organoids

MDPI

Review

Precision Medicine for Gastric Cancer: Current State of Organoid Drug Testing

Tharindie N. Silva [1], Josephine A. Wright [2], Daniel L. Worthley [3] and Susan L. Woods [1,2,*]

[1] Adelaide Medical School, University of Adelaide, Adelaide, SA 5000, Australia
[2] Precision Cancer Medicine Theme, South Australia Health and Medical Research Institute, Adelaide, SA 5000, Australia
[3] Colonoscopy Clinic, Brisbane, QLD 4000, Australia
* Correspondence: susan.woods@adelaide.edu.au

Abstract: Gastric cancer (GC) presents a significant health challenge and ranks as the fifth most common cancer in the world. Unfortunately, most patients with GC exhaust standard care treatment options due to late diagnosis and tumour heterogeneity that leads to drug resistance, resulting in poor survival outcomes. Potentially, this situation can be improved by personalising treatment choice. Organoids are an emerging cell model system that recapitulates tumour heterogeneity and drug responses. Coupled with genomic analysis, organoid culture can be used to guide personalised medicine. The GC organoid field, however, lacks standardised methodologies for assessing organoid drug sensitivities. Comparing results across different GC organoid studies and correlating organoid drug responses with patient outcomes is challenging. Hence, we aim to summarise the methodologies used in GC organoid drug testing and correlation with clinical outcomes and discuss design considerations and limitations to enhance the robustness of such studies in the future.

Keywords: gastric cancer; organoids; precision medicine

Citation: Silva, T.N.; Wright, J.A.; Worthley, D.L.; Woods, S.L. Precision Medicine for Gastric Cancer: Current State of Organoid Drug Testing. *Organoids* **2024**, *3*, 266–280. https://doi.org/10.3390/organoids3040016

Academic Editors: Elizabeth Vincan, Ramanuj DasGupta, Somponnat Sampattavanich and Joao Ferreira

Received: 6 September 2024
Revised: 13 October 2024
Accepted: 28 October 2024
Published: 31 October 2024

1. Introduction

Gastric cancer (GC) is an important global health issue, ranking as the fourth leading cause of cancer-related mortality worldwide, with over one million cases in 2020 [1]. GC affects males at twice the rate of females and is more common in elderly people, with an average onset at 68 years of age [2]. While efforts to eradicate *H. pylori* and advancements in food preservation have marginally reduced GC incidence, our ageing population is likely to result in increased GC incidence in the future [3]. Concurrently, there is a steady, or slightly increased, incidence rate among young adults [4]. GC is a heterogeneous disease that is mostly composed of gastric adenocarcinoma, which is further sub-classified into intestinal, diffuse, unclassified, or intermediate types [5], with significant molecular heterogeneity identified by The Cancer Genome Atlas (TCGA) to include EBV-positive (EBV+), microsatellite instable (MSI), genomically stable, and chromosomally instable (CIN) subtypes [6]. This heterogeneity of GC significantly impacts survival outcomes. Exploring GC treatment options that can effectively mitigate the impact of GC is crucial for this poor prognosis cohort of patients.

Standard care treatment options for GC generally include surgery combined with neoadjuvant or adjuvant chemotherapy and/or radiotherapy, depending on the GC stage [7]. First-line standard care treatment often includes fluoropyrimidine (5-Fluorouracil, Capecitabine) combined with a platinum-based drug (Oxaliplatin) [8]. Second-line or high-risk disease treatment includes docetaxel and irinotecan in combinations such as FLOT (fluorouracil, leucovorin, oxaliplatin, docetaxel), FOLFIRI (fluorouracil, leucovorin, irinotecan), and FOLFOX (fluorouracil, leucovorin, oxaliplatin). For advanced disease patients with peritoneal metastasis, pressurised intraperitoneal aerosol chemotherapy (PIPAC), where

chemotherapy is directly administered into the peritoneum using pressurised normothermic aerosol [9], can provide a palliative option. In addition to these standard-care chemotherapeutic options, the additional treatment modalities of targeted therapy and immunotherapy have also emerged.

Targeted therapy involves drugs that target specific genes or proteins that are crucial for cancer cell growth and survival, with minimal damage to normal healthy cells. These include Trastuzumab for GC positive for human epidermal growth factor receptor 2 (HER2) and Ramucirumab for tumours with elevated vascular endothelial growth factor (VEGF) [8]. Immunotherapy instead aims to boost the patient's natural immune response to fight cancer cells [10]. Immune checkpoint inhibitors (ICI) are a type of immunotherapy drug that blocks the checkpoint proteins from binding to their respective ligands or receptors, i.e., they work to remove the brakes on the immune system. Programmed death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) inhibitors, such as pembrolizumab, are ICIs used in microsatellite instability-high (MSI-H) or tumour mutation burden (TMB) high patients with GC to prevent PD-1 from binding to PD-L1 [8,11,12]. Although these options have shown satisfactory responses in MSI and EBV+ GC subtypes, 80–85% of GCs do not respond to ICIs [13–15]. Furthermore, these therapies are not universally adopted in standard care treatment due to variability in access and resource availability. Overall, most patients exhaust available treatments due to late diagnosis and tumour heterogeneity both between and within patient tumours that result in drug resistance [16–18]. Thus, the 35%, 5-year survival rate for localised GC, further decreases to 7% for advanced GC, indicating the need for improved treatment strategies including precision medicine approaches that can be personalised to individual tumour characteristics [19].

Next-generation sequencing (NGS) technologies enable a thorough analysis of tumour (epi)genomics and transcriptomics, providing an in-depth understanding of the molecular characteristics specific to each tumour [20], including TMB, a key indicator of potential sensitivity to ICI [21]. These developments have paved the way for careful categorisation and detection of germline or tumour-specific markers. Examples include *Dihydropyrimidine Dehydrogenase* (*DPYD*) variants found in 3–5% of Caucasian, African American, and Asian populations that predict poor response to 5-FU [22], and *ARID1A* mutations that predict favourable overall survival outcomes to fluorouracil-based chemotherapeutics and pembrolizumab for PD-1 blockade [23]. Even though potential sensitivity to chemotherapeutics and targeted therapies can be identified by tumour sequencing, clinical outcomes from cancer treatment led purely by this static measure of genomic data have been largely inadequate [24]. Therefore, integrating tumour genomic profiles with functional assays, such as in vitro models, may enhance precision medicine efforts to guide personalised treatment strategies for GC.

Historically, cancer research relied on conventional cancer cell lines and patient-derived tumour xenografts (PDTXs) that have been instrumental in advancing cancer research [25]. However, there are a few major drawbacks to these methods, including cell line adaptive changes in 2D culture, low culture success rates, and mouse studies being both time-consuming and expensive while also involving mouse-specific tumour evolution [26–31]. Therefore, to overcome these limitations and better understand tumour characteristics and drug effects, Hans Clever's lab in the Netherlands popularised a powerful in vitro method called "organoids" [32].

Organoids are three-dimensional (3D) structures composed of adult stem cells or embryonic/pluripotent stem cells [33]. They are embedded in a laminin-rich extracellular matrix that mimics the in vivo extracellular microenvironment and supplied with a nutrient-rich medium to promote growth. Compared to conventional tumour cell culture, organoids can preserve genomic stability and the characteristics of the tumour from which they were derived over extended periods [34]. Research by Fujii et al. demonstrated the genomic stability of microsatellite-stable colorectal cancer organoids before and after long-term culture (more than 6 months) [35]. As a cell culture model, organoids can also be expanded, frozen, and thawed to maximise the use of organoids in experiments as required.

Furthermore, organoid cultures can be used in different applications, including genomic, transcriptomic, and proteomic analyses; genetic manipulation via the use of viruses and/or clustered regularly interspaced short palindromic repeats (CRISPR/Cas9); and drug testing, making the technique useful in both basic research and clinical studies [33].

Organoids have been extensively used in cancer research and have successfully grown from many epithelial cancers such as breast [36,37], lung [38], and colorectal cancer [39,40]. However, research on GC organoids remains limited, hindered by small sample sizes, moderate culture success rates, and limited testing of chemotherapeutics. Previous review articles have extensively covered procedures for the establishment of GC organoids, identifying both the challenges and strengths of this technology, which has significantly advanced the field [33,41–44]. A detailed review of drug-testing methodologies used in GC organoid studies is lacking. Ren et al. provided an overview of the basic technology and clinical applications of drug screening using organoids across various cancers, including colorectal, liver, gastric, pancreatic, and brain cancers [45]. Meanwhile, Verduin et al. emphasised studies comparing organoid drug responses with clinical patient outcomes [46]. Yet, the absence of standardised protocols for assessing drug sensitivity in GC organoids and the correlation of those organoid responses with patient outcomes remains a critical gap. These protocols vary widely among laboratories and researchers, posing challenges for comparing results across different GC studies.

In this review, our goal is to explore the methodologies used in drug testing using patient-derived GC organoids, focusing on correlating organoid drug responses with patient outcomes while also briefly addressing GC organoid establishment. We aim to critically evaluate these methodologies, highlight key design considerations, discuss limitations, and propose avenues for enhancing the robustness of such studies in the future.

2. Gastric Cancer-Derived Organoid Establishment

Briefly, GC organoids are established by mechanically disrupting GC tissue, followed by enzymatic digestion to yield GC cells, which are seeded in an extracellular matrix (typically Matrigel) and supplied with a medium supplemented with GC growth factors [47]. The media composition varies slightly between studies as reviewed and is refreshed every two to four days, and most studies passage organoids every two weeks to expand the lines [48]. Schmäche et al. successfully established 64 GC and nine normal organoids, achieving a culture success rate of 61% for both GC and normal organoid establishment [49]. Organoids were established at two study sites; the study site with the most experience in handling GC organoids achieved a 73% culture success rate, whereas the study site with no previous experience showed improvement over time [49]. This finding is promising, as it reflects the feasibility of establishing GC organoids and enhanced proficiency with practice. Furthermore, this is so far the highest number of GC organoids established in a study. Recently, Zhao et al. achieved a culture success rate of 78% by establishing 57 GC organoid lines from 73 patient tumour samples; however, only five organoid lines were passaged for up to 17 passages (high growth rate), while 52 were limited to 8–9 passages (low growth rate) [50]. A significant difference in gene expression was reported, with transcripts related to increased proliferation and stemness (*REG4*, *KLF4*, *ERBB3*, *HRAS*, *NOTCH1*, and *MYC*) upregulated in high growth rate organoids, while transcripts related to growth inhibition (*BAX*, *DKK3*, *TNFSF12*, *MCC*, *BNIP3*, and *TP53BP1*) were upregulated in low growth rate organoids [50]. However, given the majority of organoid lines (91%) in this study did not survive past passage 10, it suggests that there may be essential components missing in the growth media to maintain long-term growth or that some of the organoid lines may be contaminated by normal gastric organoids, as can be indicated by cystic morphology. Both studies make significant contributions to the expanding field of GC organoids, particularly through their use of larger sample cohorts.

One of the benefits of organoids over conventional cell culture is the maintenance of tumour genomic and morphological stability for extended periods. Seidlitz et al. cultured 20 organoid lines from surgically resected tumour tissues and maintained the lines continuously in culture for over a year without observing any changes in growth behaviour or morphological phenotype [51]. They also provided an in-depth analysis of four GC organoid lines via genomic and transcriptomic analysis to identify unique mutations present in each GC line to aid identification of potentially beneficial targeted therapies. While most studies have generated organoids from GC tissue, Li et al. was one of the first studies to establish all organoids from malignant ascites fluid obtained from patients with GC [52]. They demonstrated that culturing the tumour cell pellet with a moderate amount of the supernatant resulted in increased organoid growth and size in comparison to completely removing the supernatant according to the standard method [52]. Yan et al. cultured 46 GC organoid lines from 34 patients, achieving a 50% culture success rate, and uniquely cultured multiple biopsies from the same patient [53]. This enabled an analysis of subclones found within primary cancer and matched organoids using Superfreq, which identified varying degrees of heterogeneity across the tumour and metastatic lymph nodes.

A primary challenge in culturing GC organoids is controlling the overgrowth of non-malignant organoids that occurs when identical media is used to culture both normal and neoplastic tissue organoids [43]. Yan et al. used two methods to prevent the overgrowth of normal organoids, including the microscopic selection of tumour organoids and the use of Nutlin3a in growth media, to select against normal cells containing wild-type *TP53*, as many GCs have mutant *TP53* [53]. Nanki et al. cultured 37 GC organoid lines and tested four alternate growth media to prevent normal organoid overgrowth in slow-growing tumour lines. This was enabled by recognition and altered growth media based on recurrent genetic changes found in GC and resulted in an improvement of organoid establishment rate from 55 to 75% [54]. This methodology from Nanki et al. provides the current, best-practice path to GC organoid culture success, although combining alternative media with manual selection of GC organoids away from normal gastric organoids would further elevate the technique to ensure a pure population of GC organoids is established. A key outcome measure of these initial studies was culture success rate; however, only a limited number reported a detailed set of culture success metrics. Zu et al. addressed this by creating a detailed scoring system including sample acquisition method, initial sample volume, and proportion of tumour cells in the sample that may influence the quality of organoid line derivation [55]. Despite barriers like the overgrowth of normal organoids and microbial contamination issues, collectively GC organoid studies covered in Table 1 or previously reviewed [48] have reported a promising average culture success rate of 67% in establishing GC organoids, although not all studies address the problem of normal organoid overgrowth, and so this number may be slightly inflated. Verification that cultured cells are tumourigenic is an important quality assurance metric. This can be performed using the fairly time-consuming process of transplant into immunocompromised host mice and detection of tumour formation or more rapidly by validation of the presence of tumour-associated alterations via genomic sequencing. The next step in the use of GC organoids for potentially improving treatment choice decisions is to test chemotherapeutics and targeted therapy drugs to distinguish which drugs may benefit each patient and then correlate organoid-predicted responses with clinical outcomes (Figure 1).

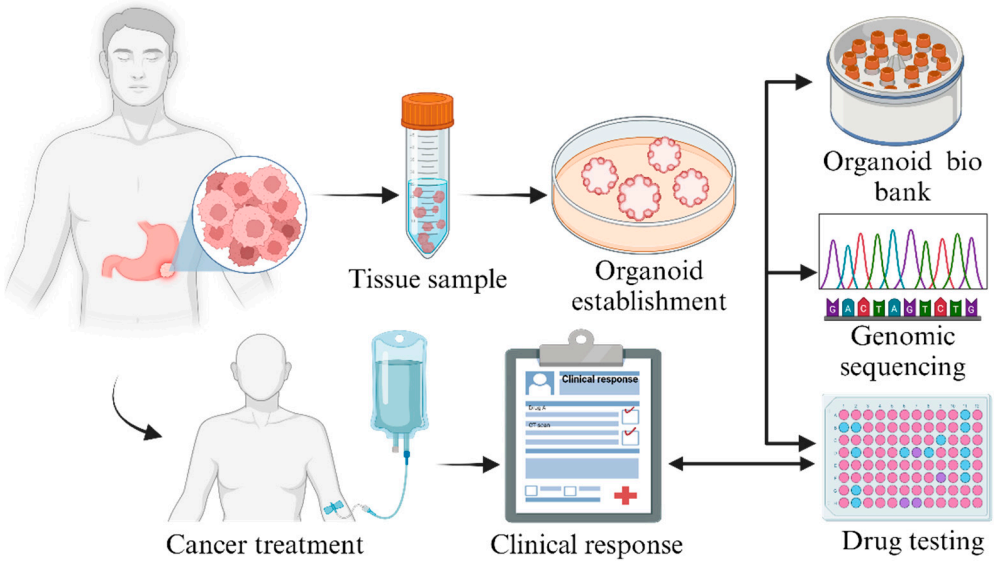

Figure 1. Schematic representation of personalised cancer treatment using GC organoids. The flow chart depicts the establishment of GC organoids from human cancer tissue, the creation of a GC organoid biobank, and the analysis performed (genomic sequencing and drug assays). These patient-derived organoid drug responses can be directly compared to patient clinical responses to assess the clinical utility of this approach and guide personalised treatment in the future.

3. Gastric Cancer Organoid Drug Testing: Summary of Current Methodologies

3.1. Patient Recruitment and Organoid Selection for Drug Assays

Since 2017, 15 studies have utilised patient-derived GC organoids to perform drug testing, with sample sizes ranging from as few as three [56] up to 120 patients (Table 1) [49]. Schmäche et al. recently expanded patient recruitment to include esophagogastric junction (EGJ) adenocarcinoma patients alongside patients with GC, resulting in a large study cohort of 120 patients [49]. The inclusion of organoids derived from the esophagogastric junction (EGJ) and stomach leverages their shared epithelial cell biology to facilitate increased patient recruitment while maintaining biologically consistent and clinically relevant drug screening [33]. While most GC organoid studies collected tumour samples during surgical resection, successful organoid establishment has also been achieved from tissue biopsies and ascites fluid [52]. A few studies have recruited treatment-naïve patients to allow assessment of organoid drug responses in the absence of acquired resistance to chemotherapeutics [49,50,53,56,57], with the largest of these generating 53 organoid lines from treatment-naïve patients [50].

An underreported aspect of GC organoid drug testing is the assessment of culture quality. This includes an evaluation of whether there is minimal contamination by normal gastric organoids and sufficient organoids for independent, replicated drug tests. The Vlachogiannis et al. study performed a variant allele frequency assessment to validate cancer cell populations rather than normal cells within organoids [39]. The culture quality metrics of Zu et al. were described previously, and only organoid lines with adequate expandability scores progressed to drug testing assays [55], whereas other studies focused on organoid lines capable of undergoing multiple passages for drug testing [50,58]. We suggest that future studies use PDOs with sufficient expansion capacity for drug testing, with the amount determined by the number of drugs being tested and the layout/size of the screening setup, and that genetic sequencing be utilised to assess the tumourigenicity of all PDOs and to confirm that they accurately represent the original patient tumour. This validation is crucial for ensuring that organoid responses model tumour responses and can effectively inform patient outcomes. Despite most studies featuring low numbers (3–13) of GC organoid lines, one study established 64 organoid lines, with 40% (26 lines) undergoing FLOT chemotherapy testing in conjunction with a clinical response assessment

to evaluate clinical utility [49]. This was similar to a recent study where 57 patient-derived GC organoid lines were successfully established, and 41 lines were drug-treated using six single and one combination chemotherapeutic regimens [50].

Table 1. Overview of patient recruitment, the efficiency of organoid culture establishment, and drug testing in GC organoid studies.

Year	Author	Tissue Acquisition	Tumour Site	Histology (Lauren Classification)	Total GC Organoids Established	Efficiency of GC Organoid Establishment	Total GC Organoids Drug Tested
2018	Gao M et al. [58]	Endoscope/Surgery	Stomach/MS	N. R	15	N. R	N. R
2018	Vlachogiannis G et al. [39]	Endoscope	GOJ/MS (liver/peritoneum)	Intestinal/Diffuse	5	N. R	5
2018	Yan H et al. [53]	Surgery	Stomach	Intestinal/Diffuse	34	50%	9 from 7 patients
2019	Seidlitz T et al. [51]	Surgery	Stomach/GOJ/MS (lung)	Intestinal/Diffuse/Mixed	20	N. R	4
2019	Steele NG et al. [29]	Surgery	Stomach	Intestinal/Diffuse/Mixed	7	N. R	6
2019	Li J et al. [52]	Ascites puncture	MA	N. R	11	92% #	7
2022	Miao X et al. [56]	Surgery	Stomach	N. R	3	N. R	N. R
2022	Li G et al. [59]	Surgery	Stomach	N. R	12	46%	4
2023	Yoon C et al. [57]	Endoscope	GOJ/stomach	N. R	13	54%	13
2023	Zhang H et al. [60]	Surgery	Stomach	N. R	30	80%	~5
2023	Zu M et al. [55]	Surgery	Stomach	Intestinal/Diffuse/Mixed	12	92% #	12
2023	McDonald H et al. [61]	Endoscope	Stomach	Intestinal/Diffuse/Mixed	8	53%	2
2024	Xu J et al. [62]	Surgery	Stomach	Intestinal/Diffuse/Mixed	21	60% (both GC and normal)	5
2024	Schmäche T et al. [49]	Endoscope	Stomach/GOJ	Intestinal/Diffuse/Mixed	64	61% (both GC and normal)	26
2024	Zhao Y et al. [50]	Surgery	Stomach	Intestinal/Diffuse/Mixed	57	78%	41
2024	Chen G et al. [63]	Surgery	Stomach	Intestinal/Diffuse	28	56%	N. R

N. R not reported; GOJ, gastroesophageal junction; MS, metastatic sites; MA, Malignant ascites; #, noting that cystic, normal-like morphology of organoids in the studies may suggest that the % is slightly inflated.

3.2. Duration of Drug Treatment

Following GC organoid dissociation and plating, most studies initiated drug treatment three days post-seeding of cells (range 24 h to five days) (Table 2) [39,52,57,59]. Treatment duration commonly ranged from two to six days [39]; however, Li et al. extended treatment of ascites-derived GC organoids with mono-chemotherapeutics out to 10 days, then removed drugs from the culture medium and assessed organoid recovery up to day 18 [52]. This method attempts to measure the recovery of drug-resistant populations, but the clinical validity of this approach remains to be tested with paired patient response data. While the field awaits larger studies reporting GC organoid drug responses and clinical outcomes to standardise these procedures, future studies could aim to initiate drug treatment 2–3 days post-seeding and continue treatments for 4–6 days. These protocols align with the GC organoid studies that have validated PDO drug responses against clinical outcomes thus far.

Table 2. Summary of chemotherapy and targeted drugs tested on GC organoids.

Year	Author	Days Post-Seeding When Drugs Were Administered *	Number of Days Drug-Treated	Single Chemotherapeutics Tested	Combination Chemotherapeutics Tested	Targeted Therapeutics Tested
2018	Gao M et al. [58]	2	2	Cisplatin, oxaliplatin, and irinotecan	N/A	N/A
2018	Vlachogiannis G et al. [39]	3	~6–8	5FU, irinotecan, oxaliplatin, etc.	5FU+ cisplatin	Regorafenib, Lapatinib, Erlotinib, etc.
2018	Yan H et al. [53]	1	6	5FU, carboplatin, doxorubicin, etc.	5FU + cisplatin	Afatinib, Alpelisib, Crizotinib. Etc.

Table 2. *Cont.*

Year	Author	Days Post-Seeding When Drugs Were Administered *	Number of Days Drug-Treated	Single Chemotherapeutics Tested	Combination Chemotherapeutics Tested	Targeted Therapeutics Tested
2019	Seidlitz T et al. [51]	1	1–3	5-FU, oxaliplatin, irinotecan, Epirubicin, and docetaxel	5FU + trastuzumab	Trastuzumab, palbociclib, and imatinib
2019	Steele NG et al. [29]	Unknown	2	Epirubicin, oxaliplatin, and 5FU	Epirubicin + oxaliplatin + 5FU	Mubritinib (as a combination)
2019	Li J et al. [52]	3	~9	Oxaliplatin, 5-FU, cis-platinum, docetaxel, irinotecan, Epirubicin, and paclitaxel	N/A	N/A
2022	Miao X et al. [56]	1	4	Paclitaxel, oxaliplatin and 5FU	N/A	N/A
2022	Li G et al. [59]	3	3	5-FU, oxaliplatin, irinotecan, and docetaxel	N/A	N/A
2023	Yoon C et al. [57]	3	4	5FU	FLOT and FOLFOX	N/A
2023	Zhang H et al. [60]	Unknown	3	5FU	N/A	Trastuzumab
2023	Zu M et al. [55]	4	3	5FU, oxaliplatin, cisplatin, irinotecan, paclitaxel, docetaxel, epirubicin, etc.	N/A	Entrectinib, Larotrectinib, DS-8201, and trastuzumab
2023	McDonald H et al. [61]	2–3	2	N/A	ECF, FLOT, FOLFIRI, and FOLFOX.	N/A
2024	Xu J et al. [62]	2	4	5-FU, paclitaxel, oxaliplatin, irinotecan, and epirubicin	N/A	Napabucasin, afatinib, erlotinib, trametinib, flavopiridol, etc.
2024	Schmäche T et al. [49]	1	6	5-FU, oxaliplatin and docetaxel	FLOT	N/A
2024	Zhao Y et al. [50]	1	6	5-FU, oxaliplatin, cisplatin, paclitaxel, doxorubicin, and irinotecan	5-FU + oxaliplatin	N/A
2024	Chen G et al. [63]	Unknown	Unknown	5-FU + oxaliplatin + docetaxel	5-FU + veliparib	Veliparib

* Post-seeding day references vary between studies; some begin with day 0, others with day 1, leading to differences in how days are described (e.g., "2nd day" versus "2 days post-seeding"), N/A not applicable.

3.3. Measurement and Analysis of Drug Sensitivity in Organoids

Following the treatment of GC organoids with chemotherapeutics or targeted drugs, cell viability has typically been measured using CellTiter-Glo (Promega, Madison, WI, USA), Resazurin, or formazan dye assays. Image-based analysis of changes in organoid morphology, size, and quantity has also been used and can be very cost-effective [56,60]. Most GC organoid drug assays have been conducted with three technical replicates, with independent biological replicates uncommon but exhibiting high correlation when performed over two passages of the same line (Pearson correlation $R^2 > 0.87$) [50,53]. Nearly all GC organoid studies used GraphPad Prism software (5.0, 8.0, 9.0 or 10.0) to analyse drug response data, with data normalised to the vehicle control and presented as a dose–response curve. In general, fairly standard methods are used to assess cell viability and present drug sensitivity data across GC organoid drug treatment studies to date.

3.4. Composition of Chemotherapeutic Drugs Being Tested

Most GC organoid studies investigated treatment with single chemotherapies commonly used in the treatment of primary or advanced GC, including 5FU, oxaliplatin, cisplatin, doxorubicin, irinotecan, paclitaxel, epirubicin, and docetaxel (Table 2). Some also included alternative chemotherapeutic drugs that are not commonly used in standard care practice, such as hydroxy camptothecin, vincristine, pirarubicin, semustine, nimustine, and etoposide (Table 2) [39,53,55,63]. The drug concentrations used to treat GC organoids varied in each study, with most studies using a concentration range that spans therapeutic dosing [39,49,51–53,59]. Occasionally, a few studies used single drug doses that far exceed

the Cmax value of the drug obtainable in a patient, calling into question the translational validity of the results obtained [56].

Patients with GC are usually treated with combination therapies; however, only a few studies have concurrently treated GC organoids with combination regimens, and yet again, there is no standardised protocol. For example, Vlachogiannis et al. used equimolar (1:1) dosing of cisplatin to 5-FU to treat GC organoids over a concentration range (0.06–4 μM) and identified a ~10-fold difference in the drug concentration required to inhibit organoid cell growth by 50% (GI50) in organoids derived from a chemo-sensitive and a chemo-resistant patient [39]. In comparison, Yan et al. used drug doses that mimicked the concentration of each drug in the plasma of patients treated with 5-FU (2.46 μM) and cisplatin (11 μM) alone. Using this method, organoid responses to combined 5-FU/cisplatin treatment correlated with clinical response for two patients where tumour response was measured via PET-CT scans [53]. Steele et al. used a different approach in performing combination regimen testing, treating GC organoids with a combination of epirubicin, oxaliplatin, and 5-FU using IC50 drug concentrations calculated for each drug on each organoid line [29]. This resulted in the use of 5-FU doses above those obtainable in the plasma of patients. The correlation to clinical response was limited to 2 organoid lines, of which the in vitro response of only one line mirrored the patient's clinical response [29]. Schmäche et al. investigated organoid sensitivity to a more common chemotherapeutic regimen, FLOT, by calculating the mean IC50 value for each chemotherapeutic alone across a panel of GC organoid lines [49]. Organoids derived from patients who were not responsive to FLOT were the least sensitive to FLOT in vitro in this study [49]. In clinical settings, FLOT is typically administered in a ratio of 5-FU–Leucovorin–oxaliplatin–docetaxel at 52:4:1.7:1, respectively [64]. Yoon et al. used the ratio method to treat organoids with FLOT, i.e., the concentration of each drug in the combination was designed to mimic the ratio administered to patients [57]. McDonald et al. also tested four different combination therapies: FLOT, FOLFIRI, FOLFOX, and ECF (epirubicin, cisplatin, and 5FU) using the ratio method and found one GC organoid line was most sensitive to ECF, while a second was most sensitive to FLOT [61]. Coincidentally, the patients from which these organoids were derived received the drug combination to which their organoids were most sensitive and showed no evidence of recurrence for three years following gastrectomy [61]. Overall, GC organoid responses to combination drug treatment in vitro across these studies were similar to patient responses. However, only a very small number of patients with known clinical response data were included, thus confirmation of the predictive utility of GC organoid drug testing in the clinic awaits further testing in larger patient cohorts. Concurrently, it is essential to standardise methods when treating PDOs with chemotherapeutics. Utilising a concentration range that spans therapeutic dosing is crucial for both monochemotherapeutics and combinations. Additionally, when conducting combination chemotherapy regimens on PDOs, agents should be added together instead of evaluating the responses of these agents individually, as this requires a smaller amount of GC organoid starting material to speed up the process [65].

3.5. Testing Molecularly Targeted Therapies

A handful of studies tested the sensitivity of GC organoids to molecularly targeted drugs, ranging from the evaluation of 1–3 drugs in clinical use for GC to investigational panels of over 30 targeted drugs [53,62,63]. Many provided convincing evidence that GC organoids can predict responses to targeted therapies by confirmation of known sensitivities aligned with the underlying molecular targets of the therapy [29,51,55,60,62]. For example, Steele et al. pretreated GC organoids with mubritinib (HER 2-inhibitor) at varying concentrations (0–200 mmol/L) for 2 h prior to treating with individual chemotherapeutic drugs epirubicin, oxaliplatin, or 5-FU [29]. Pre-treatment with mubritinib enhanced the sensitivity of chemotherapy-resistant, HER2-positive organoid lines to chemotherapeutic agents, resulting in reduced IC50 values compared to HER2-negative lines [29]. Similarly,

GC organoids containing an *ARID1A* mutation were more sensitive to ATR inhibitor VE822, a known synthetic lethal combination [66]. Such studies have the potential to inform the development of novel therapeutic agents and suggest potential tumour-specific sensitivities for patients with GC.

4. Methods of Comparing Organoid Drug Response to Patient Response in the Clinic

One of the primary goals of organoid drug testing is to assess its clinical usefulness in guiding precision medicine by comparing organoid responses with patient responses to the same drugs. Unlike limited studies in other cancers, to date, there are no reports of GC organoid studies that have prospectively changed patient treatment based on organoid testing. Here, we highlight differing approaches used by researchers to compare GC organoid drug responses retrospectively to patient clinical responses (Figure 2). Yan et al. reported anecdotal data from three patients using PET/CT scan results and compared these to the cell viability data of organoids [53]. In contrast, Steele et al. used Becker's criteria to assess patient responses to oxaliplatin, epirubicin, and 5-FU and compared these with the percentage of dead cells following drug treatment of corresponding patient-derived GC organoid lines [29]. The four-tiered Becker's histopathological tumour regression grade method evaluates patient response based on the percentage of remaining tumour tissue after treatment [67]. Of the seven patient-derived organoid lines, comparison with clinical treatment response was only possible for two lines. One showed the highest percentage of dead organoids when treated with oxaliplatin, epirubicin, and 5-FU, correlating with a near-complete pathological response in the patient. In contrast, the other PDO was relatively sensitive to treatment, which is inconsistent with the patient's lack of response to chemotherapy [29]. This discrepancy was explained by the lack of immune components in PDOs and that the patient may have high levels of infiltrating myeloid suppressor cells that suppress T cell activation and can lead to poor tumour response. In the future, this may be addressed with the inclusion of immune cell populations in coculture with PDOs [68]. With a similarly limited number of patients with treatment response data, Vlachogiannis et al. compared five metastatic GC organoid responses with corresponding patient responses using Response Evaluation Criteria in Solid Tumours (RECIST) [39]. This included creating organoid lines before and after paclitaxel treatment from a patient that was initially paclitaxel-sensitive, with the corresponding pre-treatment organoids more sensitive (lower IC50) to paclitaxel than organoids generated at progression, or organoids from two other paclitaxel-resistant patients, consistent with acquired resistance [39]. Understandably, no metrics were given in these seminal early studies to define the level of organoid viability reduction that may predict a patient response, given the small number of patients with clinical response data included.

More recently, Zhao et al. used progression-free survival (PFS) per RECIST [69] to assess the response to 5FU: oxaliplatin treatment for 12 patients with GC. The 6.05 month median PFS from the chemotherapy alone group of the CheckMate 649 trial in patients with advanced GC, gastro-oesophageal junction, and oesophageal cancer [50] was used to dichotomise patients in their cohort into those with 'poor' clinical response (recurrence within 6.05 months, 1/12 patients) versus 'good' response (no recurrence within 6.05 months, 11/12 patients) [50]. AUC values from 5FU: oxaliplatin organoid treatment was used to categorise organoids as resistant (AUC > 50%) or sensitive (AUC < 50%), with all 12 GC organoid lines reported as sensitive to treatment. As such, the correlation between clinical and organoid response to treatment was high (11/12 patients), although further granular detail on the methodology for generation of normalised organoid AUC data and utility of the data to predict relative time to recurrence within the cohort would be informative but was not reported [50].

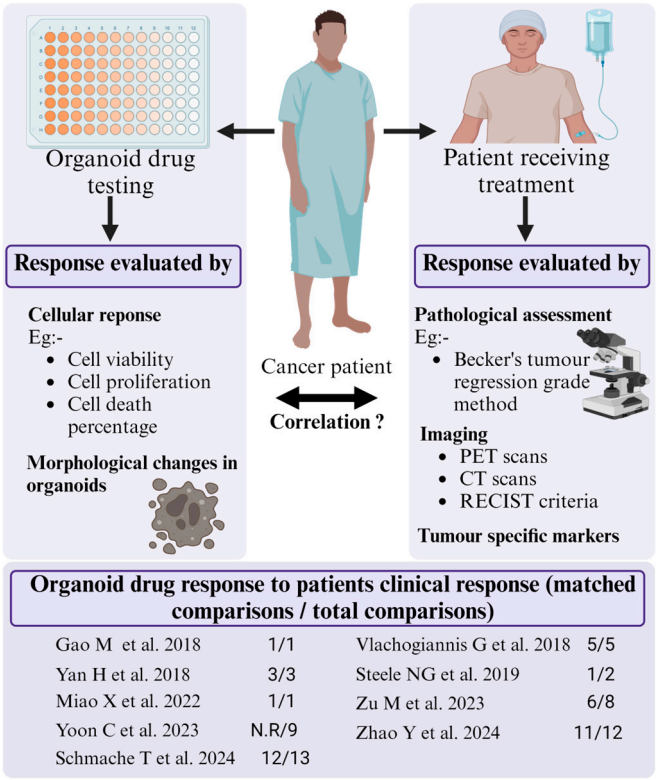

Figure 2. Summary of patient to organoid drug response correlation in GC studies. Patient-derived organoids were treated using various chemotherapeutic and targeted drugs, and responses were evaluated by their cellular responses (cell viability, proliferation, and death percentage) and morphological changes. In contrast, patient clinical responses were evaluated using pathological assessments, imaging, and tumour-specific markers. Patient clinical responses and organoid responses to drug treatment were compared to evaluate the utility of using organoids as a pre-clinical model to guide personalised chemotherapy in GC studies [29,39,49,50,53,55–58]. N.R. not reported.

In a recent pivotal study, Schmäche et al. generated organoids from pre-treatment primary tumour biopsies from patients with esophagogastric cancer (n = 13 exploratory cohort, n = 13 validation cohort with matched clinical response data). Patient clinical response to neoadjuvant FLOT was subsequently categorised using Becker's method into responders (Becker 1a and 1b) and non-responders (Becker 2 and 3) [49]. In vitro, organoid response to combination FLOT treatment was assessed using relative AUC values from organoid dose–response curves [49] and compared to clinical response. A threshold AUC value was applied that correctly predicted FLOT response in all patients in the exploratory cohort. Excitingly, using this methodology and the AUC threshold value enabled the differentiation of responders from non-responders to FLOT in the validation cohort with high sensitivity (90%), specificity (100%), and accuracy (92%) [49]. Of note, the predictive value of in vitro combination treatment of organoids with FLOT outperformed treatment with individual agents from the FLOT regimen. This is the largest study to date with matched clinical response data and one of the few studies with detailed metrics to enable evaluation of the predictive ability of organoid drug testing.

Studies comparing organoid and patient drug responses have primarily relied on imaging or pathological reports for patient evaluation, alongside metrics like AUC from dose–response curves or cell viability percentages for organoid response evaluation. As the field matures, this highlights the necessity for standardised approaches in assessing patient responses through pathology or imaging and evaluation of organoid drug responses. Moreover, larger organoid cohorts are essential to accurately gauge the predictive capability of organoids to guide clinical treatment decisions.

An important aspect to explore is also the timeliness of drug assay result production using PDOs, as this is crucial to guiding individualised treatments. While initial studies did not specify the time required to expand GC PDOs and deliver drug results, more recent research has started to offer some insights. From the few recent studies, organoid drug screening was achieved within 2–3 weeks; however, it remains unclear whether this timeframe includes the entire duration for testing all drugs and if it accounts for the time needed for sample processing and expansion [49,50,53,57]. Studies involving other organoid models have successfully utilised fully automated robotic systems to conduct drug assays [70,71], which could help reduce expansion time, reduce the number of organoids required in smaller plating volumes, minimise the need for Matrigel to decrease costs and variability, and enhance reproducibility.

5. Limitations and Future Directions

GC patient recruitment for organoid studies has improved markedly in the past decade, with a recent study recruiting more than 100 patients [49]. This expansion and inclusion of multiple study sites globally covering a variety of racial backgrounds is promising and will lead to enhanced diversity and representation in patient cohorts. However, the GC organoid culture success rate remains relatively low (67%) due to factors such as culture contamination, normal organoid overgrowth, and limited starting tissue [48], which needs to be taken into account in the design of new studies. Methodological improvements should continue to be integrated into current platforms, including automation and matrix formulations, to improve variability and reduce starting organoid material requirements [72]. Similar to calls in other areas of organoid research, the GC organoid field lacks standardised culture quality metrics to ensure consistency, reliability, and comparability of research outcomes across different studies and laboratories [73].

Thus far, GC organoids have primarily been treated with a limited number of standard-care chemotherapeutics and targeted drugs, while GC organoid treatment with combination chemotherapeutic regimens remains much lower. Current studies have used various methods to perform combination drug testing, with inconsistencies in the definition of drug sensitivity or resistance across organoid lines and patients due to varied metrics used in different studies. Despite this, most organoid combination treatment responses appear consistent with clinical treatment responses; however, the limited number of patients in these studies to date makes it challenging to standardise to a single approach currently, although recent reports are leading the way [49]. These limitations highlight the need for GC organoid studies with larger patient cohorts, linked clinical treatment response data, detailed organoid culture quality scoring, and in vitro treatment response metrics to enable meaningful evaluation of the efficacy of patient-derived organoids in predicting drug treatments in vivo. In the future, the incorporation of additional immune and stromal cell populations into organoid methodologies is challenging but may enable response prediction for therapies targeting the tumour microenvironment [74,75].

6. Conclusions

In recent years, GC patient-derived organoids have shown improved efficiency in culture success rates and drug screening with much refined techniques. However, the current studies are limited in their sample numbers and lack standardised protocols to perform drug testing, making it challenging to evaluate the efficacy of organoids in predicting patient responses. By addressing these limitations, researchers can further advance the GC organoid field, aiding in high-throughput drug screening approaches to guide personalised treatment.

Author Contributions: T.N.S. and S.L.W. contributed to the manuscript conception, and T.N.S. wrote the manuscript. J.A.W., D.L.W. and S.L.W. reviewed and/or edited the manuscript before submission and approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by Tour de Cure (RSP-271-FY2023 to S.L.W); Greaton and Haneco (S.L.W.); the Gastroenterological Society of Australia Bushell Post-Doctoral Research Fellowship (S.L.W.); the Faculty of Health Science at the University of Adelaide (S.L.W.); and the South Australian Health and Medical Research Institute (S.L.W.). T.N.S. is supported by the University of Adelaide Research Scholarship.

Data Availability Statement: Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Acknowledgments: This work was supported by Tour de Cure (RSP-271-FY2023 to S.L.W); Greaton and Haneco (S.L.W.); the Gastroenterological Society of Australia Bushell Post-Doctoral Research Fellowship (S.L.W.); the Faculty of Health Science at the University of Adelaide (S.L.W.); and the South Australian Health and Medical Research Institute (S.L.W.). T.N.S. is supported by the University of Adelaide Research Scholarship. Figures were created with BioRender.com.

Conflicts of Interest: S.L.W. and D.L.W have equity in GenCirq Inc., which focuses on bacterial cancer therapeutics. All other authors declare no financial or non-financial competing interests.

References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef]
2. De, B.; Rhome, R.; Jairam, V.; Özbek, U.; Holcombe, R.F.; Buckstein, M.; Ang, C. Gastric adenocarcinoma in young adult patients: Patterns of care and survival in the United States. *Gastric Cancer* **2018**, *21*, 889–899. [CrossRef]
3. Smyth, E.C.; Nilsson, M.; Grabsch, H.I.; van Grieken, N.C.T.; Lordick, F. Gastric cancer. *Lancet* **2020**, *396*, 635–648. [CrossRef]
4. Merchant, S.J.; Kim, J.; Choi, A.H.; Sun, V.; Chao, J.; Nelson, R. A rising trend in the incidence of advanced gastric cancer in young Hispanic men. *Gastric Cancer* **2017**, *20*, 226–234. [CrossRef]
5. Laurén, P. The Two Histological Main Types of Gastric Carcinoma: Diffuse and So-Called Intestinal-Type Carcinoma. *Acta Pathol. Microbiol. Scand.* **1965**, *64*, 31–49. [CrossRef]
6. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* **2014**, *513*, 202–209. [CrossRef]
7. Bonelli, P.; Borrelli, A.; Tuccillo, F.M.; Silvestro, L.; Palaia, R.; Buonaguro, F.M. Precision medicine in gastric cancer. *World J. Gastrointest. Oncol.* **2019**, *11*, 804–829. [CrossRef]
8. Guan, W.-L.; He, Y.; Xu, R.-H. Gastric cancer treatment: Recent progress and future perspectives. *J. Hematol. Oncol.* **2023**, *16*, 57. [CrossRef]
9. Girshally, R.; Demtröder, C.; Albayrak, N.; Zieren, J.; Tempfer, C.; Reymond, M.A. Pressurized intraperitoneal aerosol chemotherapy (PIPAC) as a neoadjuvant therapy before cytoreductive surgery and hyperthermic intraperitoneal chemotherapy. *World J. Surg. Oncol.* **2016**, *14*, 253. [CrossRef]
10. Takei, S.; Kawazoe, A.; Shitara, K. The New Era of Immunotherapy in Gastric Cancer. *Cancers* **2022**, *14*, 1054. [CrossRef]
11. Marabelle, A.; Le, D.T.; Ascierto, P.A.; Di Giacomo, A.M.; De Jesus-Acosta, A.; Delord, J.-P.; Geva, R.; Gottfried, M.; Penel, N.; Hansen, A.R.; et al. Efficacy of Pembrolizumab in Patients with Noncolorectal High Microsatellite Instability/Mismatch Repair–Deficient Cancer: Results from the Phase II KEYNOTE-158 Study. *J. Clin. Oncol.* **2019**, *38*, 1–10. [CrossRef]
12. Marabelle, A.; Fakih, M.; Lopez, J.; Shah, M.; Shapira-Frommer, R.; Nakagawa, K.; Chung, H.C.; Kindler, H.L.; Lopez-Martin, J.A.; Miller, W.H.; et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: Prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. *Lancet Oncol.* **2020**, *21*, 1353–1365.
13. Sohn, B.H.; Hwang, J.-E.; Jang, H.-J.; Lee, H.-S.; Oh, S.C.; Shim, J.-J.; Lee, K.-W.; Kim, E.H.; Yim, S.Y.; Lee, S.H.; et al. Clinical Significance of Four Molecular Subtypes of Gastric Cancer Identified by The Cancer Genome Atlas Project. *Clin. Cancer Res.* **2017**, *23*, 4441–4449. [CrossRef]
14. Kohlruss, M.; Grosser, B.; Krenauer, M.; Slotta-Huspenina, J.; Jesinghaus, M.; Blank, S.; Novotny, A.; Reiche, M.; Schmidt, T.; Ismani, L.; et al. Prognostic implication of molecular subtypes and response to neoadjuvant chemotherapy in 760 gastric carcinomas: Role of Epstein–Barr virus infection and high- and low-microsatellite instability. *J. Pathol. Clin. Res.* **2019**, *5*, 227–239. [CrossRef]
15. Smyth, E.C.; Wotherspoon, A.; Peckitt, C.; Gonzalez, D.; Hulkki-Wilson, S.; Eltahir, Z.; Fassan, M.; Rugge, M.; Valeri, N.; Okines, A.; et al. Mismatch Repair Deficiency, Microsatellite Instability, and Survival. *JAMA Oncol.* **2017**, *3*, 1197. [CrossRef]
16. Sexton, R.E.; Hallak, M.N.A.; Uddin, M.H.; Diab, M.; Azmi, A.S. Gastric Cancer Heterogeneity and Clinical Outcomes. *Technol. Cancer Res. Treat.* **2020**, *19*, 1533033820935477. [CrossRef]
17. Li, Y.; Feng, A.; Zheng, S.; Chen, C.; Lyu, J. Recent Estimates and Predictions of 5-Year Survival in Patients with Gastric Cancer: A Model-Based Period Analysis. *Cancer Control* **2022**, *29*, 10732748221099227.
18. Alsina, M.; Arrazubi, V.; Diez, M.; Tabernero, J. Current developments in gastric cancer: From molecular profiling to treatment strategy. *Nat. Rev. Gastroenterol. Hepatol.* **2023**, *20*, 155–170. [CrossRef]
19. Siegel, R.L.; Giaquinto, A.N.; Jemal, A. Cancer statistics, 2024. *CA Cancer J. Clin.* **2024**, *74*, 12–49. [CrossRef]

20. Onoyama, T.; Ishikawa, S.; Isomoto, H. Gastric cancer and genomics: Review of literature. *J. Gastroenterol.* **2022**, *57*, 505–516. [CrossRef]
21. Berger, M.F.; Mardis, E.R. The emerging clinical relevance of genomics in cancer medicine. *Nat. Rev. Clin. Oncol.* **2018**, *15*, 353–365. [CrossRef]
22. Morel, A.; Boisdron-Celle, M.; Fey, L.; Soulie, P.; Craipeau, M.C.; Traore, S.; Gamelin, E. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. *Mol. Cancer Ther.* **2006**, *5*, 2895–2904. [CrossRef]
23. Gu, Y.; Zhang, P.; Wang, J.; Lin, C.; Liu, H.; Li, H.; He, H.; Li, R.; Zhang, H.; Zhang, W. Somatic ARID1A mutation stratifies patients with gastric cancer to PD-1 blockade and adjuvant chemotherapy. *Cancer Immunol. Immunother.* **2023**, *72*, 1199–1208. [CrossRef]
24. Brittain, H.K.; Scott, R.; Thomas, E. The rise of the genome and personalised medicine. *Clin. Med.* **2017**, *17*, 545–551. [CrossRef]
25. De Thaye, E.; Van De Vijver, K.; Van Der Meulen, J.; Taminau, J.; Wagemans, G.; Denys, H.; Van Dorpe, J.; Berx, G.; Ceelen, W.; Van Bocxlaer, J.; et al. Establishment and characterization of a cell line and patient-derived xenograft (PDX) from peritoneal metastasis of low-grade serous ovarian carcinoma. *Sci. Rep.* **2020**, *10*, 6688. [CrossRef]
26. Katt, M.E.; Placone, A.L.; Wong, A.D.; Xu, Z.S.; Searson, P.C. In Vitro Tumor Models: Advantages, Disadvantages, Variables, and Selecting the Right Platform. *Front. Bioeng. Biotechnol.* **2016**, *4*, 12. [CrossRef]
27. Domcke, S.; Sinha, R.; Levine, D.A.; Sander, C.; Schultz, N. Evaluating cell lines as tumour models by comparison of genomic profiles. *Nat. Commun.* **2013**, *4*, 2126. [CrossRef]
28. Ertel, A.; Verghese, A.; Byers, S.W.; Ochs, M.; Tozeren, A. Pathway-specific differences between tumor cell lines and normal and tumor tissue cells. *Mol. Cancer* **2006**, *5*, 55. [CrossRef]
29. Steele, N.G.; Chakrabarti, J.; Wang, J.; Biesiada, J.; Holokai, L.; Chang, J.; Nowacki, L.M.; Hawkins, J.; Mahe, M.; Sundaram, N.; et al. An Organoid-Based Preclinical Model of Human Gastric Cancer. *Cell. Mol. Gastroenterol. Hepatol.* **2019**, *7*, 161–184. [CrossRef]
30. Tentler, J.J.; Tan, A.C.; Weekes, C.D.; Jimeno, A.; Leong, S.; Pitts, T.M.; Arcaroli, J.J.; Messersmith, W.A.; Eckhardt, S.G. Patient-derived tumour xenografts as models for oncology drug development. *Nat. Rev. Clin. Oncol.* **2012**, *9*, 338–350. [CrossRef]
31. Drost, J.; Clevers, H. Organoids in cancer research. *Nat. Rev. Cancer* **2018**, *18*, 407–418. [CrossRef]
32. Bender, E. Q&A: Hans Clevers. *Nature* **2015**, *521*, S15.
33. Seidlitz, T.; Koo, B.-K.; Stange, D.E. Gastric organoids—An in vitro model system for the study of gastric development and road to personalized medicine. *Cell Death Differ.* **2021**, *28*, 68–83. [CrossRef]
34. Song, H.; Park, J.Y.; Kim, J.-H.; Shin, T.-S.; Hong, S.A.; Huda, M.N.; Kim, B.J.; Kim, J.G. Establishment of Patient-Derived Gastric Cancer Organoid Model from Tissue Obtained by Endoscopic Biopsies. *J. Korean Med. Sci.* **2022**, *37*, e220. [CrossRef]
35. Fujii, M.; Shimokawa, M.; Date, S.; Takano, A.; Matano, M.; Nanki, K.; Ohta, Y.; Toshimitsu, K.; Nakazato, Y.; Kawasaki, K.; et al. A Colorectal Tumor Organoid Library Demonstrates Progressive Loss of Niche Factor Requirements during Tumorigenesis. *Cell Stem Cell* **2016**, *18*, 827–838. [CrossRef]
36. Sachs, N.; Papaspyropoulos, A.; Zomer-Van Ommen, D.D.; Heo, I.; Böttinger, L.; Klay, D.; Weeber, F.; Huelsz-Prince, G.; Iakobachvili, N.; Amatngalim, G.D.; et al. Long-term expanding human airway organoids for disease modeling. *EMBO J.* **2019**, *38*, e100300. [CrossRef]
37. Dekkers, J.F.; Whittle, J.R.; Vaillant, F.; Chen, H.-R.; Dawson, C.; Liu, K.; Geurts, M.H.; Herold, M.J.; Clevers, H.; Lindeman, G.J.; et al. Modeling Breast Cancer Using CRISPR-Cas9–Mediated Engineering of Human Breast Organoids. *JNCI J. Natl. Cancer Inst.* **2020**, *112*, 540–544. [CrossRef]
38. Kim, M.; Mun, H.; Sung, C.O.; Cho, E.J.; Jeon, H.-J.; Chun, S.-M.; Jung, D.J.; Shin, T.H.; Jeong, G.S.; Kim, D.K.; et al. Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening. *Nat. Commun.* **2019**, *10*, 3991. [CrossRef]
39. Vlachogiannis, G.; Hedayat, S.; Vatsiou, A.; Jamin, Y.; Fernández-Mateos, J.; Khan, K.; Lampis, A.; Eason, K.; Huntingford, I.; Burke, R.; et al. Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* **2018**, *359*, 920–926. [CrossRef]
40. Narasimhan, V.; Wright, J.A.; Churchill, M.; Wang, T.; Rosati, R.; Lannagan, T.R.M.; Vrbanac, L.; Richardson, A.B.; Kobayashi, H.; Price, T.; et al. Medium-throughput Drug Screening of Patient-derived Organoids from Colorectal Peritoneal Metastases to Direct Personalized Therapy. *Clin. Cancer Res.* **2020**, *26*, 3662–3670. [CrossRef]
41. Huo, C.; Zhang, X.; Gu, Y.; Wang, D.; Zhang, S.; Liu, T.; Li, Y.; He, W. Organoids: Construction and Application in Gastric Cancer. *Biomolecules* **2023**, *13*, 875. [CrossRef]
42. Wallaschek, N.; Niklas, C.; Pompaiah, M.; Wiegering, A.; Germer, C.-T.; Kircher, S.; Brändlein, S.; Maurus, K.; Rosenwald, A.; Yan, H.H.N.; et al. Establishing Pure Cancer Organoid Cultures: Identification, Selection and Verification of Cancer Phenotypes and Genotypes. *J. Mol. Biol.* **2019**, *431*, 2884–2893. [CrossRef]
43. Pang, M.-J.; Burclaff, J.R.; Jin, R.; Adkins-Threats, M.; Osaki, L.H.; Han, Y.; Mills, J.C.; Miao, Z.-F.; Wang, Z.-N. Gastric Organoids: Progress and Remaining Challenges. *Cell. Mol. Gastroenterol. Hepatol.* **2022**, *13*, 19–33. [CrossRef]
44. Liu, Y.Y.; Wu, D.K.; Chen, J.B.; Tang, Y.M.; Jiang, F. Advances in the study of gastric organoids as disease models. *World J. Gastrointest. Oncol.* **2024**, *16*, 1725–1736. [CrossRef]
45. Ren, X.; Chen, W.; Yang, Q.; Li, X.; Xu, L. Patient-derived cancer organoids for drug screening: Basic technology and clinical application. *J. Gastroenterol. Hepatol.* **2022**, *37*, 1446–1454. [CrossRef]

46. Verduin, M.; Hoeben, A.; De Ruysscher, D.; Vooijs, M. Patient-Derived Cancer Organoids as Predictors of Treatment Response. *Front. Oncol.* **2021**, *11*, 641980. [CrossRef]
47. Bartfeld, S.; Bayram, T.; van de Wetering, M.; Huch, M.; Begthel, H.; Kujala, P.; Vries, R.; Peters, P.J.; Clevers, H. In vitro expansion of human gastric epithelial stem cells and their responses to bacterial infection. *Gastroenterology* **2015**, *148*, 126–136.e6. [CrossRef]
48. Jiang, K.L.; Wang, X.X.; Liu, X.J.; Guo, L.K.; Chen, Y.Q.; Jia, Q.L.; Yang, K.M.; Ling, J.H. Success rate of current human-derived gastric cancer organoids establishment and influencing factors: A systematic review and meta-analysis. *World J. Gastrointest. Oncol.* **2024**, *16*, 1626–1646. [CrossRef]
49. Schmäche, T.; Fohgrub, J.; Klimova, A.; Laaber, K.; Drukewitz, S.; Merboth, F.; Hennig, A.; Seidlitz, T.; Herbst, F.; Baenke, F.; et al. Stratifying esophago-gastric cancer treatment using a patient-derived organoid-based threshold. *Mol. Cancer* **2024**, *23*, 10. [CrossRef]
50. Zhao, Y.; Li, S.; Zhu, L.; Huang, M.; Xie, Y.; Song, X.; Chen, Z.; Lau, H.C.-H.; Sung, J.J.-Y.; Xu, L.; et al. Personalized drug screening using patient-derived organoid and its clinical relevance in gastric cancer. *Cell Rep. Med.* **2024**, *5*, 101627. [CrossRef]
51. Seidlitz, T.; Merker, S.R.; Rothe, A.; Zakrzewski, F.; Von Neubeck, C.; Grützmann, K.; Sommer, U.; Schweitzer, C.; Schölch, S.; Uhlemann, H.; et al. Human gastric cancer modelling using organoids. *Gut* **2019**, *68*, 207–217. [CrossRef]
52. Li, J.; Xu, H.; Zhang, L.; Song, L.; Feng, D.; Peng, X.; Wu, M.; Zou, Y.; Wang, B.; Zhan, L.; et al. Malignant ascites-derived organoid (MADO) cultures for gastric cancer in vitro modelling and drug screening. *J. Cancer Res. Clin. Oncol.* **2019**, *145*, 2637–2647. [CrossRef]
53. Yan, H.H.N.; Siu, H.C.; Law, S.; Ho, S.L.; Yue, S.S.K.; Tsui, W.Y.; Chan, D.; Chan, A.S.; Ma, S.; Lam, K.O.; et al. A Comprehensive Human Gastric Cancer Organoid Biobank Captures Tumor Subtype Heterogeneity and Enables Therapeutic Screening. *Cell Stem Cell* **2018**, *23*, 882–897.e11. [CrossRef]
54. Nanki, K.; Toshimitsu, K.; Takano, A.; Fujii, M.; Shimokawa, M.; Ohta, Y.; Matano, M.; Seino, T.; Nishikori, S.; Ishikawa, K.; et al. Divergent Routes toward Wnt and R-spondin Niche Independency during Human Gastric Carcinogenesis. *Cell* **2018**, *174*, 856–869.e17. [CrossRef]
55. Zu, M.; Hao, X.; Ning, J.; Zhou, X.; Gong, Y.; Lang, Y.; Xu, W.; Zhang, J.; Ding, S. Patient-derived organoid culture of gastric cancer for disease modeling and drug sensitivity testing. *Biomed. Pharmacother.* **2023**, *163*, 114751. [CrossRef]
56. Miao, X.; Wang, C.; Chai, C.; Tang, H.; Hu, J.; Zhao, Z.; Luo, W.; Zhang, H.; Zhu, K.; Zhou, W.; et al. Establishment of gastric cancer organoid and its application in individualized therapy. *Oncol. Lett.* **2022**, *24*, 447. [CrossRef]
57. Yoon, C.; Lu, J.; Kim, B.-J.; Cho, S.-J.; Kim, J.H.; Moy, R.H.; Ryeom, S.W.; Yoon, S.S. Patient-Derived Organoids from Locally Advanced Gastric Adenocarcinomas Can Predict Resistance to Neoadjuvant Chemotherapy. *J. Gastrointest. Surg.* **2023**, *27*, 666–676. [CrossRef]
58. Gao, M.; Lin, M.; Rao, M.; Thompson, H.; Hirai, K.; Choi, M.; Georgakis, G.V.; Sasson, A.R.; Bucobo, J.C.; Tzimas, D.; et al. Development of Patient-Derived Gastric Cancer Organoids from Endoscopic Biopsies and Surgical Tissues. *Ann. Surg. Oncol.* **2018**, *25*, 2767–2775. [CrossRef]
59. Li, G.; Ma, S.; Wu, Q.; Kong, D.; Yang, Z.; Gu, Z.; Feng, L.; Zhang, K.; Cheng, S.; Tian, Y.; et al. Establishment of gastric signet ring cell carcinoma organoid for the therapeutic drug testing. *Cell Death Discov.* **2022**, *8*, 6. [CrossRef]
60. Zhang, H.; Qin, Y.; Jia, M.; Li, L.; Zhang, W.; Li, L.; Zhang, Z.; Liu, Y. A gastric cancer patient-derived three-dimensional cell spheroid culture model. *Am. J. Cancer Res.* **2023**, *13*, 964–975.
61. McDonald, H.G.; Harper, M.M.; Hill, K.; Gao, A.; Solomon, A.L.; Bailey, C.J.; Lin, M.; Barry-Hundeyin, M.; Cavnar, M.J.; Mardini, S.H.; et al. Creation of EGD-Derived Gastric Cancer Organoids to Predict Treatment Responses. *Cancers* **2023**, *15*, 3036. [CrossRef]
62. Xu, J.; Gong, J.; Li, M.; Kang, Y.; Ma, J.; Wang, X.; Liang, X.; Qi, X.; Yu, B.; Yang, J. Gastric cancer patient-derived organoids model for the therapeutic drug screening. *Biochim. Biophys. Acta (BBA)-Gen. Subj.* **2024**, *1868*, 130566. [CrossRef] [PubMed]
63. Chen, G.; Han, R.; Wang, L.; Ma, W.; Zhang, W.; Lu, Z.; Wang, L. Establishment of patient-derived organoids and a characterization based drug discovery platform for treatment of gastric cancer. *Cancer Cell Int.* **2024**, *24*, 489. [CrossRef] [PubMed]
64. Al-Batran, S.E.; Homann, N.; Pauligk, C.; Goetze, T.O.; Meiler, J.; Kasper, S.; Kopp, H.G.; Mayer, F.; Haag, G.M.; Luley, K.; 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*, 1948–1957. [PubMed]
65. Wensink, G.E.; Elias, S.G.; Mullenders, J.; Koopman, M.; Boj, S.F.; Kranenburg, O.W.; Roodhart, J.M.L. Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. *npj Precis. Oncol.* **2021**, *5*, 30. [CrossRef]
66. Williamson, C.T.; Miller, R.; Pemberton, H.N.; Jones, S.E.; Campbell, J.; Konde, A.; Badham, N.; Rafiq, R.; Brough, R.; Gulati, A.; et al. ATR inhibitors as a synthetic lethal therapy for tumours deficient in ARID1A. *Nat. Commun.* **2016**, *7*, 13837. [CrossRef]
67. Becker, K.; Mueller, J.D.; Schulmacher, C.; Ott, K.; Fink, U.; Busch, R.; Böttcher, K.; Siewert, J.R.; Höfler, H. Histomorphology and grading of regression in gastric carcinoma treated with neoadjuvant chemotherapy. *Cancer* **2003**, *98*, 1521–1530. [CrossRef]
68. Magré, L.; Verstegen, M.M.A.; Buschow, S.; van der Laan, L.J.W.; Peppelenbosch, M.; Desai, J. Emerging organoid-immune co-culture models for cancer research: From oncoimmunology to personalized immunotherapies. *J. ImmunoTher. Cancer* **2023**, *11*, e006290. [CrossRef]

69. Janjigian, Y.Y.; Shitara, K.; Moehler, M.; Garrido, M.; Salman, P.; Shen, L.; Wyrwicz, L.; Yamaguchi, K.; Skoczylas, T.; Campos Bragagnoli, A.; et al. First-line nivolumab plus chemotherapy versus chemotherapy alone for advanced gastric, gastro-oesophageal junction, and oesophageal adenocarcinoma (CheckMate 649): A randomised, open-label, phase 3 trial. *Lancet* **2021**, *398*, 27–40. [CrossRef]
70. Du, Y.; Li, X.; Niu, Q.; Mo, X.; Qui, M.; Ma, T.; Kuo, C.J.; Fu, H. Development of a miniaturized 3D organoid culture platform for ultra-high-throughput screening. *J. Mol. Cell Biol.* **2020**, *12*, 630–643. [CrossRef]
71. Hirt, C.K.; Booij, T.H.; Grob, L.; Simmler, P.; Toussaint, N.C.; Keller, D.; Taube, D.; Ludwig, V.; Goryachkin, A.; Pauli, C.; et al. Drug screening and genome editing in human pancreatic cancer organoids identifies drug-gene interactions and candidates for off-label treatment. *Cell Genom.* **2022**, *2*, 100095. [CrossRef] [PubMed]
72. Tebon, P.J.; Wang, B.; Markowitz, A.L.; Davarifar, A.; Tsai, B.L.; Krawczuk, P.; Gonzalez, A.E.; Sartini, S.; Murray, G.F.; Nguyen, H.T.L.; et al. Drug screening at single-organoid resolution via bioprinting and interferometry. *Nat. Commun.* **2023**, *14*, 3168. [CrossRef] [PubMed]
73. Sandoval, S.O.; Cappuccio, G.; Kruth, K.; Osenberg, S.; Khalil, S.M.; Méndez-Albelo, N.M.; Padmanabhan, K.; Wang, D.; Niciu, M.J.; Bhattacharyya, A.; et al. Rigor and reproducibility in human brain organoid research: Where we are and where we need to go. *Stem Cell Rep.* **2024**, *19*, 796–816. [CrossRef] [PubMed]
74. Recaldin, T.; Steinacher, L.; Gjeta, B.; Harter, M.F.; Adam, L.; Kromer, K.; Mendes, M.P.; Bellavista, M.; Nikolaev, M.; Lazzaroni, G.; et al. Human organoids with an autologous tissue-resident immune compartment. *Nature* **2024**, *633*, 165–173. [CrossRef]
75. Dijkstra, K.K.; Cattaneo, C.M.; Weeber, F.; Chalabi, M.; Van De Haar, J.; Fanchi, L.F.; Slagter, M.; Van Der Velden, D.L.; Kaing, S.; Kelderman, S.; et al. Generation of Tumor-Reactive T Cells by Co-culture of Peripheral Blood Lymphocytes and Tumor Organoids. *Cell* **2018**, *174*, 1586–1598.e12. [CrossRef]