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Review

Precision Medicine for Peritoneal Carcinomatosis—Current Advances in Organoid Drug Testing and Clinical Applicability

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Abstract: Peritoneal carcinomatosis from gastrointestinal tumours is considered a poor prognostic factor, with a median overall survival of six to nine months in the absence of intervention. The advent of patient-derived organoid cultures (PDOs) has provided a breakthrough in personalised medicine, allowing researchers and clinicians to model the complexity and heterogeneity of individual tumours in vitro. PDOs hold great promise in this field, as variations in the management of peritoneal carcinomatosis due to differences in the method of delivery of chemotherapeutics, drug selection, exposure duration, and tumour pathology make it impractical to use a single, standardised treatment regimen. We aim to summarise the methodologies and limitations of studies encapsulating organoids derived from peritoneal metastases to encourage design considerations that may improve future clinical relevance, standardise protocols, and address translational challenges in personalising treatment strategies.

Keywords: peritoneal metastasis; organoids; precision medicine

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1. Introduction

Peritoneal carcinomatosis arising from gastrointestinal cancers is a challenging clinical scenario, associated with a bleak prognosis, often presenting a median overall survival of 6 to 9 months in the absence of intervention [1]. However, advancements in therapeutic approaches have brought about notable improvements in outcomes. The introduction of cytoreductive surgery combined with intraperitoneal chemotherapy has significantly enhanced prognosis, with reported median survival rates ranging from 27 to 41 months [2–4].

As early as 1955, Weisberger et al. explored the direct infusion of chemotherapy into the peritoneal cavity [5]. This method allows for higher intraperitoneal drug concentrations compared to intravenous administration, as the effectiveness of systemic chemotherapy within the peritoneal cavity is hindered by the presence of the peritoneal–plasma barrier [6–8]. This barrier constitutes an intricate three-dimensional structure involving peritoneal cells, interstitial tissue space, and microvessels that are responsible for regulating intraperitoneal homeostasis [8]. Moreover, the peritoneal–plasma barrier acts as a protective shield, limiting drug absorption into the systemic circulation. This dual effect reduces the systemic toxicity of drugs administered into the peritoneum, while also extending the exposure of tumour cells to the therapeutic agent.

The peritoneal carcinomatosis index (PCI) is a measure of the spread of the disease in the peritoneum. Patients with a low PCI typically undergo an aggressive therapeutic approach, involving cytoreductive surgery, peritonectomy, and a multi-visceral resection, coupled with the administration of hyperthermic intraperitoneal chemotherapy (HIPEC). This comprehensive treatment regimen is aimed at achieving a complete curative response. On the other hand, in patients with a high PCI, where a curative outcome is less feasible, palliative intraperitoneal chemotherapy can be considered alongside systemic chemotherapy [9]. One form of experimental treatment is known as pressurised intraperitoneal aerosol chemotherapy (PIPAC) [1]. This is a minimally invasive technique that delivers chemotherapy directly to the peritoneal cavity as an aerosol, allowing for the targeted and concentrated treatment of peritoneal metastases (PMs). It involves three or more cycles of intraperitoneal chemotherapy and primarily aims to relieve symptoms such as abdominal pain and ascites, control tumour volume, improve quality of life, and prolong progression-free survival (PFS) in patients with unresectable disease [10].

Currently, classes of chemotherapeutics that have been evaluated for intraperitoneal use include taxanes (paclitaxel, Nab-paclitaxel), topoisomerase inhibitors (doxorubicin, irinotecan), platinum-based agents (cisplatin, oxaliplatin), and antimetabolites (5-Fluorouracil, Gemcitabine) [11]. There are many characteristics that make these compounds desirable for intraperitoneal use. This includes limited absorption of these drugs into the systemic circulation via the peritoneal–plasma barrier that limits systemic toxicity and physical properties, such as relatively high molecular weight, hydrophilic characteristics, and ionisation that hinder the rapid clearance of the drug by the peritoneal barrier [12]. Furthermore, these agents are administered at a maximum tolerable dose that is constrained by systemic toxicity, rather than local toxicity, allowing for use of higher concentrations intra-peritoneally [12–14].

Assessing the chemosensitivity of these drugs in vitro is essential for the efficient translation of treatments into affected patients as recurrence rates can be as high as 82% for CRC and up to 32% for appendiceal tumours following cytoreductive surgery [15,16]. Tumour cells, whether cultured or presented as xenografts, have proven to be valuable; however, they fail to faithfully reproduce the complexity of human cancers. Two-dimensional cell line models perform poorly in preserving cell polarity and heterogeneity, simulating tumour–stromal or cell–cell interactions, and capturing the dynamic interactions between tumour cells and the surrounding extracellular matrix or tumour microenvironment (TME) [17,18]. Additionally, genetic variations accumulate during prolonged in vitro maintenance and passage [17]. Patient-derived xenograft models (PDXs) while reproducible, present long-term genomic stability along with clinical applicability; however, they encounter limitations such as sample accessibility, the time required to generate xenograft models, economic constraints, and ethical concerns, hindering their extensive use in basic research and personalised medicine [18,19]. Considering the limitations of traditional models like 2D cell lines and PDXs, organoids offer a promising alternative for assessing drug sensitivity and advancing personalised therapy. Patient-derived organoid cultures are three-dimensional structures grown from patient tissue samples, obtained through a biopsy or surgery. These organoids retain genetic, histological, and functional characteristics of the epithelial compartment over multiple passages and can be cryopreserved for storage without losing their fidelity to the original tissue [20].

2. Overview of Organoid Technology and Its Relevance to Personalised Therapy

Organoids are three-dimensional in vitro tissue analogues originating from human stem cells, organ-specific progenitor cells, or dissociated tumour tissues that have gained

prominence across various scientific disciplines, with a particular focus on their applications in cancer and disease research. Cultivated in an ECM-based medium with high success rates for gastrointestinal tissues, organoids closely mimic the epithelial component of primary tissues. They retain the histopathological features of the epithelial lineage, genetic profiles, mutational landscapes, and responses to therapy, making them unique tools for investigating tumourigenesis and cancer progression in vitro [20–23]. Organoids offer significant potential for translational studies, having been successfully established for various human tumour pathologies. Notably, compared to patient-derived xenograft models, organoid establishment is more time-efficient, requires less tissue, and ensures the maintenance of primary tumour characteristics following prolonged passages [24].

The structural makeup of organoids is a key determinant of their success in modelling complex biological systems. Organoids typically consist of progenitor and differentiated cell types arranged in a manner that recapitulates the architecture of the original tissue. This cellular diversity is crucial for capturing the intricate cellular interactions and functionalities observed in vivo. The process of organoid formation involves the self-organisation of cells into tissue-specific structures. This self-assembly is driven by cell signalling pathways and interactions within the microenvironment. The resulting organoids can range from simple structures, such as intestinal crypts, to more complex and organ-like formations such as brain organoids [25]. In the realm of cancer research, organoids have emerged as invaluable tools for studying tumour development, progression, and response to therapy. Patient-derived organoids (PDOs) are generated from individual patient tumour tissues, allowing personalised models that retain the genetic and molecular characteristics of the original cancer along with providing a platform for preclinical testing and biobanking (Figure 1).

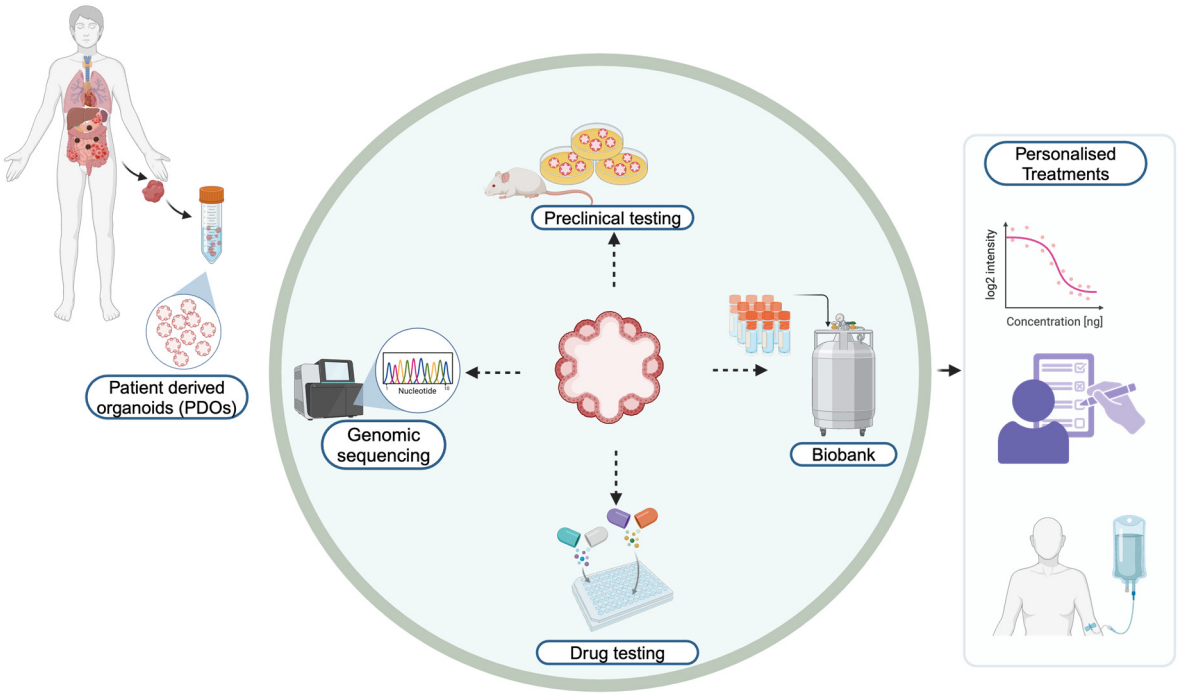

Figure 1. Illustration depicting patient-derived organoids (PDOs) and their potential in personalising treatments for patients by providing an ex vivo platform for genomic sequencing, drugs, and preclinical testing. Created with Biorender.com.

Numerous investigations have utilised colorectal (CRC) and PM organoids to explore tumour development [26–28]. Organoids originating from CRC-PM and metastatic gastroesophageal cancer exhibit a remarkable similarity to their respective biopsies, en-

compassing morphology, mutational spectrum, and expression patterns of crucial clinical diagnosis markers like caudal-related homeobox 2 (CDX2) and cytokeratin 7 (CK7) [23,29]. Recently, our team demonstrated a 68% success rate in establishing organoids from PM from 28 patients [29]. Similarly to other gastrointestinal tract cancer organoid studies, we demonstrated that cellular and genomic heterogeneity present in the original tumour was preserved in the generated organoids [29]. Investigators from the Utrecht Cancer Centre in the Netherlands also generated organoids from peritoneal tumour samples and drug-tested these with common HIPEC agents including oxaliplatin and mitomycin C [30]. They attempted to evaluate cell responses to existing HIPEC regimens and correlate these findings with patient responses. They concluded that this model was a robust in vitro system capable of serving as a preclinical model to study HIPEC protocols, predict patient responses, and test novel combination strategies that may augment the efficacy of HIPEC [30].

3. Current Methodologies for Organoid Drug Testing

3.1. Mimicking Hyperthermic Drug Treatment In Vitro

To test the utility of organoid cultures to predict responses to HIPEC, mimicking hyperthermic chemotherapy is a critical aspect of experimental design to replicate clinical conditions accurately. Hyperthermia not only enhances the cytotoxic effects of chemotherapeutic agents but also influences drug penetration and tumour response (Figure 2) [31]. It is believed to achieve this through several mechanisms, including promoting greater uptake of therapeutic agents into cells, increasing integration into DNA, and disrupting normal DNA repair processes (Figure 2) [32]. Equally, the choice of chemotherapeutic drug is also essential to consider, as different drugs used for HIPEC have varying efficacy and mechanisms of action under hyperthermic conditions. Additionally, the duration of organoid exposure to both heat and chemotherapy to model the clinical HIPEC procedure is warranted, as it may impact drug efficacy [31]. Currently, there are two primary chemotherapy protocols, oxaliplatin and mitomycin C (MMC), that are employed for intraperitoneal chemotherapy use in HIPEC as their large molecular weights allow for higher concentrations within the peritoneal cavity while maintaining lower systemic levels [33,34]. Peritoneal Cmax values for MMC range from approximately 10 to 50 μg/mL when administering MMC at doses of 30–40 mg/m^2 and oxaliplatin values of 200 to 400 μg/mL when administering oxaliplatin at doses of 200–460 mg/m^2 for a 30–120 min duration [33,35–37]. Thus, significant variability exists across institutions regarding drug selection, drug doses, and HIPEC duration [38].

Various studies have used organoid models from PM to investigate chemotherapy responses (Table 1). Eight of the sixteen studies have evaluated and mirrored the effects of hyperthermic chemotherapy on PDOs (Table 1). HIPEC involves the delivery of heated chemotherapy, typically at 42 °C directly into the peritoneal cavity to enhance chemotherapeutic efficacy [31]. Heat is a distinctive feature of HIPEC and is not present in PIPAC or other forms of intraperitoneal chemotherapy. Mimicking this condition in vitro may be important to ensure an accurate representation of the in vivo treatment regime [15,16]. Forsythe et al. demonstrated differences in response to chemotherapeutics in hyperthermic and normothermic conditions by mapping DNA damage responses in patient-derived organoids (PDOs) from peritoneal mesothelioma, where drug efficacy and DNA damage differed significantly in the presence of heat [39]. These results were also mirrored by Varinelli et al., who demonstrated that hyperthermia amplified the efficacy of chemotherapy in CRC-PM organoids in comparison to normothermia [31]. PDOs were treated with chemotherapeutic agents, including oxaliplatin at concentrations ranging from 100 to 300 μg/mL and MMC at 10–30 μg/mL. Patient-derived organoids were exposed to these

drugs under hyperthermic and normothermic conditions, 37 °C and 42.5 °C, respectively, for durations of 30 to 90 min to mimic the clinical HIPEC regimen. Organoid drug responses were analysed using IC50 values to identify dose-dependent cytotoxic effects, with their correlation to clinical outcomes assessed as trends in survival [31].

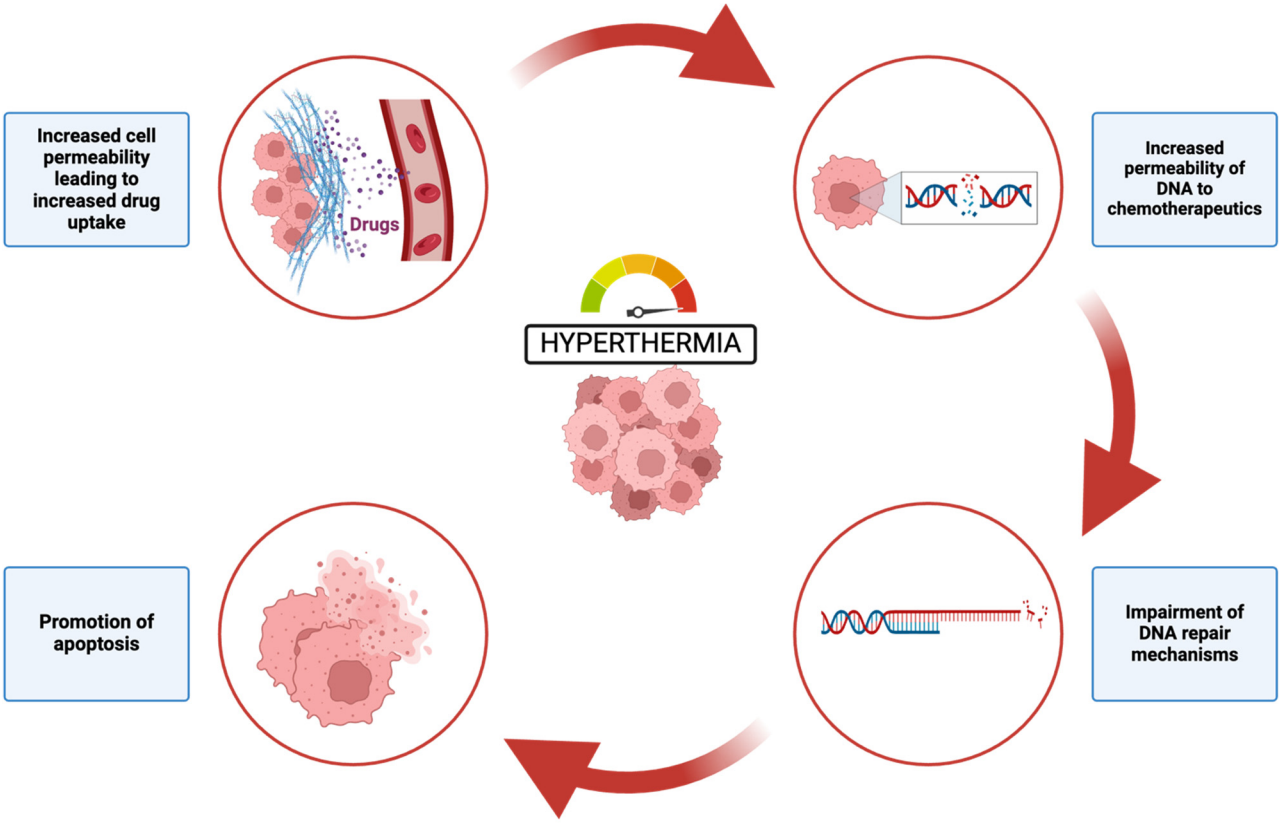

Figure 2. Mechanism of hyperthermia to enhance chemotherapeutic efficacy. Hyperthermic conditions increase cell membrane permeability, promoting greater drug uptake and the integration of chemotherapeutic agents into DNA strands [32]. Additionally, hyperthermia disrupts normal DNA repair processes, including the inhibition of repair enzymes and the accumulation of DNA damage. These effects sensitise tumour cells to therapy, induce cellular stress, and drive apoptosis, providing a synergistic therapeutic effect when combined with chemotherapy. Created with BioRender.com.

Roy et al. also exposed CRC organoids to oxaliplatin, mitomycin C, irinotecan, and paclitaxel at 37 °C or 43 °C for 30/60/90 min, to demonstrate that in vitro hyperthermia significantly enhanced chemotherapy efficacy, but the duration of chemotherapy treatment did not. High-dose hyperthermic oxaliplatin (460 mg/m^2) was the most effective cytotoxic treatment assessed with repeated incubations every 3 days, for 9 days, mimicking intraperitoneal cycles [38]. An alternate study investigated HIPEC regimens using colorectal and appendiceal peritoneal tumour PDOs cultured in a collagen–hyaluronic acid matrix for seven days [40]. PDOs were treated with either 37 °C or 42 °C oxaliplatin (200 mg/m^2) and mitomycin C (40 mg/L) for 2 h, or high-dose oxaliplatin (460 mg/m^2) for 30 min, mimicking the Prodigé 7 trial protocol [41]. Hyperthermia was shown to significantly enhance cytotoxicity in both primary tumour PDO types for mitomycin C and high-dose oxaliplatin (19% vs. 54%, $p < 0.001$ and 27% vs. 53%, $p = 0.002$, respectively), but surprisingly high-dose oxaliplatin was less effective than the low-dose regimen in both primary tumour PDO types (25% vs. 54%, $p < 0.001$ and 31% vs. 53%, $p = 0.008$, respectively) [40].

Presumably, the close mimicking of in vivo conditions will yield more clinically relevant drug responses in organoids; however, this hypothesis requires validation in larger studies with additional clinical outcome data. Normothermic drug delivery may not en-

tirely replicate the therapeutic environment or drug responses observed during HIPEC and therefore could be a major limitation to experimental study design. Mimicking hyperthermic conditions will be beneficial in the optimisation of drug combinations and concentrations for personalised therapy and may be critical to enhance the translational relevance of preclinical findings [38,39].

3.2. Optimising Drug Dose In Vitro for PDO Drug Sensitivity Testing

There is variation in methodology across PDO drug testing studies regarding drug dosages used. Commonly either a standardised dose across all PDO samples is used for comparison or IC50 values specific to the PDO being evaluated are used to evaluate organoid responses to treatment. Using standardised drug doses that mimic clinically relevant concentrations used in patients offers a direct assessment of whether the chemotherapy achieves its expected effect at the therapeutic clinical dose. This mirrors the HIPEC procedure where the peritoneal cavity is directly perfused with heated chemotherapy and replicates the localised drug exposure seen in treatment. In contrast, IC50 values determine the concentration of a drug required to inhibit cell viability by 50% and aim to assess drug sensitivity quantitatively, comparing the efficacy of different agents or combinations. This test provides detailed pharmacodynamic data, which can identify sensitive and resistant tumour subpopulations and optimise dose selection for potential clinical application. In a study by Ubink et al., the clinical doses of MMC and oxaliplatin used in patients appeared to be insufficient to eliminate all tumour cells completely based on IC50 values for the five CRC peritoneal tumour PDOs tested [30]. This suggested the current HIPEC dosing regimen may be insufficient to eradicate residual microscopic disease, consistent with the high recurrence rates seen with CRPM receiving CRS-HIPEC [41,42]. Similarly, a second study used 22 lines of PDOs generated from CRC-PM to tailor HIPEC [31]. PDOs were tested against five different HIPEC regimens and efficacy was assessed through cell viability assays to generate drug IC50 values for comparison between lines. Results showed that two particular PDO lines (C3 and PM4) were highly sensitive, retaining only ~5% viable cells, while others were resistant to all HIPEC regimens [31]. Treatment with mitomycin C (MMC) alone, or in combination with cisplatin or low-dose oxaliplatin, reduced cell viability most consistently and effectively across PDO lines, in comparison to high-dose oxaliplatin and a cisplatin/doxorubicin combination. Despite these promising results, clinically relevant doses failed to eliminate all tumour cells, highlighting the limitations in current HIPEC regimens and the value of PDOs in assessing this shortfall [31]. The insufficiency of clinical HIPEC doses in eradicating residual microscopic disease has also prompted further investigations into optimising treatment regimens, including increased MMC doses in trials such as GECOP-MMC [43]. Another study optimised drug screening methods using PDOs from 23 CRC metastases to correlate organoid responses with clinical outcomes. PDOs were exposed to 5-fluorouracil (5-FU), irinotecan, and oxaliplatin-based chemotherapy, with optimisation involving the exclusion of N-acetylcysteine (NAC) from the medium, biphasic curve fitting for combination screens, and testing different readouts and treatment setups. The area under the curve was identified as the most robust drug response metric, and correlations between PDO sensitivity and patient response were demonstrated (coefficients of 0.58 for 5-FU, 0.61 for irinotecan, and 0.60 for oxaliplatin). PDO resistance to oxaliplatin was associated with shorter patient progression-free survival (3.3 vs. 10.9 months), and prior patient exposure to 5-FU/capecitabine was reflected in PDO resistance ($p = 0.003$) [44].

Assessing drug efficacy using tumour organoids poses challenges, yet this methodology may be able to overcome questions that are difficult to answer safely in patients. In vitro modelling of standardised HIPEC drug doses across PDOs can potentially identify

a correlation between in vitro findings and clinical outcomes, while the use of IC50 doses can help assess drug sensitivity and optimal dosing specific to the patient and tumour for personalised treatment strategies [23]. However, a significant limitation of many studies is the reliance on standardised clinical drug dosing, which may not accurately reflect the variable pharmacokinetics and pharmacodynamics in different patient-specific contexts. There is currently insufficient clinical outcome data to determine which metric is more useful for predicting patient outcomes. Therefore, we recommend a combined approach where possible for PM PDO drug testing to effectively replicate in vitro HIPEC conditions and assess patient and tumour-specific responses.

3.3. Assessing the Efficacy of Experimental Drugs Using PDO

Given the poor prognosis for peritoneal carcinomatosis, research is ongoing to improve treatment regimens for this disease. Organoids have the potential to provide a critical first step in developing novel treatments or assessing patient- and tumour-specific drug responses. The following studies explore the use of patient-derived organoids (PDOs) to evaluate the efficacy of experimental drugs and drug combinations in the treatment of PM disease.

Liu and colleagues 2024 investigated the sensitivity of CRC organoids to HIPEC with a non-standard platin-based compound, lobaplatin, and standard treatment with oxaliplatin in vitro. Thirty-two PDOs generated from CRC peritoneal metastases were subjected to six drug concentrations of oxaliplatin and lobaplatin at 42 °C, with exposure times of 30 min and 60 min, respectively. By comparing the average organoid growth inhibition rate, these authors were able to demonstrate a heightened sensitivity to lobaplatin [89.7% (95%CI: 87.0–92.3%)] when compared to oxaliplatin [39.6% (95%CI: 32.1–47.0%)] [45]. Similarly, Ubink et al. developed PDOs from colorectal peritoneal metastases or ascites to evaluate HIPEC with oxaliplatin or MMC at 42 °C. To enhance MMC efficacy, investigational Ataxia Telangiectasia and Rad3-related (ATR) inhibitors (VE-821 and VX-970) were successfully combined with MMC, supporting a model where ATR inhibition disrupts the DNA replication checkpoint, allowing unrestrained replication despite MMC-induced DNA damage, thereby increasing cytotoxicity [30]. The combined efficacy of ATR inhibitors with MMC is yet to be explored in clinical trials and no biomarkers of response to this combination treatment using organoids were presented in this study. ATR inhibitors such as ART0380, have progressed into clinical trials but the primary focus has been on solid tumours and DNA damage response pathways, rather than in combination with MMC in HIPEC or related contexts [46,47]. Zeng et al. also demonstrated enhanced hyperthermic synergism with raltitrexed in CRC-derived PDOs observed in 11/22 of their tested organoid lines when treated under hyperthermic (43 °C), as compared to normothermic conditions (37 °C). Raltitrexed is an anti-metabolic folate analogue that specifically inhibits thymidylate synthase (TS), a key enzyme in thymidine triphosphate synthesis, leading to DNA breakage, apoptosis, and enhanced TS inhibition with prolonged effects [48,49]. This agent has also previously been shown to provide a clinical benefit in some patients who have failed initial standard-care systemic chemotherapy [48]. This enhanced potency under hyperthermic conditions supports the use of PDOs as robust in vitro models to evaluate the synergistic effects of hyperthermic chemotherapies [50].

Oxaliplatin resistance mechanisms were investigated using ten colorectal peritoneal tumours and primary tumour PDOs derived from malignant ascites collected during PIPAC. PDOs exhibited various hallmarks of aggressive CRC biology, with the majority of both PM and paired primary tumours classified as Consensus Molecular Subtype 4 (CMS4) [51]. PM-derived organoid cultures showed resistance to oxaliplatin, thought to be a result of high expression of glutamate-cysteine ligase (GCLC), which promoted

the detoxification of oxaliplatin through glutathione synthesis. HIPEC conditions (1 h at 42 °C) were mimicked with oxaliplatin ± L-buthionine-sulfoximine (BSO). Results demonstrated enhanced oxaliplatin efficacy when glutathione synthesis was inhibited via a glutamate–cysteine ligase (GCLC) blockade by BSO, highlighting a potential therapeutic strategy to overcome resistance [51].

Building on the use of organoids to assess experimental therapies, it is important to acknowledge the limitations of these models. While organoids offer specific advantages, they do not fully capture tissue and cellular complexity present in vivo. Major limitations in translating in vitro results to the clinical picture are the absence of a tumour microenvironment and immune cells, systemic circulation, and drug metabolism, limiting the assessment of phenomena such as pharmacokinetics, pharmacodynamics, and immune responses [52]. Several studies have successfully developed animal HIPEC models, utilising both closed and open coliseum techniques, to evaluate treatment efficacy in peritoneal carcinomatosis [53–55]. Of note, no in vivo HIPEC model has incorporated PDOs to date. Thus, the potential for PDOs to improve the predictive accuracy of treatment outcomes in preclinical models, albeit commonly using immunocompromised animals but with the potential to use humanised mouse models, remains untapped. Combining the precision of patient-derived organoids (PDOs) with the physiological relevance of animal models may drive the improved translation of preclinical findings when assessing the efficacy of novel combination and experimental drugs.

3.4. Intra-Patient Peritoneal Tumour Heterogeneity

The peritoneal cavity presents unique challenges, as aggressive metastatic deposits can harbour diverse subpopulations of tumour cells [39]. In patients with metastatic peritoneal disease, caution must be exercised when interpreting chemotherapeutic responses based on testing from a single site, as metastatic lesions can exhibit significant heterogeneity [39]. This heterogeneity reflects variations in genetic, molecular, and microenvironmental characteristics of the metastatic deposits, vascularity, and exposure to chemotherapeutic agents [39]. The transcriptional heterogeneity within CRC peritoneal metastases isolated from the same patient has been investigated and illustrated that variations in gene expression, even from different sites within the peritoneum and primary tumour, can influence therapeutic targets and resistance [56]. Next-generation sequencing (NGS) revealed that these peritoneal metastases displayed a complex interplay of genomic and transcriptomic alterations, contributing to poor therapeutic response and highlighting the need for precision medicine approaches tailored to the intra-tumoural variability of each patient [56]. This was consistent with the varied molecular alterations reported across different regions of tumours in treatment-naïve CRC patients with peritoneal carcinomatosis. Such heterogeneity challenges the development of effective treatments as it creates variability in tumour progression and drug response and may explain the varied clinical outcomes during HIPEC [57]. These findings reinforce the potential of PDOs as a bridge between genomic insights and clinical application. PDOs can be tailored to reflect tumour heterogeneity, especially if generated from multiple intrapatient sample sites, allowing for the testing of personalised therapies that account for variable molecular profiles.

Most studies listed in Table 1 have relied on single-site tumour sampling, limiting their ability to capture the full extent of intrapatient intermetastatic heterogeneity. In contrast, Radomski et al. generated PDOs from 31 patients from a variety of metastatic peritoneal sites obtained from appendiceal (n = 6), colon (n = 3), small bowel (n = 2), gastric (n = 1), and adrenal (n = 1). The viability of PDOs was tested following exposure to various chemotherapeutics and demonstrated intrapatient drug response heterogeneity, thought to reflect differences in molecular and microenvironment across sample sites;

however, the molecular mechanisms governing this were not evaluated [58]. Similarly, 16 PDO lines were developed from mesothelioma samples from seven patients in a separate study, derived from different tumour locations accessed during surgical procedures. PDOs were then exposed to cisplatin and MMC at 37 °C and 42 °C [39]. PM deposits derived from the colon or ovary from a single patient demonstrated distinct differences in their responses to chemotherapy. The colon-derived PDO demonstrated high sensitivity to both normothermic and hyperthermic cisplatin and MMC treatments when compared to controls ($p < 0.05$), whereas the ovary-derived PDO was selectively sensitive to cisplatin and resistant to MMC ($p < 0.05$) [39]. These studies highlight the heterogeneity of tumour responses within a single patient, as anatomical sites from multiple patients exhibited significant disease variability in treatment responses suggesting underlying disease clonality [39]. Narasimhan et al. generated patient-derived organoids (PDOs) from two distinct tumour sites per patient to better represent this heterogeneity and predict therapy responses [29]. Organoids were successfully generated for 19 of 28 patients (68%) and drug screening was performed using a USA clinically certified drug panel test. This initial screen included up to 87 drugs, later refined to a CRC-specific panel of 35 drugs. This study emphasised that multiple-tumour sampling could be incorporated whenever feasible to guide the selection of therapies effectively across potentially heterogeneous disease sites, thereby improving personalised treatment strategies [29].

In summary, caution should be exercised when interpreting results derived from a single tumour harvest site in patients with peritoneal disease. Experimental design should account for this variability and consider sample acquisitions from multiple tumour sites [39,58].

4. Correlating Patient Clinical Outcomes with Organoid Drug Responses

Assessing the correlation of PDO drug responses in vitro with clinical outcomes for peritoneal disease following treatment presents significant challenges, largely due to varying extents of cytoreductive surgery (CRS) completion, types of chemotherapeutics used, the influence of residual disease on progression, limitations in imaging modalities, and differential responses to HIPEC and systemic treatments depending on the site of the metastatic deposits [39,59]. Additionally, factors like tumour microenvironment interactions, genetic heterogeneity, and the complexity of patient-specific factors may contribute to discrepancies between organoid results and patient outcomes [60]. While there is no universally accepted standardised method to correlate PDO responses with clinical outcomes, several commonly employed strategies provide valuable insights. These include the use of progression-free survival (PFS) and overall survival (OS) as key clinical endpoints, reflecting the time a patient remains disease-free or survives following treatment (Table 1). Additionally, RECIST (Response Evaluation Criteria in Solid Tumours) is frequently used to evaluate radiologic tumour responses, providing an objective measure of treatment efficacy [61]. However, peritoneal metastases may not be measurable lesions on staging CT, and therefore, applying the RECIST criteria is not always feasible or uniform [62,63]. In the absence of a reliable clinical measurement of response, establishing correlations with PDO responses will always be challenging. Alternative imaging tools such as diffuse weight magnetic resonance imaging (DW-MRI) or fibroblast activation protein inhibitor positron emission tomography (FAPI-PET) may be better suited to detect PM and should be considered as a possible solution in future studies. These metrics can be combined with drug sensitivity assays performed on PDOs, to form the basis for assessing whether in vitro findings align with patient outcomes and hence the potential predictive value of PDO drug testing for guiding treatment choice.

Recent studies have made significant strides in establishing PDO models from peritoneal disease samples to explore personalised treatment strategies by correlating results with patient outcomes. Most studies correlated organoid drug responses with PFS and OS data, but a select few assessed PM PDO drug responses in comparison to patient radiologic findings [1,31,50,58,64–66]. Zeng et al. reported the case of a patient with sigmoid cancer and liver metastases who underwent systemic therapy to reduce tumour size before surgical intervention [50]. During surgery, peritoneal thickening was observed, leading to a palliative resection of omental nodules, liver metastasis, and an abdominal wall tumour [50]. The patient then underwent five rounds of HIPEC, after which a CT scan indicated a reduction in the size of the remaining nodules. However, a notable limitation of this study is the absence of RECIST to objectively quantify the changes in nodule sizes. The absence of RECIST for radiological evaluation faces a significant limitation in the objective assessment of treatment responses as RECIST provides a standardised and widely accepted framework to evaluate changes in tumour size, offering quantifiable criteria for partial response, stable disease, or progressive disease [61]. Thus, a lack of RECIST makes it challenging to compare results across studies or correlate in vitro findings from organoid models with clinical outcomes in a reproducible manner. In contrast, Liu et al., (2022) reported a patient with mucinous appendiceal adenocarcinoma, where organoid testing was used to predict treatment response [66]. Radiological responses were measured using RECIST (Response Evaluation Criteria in Solid Tumours), supporting a correlation between organoid sensitivity and patient outcomes [66]. Building on this, Prieto et al. (2023) also correlated organoid sensitivity with patient outcomes by assessing radiologic response via computed tomography imaging RECIST and clinical follow up, showing a positive association between in vitro sensitivity and clinical response [1]. Thus, the use of RECIST is an important measure to provide an objective measure of treatment efficacy.

Additional metrics such as PFS and OS are more commonly used to correlate PM patient outcomes, given that there is not always a useful tumour deposit to track via imaging for RECIST and imaging may poorly estimate disease burden for PM. Narasimhan et al. (2020) monitored changes in nine patient outcomes such as progression-free survival (PFS) and overall survival to assess potential correlations between peritoneal CRC metastatic PDO drug sensitivity and disease status [29]. In two cases, PDO-guided therapy was prospectively used to guide treatment choice, resulting in a treatment change for both patients, one of whom had a partial response despite previously progressing on multiple rounds of standard-care chemotherapy [29]. In a separate study focused on peritoneal mesothelioma, Forsythe et al. (2020) effectively correlated PFS and disease progression with CRC-PM organoid models tested with cisplatin and mitomycin C to explore responses in five patients [40]. The first patient remained disease free at 22 months post-MMC perfusion with 19% organoid viability upon drug testing in vitro. Another patient perfused with cisplatin, had a 23% organoid viability during drug testing in vitro, and remained progression-free for one year. One patient with incomplete cytoreduction with cisplatin perfusion, showed 47% organoid viability, and died seven months post-surgery, while another patient showed high organoid viability at 47% and died 5 months post-surgery despite receiving a complete cytoreductive surgery [39]. Varinelli et al. also focused on organoids from CRC-PM specifically to tailor HIPEC based on a variety of HIPEC schemes including MMC, oxaliplatin high- and low-dose monotherapies, and combination therapies of MMC + Cisplatin and Doxorubicin + Cisplatin under hyperthermic conditions (42.5 °C) [31]. Organoid drug sensitivities were used to direct chemotherapeutic agent choice for HIPEC in five of twelve patients. Patient response was assessed through follow-up imaging and PFS and three of five patients remained recurrence-free [31]. Although limited in number, these studies collectively highlight the predictive potential of organoid

models for both standard chemotherapy and targeted therapies by directly correlating organoid drug responses with patient PFS and OS. However, these studies are limited by the small number of patients and clinical outcome data available to date. Larger studies are needed to fully assess the true value of patient-derived organoid drug testing in guiding treatment choices for these patients.

A reliable comparison of PDO drug testing results to patient outcomes requires several key strategies. First, standardised protocols for organoid culture and drug testing are essential to ensure consistency across studies, minimising technical variability. Patient-specific factors, including genetic background, tumour heterogeneity, clinical staging, and prior treatment responses, should be carefully documented to enable meaningful comparisons. Direct correlations and longitudinal tracking between organoid results and clinical endpoints such as overall survival, progression-free survival, recurrence rates, and radiologic assessments using RECIST criteria are critical for validating the predictive potential of PDOs [61]. Incorporating tumour microenvironment components, such as stromal and immune cells, into organoid models can also improve their relevance by better mimicking in vivo conditions. By integrating these strategies, organoid models can be more reliably used to predict clinical responses, making them a valuable tool for personalising treatment strategies.

Table 1. Studies investigating drug sensitivities using PDOs derived from peritoneal carcinomatosis.

Year	Author	Source of Tissue Acquisition	Histology	Organoids Established	Establishment Success Rate	Clinical Response Correlation	No. of Patients Assessed for Clinical Response/Total No. of Recruited Patients	Total No. of Drugs Tested	Conditions
2024	Liu et al. [45]	Resected tumour	Colorectal adenocarcinoma, peritoneal metastases	32	55%	NR	NA	2—Lobaplatin and oxaliplatin	Hyperthermic—42 °C for 30 and 60 min
2024	Varinelli et al. [31]	Resected tumour	Colorectal and signet cell mucinous adenocarcinoma	22	78.6%	Yes–Progression-free survival (PFS), overall survival (OS), and radiological evidence	5/12	4–Oxaliplatin, cisplatin/mitomycin C, mitomycin C and doxorubicin/cisplatin	All 4 chemotherapeutics were delivered at 37 °C and 42.5 °C
2024	Martinez-Quintanilla et al. [67]	Resected tumour	Pseudomyxoma peritonei	50	49%	NR (correlated to drug response of organoids grown in mouse xenograft models only)	NA	3—Encorafenib and targeted therapies	Normothermia
2024	Radomski et al. [58]	Resected tumour	Appendiceal, colorectal, small bowel, gastric, and adrenal	6, 3, 2, 1 and 1	56%	Yes—Progression-free survival (PFS) and overall survival (OS)	13/13	5—Mitomycin C, irinotecan, doxorubicin, oxaliplatin, cisplatin, MMC/cisplatin, oxaliplatin/irinotecan, cisplatin/doxorubicin	Normothermia
2023	Prieto et al. [1]	Resected tumour	Colorectal adenocarcinoma and peritoneal metastases	1	100%	Yes—Progression-free survival (PFS), overall survival (OS), and RECIST	1/1	3—Oxaliplatin, 5-Flourouracil, SN-38	Normothermia
2023	Forsythe et al. [39]	Resected tumour	Peritoneal mesothelioma	16	94.1%	Yes—Progression-free survival (PFS) and overall survival (OS)	5/7	2—Mitomycin C and cisplatin	Delivered MMC (120 min) and cisplatin (90 min) at 37 °C and 42 °C
2023	Choi et al. [65]	Malignant ascites and pleural effusions	Pancreatic, gastric, and breast cancer	39, 21, and 10, respectively	48.7%, 33.3%, and 20.0%, respectively	Yes—Progression-free survival (PFS) and overall survival (OS)	58/58	9-Flourouracil, oxaliplatin, irinotecan, Gemcitabine, Nab-paclitaxel, Erlotiib, epirubicin, cisplatin, carboplatin	Normothermic
2022	Liu et al. [66]	Resected tumour	Mucinous appendiceal adenocarcinoma	1	100%	Yes—RECIST for radiological analysis	1/1	8—5-Flourouracil, oxaliplatin, SN38, Apatinib, Dasatinib, Docetaxel, Regorafenib, Everolimus	Normothermic
2022	Laoukili et al. [51]	Malignant Ascites	Appendiceal peritoneal primary	6	10.5%	NR	NA	1—Oxaliplatin	Delivered at 42 °C for 60 min
2021	Zeng et al. [50]	Resected tumour	Colorectal Adenocarcinoma Pertioneal Metastases	22	100%	Yes—Progression-free survival (PFS), overall survival (OS), and radiological data	1/22	7—Mitomycin C, oxaliplatin, raltitrexed, 5-Flourouracil, lobaplatin, Gemcitabine, Abraxane	Delivered at 43 °C for 90min

Table 1. *Cont.*

Year	Author	Source of Tissue Acquisition	Histology	Organoids Established	Establishment Success Rate	Clinical Response Correlation	No. of Patients Assessed for Clinical Response/Total No. of Recruited Patients	Total No. of Drugs Tested	Conditions
2020	Forsythe et al. [40]	Resected tumour	Colorectal adenocarcinoma and appendiceal peritoneal metastases	17	74%	NR	NA	2—MMC and oxaliplatin	Hyperthermic chemotherapy delivered for 30 min (oxaliplatin) or 120min (MMC) at 37 °C and 42 °C
2020	Narsimhan et al. [29]	Resected tumour	Colorectal adenocarcinoma and peritoneal metastases	19	68%	Yes—Progression-free survival (PFS) and overall survival (OS)	9/19	87 chemotherapeutics and targeted therapies	Normothermic
2019	Votanopoulos et al. [68]	Resected tumour	LAMN and Adenocarcinoma from appendiceal origin	6	75%	Yes—Progression-free survival (PFS) and overall survival (OS)	3/12	5—FOLFOX, FOLFIRI, Regorafenib, 5-Flourouracil, oxaliplatin	Normothermic
2019	Phan et al. [64]	Resected tumour	High-grade serous ovarian carcinoma, peritoneal high-grade serous carcinoma, and ovarian sarcoma	2, 1 and 1	NR	Yes—Progression Free Survival (PFS) and Overall survival (OS)	4/4	15	Normothermic
2019	Ubink et al. [30]	Resected tumour	Colorectal adenocarcinoma and peritoneal metastases	5	33%	NR	NA	2—Mitomycin C and oxaliplatin	Hyperthermic chemotherapy delivered for 30 min (oxaliplatin) or 90 min (MMC) at 42 °C
2017	Roy et al. [38]	Resected tumour	Colorectal adenocarcinoma and peritoneal metastases	4	NR	NR	NA	6—Mitomycin C, 5-Flourouracil, oxaliplatin, irinotecan, doxorubicin, paclitaxel	Delivered at 30/60/90 min at 37 °C and 43 °C

NR: Not reported; NA: not applicable; RECIST: response evaluation criteria in solid tumours; PFS: progression-free survival; OS: overall survival.

5. Future Directions and Limitations for Personalising Treatment for Peritoneal Disease

Each study investigating organoids generated from peritoneal metastases has a distinct experimental design, making it challenging to draw consistent conclusions [69]. Variations include the delivery of chemotherapeutics at normal versus hyperthermic temperatures, differences in drug selection and exposure durations, the pathological characteristics of the tumour, and the addition of factors such as specific growth conditions. These methodological discrepancies result in significant heterogeneity across studies, complicating the interpretation of results and the establishment of standardised experimental designs and protocols [69].

Personalising treatment for peritoneal disease faces multiple challenges. The diversity in tumour pathology and grades, such as low-grade versus high-grade, mucinous versus non-mucinous, or primary tissue of origin such as colorectal adenocarcinoma versus ovarian origin, all complicate the tailoring of therapies, as each subtype responds differently to treatment [70,71]. While PDOs offer the potential for personalised drug testing, creating these models is still time-intensive and challenging due to the complex structure of some peritoneal tumours, often delaying results needed for timely clinical decisions [59,72]. To address these limitations, droplet emulsion microfluidics with temperature control and dead-volume minimization are now emerging to generate thousands of Micro-Organospheres (MOSs) from low-volume patient tissues, providing a rapid, scalable, and clinically relevant model for precision oncology [73]. Furthermore, individual responses to therapies vary significantly, even among patients with similar diagnoses, adding complexity to prediction and personalisation which is further compounded by the limited number of studies correlating organoid drug test findings with clinical outcome data [74]. Large-scale data supporting personalised approaches for peritoneal disease are lacking, as most treatment strategies are adapted from protocols developed for other cancer types. Finally, personalised treatments are resource-intensive and costly, posing a barrier to implementation, especially in low-volume centres.

Organoid drug testing for patients with PM offers significant potential for advancing personalised therapies, but several considerations are crucial to enhancing its translational relevance. Given the heterogeneity of peritoneal tumours, sampling multiple tumour sites is essential to capture the diverse subpopulations of cells that influence therapeutic responses. Additionally, incorporating hyperthermic conditions during in vitro drug testing can accurately model the environment of HIPEC, particularly as only a handful of studies to date have utilised such conditions. To ensure clinical applicability, drug-testing results should be correlated with patient outcomes to validate predictive accuracy and refine treatment protocols. Additionally, establishing an expert consensus on a standardised set of treatment regimens, tailored to patient characteristics, expected toxicities, and local clinical practises, can help reduce variability and improve standardisation. These strategies collectively aim to bridge the gap between preclinical findings and effective, individualised patient care [69].

6. Conclusions

The emergence of patient-derived organoid cultures (PDOs) represents a significant advancement in personalised medicine [75]. It enables researchers and clinicians to recapitulate the intricate and diverse characteristics of individual tumours in a laboratory setting [76]. Future research and clinical trials on organoids and intraperitoneal chemotherapy for PM should emphasise multicentre collaborations to pool resources, standardise methodologies, and increase the diversity of patient-derived organoid models. Clinical trials should integrate organoid-based drug sensitivity testing to personalise HIPEC proto-

cols and validate the predictive accuracy of organoids in treatment outcomes. Establishing collaborative frameworks will facilitate the translation of organoid research into clinical practice, ensuring that novel therapies reach patients safely and efficiently.

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