;131:58–67.
47. Aggarwal V, Workman CJ, Vignali DAA. LAG-3 as the third checkpoint inhibitor. Nat Immunol. 2023;24:1415–22.
48. Cai L, Li Y, Tan J, Xu L, Li Y. Targeting LAG-3, TIM-3, and TIGIT for cancer immunotherapy. J Hematol Oncol. 2023;16:101.
49. Chiang EY, Mellman I. TIGIT-CD226-PVR axis: advancing immune checkpoint Blockade for cancer immunotherapy. J Immunother Cancer. 2022;10:e004711.
50. Cascone T, William WN Jr., Weissferdt A, Leung CH, Lin HY, Pataer A, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in operable non-small cell lung cancer: the phase 2 randomized NEOSTAR trial. Nat Med. 2021;27:504–14.
51. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020;577:549–55.
52. Liu D, Schilling B, Liu D, Sucker A, Livingstone E, Jerby-Arnon L, et al. Integrative molecular and clinical modeling of clinical outcomes to PD1 Blockade in patients with metastatic melanoma. Nat Med. 2019;25:1916–27.
53. Comprehensive molecular. Characterization of gastric adenocarcinoma. Nature. 2014;513:202–9.
54. Puliga E, Corso S, Pietrantonio F, Giordano S. Microsatellite instability in gastric cancer: between lights and shadows. Cancer Treat Rev. 2021;95:102175.
55. Kwon M, An M, Klempner SJ, Lee H, Kim KM, Sa JK, et al. Determinants of response and intrinsic resistance to PD-1 Blockade in microsatellite Instability-High gastric Cancer. Cancer Discov. 2021;11:2168–85.
56. Samstein RM, Lee CH, Shoushtari AN, Hellmann MD, Shen R, Janjigian YY, et al. Tumor mutational load predicts survival after immunotherapy across multiple cancer types. Nat Genet. 2019;51:202–6.
57. André T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in Microsatellite-Instability-High advanced colorectal Cancer. N Engl J Med. 2020;383:2207–18.
58. Wainberg ZA, Fuchs CS, Tabernero J, Shitara K, Muro K, Van Cutsem E, et al. Efficacy of pembrolizumab monotherapy for advanced gastric/gastroesophageal junction Cancer with programmed death ligand 1 combined positive score ≥ 10. Clin Cancer Res. 2021;27:1923–31.
59. Holder AM, Dedeilia A, Sierra-Davidson K, Cohen S, Liu D, Parikh A, et al. Defining clinically useful biomarkers of immune checkpoint inhibitors in solid tumours. Nat Rev Cancer. 2024;24:498–512.
60. Janjigian YY, Ajani JA, Moehler M, Shen L, Garrido M, Gallardo C, et al. First-Line nivolumab plus chemotherapy for advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma: 3-Year Follow-Up of the phase III checkmate 649 trial. J Clin Oncol. 2024;42:2012–20.
61. Shen L, Bai Y, Lin X, Li W, Wang J, Zhang X, et al. First-line (1L) nivolumab (NIVO) plus chemotherapy (chemo) vs chemo in patients (pts) with advanced gastric cancer, gastroesophageal junction cancer, and esophageal adenocarcinoma (GC/GEJC/EAC): 5-year (y) follow-up of Chinese Pts from checkmate 649. J Clin Oncol. 2025;43:392.
62. Shitara K, Van Cutsem E, Bang YJ, Fuchs C, Wyrwicz L, Lee KW, et al. Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with First-line, advanced gastric cancer: the KEYNOTE-062 phase 3 randomized clinical trial. JAMA Oncol. 2020;6:1571–80.
63. Yamaguchi H, Hsu JM, Sun L, Wang SC, Hung MC. Advances and prospects of biomarkers for immune checkpoint inhibitors. Cell Rep Med. 2024;5:101621.
64. Wang X, Lamberti G, Di Federico A, Alessi J, Ferrara R, Sholl ML, et al. Tumor mutational burden for the prediction of PD-(L)1 Blockade efficacy in cancer: challenges and opportunities. Ann Oncol. 2024;35:508–22.
65. André T, Tougeron D, Piessen G, de la Fouchardière C, Louvet C, Adenis A, et al. Neoadjuvant nivolumab plus ipilimumab and adjuvant nivolumab in localized deficient mismatch repair/microsatellite Instability-High gastric or

esophagogastric junction adenocarcinoma: the GERCOR NEONIPIGA phase II study. J Clin Oncol. 2023;41:255–65.
66. Raimondi A, Lonardi S, Murgioni S, Cardellino GG, Tamberi S, Strippoli A, et al. Tremelimumab and durvalumab as neoadjuvant or non-operative management strategy of patients with microsatellite instability-high resectable gastric or gastroesophageal junction adenocarcinoma: the INFINITY study by GONO. Ann Oncol. 2025;36:285–96.
67. Yasuda T, Wang YA. Gastric cancer immunosuppressive microenvironment heterogeneity: implications for therapy development. Trends Cancer. 2024;10:627–42.
68. Guan WL, He Y, Xu RH. Gastric cancer treatment: recent progress and future perspectives. J Hematol Oncol. 2023;16:57.
69. Durinikova E, Buzo K, Arena S. Preclinical models as patients' avatars for precision medicine in colorectal cancer: past and future challenges. J Exp Clin Cancer Res. 2021;40:185.
70. Liabeuf D, Oshima M, Stange DE, Sigal M, Stem, Cells. Helicobacter pylori, and mutational landscape: utility of preclinical models to understand carcinogenesis and to direct management of gastric Cancer. Gastroenterology. 2022;162:1067–87.
71. Seidlitz T, Merker SR, Rothe A, Zakrzewski F, von Neubeck C, Grützmann K, et al. Human gastric cancer modelling using organoids. Gut. 2019;68:207–17.
72. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459:262–5.
73. Lancaster MA, Renner M, Martin CA, Wenzel D, Bicknell LS, Hurles ME, et al. Cerebral organoids model human brain development and microcephaly. Nature. 2013;501:373–9.
74. Jacob F, Salinas RD, Zhang DY, Nguyen PTT, Schnoll JG, Wong SZH, et al. A Patient-Derived glioblastoma organoid model and biobank recapitulates Inter- and Intra-tumoral heterogeneity. Cell. 2020;180:188–204.
75. Bartfeld S, Bayram T, van de Wetering M, Huch M, Begthel H, Kujala P, et al. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. Gastroenterology. 2015;148:126–36.
76. Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, van Es JH, et al. Lgr5(+ve) stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. Cell Stem Cell. 2010;6:25–36.
77. McCracken KW, Catá EM, Crawford CM, Sinagoga KL, Schumacher M, Rockich BE, et al. Modelling human development and disease in pluripotent stem-cell-derived gastric organoids. Nature. 2014;516:400–4.
78. Schlaermann P, Toelle B, Berger H, Schmidt SC, Glanemann M, Ordemann J, et al. A novel human gastric primary cell culture system for modelling Helicobacter pylori infection in vitro. Gut. 2016;65:202–13.
79. McCracken KW, Aihara E, Martin B, Crawford CM, Broda T, Treguier J, et al. Wnt/β-catenin promotes gastric fundus specification in mice and humans. Nature. 2017;541:182–7.
80. Lo YH, Kolahi KS, Du Y, Chang CY, Krokhotin A, Nair A, et al. A CRISPR/Cas9-Engineered ARID1A-Deficient human gastric Cancer organoid model reveals essential and nonessential modes of oncogenic transformation. Cancer Discov. 2021;11:1562–81.
81. Kasagi Y, Chandramouleeswaran PM, Whelan KA, Tanaka K, Giroux V, Sharma M, et al. The esophageal organoid system reveals functional interplay between Notch and cytokines in reactive epithelial changes. Cell Mol Gastroenterol Hepatol. 2018;5:333–52.
82. Barkauskas CE, Chung MI, Fioret B, Gao X, Katsura H, Hogan BL. Lung organoids: current uses and future promise. Development. 2017;144:986–97.
83. Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell. 2015;160:299–312.
84. Li X, Nadauld L, Ootani A, Corney DC, Pai RK, Gevaert O, et al. Oncogenic transformation of diverse Gastrointestinal tissues in primary organoid culture. Nat Med. 2014;20:769–77.
85. Cruz NM, Song X, Czerniecki SM, Gulieva RE, Churchill AJ, Kim YK, et al. Organoid cystogenesis reveals a critical role of microenvironment in human polycystic kidney disease. Nat Mater. 2017;16:1112–9.
86. Pringle S, Maimets M, van der Zwaag M, Stokman MA, van Gosliga D, Zwart E, et al. Human salivary gland stem cells functionally restore radiation damaged salivary glands. Stem Cells. 2016;34:640–52.
87. Maenhoudt N, Defraye C, Boretto M, Jan Z, Heremans R, Boeckx B, et al. Developing organoids from ovarian Cancer as experimental and preclinical models. Stem Cell Rep. 2020;14:717–29.
88. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, et al. A living biobank of breast Cancer organoids captures disease heterogeneity. Cell. 2018;172:373–86.
89. van de Wetering M, Francies HE, Francis JM, Bounova G, Iorio F, Pronk A, et al. Prospective derivation of a living organoid biobank of colorectal cancer patients. Cell. 2015;161:933–45.
90. Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014;159:176–87.
91. Schutgens F, Clevers H. Human organoids: tools for Understanding biology and treating diseases. Annu Rev Pathol. 2020;15:211–34.
92. Luckett KA, Ganesh K. Engineering the immune microenvironment into organoid models. Annual Rev Cancer Biology. 2023;7:171–87.
93. Clevers H, Tuveson DA. Organoid models for Cancer research. Annual Rev Cancer Biology. 2019;3:223–34.
94. Yan HHN, Chan AS, Lai FP, Leung SY. Organoid cultures for cancer modeling. Cell Stem Cell. 2023;30:917–37.
95. Zou Z, Lin Z, Wu C, Tan J, Zhang J, Peng Y, et al. Micro-Engineered Organoid-on-a-Chip based on mesenchymal stromal cells to predict immunotherapy responses of HCC patients. Adv Sci (Weinh). 2023;10:e2302640.
96. Senkowski W, Gall-Mas L, Falco MM, Li Y, Lavikka K, Kriegbaum MC, et al. A platform for efficient establishment and drug-response profiling of high-grade serous ovarian cancer organoids. Dev Cell. 2023;58:1106–21.
97. Zhou Z, Van der Jeught K, Li Y, Sharma S, Yu T, Moulana I, et al. A T Cell-Engaging tumor organoid platform for pancreatic Cancer immunotherapy. Adv Sci (Weinh). 2023;10:e2300548.
98. Ma S, Wang W, Zhou J, Liao S, Hai C, Hou Y, et al. Lamination-based organoid spatially resolved transcriptomics technique for primary lung and liver organoid characterization. Proc Natl Acad Sci U S A. 2024;121:e2408939121.
99. Zhong Z, Quiñones-Pérez M, Dai Z, Juarez VM, Bhatia E, Carlson CR, et al. Human immune organoids to Decode B cell response in healthy donors and patients with lymphoma. Nat Mater. 2024;24:297–311.
100. Santos AJM, van Unen V, Lin Z, Chirieleison SM, Ha N, Batish A, et al. A human autoimmune organoid model reveals IL-7 function in coeliac disease. Nature. 2024;632:401–10.
101. Reynolds DE, Sun Y, Wang X, Vallapureddy P, Lim J, Pan M, et al. Live Organoid Cycl Imaging Adv Sci (Weinh). 2024;11:e2309289.
102. Marchal I. An improved organoid model of the human cerebellum. Nat Biotechnol. 2024;42:184.
103. Afting C, Walther T, Drozdowski OM, Schlagheck C, Schwarz US, Wittbrodt J, et al. DNA microbeads for spatio-temporally controlled morphogen release within organoids. Nat Nanotechnol. 2024;19:1849–57.
104. Hendriks D, Pagliaro A, Andreatta F, Ma Z, van Giessen J, Massalini S, et al. Human fetal brain self-organizes into long-term expanding organoids. Cell. 2024;187:712–32.
105. Lorenzo-Martín LF, Broguiere N, Langer J, Tillard L, Nikolaev M, Coukos G et al. Patient-derived mini-colons enable long-term modeling of tumor-microenvironment complexity. Nat Biotechnol. 2024.
106. Lo YH, Karlsson K, Kuo CJ. Applications of organoids for Cancer biology and precision medicine. Nat Cancer. 2020;1:761–73.
107. Yuki K, Cheng N, Nakano M, Kuo CJ. Organoid models of tumor immunology. Trends Immunol. 2020;41:652–64.
108. Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, et al. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nat Med. 2009;15:701–6.
109. DiMarco RL, Su J, Yan KS, Dewi R, Kuo CJ, Heilshorn SC. Engineering of three-dimensional microenvironments to promote contractile behavior in primary intestinal organoids. Integr Biol (Camb). 2014;6:127–42.
110. Neal JT, Li X, Zhu J, Giangarra V, Grzeskowiak CL, Ju J, et al. Organoid modeling of the tumor immune microenvironment. Cell. 2018;175:1972–88.
111. Finnberg NK, Gokare P, Lev A, Grivennikov SI, MacFarlane AWt, Campbell KS, et al. Application of 3D tumoroid systems to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signatures. Oncotarget. 2017;8:66747–57.
112. Verissimo CS, Overmeer RM, Ponsioen B, Drost J, Mertens S, Verlaan-Klink I, et al. Targeting mutant RAS in patient-derived colorectal cancer organoids by combinatorial drug screening. Elife. 2016;5:e18489.
113. Veninga V, Voest EE. Tumor organoids: opportunities and challenges to guide precision medicine. Cancer Cell. 2021;39:1190–201.
114. Drost J, Clevers H. Organoids in cancer research. Nat Rev Cancer. 2018;18:407–18.

115. Cartry J, Bedja S, Boilève A, Mathieu JRR, Gontran E, Annereau M, et al. Implementing patient derived organoids in functional precision medicine for patients with advanced colorectal cancer. J Exp Clin Cancer Res. 2023;42:281.
116. Boilève A, Cartry J, Goudarzi N, Bedja S, Mathieu JRR, Bani MA, et al. Organoids for functional precision medicine in advanced pancreatic Cancer. Gastroenterology. 2024;167:961–76.
117. Goto N, Westcott PMK, Goto S, Imada S, Taylor MS, Eng G, et al. SOX17 enables immune evasion of early colorectal adenomas and cancers. Nature. 2024;627:636–45.
118. Deng L. Artificial [perspectives]ntelligence [perspectives]n [perspectives] he [perspectives]ising wave of deep learning: [perspectives]he historical [perspectives]ath and future outlook [Perspectives]. IEEE Signal Process Mag. 2018;35:180–77.
119. Antonopoulos I, Robu V, Robu V, Couraud B, Kirli D, Norbu S et al. Artificial intelligence and machine learning approaches to energy demand-side response: A systematic review. Renew Sustain Energy Rev. 2020.
120. Abbasi Moud A. Recent advances in utility of artificial intelligence towards multiscale colloidal based materials design. Colloid Interface Sci Commun. 2022;47:100595.
121. Takahashi S, Asada K, Takasawa K, Shimoyama R, Sakai A, Bolatkan A et al. Predicting Deep Learning Based Multi-Omics Parallel Integration Survival Subtypes in Lung Cancer Using Reverse Phase Protein Array Data. Biomolecules. 2020;10.
122. Renner H, Schöler HR, Bruder JM. Combining automated organoid workflows with artificial Intelligence-Based analyses: opportunities to build a new generation of interdisciplinary High-Throughput screens for Parkinson's disease and beyond. Mov Disord. 2021;36:2745–62.
123. Verheyen CA, Uzel SGM, Kurum A, Roche ET, Lewis JA. Integrated data-driven modeling and experimental optimization of granular hydrogel matrices. Matter. 2023;6:1015–36.
124. Bai L, Wu Y, Li G, Zhang W, Zhang H, Su J. AI-enabled organoids: construction, analysis, and application. Bioact Mater. 2024;31:525–48.
125. Suwardi A, Wang F, Xue K, Han MY, Teo P, Wang P, et al. Machine Learning-Driven biomaterials evolution. Adv Mater. 2022;34:e2102703.
126. Kaufman HL, Atkins MB, Subedi P, Wu J, Chambers J, Joseph Mattingly T. The promise of Immuno-oncology: implications for defining the value of cancer treatment. J Immunother Cancer. 2019;7:129. 2nd et al.
127. Beaulieu E, Spanjaart A, Roes A, Rachet B, Dalle S, Kersten MJ, et al. Health-related quality of life in cancer immunotherapy: a systematic perspective, using causal loop diagrams. Qual Life Res. 2022;31:2357–66.
128. Song J, Zhu J, Jiang Y, Guo Y, Liu S, Qiao Y, et al. Advancements in immunotherapy for gastric cancer: unveiling the potential of immune checkpoint inhibitors and emerging strategies. Biochim Biophys Acta Rev Cancer. 2025;1880:189277.
129. Pitt JM, Marabelle A, Eggermont A, Soria JC, Kroemer G, Zitvogel L. Targeting the tumor microenvironment: removing obstruction to anticancer immune responses and immunotherapy. Ann Oncol. 2016;27:1482–92.
130. Pei L, Liu Y, Liu L, Gao S, Gao X, Feng Y, et al. Roles of cancer-associated fibroblasts (CAFs) in anti- PD-1/PD-L1 immunotherapy for solid cancers. Mol Cancer. 2023;22:29.
131. Zou W, Green DR. Beggars banquet: metabolism in the tumor immune microenvironment and cancer therapy. Cell Metab. 2023;35:1101–13.
132. Rauth S, Malafa M, Ponnusamy MP, Batra SK. Emerging trends in Gastrointestinal Cancer targeted therapies: Harnessing tumor microenvironment, immune factors, and metabolomics insights. Gastroenterology. 2024;167:867–84.
133. de Visser KE, Joyce JA. The evolving tumor microenvironment: from cancer initiation to metastatic outgrowth. Cancer Cell. 2023;41:374–403.
134. Ugai T, Yao Q, Ugai S, Ogino S. Advancing precision oncology: insights into the tumor microenvironment and immunotherapy outcomes. Innov (Camb). 2024;5:100656.
135. Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, Merad M, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. Nat Med. 2018;24:541–50.
136. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our Understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20:174–86.
137. Palucka AK, Coussens LM. The basis of oncoimmunology. Cell. 2016;164:1233–47.
138. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Clin Oncol. 2017;14:717–34.
139. Polak R, Zhang ET, Kuo CJ. Cancer organoids 2.0: modelling the complexity of the tumour immune microenvironment. Nat Rev Cancer. 2024;24:523–39.
140. Moynihan KD, Opel CF, Szeto GL, Tzeng A, Zhu EF, Engreitz JM, et al. Eradication of large established tumors in mice by combination immunotherapy that engages innate and adaptive immune responses. Nat Med. 2016;22:1402–10.
141. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene Laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33:2780–8.
142. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369:122–33.
143. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365:725–33.
144. Melero I, Gaudernack G, Gerritsen W, Huber C, Parmiani G, Scholl S, et al. Therapeutic vaccines for cancer: an overview of clinical trials. Nat Rev Clin Oncol. 2014;11:509–24.
145. Zitvogel L, Galluzzi L, Kepp O, Smyth MJ, Kroemer G. Type I interferons in anticancer immunity. Nat Rev Immunol. 2015;15:405–14.
146. Huang Y, Yang Y, Zhao Y, Zhao H, Zhou N, Zhang Y, et al. QL1706 (anti-PD-1 IgG4/CTLA-4 antibody) plus chemotherapy with or without bevacizumab in advanced non-small cell lung cancer: a multi-cohort, phase II study. Signal Transduct Target Ther. 2024;9:23.
147. Li Q, Liu J, Zhang Q, Ouyang Q, Zhang Y, Liu Q, et al. The anti-PD-L1/CTLA-4 bispecific antibody KN046 in combination with nab-paclitaxel in first-line treatment of metastatic triple-negative breast cancer: a multicenter phase II trial. Nat Commun. 2024;15:1015.
148. Li Y, Pan C, Gao Y, Zhang L, Ji D, Cui X, et al. Total neoadjuvant therapy with PD-1 Blockade for High-Risk proficient mismatch repair rectal Cancer. JAMA Surg. 2024;159:529–37.
149. Mao C, Xiong A, Qian J, Wang W, Liu Y, Zhang T, et al. Dual Inhibition of LAG-3 and PD-1 with IBI110 and sintilimab in advanced solid tumors: the first-in-human phase Ia/Ib study. J Hematol Oncol. 2024;17:132.
150. Qi C, Liu C, Gong J, Liu D, Wang X, Zhang P, et al. Claudin18.2-specific CAR T cells in Gastrointestinal cancers: phase 1 trial final results. Nat Med. 2024;30:2224–34.
151. Qi C, Xie T, Zhou J, Wang X, Gong J, Zhang X, et al. CT041 CAR T cell therapy for Claudin18.2-positive metastatic pancreatic cancer. J Hematol Oncol. 2023;16:102.
152. Shalhout SZ, Miller DM, Emerick KS, Kaufman HL. Therapy with oncolytic viruses: progress and challenges. Nat Rev Clin Oncol. 2023;20:160–77.
153. Braun DA, Moranzoni G, Chea V, McGregor BA, Blass E, Tu CR et al. A neoantigen vaccine generates antitumour immunity in renal cell carcinoma. Nature. 2025.
154. Guo Z, Yuan Y, Chen C, Lin J, Ma Q, Liu G, et al. Durable complete response to neoantigen-loaded dendritic-cell vaccine following anti-PD-1 therapy in metastatic gastric cancer. NPJ Precis Oncol. 2022;6:34.
155. Propper DJ, Balkwill FR. Harnessing cytokines and chemokines for cancer therapy. Nat Rev Clin Oncol. 2022;19:237–53.
156. Yi M, Li T, Niu M, Zhang H, Wu Y, Wu K, et al. Targeting cytokine and chemokine signaling pathways for cancer therapy. Signal Transduct Target Ther. 2024;9:176.
157. Chen DS, Mellman I. Oncology Meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1–10.
158. Zhou Z, Pang Y, Ji J, He J, Liu T, Ouyang L, et al. Harnessing 3D in vitro systems to model immune responses to solid tumours: a step towards improving and creating personalized immunotherapies. Nat Rev Immunol. 2024;24:18–32.
159. Cattaneo CM, Dijkstra KK, Fanchi LF, Kelderman S, Kaing S, van Rooij N, et al. Tumor organoid-T-cell coculture systems. Nat Protoc. 2020;15:15–39.
160. Ji B, Wei M, Yang B. Recent advances in nanomedicines for photodynamic therapy (PDT)-driven cancer immunotherapy. Theranostics. 2022;12:434–58.
161. Yang J, Xu J, Wang W, Zhang B, Yu X, Shi S. Epigenetic regulation in the tumor microenvironment: molecular mechanisms and therapeutic targets. Signal Transduct Target Ther. 2023;8:210.
162. An M, Mehta A, Min BH, Heo YJ, Wright SJ, Parikh M, et al. Early immune remodeling steers clinical response to First-Line chemoimmunotherapy in advanced gastric Cancer. Cancer Discov. 2024;14:766–85.
163. Li Z, Wu Y, Wang C, Zhang M. Mouse CD8(+)NKT-like cells exert dual cytotoxicity against mouse tumor cells and myeloid-derived suppressor cells. Cancer Immunol Immunother. 2019;68:1303–15.
164. Wang R, Dang M, Harada K, Han G, Wang F, Pool Pizzi M, et al. Single-cell dissection of intratumoral heterogeneity and lineage diversity in metastatic gastric adenocarcinoma. Nat Med. 2021;27:141–51.

165. Kumar V, Ramnarayanan K, Sundar R, Padmanabhan N, Srivastava S, Koiwa M, et al. Single-Cell atlas of lineage States, tumor microenvironment, and Subtype-Specific expression programs in gastric Cancer. Cancer Discov. 2022;12:670–91.
166. Zhao W, Jia Y, Sun G, Yang H, Liu L, Qu X, et al. Single-cell analysis of gastric signet ring cell carcinoma reveals cytological and immune microenvironment features. Nat Commun. 2023;14:2985.
167. Sun K, Xu R, Ma F, Yang N, Li Y, Sun X, et al. scRNA-seq of gastric tumor shows complex intercellular interaction with an alternative T cell exhaustion trajectory. Nat Commun. 2022;13:4943.
168. Li X, Sun Z, Peng G, Xiao Y, Guo J, Wu B, et al. Single-cell RNA sequencing reveals a pro-invasive cancer-associated fibroblast subgroup associated with poor clinical outcomes in patients with gastric cancer. Theranostics. 2022;12:620–38.
169. Kang B, Camps J, Fan B, Jiang H, Ibrahim MM, Hu X, et al. Parallel single-cell and bulk transcriptome analyses reveal key features of the gastric tumor microenvironment. Genome Biol. 2022;23:265.
170. Zhou X, Yang J, Lu Y, Ma Y, Meng Y, Li Q, et al. Relationships of tumor differentiation and immune infiltration in gastric cancers revealed by single-cell RNA-seq analyses. Cell Mol Life Sci. 2023;80:57.
171. Pickup MW, Mouw JK, Weaver VM. The extracellular matrix modulates the hallmarks of cancer. EMBO Rep. 2014;15:1243–53.
172. LeBleu VS, Kalluri R. A peek into cancer-associated fibroblasts: origins, functions and translational impact. Dis Model Mech. 2018;11.
173. Ehrlich HP, Allison GM, Leggett M. The myofibroblast, Cadherin, alpha smooth muscle actin and the collagen effect. Cell Biochem Funct. 2006;24:63–70.
174. Barrett R, Puré E. Cancer-associated fibroblasts: key determinants of tumor immunity and immunotherapy. Curr Opin Immunol. 2020;64:80–7.
175. Barbazán J, Matic Vignjevic D. Cancer associated fibroblasts: is the force the path to the dark side? Curr Opin Cell Biol. 2019;56:71–9.
176. Lakins MA, Ghorani E, Munir H, Martins CP, Shields JD. Cancer-associated fibroblasts induce antigen-specific deletion of CD8 (+) T cells to protect tumour cells. Nat Commun. 2018;9:948.
177. Schuth S, Le Blanc S, Krieger TG, Jabs J, Schenk M, Giese NA, et al. Patient-specific modeling of stroma-mediated chemoresistance of pancreatic cancer using a three-dimensional organoid-fibroblast co-culture system. J Exp Clin Cancer Res. 2022;41:312.
178. Zhao Y, Li S, Zhu L, Huang M, Xie Y, Song X, et al. Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer. Cell Rep Med. 2024;5:101627.
179. Schnalzger TE, de Groot MH, Zhang C, Mosa MH, Michels BE, Röder J et al. 3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids. Embo J. 2019;38.
180. Yu L, Li Z, Mei H, Li W, Chen D, Liu L, et al. Patient-derived organoids of bladder cancer recapitulate antigen expression profiles and serve as a personal evaluation model for CAR-T cells in vitro. Clin Transl Immunol. 2021;10:e1248.
181. Choi D, Gonzalez-Suarez AM, Dumbrava MG, Medlyn M, de Hoyos-Vega JM, Cichocki F, et al. Microfluidic organoid cultures derived from pancreatic Cancer biopsies for personalized testing of chemotherapy and immunotherapy. Adv Sci (Weinh). 2024;11:e2303088.
182. Cherne MD, Sidar B, Sebrell TA, Sanchez HS, Heaton K, Kassama FJ, et al. A synthetic hydrogel, VitroGel(®) ORGANOID-3, improves immune Cell-Epithelial interactions in a tissue chip Co-Culture model of human gastric organoids and dendritic cells. Front Pharmacol. 2021;12:707891.
183. Ding S, Hsu C, Wang Z, Natesh NR, Millen R, Negrete M et al. Patient-derived micro-organospheres enable clinical precision oncology. Cell Stem Cell. 2022;29:905– 17.e6.
184. Kroemer G, Chan TA, Eggermont AMM, Galluzzi L. Immunosurveillance in clinical cancer management. CA Cancer J Clin. 2024;74:187–202.
185. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991–8.
186. Wolf NK, Kissiov DU, Raulet DH. Roles of natural killer cells in immunity to cancer, and applications to immunotherapy. Nat Rev Immunol. 2023;23:90–105.
187. Huntington ND, Cursons J, Rautela J. The cancer-natural killer cell immunity cycle. Nat Rev Cancer. 2020;20:437–54.
188. Koebel CM, Vermi W, Swann JB, Zerafa N, Rodig SJ, Old LJ, et al. Adaptive immunity maintains occult cancer in an equilibrium state. Nature. 2007;450:903–7.
189. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001;410:1107–11.
190. Spranger S, Gajewski TF. Impact of oncogenic pathways on evasion of antitumour immune responses. Nat Rev Cancer. 2018;18:139–47.
191. Vitale I, Shema E, Loi S, Galluzzi L. Intratumoral heterogeneity in cancer progression and response to immunotherapy. Nat Med. 2021;27:212–24.
192. Galassi C, Chan TA, Vitale I, Galluzzi L. The hallmarks of cancer immune evasion. Cancer Cell 2024.
193. Ma X, Jia S, Wang G, Liang M, Guo T, Du H, et al. TRIM28 promotes the escape of gastric cancer cells from immune surveillance by increasing PD-L1 abundance. Signal Transduct Target Ther. 2023;8:246.
194. Zhang J, Liu H, Hou L, Wang G, Zhang R, Huang Y, et al. Circular RNA_LARP4 inhibits cell proliferation and invasion of gastric cancer by sponging miR-424-5p and regulating LATS1 expression. Mol Cancer. 2017;16:151.
195. Tang W, Fu K, Sun H, Rong D, Wang H, Cao H. CircRNA microarray profiling identifies a novel Circulating biomarker for detection of gastric cancer. Mol Cancer. 2018;17:137.
196. Thomson DW, Dinger ME. Endogenous MicroRNA sponges: evidence and controversy. Nat Rev Genet. 2016;17:272–83.
197. Miao Z, Li J, Wang Y, Shi M, Gu X, Zhang X, et al. Hsa_circ_0136666 stimulates gastric cancer progression and tumor immune escape by regulating the miR-375/PRKDC Axis and PD-L1 phosphorylation. Mol Cancer. 2023;22:205.
198. Chakrabarti J, Koh V, Steele N, Hawkins J, Ito Y, Merchant JL et al. Disruption of Her2-Induced PD-L1 Inhibits Tumor Cell Immune Evasion in Patient-Derived Gastric Cancer Organoids. Cancers (Basel). 2021;13.
199. An international association between. Helicobacter pylori infection and gastric cancer. The EUROGAST study group. Lancet. 1993;341:1359–62.
200. Eisele Y, Mallea PM, Gigic B, Stephens WZ, Warby CA, Buhrke K, et al. Fusobacterium nucleatum and clinicopathologic features of colorectal cancer: results from the ColoCare study. Clin Colorectal Cancer. 2021;20:e165–72.
201. DeStefano Shields CE, White JR, Chung L, Wenzel A, Hicks JL, Tam AJ, et al. Bacterial-Driven inflammation and mutant BRAF expression combine to promote murine Colon tumorigenesis that is sensitive to immune checkpoint therapy. Cancer Discov. 2021;11:1792–807.
202. Tzeng A, Sangwan N, Jia M, Liu CC, Keslar KS, Downs-Kelly E, et al. Human breast Microbiome correlates with prognostic features and immunological signatures in breast cancer. Genome Med. 2021;13:60.
203. Xu Q, Lu X, Li J, Feng Y, Tang J, Zhang T, et al. Fusobacterium nucleatum induces excess methyltransferase-like 3-mediated microRNA-4717-3p maturation to promote colorectal cancer cell proliferation. Cancer Sci. 2022;113:3787–800.
204. Tsoi H, Chu ESH, Zhang X, Sheng J, Nakatsu G, Ng SC et al. Peptostreptococcus anaerobius Induces Intracellular Cholesterol Biosynthesis in Colon Cells to Induce Proliferation and Causes Dysplasia in Mice. Gastroenterology. 2017;152:1419-33.e5.
205. Pushalkar S, Hundeyin M, Daley D, Zambirinis CP, Kurz E, Mishra A, et al. The pancreatic Cancer Microbiome promotes oncogenesis by induction of innate and adaptive immune suppression. Cancer Discov. 2018;8:403–16.
206. Jin C, Lagoudas GK, Zhao C, Bullman S, Bhutkar A, Hu B, et al. Commensal microbiota promote lung Cancer development via Γδ T cells. Cell. 2019;176:998–e101316.
207. Parida S, Wu S, Siddharth S, Wang G, Muniraj N, Nagalingam A, et al. A procarcinogenic Colon microbe promotes breast tumorigenesis and metastatic progression and concomitantly activates Notch and β-Catenin axes. Cancer Discov. 2021;11:1138–57.
208. Liu LN, Ding SG, Shi YY, Zhang HJ, Zhang J, Zhang C. Helicobacter pylori with high thioredoxin-1 expression promotes stomach carcinogenesis in Mongolian gerbils. Clin Res Hepatol Gastroenterol. 2016;40:480–6.
209. Thomas SC, Madaan T, Kamble NS, Siddiqui NA, Pauletti GM, Kotagiri N. Engineered Bacteria enhance immunotherapy and targeted therapy through stromal remodeling of tumors. Adv Healthc Mater. 2022;11:e2101487.
210. Bossuet-Greif N, Vignard J, Taieb F, Mirey G, Dubois D, Petit C et al. The colibactin genotoxin generates DNA interstrand Cross-Links in infected cells. mBio. 2018;9.
211. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. Proc Natl Acad Sci U S A. 2011;108:15354–9.
212. Guerra L, Guidi R, Frisan T. Do bacterial genotoxins contribute to chronic inflammation, genomic instability and tumor progression? Febs J. 2011;278:4577–88.
213. Dejea CM, Fathi P, Craig JM, Boleij A, Taddese R, Geis AL, et al. Patients with Familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. Science. 2018;359:592–97.

214. Hsiao YC, Liu CW, Chi L, Yang Y, Lu K. Effects of gut Microbiome on carcinogenic DNA damage. Chem Res Toxicol. 2020;33:2130–38.
215. Wang J, Deng Z, Lang X, Jiang J, Xie K, Lu S, et al. Meta-Analysis of the prognostic and predictive role of the CpG Island methylator phenotype in colorectal Cancer. Dis Markers. 2022;2022:4254862.
216. Park HE, Kim JH, Cho NY, Lee HS, Kang GH. Intratumoral Fusobacterium nucleatum abundance correlates with macrophage infiltration and CDKN2A methylation in microsatellite-unstable colorectal carcinoma. Virchows Arch. 2017;471:329–36.
217. Koi M, Okita Y, Carethers JM. Fusobacterium nucleatum infection in colorectal cancer: linking inflammation, DNA mismatch repair and genetic and epigenetic alterations. J Anus Rectum Colon. 2018;2:37–46.
218. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms, and consequences. Immunity. 2019;51:27–41.
219. Qiao H, Li H, Wen X, Tan X, Yang C, Liu N. Multi-Omics integration reveals the crucial role of Fusobacterium in the inflammatory immune microenvironment in head and neck squamous cell carcinoma. Microbiol Spectr. 2022;10:e0106822.
220. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157:121–41.
221. Wong-Rolle A, Wei HK, Zhao C, Jin C. Unexpected guests in the tumor microenvironment: Microbiome in cancer. Protein Cell. 2021;12:426–35.
222. Yamamura K, Baba Y, Nakagawa S, Mima K, Miyake K, Nakamura K, et al. Human Microbiome Fusobacterium Nucleatum in esophageal Cancer tissue is associated with prognosis. Clin Cancer Res. 2016;22:5574–81.
223. Le Noci V, Guglielmetti S, Arioli S, Camisaschi C, Bianchi F, Sommariva M, et al. Modulation of pulmonary microbiota by antibiotic or probiotic aerosol therapy: A strategy to promote immunosurveillance against lung metastases. Cell Rep. 2018;24:3528–38.
224. Hardbower DM, Peek RM Jr., Wilson KT. At the bench: Helicobacter pylori, dysregulated host responses, DNA damage, and gastric cancer. J Leukoc Biol. 2014;96:201–12.
225. Baj J, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, et al. Helicobacter pylori virulence Factors-Mechanisms of bacterial pathogenicity in the gastric microenvironment. Cells. 2020;10:27.
226. Soond SM, Zamyatnin AA. Jr. Helicobacter pylori and gastric cancer: a lysosomal protease perspective. Gastric Cancer. 2022;25:306–24.
227. Nie S, Wang A, Yuan Y. Comparison of clinicopathological parameters, prognosis, micro-ecological environment and metabolic function of gastric Cancer with or without Fusobacterium Sp. Infection. J Cancer. 2021;12:1023–32.
228. Zhong M, Xiong Y, Zhao J, Gao Z, Ma J, Wu Z, et al. Candida albicans disorder is associated with gastric carcinogenesis. Theranostics. 2021;11:4945–56.
229. Yu D, Liu Z. The research progress in the interaction between Candida albicans and cancers. Front Microbiol. 2022;13:988734.
230. Fu K, Cheung AHK, Wong CC, Liu W, Zhou Y, Wang F et al. Streptococcus anginosus promotes gastric inflammation, atrophy, and tumorigenesis in mice. Cell. 2024;187:882– 96.e17.
231. Puschhof J, Pleguezuelos-Manzano C, Clevers H. Organoids and organs-on-chips: insights into human gut-microbe interactions. Cell Host Microbe. 2021;29:867–78.
232. Fu A, Yao B, Dong T, Chen Y, Yao J, Liu Y, et al. Tumor-resident intracellular microbiota promotes metastatic colonization in breast cancer. Cell. 2022;185:1356–72. e26.
233. Kuck D, Kolmerer B, Iking-Konert C, Krammer PH, Stremmel W, Rudi J. Vacuolating cytotoxin of Helicobacter pylori induces apoptosis in the human gastric epithelial cell line AGS. Infect Immun. 2001;69:5080–7.
234. Capurro MI, Greenfield LK, Prashar A, Xia S, Abdullah M, Wong H, et al. VacA generates a protective intracellular reservoir for Helicobacter pylori that is eliminated by activation of the lysosomal calcium channel TRPML1. Nat Microbiol. 2019;4:1411–23.
235. Galmiche A, Rassow J. Targeting of Helicobacter pylori VacA to mitochondria. Gut Microbes. 2010;1:392–5.
236. Kim IJ, Blanke SR. Remodeling the host environment: modulation of the gastric epithelium by the Helicobacter pylori vacuolating toxin (VacA). Front Cell Infect Microbiol. 2012;2:37.
237. Son YS, Kwon YH, Lee MS, Kwon O, Jeong YJ, Mun SJ, et al. Helicobacter pylori VacA-induced mitochondrial damage in the gastric pit cells of the antrum and therapeutic rescue. Biomaterials. 2025;314:122842.
238. Driehuis E, Kretzschmar K, Clevers H. Establishment of patient-derived cancer organoids for drug-screening applications. Nat Protoc. 2020;15:3380–409.
239. Wadman M. FDA no longer has to require animal testing for new drugs. Science. 2023;379:127–28.
240. Vlachogiannis G, Hedayat S, Vatsiou A, Jamin Y, Fernández-Mateos J, Khan K, et al. Patient-derived organoids model treatment response of metastatic Gastrointestinal cancers. Science. 2018;359:920–26.
241. Yang R, Qi Y, Kwan W, Du Y, Yan R, Zang L, et al. Paired organoids from primary gastric cancer and lymphatic metastasis are useful for personalized medicine. J Transl Med. 2024;22:754.
242. Schmäche T, Fohgrub J, Klimova A, Laaber K, Drukewitz S, Merboth F, et al. Stratifying esophago-gastric cancer treatment using a patient-derived organoid-based threshold. Mol Cancer. 2024;23:10.
243. Jin RU, Mills JC. Tumor organoids to study gastroesophageal cancer: a primer. J Mol Cell Biol. 2020;12:593–606.
244. Wang T, Song W, Meng Q, Qu C, Guo S, Wang Y, et al. Tumorigenicity and prediction of clinical prognosis of patient-derived gastric cancer organoids. Clin Transl Med. 2024;14:e1588.
245. Yang R, Kwan W, Du Y, Yan R, Zang L, Li C, et al. Drug-induced senescence by aurora kinase inhibitors attenuates innate immune response of macrophages on gastric cancer organoids. Cancer Lett. 2024;598:217106.
246. Ukai S, Honma R, Sakamoto N, Yamamoto Y, Pham QT, Harada K, et al. Molecular biological analysis of 5-FU-resistant gastric cancer organoids; KHDRBS3 contributes to the attainment of features of cancer stem cell. Oncogene. 2020;39:7265–78.
247. Ouyang S, Li H, Lou L, Huang Q, Zhang Z, Mo J, et al. Inhibition of STAT3-ferroptosis negative regulatory axis suppresses tumor growth and alleviates chemoresistance in gastric cancer. Redox Biol. 2022;52:102317.
248. Tan Z, Pan K, Sun M, Pan X, Yang Z, Chang Z, et al. CCKBR^{+} cancer cells contribute to the intratumor heterogeneity of gastric cancer and confer sensitivity to FOXO Inhibition. Cell Death Differ. 2024;31:1302–17.
249. Fei Y, Cao D, Li Y, Wang Z, Dong R, Zhu M, et al. Circ_0008315 promotes tumorigenesis and cisplatin resistance and acts as a nanotherapeutic target in gastric cancer. J Nanobiotechnol. 2024;22:519.
250. Gopee NH, Winheim E, Olabi B, Admane C, Foster AR, Huang N, et al. A prenatal skin atlas reveals immune regulation of human skin morphogenesis. Nature. 2024;635:679–89.

Publisher's note