Xenograft and organoid models in developing precision medicine for gastric cancer (Review)

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Abstract. Gastric cancer (GC), a highly heterogeneous disease, has diverse histological and molecular subtypes. For precision medicine, well-characterized models encompassing the full spectrum of subtypes are necessary. Patient-derived tumor xenografts and organoids serve as important preclinical models in GC research. The main advantage of these models is the retention of phenotypic and genotypic heterogeneity present in parental tumor tissues. Utilizing diverse sequencing techniques and preclinical models for GC research facilitates accuracy in predicting personalized clinical responses to anti-cancer treatments. The present review summarizes the latest advances of these two preclinical models in GC treatment and drug response assessment.

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1. Introduction

Gastric cancer (GC) ranks fifth in malignancy incidence worldwide and is the fourth leading cause of cancer-related death globally (1). GC is a heterogeneous disease with multiple histological and molecular subtypes (2), necessitating, for optimal investigation of GC initiation and progression, the establishment of reliable preclinical model systems that reflect the heterogeneity of primary tumors. Individual treatment of GC, due to disease heterogeneity, varies greatly in clinical practice (3). In addition, the mechanisms of GC development remain to be fully elucidated. Although multiple anti-cancer drugs have been evaluated in Phase I clinical trial safety testing, only a small number have been successful in Phase II and III clinical trial efficacy testing (4). The high failure rates observed in clinical trials highlight the importance of good preclinical models to better predict clinical outcomes. Patient-derived tumor xenografts (PDXs), in which tumor fragments from cancer patients are transplanted directly into immunodeficient mice, are one such model used in precision medicine. Patient-derived organoids (PDOs), established by three-dimensional (3D) culture in a matrix, also function well as an *in vitro* model for cancer treatment. Intra- and inter-tumor heterogeneity of the primary tumor is largely conserved in PDX and PDO model systems (5-10), which can retain the morphologic and genetic features of the original tumors. Therefore, PDX and PDO models have great potential as preclinical research tools for studying individualized tumor progression and therapy resistance. Given recent advances in both scientific understanding and technology, PDO and PDX models for GC have facilitated more in-depth research and individualized precision treatment (Fig. 1). Important discoveries made in GC research using these preclinical models are summarized in Table I. These models have enhanced our comprehension of GC progression and metastasis mechanisms and have been used to forecast patient therapeutic response to anti-cancer compounds, including immunotherapy drugs (Table II).

2. GC PDX models

Mouse strains. Animal models have a crucial role in studying the biological behavior and molecular mechanisms of carcinogenesis and evaluating drug effectiveness. Cancer cell lines

are often transplanted into immunodeficient mice to generate a model with easy manipulation and accessibility. However, this model loses the phenotypic and genetic heterogeneity, as well as the tumor microenvironment (TME) of the original tumors. A main advantage of PDXs in cancer research is that the tumor's histopathological architecture, cancer cells and surrounding stromal cells are largely preserved. Evidence suggests that the characteristics of PDX models are highly similar to those of parental tumors and their response to anti-cancer drugs is also similar to that of patients. PDXs were first reported in 1969 when the Danish scholar Rygaard transplanted human colon adenocarcinoma masses into nude mice (11). As with other tumor PDX models, immunodeficient mice have been used to establish PDX models of GC. The immunocompromised mouse strains widely used for PDX models are as follows: i) Nude mice, which lack a thymus and are unable to produce T cells, resulting in defective adaptive immune responses (12); ii) severe combined immune deficiency (SCID) mice, which lack both functional T and B lymphocytes (13). Human tumor engraftment efficiency is higher in SCID mice than in nude mice (14). Furthermore, SCID/beige mice, in addition to lacking T and B cells, have a severe deficiency of natural killer (NK) cell function, so the engraftment rate of human cancer cells is enhanced in SCID/beige mice compared to SCID mice (15,16); iii) nonobese diabetic (NOD)/SCID, interleukin 2 receptor (IL2R)-γ^{null} (NSG or NOG) mice and NOD/SCID Jak3^{null} (NOJ), in which T-, B- and NK-cell activity are completely absent, may markedly improve the efficiency of xenotransplantation (17-19); iv) BALB/c Rag-2^{null}/IL2R-γ^{null} and Rag-2^{null}/Jak3^{null}, in which macrophage-mediated phagocytosis of human cells may be reduced (20-22); and v) nude Rag-2^{null}/Jak3^{null} mice, established by crossing BALB/c Rag-2^{null}/Jak3^{null} mice and BALB/c nude mice, all serve as powerful tools for evaluating human tumor-host interactions (23). Choi *et al* (24) successfully established 15 GC PDX models with passaging to maintain tumors in nude or NOG mice (24.2%, 15/62); the genetic and histological characteristics of the primary tumors and PDX models were highly consistent. Karalis *et al* (25) established 23 PDX models from Western patients with GC with various ethnic backgrounds. In theory, highly immunosuppressed mouse strains may allow for higher tumor engraftment rates, but tumors implanted into NSG (16%) and nude (21%) mice had a similar engraftment rate, possibly because, as the immunodeficiency level increases in the recipient mice, the likelihood of developing B-cell lymphoma also increases, and the presence of B-cell lymphoma hinders the generation of solid tumor PDXs. Corso *et al* (10) generated a wide, multilevel platform of GC models, including 100 PDXs, organoids and primary cell lines. This PDX platform was the widest in an academic institution, and included all GC histologic and molecular types identified by The Cancer Genome Atlas. They also conducted a transcriptomic analysis of PDXs to identify a microsatellite instable (MSI) signature with the potential to assist in the development of precision medicine for GC (10).

Humanized mice. The TME includes the extracellular matrix and stromal cells, which include cancer-associated fibroblasts (CAFs), immune cells, pro-inflammatory cells and other components. The interaction between the TME and tumor cells has a prominent role in tumor progression, metastasis and therapeutic response. However, during xenograft growth, human stromal cells originally present in patient-derived tumors are gradually replaced by murine counterparts, which may hinder the analysis of tumor-stroma interaction in humans, as certain cytokines from mouse stroma may not have an impact on human carcinoma cells in PDX models (26). To overcome this limitation in PDX models, humanized mouse models have been generated. Researchers engrafted the human immune system and human tumor tissues in animal models, allowing the human immune system to reconstitute in the immunodeficient mice with patient tumor engraftment. Improved humanized mouse models have also been developed, such as i) the human peripheral blood lymphocytes (Hu-PBL) model, ii) the Hu-CD34^{+} model and iii) the bone marrow-liver-thymus (BLT) mice model. In 1988, Mosier *et al* (27) established the first of these, the Hu-PBL model, by injecting peripheral blood mononuclear cells (PBMCs) intraperitoneally (i.p.) or intravenously (i.v.) into SCID mice. After the transplantation of PBMC, human CD3^{+} T cells could be detected within one week, and ~50% of human CD45^{+} cells could be detected in the peripheral blood of mice after approximately four weeks. The advantage of this approach was that PBMCs were readily available and easy to manipulate, but the transplanted mice developed lethal graft vs. host disease (GVHD) within 2-3 weeks caused by the human T cells attacking mouse tissue, limiting the model's utility. More importantly, these mice are incapable of mounting adaptive immune responses with their engrafted immune systems. With the Hu-CD34^{+} model, immunodeficient mice were first given sublethal irradiation to deplete mouse hematopoietic stem cells (HSCs). Then, human CD34^{+} HSCs from human umbilical cord blood, adult bone morrow, granulocyte colony-stimulating factor-mobilized PBMCs or fetal liver was injected i.v. or i.p. into newborn or adult immunodeficient mice. In this model, the CD34^{+} HSCs can differentiate into various mature blood cells, such as T cells, B cells, NK cells or myeloid cells. However, human-derived T-cell development was low due to the lack of a human thymus. To address this problem, Lan *et al* (28) established the Hu-BLT model in 2006, in which immunodeficient mice (NOD/SCID) were also treated with sublethal (2-3 Gy) whole-body irradiation, after which human fetal liver and thymus tissue were transplanted into the subrenal capsule of adult immunodeficient mice, and autologous CD34^{+} human HSCs from the same fetal liver or bone marrow were injected i.v. into the mice, resulting in a stable model 3-4 months after transplantation. This method achieved a significant reduction in GVHD symptoms, but the limited donor source and the complexity of establishment have restricted the use of this model to study the human immune microenvironment and infectious diseases. To our knowledge, GC PDX models using humanized mice have not been reported thus far. However, a cell-derived xenograft (CDX) model of GC using humanized mice has been used to evaluate the biological roles of Zinc Finger Protein 64 (ZFP64) in GC for nab-paclitaxel resistance (29). In this study, 3-week-old NSG mice were injected with cord blood-derived CD34^{+}HSCs; subsequently, human GC HGC-27 cells were subcutaneously implanted in the humanized mice. The integration of tumor progression analysis and humanized mouse models offered a novel approach for evaluating tumor cell drug resistance, as

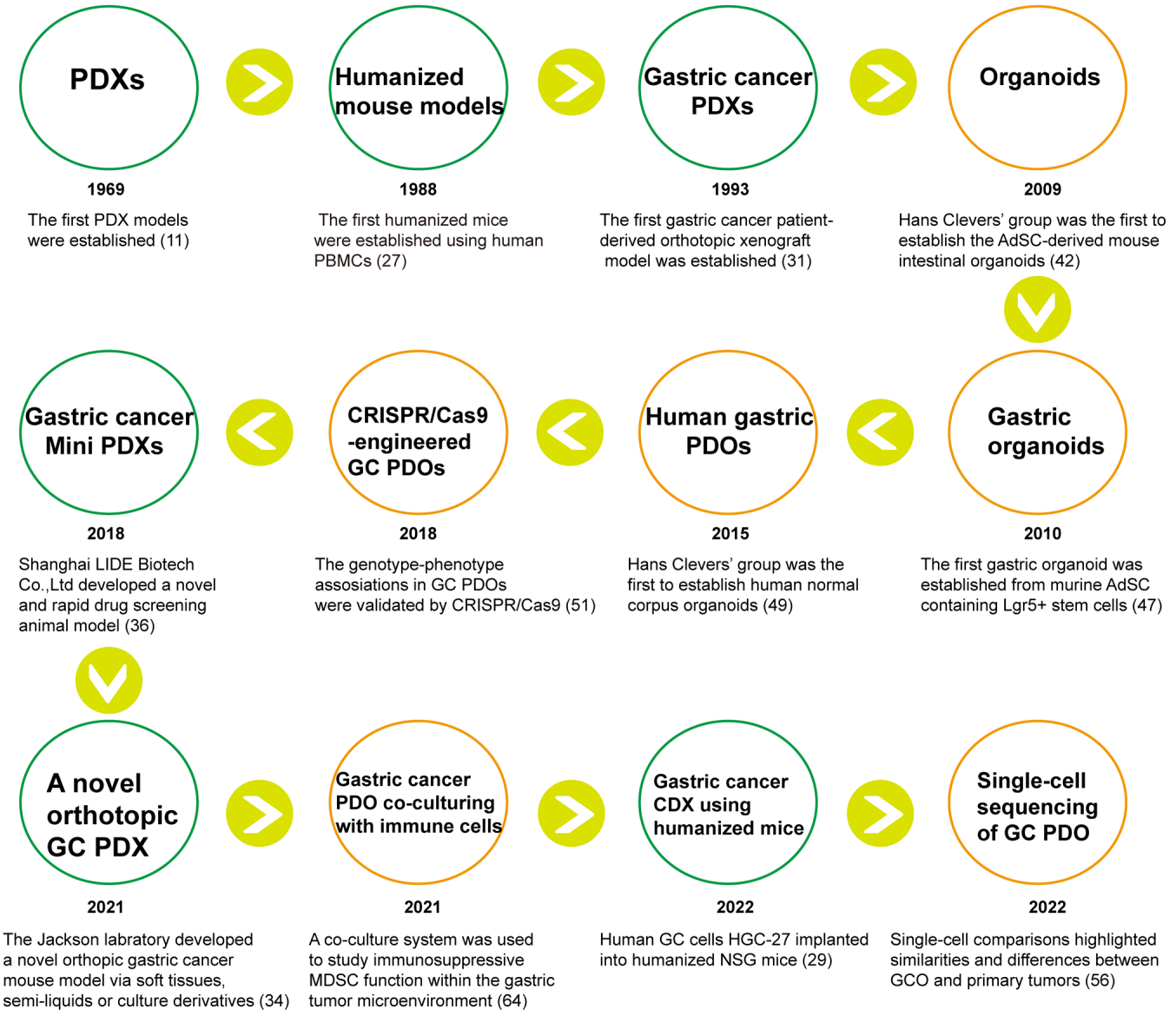

Figure 1. Evolution of GC PDX and PDO models. The timeline shows the available reports of the development of PDX and PDO models of GC. The first PDX model was successfully established in 1969. An orthotopic PDX model for GC was also successfully established in 1993. At the same time, miniPDXs and humanized mice were also rapidly developing. Hans Clevers' group was the first to establish the AdSC-derived mouse intestinal organoids in 2009; soon, murine and human-derived organoids were also successfully established. Scientists studied the organoids via CRISPR/Cas9, co-cultured systems and single-cell sequencing to investigate the mechanism and treatment of GC. GC, gastric cancer; PDX, patient-derived xenograft; PDO, patient-derived organoid; AdSC, adult-derived stem cell; CRISPR, cluster regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; PBMC, peripheral blood mononuclear cell; MDSC, myeloid-derived suppressor cell; CDX, cell-derived xenograft; GCO, gastric cancer organoid.

well as the role of the immune system in response to chemotherapy.

Heterotopic vs. orthotopic implantation. PDX models can be established by orthotopic or heterotopic (e.g. subcutaneous, intravenous or intraperitoneal injection) implantation. Heterotopic engraftments with subcutaneous injection of patient-derived cancer tissues have been widely used, as it is easier to manipulate and to monitor tumor growth. However, compared with heterotopic engraftments, orthotopic models are more clinically relevant and more suitable for the interpretation of the mechanisms of cancer metastasis, development and progression (30). In 1993, Furukawa *et al* (31) established the first patient-derived orthotopic nude mouse models of GC. In total, tissues from 36 patients with advanced GC were transplanted orthotopically into nude mice, yielding 20 tumors (56%, 20/36). GC commonly metastasizes to the liver, lymph nodes, peritoneum, lung and bone, either through direct invasion or via distant metastases by lymphatic, hematogenous or intraperitoneal spread (32). Hepatic metastases, observed in ~50% of patients with GC, are the most common distant metastases, with a survival rate of 4% at five years (33). In one study, the tumor tissues of five patients with clinical liver metastases also developed liver metastases in nude mice (31). Existing orthotopic implantation methods of GC are used to establish orthotopic stomach tumor models for studying cancer biology or organ metastasis. However, only certain types of malignant material have been successfully transplanted, such as single-cell suspensions or a firm fragment of tumor. In 2021, the Jackson Laboratory developed a novel, completely closed,

Table I. Overview of CDX, PDX and PDO model systems for gastric cancer studies.

A, CDX models

Study type	Key findings	(Refs.)
GC cell line HGC-27 was implanted into NSG mice injected with human $CD34^+$ HSCs	*ZFP64* has a pivotal role in GC progression by simultaneously promoting cellular chemotherapy resistance and tumor immunosuppression	(29)

B, PDX models

Study type	Key findings	(Refs.)
GC PDX models were established using nude mice	The first GC PDX orthotopic models	(31)
GC PDX models were generated by implanting intestinal-type tissues into nude mice	PDX models were generated with different degrees of differentiation to maintain the heterogeneity of primary tumors	(70)
50 GC PDX models from patients with advanced GC	PDX models with defined molecular signature are useful for preclinical studies with targeted drugs	(72)
PDX models from various ethnic backgrounds	Highly immunosuppressed mouse strains do not necessarily have higher transplantation rates	(25)
GC orthotopic PDX models were generated in NSG mice using various materials, such as soft tissues, semi-liquids or culture derivatives	This method overcame the weakness that engraftment materials only used single-cell suspensions or a firm tumor tissue	(34)
Establishing a rapid drug screening model named miniPDXs	miniPDXs produced drug screen outcomes in 7 days and had a similar response to the GC PDXs	(36-41)

C, PDO models

Study type	Key findings	(Refs.)
Development of long-term gastric organoids from human gastric corpus tissues	The models are able to be used for studying *H. pylori* infection or other gastric pathologies	(49)
Human PDO models from patients of phase I/II clinical trials	The first GC PDO models	(50)
GC PDO biobanks	Living organoid biobanks can be used for precision medicine	(9, 50-52)
5-FU-resistant GC PDO models	*KHDRBS3* has an important role in the acquisition of characteristics of cell stem cells in GC	(80)
Oxaliplatin-resistant GC PDO models	Myoferlin is highly involved in oxaliplatin resistance and tumor progression in GC	(81)
Modifying organoids via CRISPR-Cas9	Demonstrated genotype-phenotype associations in GC	(51,55)
Reconstruction of the immune microenvironment in GC organoids	GC organoid models with preserved TME component were established and used to study immune checkpoint inhibitors and interactions between tumor and TME	(62-64)
Single-cell RNA sequencing	A series of genetically-edited GC organoids in mice were generated and used to validate the interaction between tumor cells and macrophages	(69)
Single-cell RNA sequencing	Similarities and differences between primary GC tumors and organoids	(56)

D, Combined use of multiple models

Study type	Key findings	(Refs.)
GC CDX and PDX models	*CDK12*/*PAK2* may serve as a novel therapeutic target for patients with GC	(73)

Table I. Continued.

D, Combined use of multiple models		
Study type	Key findings	(Refs.)
GC PDO and PDX models	GC PDOs and PDXs are reliable tools for predicting nanoformulation efficacy	(75)
PDO/PDOX models: SRCC organoids and organoid-derived xenografts	SRCC organoids were highly similar to the primary tumors and may be used as a living biobank for drug screening	(77)
CDX/PDX/PDO models		
Establishing multilevel platform of GC models, including 100 PDXs, organoids and cell lines	Platform is the widest in an academic institution, including all GC histologic and molecular types	(10)
GC CDX, organoid models and PDX models	*STAT3* acts as a key negative regulator of ferroptosis in GC, and the study developed a powerful *STAT3* inhibitor, W1131	(79)

CDX, cell-derived xenograft; PDX, patient-derived xenograft; PDO, patient-derived organoid; PDOX, patient organoid-derived xenograft; GC, gastric cancer; HSC, hematopoietic stem cell; ZFP64, zinc finger protein 64; 5-FU, 5-fluorouracil; CRISPR, cluster regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9; TME, tumor microenvironment; SRCC, signet-ring cell carcinoma; STAT3, signal transducer and activator of transcription 3.

orthotopic GC animal model in NSG mice using diverse tumor materials, such as soft tissues, semi-liquids or culture derivatives (34). This novel method overcame the weaknesses of the existing methodologies that supported using only single-cell suspensions or a firm tumor fragment. Although their approach required advanced surgical techniques, this procedure can generate an appropriate animal model for numerous research purposes, including exploration of biomarker functions, testing the efficacy of anti-tumor drugs and utilizing GC organoids.

MiniPDX. PDXs have emerged as valuable models for predicting drug responses in GC treatment. However, their limitations, including being time-consuming and having a lower engraftment rate, hinder their clinical application in patients with advanced GC due to rapid disease progression. Thus, there is an urgent need for a rapid and dependable alternative approach to evaluating drug sensitivity. The hollow fiber assay has been proposed as a preliminary screening tool for anticancer agents to identify sensitive tumor cell lines (35), but this approach did not have high similarity with clinical results. In 2018, Shanghai LIDE Biotech Co., Ltd. developed a rapid drug screening model named OncoVee® MiniPDX (36). In this model, hollow fiber capsules were filled with patient-derived GC tumors and then implanted subcutaneously into mice, and they are permitted to grow for 7 days. The system has shown high similarity between compound responses of miniPDX and corresponding PDX. Several study groups have also reported the use of miniPDX models for the treatment of GC and found that drug screening through this system can provide significant benefits for patients with GC (37-41). MiniPDX in combination with next-generation sequencing (NGS) can be used to rapidly evaluate drug sensitivity in patients with GC and identify key genetic mutations (39). A single-arm, open-label phase I clinical study utilizing miniPDX models to evaluate HER2-negative medium-advanced GC/gastroesophageal junction cancer chemotherapy regimens and targeted agents resulted in favorable antitumor activity and safety outcomes (41). In the future, it will presumably be possible to co-transfer fresh cancer tissues with autoimmune cells (PBMCs or tumor-infiltrating lymphocytes) from the same patient with GC into minicapsules and engraft them into immunodeficient mice, which can capture the human TME to a maximum extent, allowing for the evaluation of the efficacy of immunotherapy drugs.

3. GC PDO models

In the last decade, organoids have been established successfully, serving as a 3D cell cultivation system derived from adult stem cells (AdSCs) or pluripotent stem cells. In 2009, Hans Clevers' group was the first to establish the AdSC-derived organoid system, in which mouse intestinal organoids were cultured in medium containing the specific growth factors required for growth of intestinal stem cells (42). Since then, organoid research has expanded to various organs or corresponding tumors, including liver (43), kidney (44), lung cancer (45), breast cancer (8) and pancreatic cancer (46). The first gastric organoid culture derived from murine adult stem cells was established using antrum glands containing leucine rich repeat containing G protein-coupled receptor 5-positive stem cells. In these cultures, markers of chief cells (pepsinogen C) and mucus neck cells (mucin 6) were observed (47). The same conditions were used for murine corpus organoids derived from $Troy^+$ stem cells, also resulting in expression of chief cell and mucus neck cell markers (48). Subsequently, human gastric corpus organoid culture protocols were established based on the murine protocol (49). These normal gastric organoids, with characteristics similar to those of parental tissues, are a useful tool to study *Helicobacter pylori* infection (49). Vlachogiannis *et al* (50) reported the first human GC PDO biobank from patients with metastatic, heavily pretreated colorectal and gastroesophageal cancer recruited from

Table II. Overview of drug candidates screened in PDX and PDO models of gastric cancer.

Target	Monoclonal antibody	Small molecule inhibitor	ADC	CAR-T	CAR-NK	Nanobody	Natural product
ABI/SRC/C-Kit	-	Dasatinib[a] (9)	-	-	-	-	-
AKT	-	MK-2206[a] (50); AZD5363 (82)	-	-	-	-	-
ARID1A	-	GSK126[a] (9)	-	-	-	-	-
ATR	-	VE-821/ VE-822[a] (9)	-	-	-	-	-
B-raf	-	Sorafenib[a] (9); vemurafenib[a] (50)	-	-	-	-	-
CDK	-	Palbociclib[a] (50,52); abemaciclib (9); AZD5438 (72); procaterol (73); SHR6390 (83)	-	-	-	-	-
CHK1	-	LY2606368 (84)	-	-	-	-	-
C-kit	-	Imatinib[a] (52)	-	-	-	-	-
Claudin 18.2	-	-	-	Claudin 18.2-CAR-T (85)	-	^{68}Ga/^{18}F/^{64}Cu-labeled (86)	-
C-met	-	Crizotinib[b] (9,71); foretinib[a] (9); volitinib (72,87); JNJ605 (10); INC280 (88); ABN401 (89)	-	-	-	-	-
C-met/AKT/ERK	-	Luteolin (90)	-	-	-	-	-
C-myc	-	JQ1 (83)	-	-	-	-	-
DUSP6	-	BCI (91)	-	-	-	-	-
EGFR	Cetuximab[b] (10,50,72,92,93); BK011 (72); GC1118 (94)	Gefitinib[a] (9)	-	EGFR-CAR-T (95)	-	-	-
EGFR/HER2	-	Lapatinib[b] (9,50,96); afatinib[b] (9,72,97)	-	-	-	-	-
FGFR	-	AZD4547 (71,98); LLM871/ BGJ398 (88); derazantinib (99)	-	-	-	-	-
GRK3	-	LD2 (100)	-	-	-	-	-
Hedgehog/SMO	-	Vismodegib[a] (9)	-	-	-	-	-
HER2	Trastuzumab[b] (10,52,71,83, 88,92,96,101); pertuzumab (96)	Mubritinib[a] (63)	DS-8201a (102); RC48 (103); LCB-ADC (104)	-	-	-	-
HER3	CAN017 (83);LJM716 (88); 1A5-3D4 (105)	-	-	-	-	-	-
IGF1R	Figitumumab (88)	-	-	-	-	-	-
JAK2/SRC	-	Vortioxetine hydrobromide (106)	-	-	-	-	-
MEK	-	Trametinib[b] (9,83,107); tegaserod maleate (107); binimetinib (88)	-	-	-	-	-

Table II. Contnued.

Target	Monoclonal antibody	Small molecule inhibitor	ADC	CAR-T	CAR-NK	Nanobody	Natural product
Mesothelin	-	-	-	MSLN-CAR-T (108)	MSLN-CAR-NK (109)	-	-
mTOR	-	Vistusertib[a] (9); everolimus[b] (9,63,88); rapamycin[a] (9,64); temsirolimus (110)	-	-	-	-	-
PARP	-	Olaparib[b] (9,111); Veliparib[a] (9); BMN673 (84)	-	-	-	-	-
PD-1	Nivolumab[a] (63,64)		-	-	-	-	-
PI3K	-	Alpelisib[a] (9); GDC-0980[a] (50); CLR457/BKM120/BYL719 (88); pictilisib (112)	-	-	-	-	-
PI3K/mTOR	-	BEZ235 (83)	-	-	-	-	-
PLK	-	CFI-400945[a] (9)	-	-	-	-	-
STAT3	-	Napabucasin[a] (9); W1131[b] (79)	-	-	-	-	XYA-2 (113)
TTK	-	CFI-402257-18[a] (9)	-	-	-	-	-
VEGF	Bevacizumab (92,93)	-	-	-	-	-	-
VEGFR	-	Regorafenib[a] (9); cabozantinib[a] (63,64); apatinib (72,93); lenvatinib (25)	-	-	-	-	-
Wee1	-	Adavosertib (114)	-	-	-	-	-
Wnt	-	IWP2/LGK-974[a] (9)	-	-	-	-	-
YAP1	-	CA3 (115)	-	-	-	-	-

[a]Drug screening in the PDO models, [b]drug screening in the PDO and PDX models, and the rest were screened in the PDX models. PDX, patient-derived xenograft; PDO, patient-derived organoid; ADC, antibody-drug-conjugate; CAR-T, chimeric antigen receptor T cell; NK cell, natural killer cell; CAR-NK, chimeric antigen receptor NK cell; AKT, serine/threonine kinase; ARID1A, AT-rich interaction domain 1A; ATR, ATM and Rad3 related protein; CDK, cyclin-dependent kinase; DUSP6, dual specificity phosphatase 6; EGFR, epidermal growth factor receptor; HER2, ErbB2 receptor tyrosine kinase 2; FGFR, fibroblast growth factor receptor; GRK3, G Protein-coupled receptor kinase 3; SMO, smoothened, frizzled class receptor; HER3, ErbB2 receptor tyrosine kinase 3; IGF1R, insulin like growth factor 1 receptor; JAK2, Janus kinase 2; mTOR, mammalian target of rapamycin; PARP, poly(ADP-ribose) polymerase; PD-1, programmed cell death protein 1; PI3K, phosphoinositide-3-kinase; PLK, polo-like kinase; STAT3, signal transducer and activator of transcription 3; VEGFR, vascular endothelial growth factor receptor; YAP1, Yes1-associated transcriptional regulator.

Phase I/II clinical trials. In their study, patient drug responses in the clinic were also observed in the PDOs, indicating their potential use in personalized medicine. As organoid technologies have matured, several independent study groups have successfully generated patient-derived GC organoids (9,51,52). Yan *et al* (9) established the largest GC biobank consisting of 46 molecularly characterized GC PDOs, including most known molecular subtypes of GC, such as Epstein-Barr virus-positive, MSI, intestinal/chromosomal unstable and diffuse/genomically stable. The cluster regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas 9) system, originally identified in bacteria as a defense mechanism against phage infection and plasmid transfer, has been repurposed as a potent RNA-guided DNA genome editing technology for various applications, such as gene editing, epigenome editing and transcriptional perturbation (53). Nanki *et al* (51) established a living biobank of 37 patient-derived GC organoid lines that included diverse histological and genetic subtypes. They demonstrated genotype-phenotype associations in GC organoids and validated their findings through the CRISPR-Cas9-engineered gastric organoids with different GC mutations (51). AT-rich interaction domain 1A (*ARID1A*) helps regulate gene expression that drives oncogenesis or tumor suppression (54). However, the oncogenic consequences of *ARID1A* mutation in human cells remain poorly defined due to a lack of accurate genetic models. Lo *et al* (55) used CRISPR/Cas9 to knock out *ARID1A* in primary $TP53^{-/-}$ GC, causing morphologic dysplasia, tumorigenicity and mucinous differentiation. When Wnt/β-catenin was activated genetically, mucinous differentiation was rescued, but not hyperproliferation. This phenotype-genotype association suggests alternative pathways of *ARID1A*-mediated transformation. An independent research group confirmed the association of *ARID1A* loss with the induction of a mucinous phenotype (56).

Tumor environment in GC PDO models. The presence of stromal cells in the TME, such as endothelial cells, immune cells and CAFs, contributes significantly to tumorigenesis, metastasis and treatment resistance (57). Tumors expressing programmed cell death-ligand 1 (PD-L1) interact with $CD8^+$ cytotoxic T lymphocytes (CTLs) expressing programmed cell death protein 1 (PD-1) to inhibit CTL proliferation and survival, leading to tumor evasion of immune surveillance, which in turn leads to increased proliferation of cancer cells (58,59). More than 40% of patients with GC have tumors that express PD-L1 (60). However, only 22% (8/36) of patients with GC have had an overall response to anti-PD-1 antibody pembrolizumab (61). Therefore, improved preclinical models are needed that can predict the efficacy of immune therapies to enhance the survival of patients with GC. In most organoid models, these crucial components of the TME are absent. Given the lack of immune cells, a co-culture model system was established to overcome this drawback. Co-culturing cancer organoids with immune cells or fibroblasts provided a valuable tool for investigating the TME and molecular interactions in cancer treatment. Current checkpoint blockade immunotherapy has shown remarkable efficacy in unblocking T cells that are negatively controlled, leading to T cell-mediated anticancer responses. Several studies of cancer precision medicine have utilized co-culturing of GC PDOs with immune cells in combination with checkpoint blockade inhibitors. Chakrabarti *et al* (62) established a GC patient-derived organoids/immune cell co-culturing system. Before co-culturing organoids with CTLs, researchers pulsed antigen-presenting dendritic cells (DCs) with tumor antigens and then cultured autologous CTLs with the DCs to increase cytolytic activity and proliferation of tumor-specific T lymphocytes. Using this autologous organoid/immune cell co-culture system, they found that HER2 expression may promote immune evasion in GC that was mediated by PD-L1. This co-culturing strategy provided a suitable preclinical model for studying the effect of anti-HER2-targeted therapy in combination with anti-PD-L1 immunotherapy for patients with GC (63). In addition, this system was used to investigate the differentiation and immunosuppressive function of myeloid-derived suppressor cells (MDSCs) (64). In another study, PDO/immune cell co-cultures demonstrated that gastric organoids expressing PD-L1 were not responsive to nivolumab *in vitro* when PMN-MDSCs were present. However, when PMN-MDSCs were depleted in these co-cultures, the organoids became sensitive to anti-PD-1/PD-L1-induced cancer cell death (64), suggesting that MDSCs with immunosuppressive function had an important role in the TME of GC. These studies have provided valuable insight into predicting alternative drug regimens and studying the GC microenvironment using GC PDOs. Thus, advances in co-culture organoid techniques may yield additional clinical treatment strategies using targeted therapy and immunotherapy. This platform can also benefit patients with GC by generating individualized therapy data more rapidly than animal models.

Single-cell sequencing-a tool to better understand GC. Single-cell RNA sequencing (scRNA-seq) is a valuable approach that enables analysis of cancer expression profiles at the single-cell level, allowing identification and characterization of unique subpopulations with specific biological behaviors (65). Numerous studies examining the heterogeneity of tumor cells and comprehensive dynamics in the TME have been performed using scRNA-seq in GC (66-68). Jiang *et al* (66) were the first to evaluate the heterogeneity of GC primary tumors and metastases in different organs at the single-cell level, demonstrating the characteristics of different organ-tropism metastases of GC and identifying effective therapeutic targets. Li *et al* (67) utilized scRNA-seq to study the role of CAFs in the GC TME, including their classification, function, origin, interaction with other cell subsets and spatial distribution in different pathological types. They found distinct roles of CAFs in regulating various aspects of TME biology, including immune modulation, invasion, migration and angiogenesis. Of note, their study demonstrated that a specific type of CAFs, known as extracellular matrix CAFs, exhibited an enhanced chemotaxis ability for attracting M2 macrophages and their presence was associated with poor prognosis of patients with GC (67). GC commonly metastasizes to lymph nodes. Qian *et al* (68) conducted a comprehensive analysis of the transcriptome profiles of GC tissues of primary tumors and metastatic lymph nodes (MLNs) at the single-cell level. They discovered that dysfunctional neutrophil polarization and maturation had a vital role in lymph node metastasis of

GC. In addition, secreted phosphoprotein 1 (SPP1) signaling, an immune checkpoint, can be activated in MLNs. Hence, targeting the disordered neutrophils and SPP1 signaling may be novel strategies to treat and prevent lymph node metastasis of GC.

Among the platforms to study GC, patient-derived organoids have emerged as a promising system for investigating tumor behavior and the influence of TME components. To elucidate the stepwise progression of the disease from dysplasia to different stages of adenocarcinoma, including well-differentiated, poorly-differentiated and metastatic, Lu *et al* (69) generated a series of genetically-edited gastric organoids in mice. Through scRNA-seq analyses and functional studies, they identified an interaction between tumor cells and macrophages, facilitated by integrin α6/β4 and fibronectin 1, which had an important role in promoting GC progression and metastasis (69). To study the extent to which GC organoid *in vitro* culture affects transcriptional lineage states or cellular proportions compared with primary cancer cells *in vivo*, Kumar *et al* (56) performed an overall analysis of cell states between primary GC cells and organoids by scRNA-seq and found similarities and differences between primary GC tissues and organoids. Similar to primary tumors, tumor PDO epithelial cells showed upregulation of cancer-associated modules and GC-related genes compared with normal PDO epithelial cells (56). In addition, they found differences between primary tumors and PDOs; for instance, stromal and epithelial cell clusters were significantly enriched in PDOs, while lymphoid and plasma cell clusters were depleted (56). A gene-expression comparison between PDO and primary samples found that plasma cells exhibited the most significant differences in gene expression profile in PDO models, whereas epithelial signatures were relatively more conserved. Altogether, gastric organoids are soon expected to use combinatorial single-cell methods, including epigenetic, genetic and transcriptional analyses, and spatial context, to further enhance our understanding of the mechanisms underlying GC development.

4. Drug screens and personalized medicine using GC PDX and PDO models

GC PDX models have the potential to emerge as effective screening platforms for predicting clinical drug response and determining biomarkers for drug sensitivity and resistance. Venkatasamy *et al* (70) implanted intestinal-type GC tissue samples into nude mice and generated five PDX models with different degrees of differentiation, including three well-differentiated, one moderately and one poorly differentiated adenocarcinoma, which maintained the heterogeneity and complexity of their primary tumors. Their data highlighted the complex response of patients to platinum-based anticancer drugs, which not only affected tumor cell proliferation but also the TME and remote tissues. Therefore, it is crucial to consider these factors when developing combination treatments or new therapeutic protocols. Wang *et al* (71) successfully established 13 PDX models, which included four with *HER2* (12.5%, 4/32), eight with *cMet* (25.0%, 8/32) and one with fibroblast growth factor receptor 2 (*FGFR2*) alterations (3.1%, 1/32). These PDX models offered an ideal platform for drug screening and efficacy evaluation for particular patients with *cMet* or *FGFR2* gene amplification who may benefit from the corresponding targeted therapies. In another study, Chen *et al* (72) generated 50 GC PDX models from patients with advanced GC and the genomic variation and molecular profile were analyzed by NGS, *in situ* hybridization and immunohistochemistry (IHC). Several drug targets, such as *MET* and cyclin E1 (*CCNE1*), were selected and validated in this study. Volitinib, a MET inhibitor, exhibited potent antitumor activity in PDX models characterized by MET overexpression or with phosphorylated MET (72), and the cyclin-dependent kinase 1/2/9 (*CDK1/2/9*) inhibitor AZD5438 displayed superior antitumor activity in two PDX models with a higher copy number of *CCNE1* (72). Liu *et al* (73) conducted IHC analysis of human GC tissues to identify the expression level of *CDK12* and then used CDX and PDX models to study the gene function and molecular interaction between CDK12 and p21-activated kinase 2 (*PAK2*). They identified that the food and drug administration-approved clinical drug procaterol may serve as a potent CDK12 inhibitor capable of inhibiting GC-cell proliferation and tumor growth in both models. Thus, *CDK12*/*PAK2* can serve as a novel therapeutic target for patients with GC.

Although PDX models have proven to be useful in drug screening and for predicting clinical outcomes, they are not appropriate for high-throughput drug screening. Compared to PDX models, PDOs have the advantage of being established and expanded more efficiently, making them suitable for conducting high-throughput drug screening. PDOs as preclinical models for identifying biomarkers and performing genotype-drug associations are a relatively new area of investigation. The limited studies conducted thus far have been promising. Chemotherapy is a primary therapeutic strategy used to treat patients with GC, but conventional chemotherapeutic agents often cause undesirable adverse effects. Nanoparticles have recently emerged as potential treatment options for GC. Compared with conventional chemotherapeutic drugs, nanoparticles can have improved therapeutic and pharmacologic features, while simultaneously reducing systemic toxicity (74). Zou *et al* (75) established, from surgically resected tumor tissues and endoscopic biopsies, nine GC PDO lines using a multiple-batch dissociation method. Two representative paclitaxel (PTX) nanoparticles were chosen for a comparative study and liposomal PTX was more effective than albumin-bound PTX in killing GC PDOs in both transcriptome and cellular levels (75). PDX models have also been used to validate the therapeutic outcomes obtained through intratumoral drug administration, which provided enhanced drug concentrations at the local site with reduced systemic toxicity (75). The evaluation of nanoparticles using GC PDOs has been crucial to both experimental and clinical design. Signet-ring cell carcinoma (SRCC) in advanced GC is defined as being present in exceeding 50% of GC tumors and was often associated with greater invasiveness and a worse prognosis compared to other cell types (76). Recently, Li *et al* (77) generated four SRCC and eight non-SRCC PDOs, performed a thorough phenotypic and genotypic analysis, and used5-fluorouracil (5-FU), oxaliplatin, docetaxel and irinotecan to treat SRCC and non-SRCC organoids. In addition, they implanted GC PDOs into immunodeficient mice and successfully formed tumors, which retained the characteristics of the primary tumors.

Table III. Characteristics of the three gastric cancer preclinical model systems.

Model type	Advantages	Limitations
CDX models	Relatively cheap	Lack of tumor heterogeneity
	Technically not complicated	Lack of tumor microenvironment
	Cells are readily available and grow rapidly	Low predictive drug response
PDX models	Preservation of the genetic and phenotypic landscape of the parental tumor	Expensive
	Tumor heterogeneity and specific traits of metastases are maintained	Not suitable for early-stage cancer
	Retention of the original tumor architecture	Replacement of human stroma with murine counterparts
	Similar drug response to that in the patients	Loss of subclone heterogeneity during passages of PDX
		Long time course of PDX tumor tissue generation
		Failure to evaluate the immune system
Humanized mice	Mimics the human tumor microenvironment	Expensive
	Evaluation of drug response in cancer	Complicated technology
	Prediction and evaluation of cancer immunotherapy	Long time for humanization and PDX generation
		Limited reconstitution of human immune system
PDO models	Higher success and ease of use	Costly niche factors supplement
	Preservation of the genomic and histological characteristics of parental tumors	Lack of tumor microenvironment
	Genetic modification	Lack of tumor-stroma interaction
	High-throughput drug screens	
	Prediction of clinical response	

CDX, cell-derived xenograft; PDX, patient-derived xenograft; PDO, patient-derived organoid.

Besides classical chemotherapeutics, GC PDOs can also be treated with targeted drugs against molecular alterations. GC organoids with *AKT* serine/threonine kinase 1 (*AKT1*) mutations were sensitive to the AKT inhibitor MK-2206 (50). Similar to clinical outcomes, GC PDOs carrying *HER2* amplification were sensitive to trastuzumab (52). Palbociclib or abemaciclib, which are both inhibitors of *CDK4/6*, can effectively suppress the proliferation of GC organoids (9,50,52). The signal transducer and activator of transcription 3 (*STAT3*) is a key oncogene, which functions both in signal transduction and transcriptional activation (78). In a recent study, Ouyang *et al* (79) found that *STAT3* negatively regulated ferroptosis in GC. They then developed a potent and selective *STAT3* inhibitor, W1131, which had powerful antitumor activity in CDX, PDX and PDO models, suggesting that W1131 may be a novel candidate drug or therapeutic strategy for advanced GC. Cancer stem cells (CSCs) have a key role in the acquisition of drug resistance. However, there is currently no biomarker capable of accurately predicting 5-FU and oxaliplatin resistance in relation to CSCs in clinical practice. Ukai *et al* (80) successfully established four 5-FU-resistant GC PDOs and performed a microarray analysis using normal gastric organoids with matched 5-FU-resistant and parental PDOs. They determined that KH domain containing, RNA binding, signal transduction associated 3 (*KHDRBS3*) may function in the acquisition of CSC-like features, including multi-drug resistance and organoid formation by regulating CD44 variant expression (80). Hence, *KHDRBS3* may be a potential marker for predicting treatment response and prognosis in patients with GC. In another study, Harada *et al* (81) established oxaliplatin-resistant GC organoids and evaluated their gene profiles using microarray analysis. They found that expression of myoferlin in GC was highly related to oxaliplatin resistance, tumor progression and unfavorable prognosis.

5. Challenges and perspectives

In the current review, the advantages and limitations of CDX, PDO and PDX preclinical models of GC in cancer research and therapy development were discussed (Table III). As organoid technologies have developed, PDO models have become robust tools for pathogenesis research. Organoids reflect the genetic and phenotypic heterogeneity of cancer patients and can be expanded rapidly and modified genetically using CRISPR-Cas9 technologies. Initially, CDX models are used for drug screening due to their uncomplicated technology

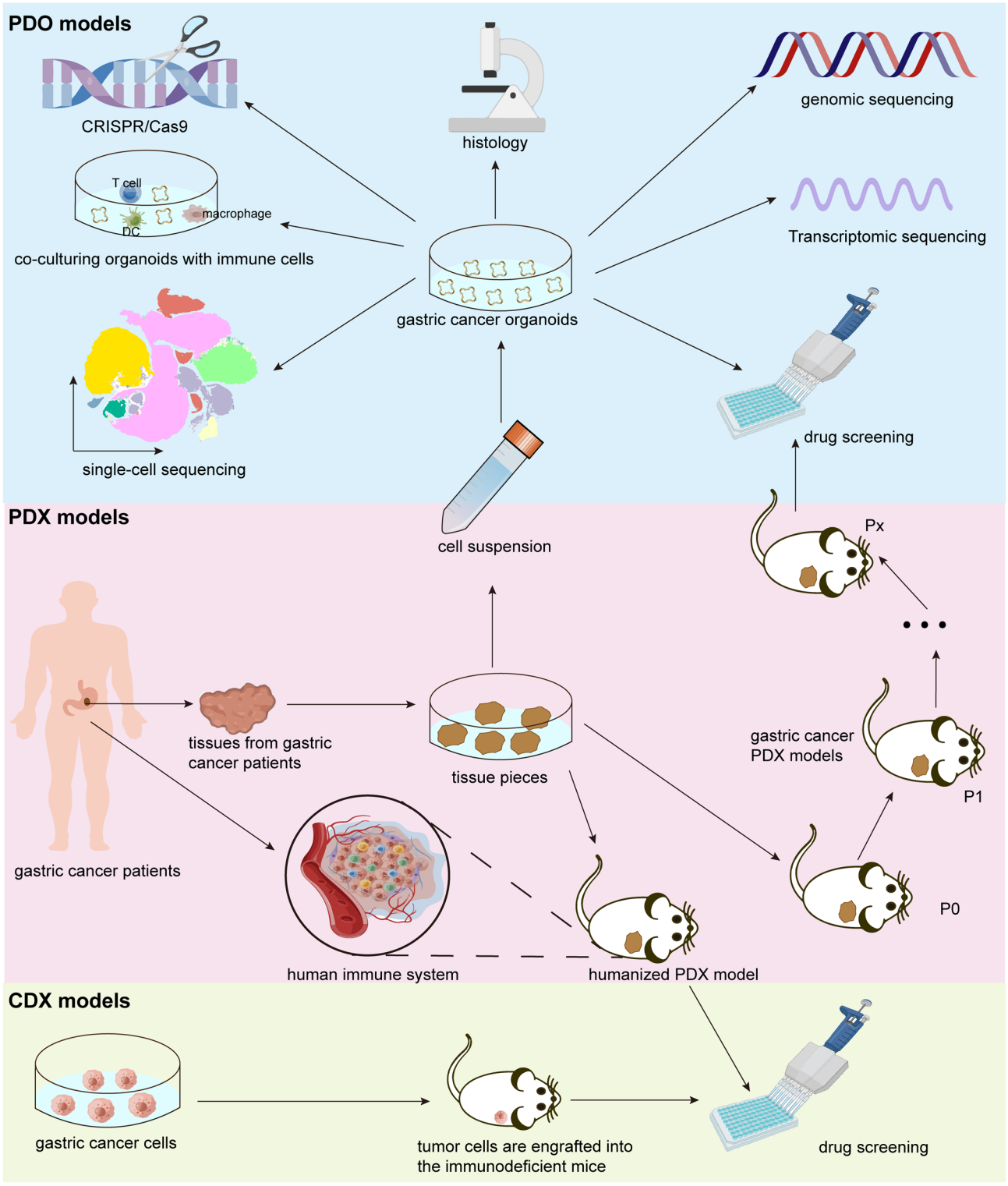

Figure 2. Establishment and application of PDO, PDX and CDX models in GC research. CDX models are established by inoculating GC cell lines into immunodeficient mice. CDX models are unlikely to retain the heterogeneous histological and genetic features of the original tumor, while PDX and PDO models are established by tumor tissues or cell suspension derived from patients with GC into immunodeficient mice. PDX and PDO models have a relatively high predictive value for clinical outcomes compared to CDX models. PDO, patient-derived organoid; PDX, patient-derived xenograft; CDX, cell-derived xenograft; GC, gastric cancer; DC, dendritic cell; CRISPR, cluster regularly interspaced short palindromic repeats; Cas9, CRISPR-associated protein 9.

and ready cell availability. However, CDX models have a low predictive value for clinical outcomes, rendering them unsuitable for personalized medicine approaches. In contrast with CDX models, PDOs are cultured in medium with various niche factors, thereby increasing cost of maintenance. Organoids have a higher success rate and operational convenience and are useful for high-throughput drug screening, compared with PDX models. However, PDX models preserve tumor heterogeneity and tumor-stromal interactions observed in patients' tumor tissues, making them more relevant for

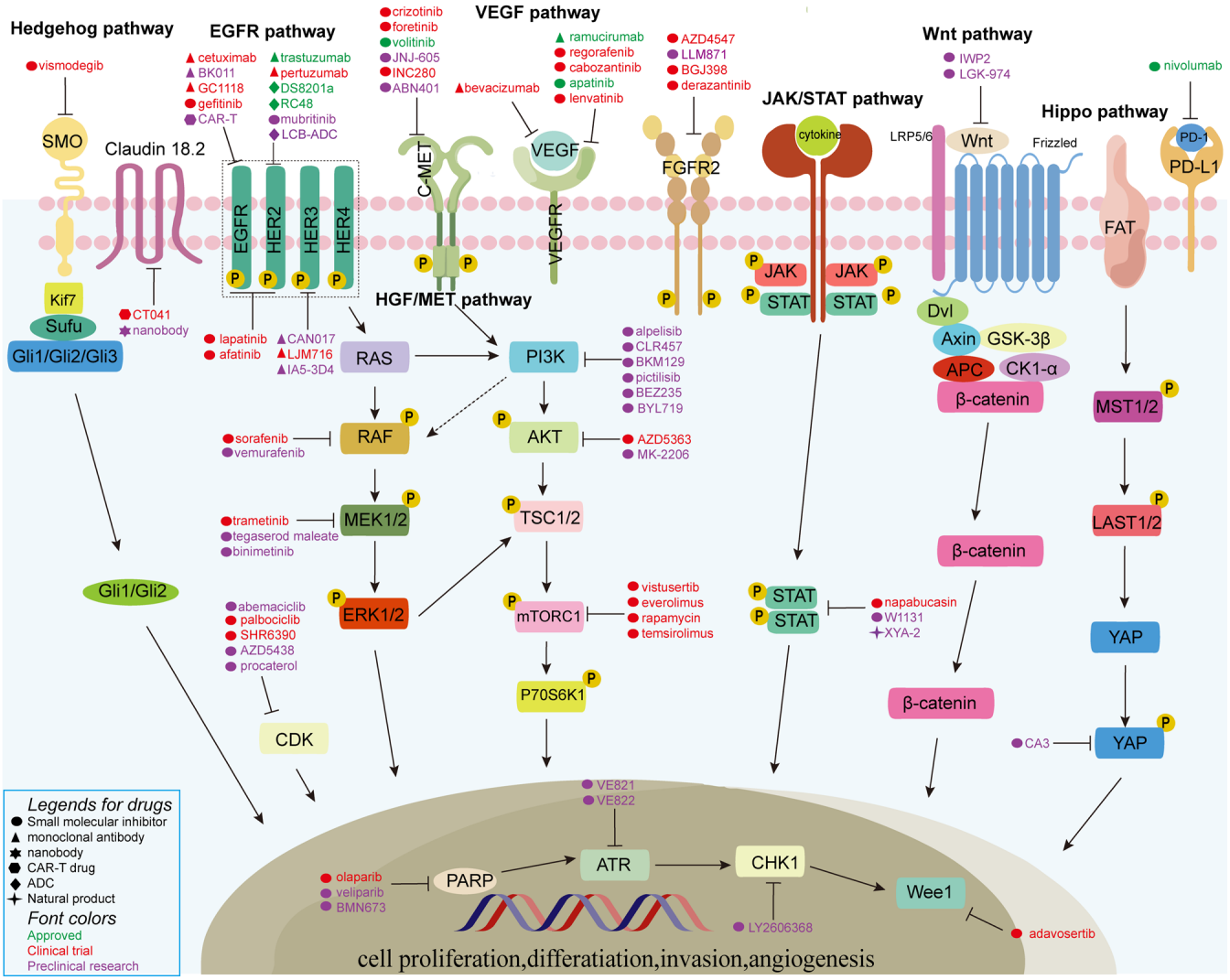

Figure 3. Overview of targeted and immunotherapeutic agents screened in GC PDX and PDO models. The major signaling and crosstalk of Hedgehog, EGFR, HGF-MET, VEGF, JAK/STAT, Wnt and Hippo pathways are illustrated. Representative targets in GC and the corresponding targeted or immunotherapeutic agents are depicted. Drugs in the figure include small molecular inhibitors, monoclonal antibodies, nanobodies, CAR-T drugs, ADCs and natural products. Targeted agents include those that have been approved (colored in green), studied in clinical trials (colored in red) and in preclinical or early phase development (colored in purple) for the treatment of gastric cancer. SMO, smoothened, frizzled class receptor; Kif7, kinesin family member 7; Sufu, SUFU negative regulator of hedgehog signaling; Gli, GLI family zinc finger; CDK, cyclin-dependent kinase; EGFR, epidermal growth factor receptor; HER2, Erb-B2 receptor tyrosine kinase 2; HGF, hepatocyte growth factor; MET, met proto-oncogene, receptor tyrosine kinase; AKT, AKT serine/threonine kinase; TSC1/2, TSC complex subunit 1/2; mTOR, mammalian target of rapamycin; P70S6K1, ribosomal protein S6 kinase B1; VEGFR, vascular endothelial growth factor receptor; FGFR2, fibroblast growth factor receptor 2; JAK, janus kinase; STAT, signal transducer and activator of transcription; APC, APC regulator of Wnt signaling pathway; MST, mitogen-activated protein kinase kinase kinase 10; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; YAP, yes-associated protein; PARP, poly ADP-ribose polymerase; ATR, ATM and Rad3 related protein; Wee1, Wee1 G2 checkpoint kinase; GC, gastric cancer; PDX, patient-derived xenograft; PDO, patient-derived organoid; CAR-T, chimeric antigen receptor T cell; ADC, antibody-drug conjugate.

studying *in vivo* cancer biology and for predicting clinical outcomes. Patient-derived xenograft models may serve as an 'avatar model', meaning that PDX models derived from cancer patients participating in a clinical trial can be subjected to the same treatment given to the patient. This approach facilitates the identification of new biomarkers for sensitivity or resistance to specific anti-cancer treatments. While tumor xenograft and organoid models lack a competent immune environment, this limitation can be addressed by transplanting HSCs and co-culturing with immune cells for PDX and PDO models, respectively.

In brief, preclinical cancer research faces the challenge of generating reliable models that closely reflect the patient's condition, including intra-tumor heterogeneity and the TME. Each model has its strengths and weaknesses, so combining different preclinical models may enable better precision cancer research. For instance, PDO models may be used for high-throughput drug screening, followed by validation of lead candidates or combinations using patient-derived tumor xenograft models. Furthermore, combining drug responsiveness data from different models can lead to more accurate predictions of drug efficacy in clinical trials. In future investigations, scientists can, on the one hand, improve organoid culture methods and techniques, and on the other hand, optimize animal models for more accurate implantation, dynamic monitoring of tumor cells and evaluating the immune system, thereby overcoming the limitations of existing models and developing better

preclinical GC models for drug discovery and personalized medicine.

6. Conclusion

In the present review, the features of the three mainstream preclinical GC models were highlighted and the establishment and application of CDX, PDX and PDO model systems in GC research were discussed (Fig. 2). GC PDX and PDO models not only reflect the morphological and genetic characteristics of primary tumor tissues, but also mimic therapeutic responses to anti-cancer treatments. Therefore, both of these preclinical models may serve to predict individual responses to diverse treatments (Fig. 3), improving personalized precision medicine. Tumor stromal cells in the PDX models are gradually replaced during xenograft passages. Researchers increasingly favor PDO models due to the rapid time to be established and utility for efficient drug screening compared to PDX models. Organoids also lack a TME. Scientists have overcome this common problem by using humanized mice and co-culturing immune cells to resemble the TME. Furthermore, with the rapid development of various sequencing and genetic editing technologies, it is possible to combine whole-exome sequencing, single-cell sequencing and CRISPR-Cas9 with PDX as well as PDO models to study the mechanisms of GC development more deeply and develop individualized treatments for patients with GC.

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Authors' contributions

JX conceptualized the study, performed the literature search and drafted the manuscript. JX and BY generated the figures. FW and JY revised the manuscript. FW and JY were responsible for project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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