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INVITED REVIEW

Experimental Modeling and Treatment Strategies for Peritoneal Carcinomatosis

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ABSTRACT

Peritoneal carcinomatosis (PC), associated with a range of gastrointestinal and gynecological malignancies, represents a significant condition characterized by the dissemination of cancer cells within the peritoneal cavity. The advancement of our comprehension of the pathophysiology and therapeutic strategies for PC hinges on utilizing experimental models. This comprehensive review provides an overview of the current experimental models employed in the examination of PC along with the current treatment strategies. The review comprehensively explores the merits and demerits of each model and their respective contributions to our understanding of peritoneal metastasis. This review will serve as a valuable resource for researchers and clinicians engaged in investigating and managing PC, offering direction for future endeavors to refine experimental modeling and clinical outcomes.

Keywords: Experimental models, peritoneal carcinomatosis, treatment

Introduction

Peritoneal carcinomatosis (PC) refers to the widespread dissemination of cancer cells in the peritoneal cavity, forming tumor nodules on the peritoneal surfaces. Since various types of cancer can spread to the peritoneum, PC is highly heterogeneous. The diversity observed in patients with cancer depends on various factors, such as differences in primary cancer treatment strategies, genetic background, age, sex, and epigenetic factors. These factors make it challenging to conduct unbiased clinical research studies.[1,2] However, experimental models can help overcome these limitations and provide insights into the molecular mechanisms implicated in cancer and the efficacy of new treatment options.

To enhance the accuracy of preclinical data, it is crucial to initiate a clear statement outlining the biological problem and provide a comprehensive description of the relevant model, incorporating its advantages and disadvantages.[3] Preclinical experimental models consist of three methods: *in vitro*, *in vivo*, and *in silico*.[4] While *in vitro* models offer some benefits, they are not fully comprehensive in accurately representing the complexity of a patient's condition. Therefore, it is crucial to consider the limitations of such models when studying diseases and developing new treatments. *Ex vivo* models are more complex and representative tools that are commonly used in the evaluation of intraperitoneal (IP) drug delivery and treatment efficacy. However, this type of model lacks several features, such as functional immunity and drug metabolism.[5] *In vivo* models, such as mice, rat, and pig models, closely mimic the patient's condition and are commonly used to study diseases.[6] Novel *in vivo* models, such as patient-derived xenograft (PDX) and transgenic mice models, are created to mimic patient tumors better.[7] To represent the molecular characteristics of tumors and to choose the best treatment option for patients with cancer, individualized preclinical, experimental models need to be generated. To this end, *in silico* methods, known as "dry labs", analyze retrospective and prospective data in computational platforms, such as genome, transcriptome, proteome, and metabolome platforms, to provide insights into the molecular phenomena of malignancies.[8]

This review provides valuable insights by outlining various experimental models used in cancer research. A comprehensive understanding of these models is crucial for developing more

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effective treatments and therapies to fight cancer. By thoroughly exploring the current research landscape, we aim to pave the way for significant advancements in cancer treatment.

Materials and Methods

This narrative review aims to provide a comprehensive overview of the experimental models and treatment strategies for PC. The review synthesizes findings from preclinical and clinical studies, focusing on *in vitro*, *ex vivo*, and *in vivo* models, as well as innovative therapeutic approaches. Given the complexity of PC and its challenging treatment landscape, a thorough examination of relevant literature was conducted using established databases, including PubMed, Scopus, and Web of Science.

Literature Search

A literature search was performed across multiple databases using a combination of relevant keywords, including "PC", "experimental models", "treatment strategies", "animal/*in vitro*/*in vivo* models", "photodynamic", "gene therapy", "IP chemotherapy", and "immunotherapy". The search covered articles published up to February 2024. Studies included preclinical models (*in vitro*, *ex vivo*, *in vivo*, and *in silico*), various treatment modalities, and emerging approaches such as targeted therapies and immunotherapy.

Inclusion and Exclusion Criteria

To ensure relevance to the topic, only articles focusing on the development and use of experimental models for PC and their application in assessing treatment efficacy were included. Both basic science and translational research articles were considered. Studies focusing on other forms of carcinomatosis or not addressing PC-specific treatments were excluded. Review articles, original research papers, and conference proceedings were evaluated for inclusion.

Synthesis of Evidence

The evidence was categorized according to model type (*in vitro*, *ex vivo*, and *in vivo*) and the treatment strategy used. Emphasis was placed on identifying the strengths, limitations, and translational relevance of each model in mimicking human PC. Therapeutic strategies were analyzed in terms of their preclinical efficacy, clinical applicability, and innovative potential.

Study Limitations

As a narrative review, this study does not involve a formal meta-analysis or systematic review process, and as such, does not employ strict quantitative data synthesis. The scope of this review is also limited to articles available in English and may not fully capture all international research.

In summary, this narrative review provides a synthesized understanding of the experimental models used to study PC and the evolving landscape of treatment strategies, with a focus on their translational potential.

Modeling for Peritoneal Carcinomatosis

In vitro Models

The conventional two-dimensional (2D) cell culture technique for cancer research remains the most widely used *in vitro* model. However, this model has several limitations in representations of the natural tumor microenvironment (TME) due to the absence of cellular communication (cell-cell) and interaction (cell-cell and cell-matrix).[9-11] An increasing amount of research indicates that tumor growth is influenced by cancer cells and the surrounding stroma, known as the TME.[12,13] The TME is crucial in enabling cancer cells to acquire key characteristics through reciprocal interaction between cancer cells and TME components, which include both cellular elements and the extracellular matrix (ECM).[14,15] The ECM within the tumor TME serves as a structural framework, composed primarily of collagens, fibronectins, proteoglycans, elastins, and laminin. Additionally, various other molecules are ensnared within this matrix. The cellular constituents of the TME consist of endothelial cells, infiltrating immune cells, pericytes, and fibroblasts.[16] The conventional *in vitro* models cannot replicate the oxygen, pH, and nutrient gradients found in *in vivo* tumors, thus leading to a lack of realistic representation.[11] There is a growing trend in research towards creating three-dimensional (3D) culture systems to address these constraints, which has become essential for advancing tumor studies.[17] In this approach, false results can be reduced, meaning the clinical translation of any novel anticancer drugs can be improved.[18] Several approaches exist to create more real-like PC models by including ECM components in the culture system. Differences between 2D and 3D models and their features are summarized in Figure 1.

Aiming at the significant effect of 3D formation on cancer cell behavior, a study by Chen et al.[19] created a 3D PC spheroid model using patient-derived cells and commercial cell lines. The results showed that the 3D spheroid model has different proliferation kinetics and anoikis resistance with various cancer lines, including YOU, PANC1, HEYA8, CHLA10, and TC71, compared with 2D culturing.[19] On the other hand, to prove the critical role of TME components, a published study by Loessner et al.[20] focused on creating an ovarian TME to replicate PC progression. The study involves ovarian cancer cell-loaded hydrogels with mesothelial cell-layered melt electrospun written scaffolds, with transcriptomic and proliferation analyses performed for the characterization. The results indicated elevated cancer cell proliferation in the co-culture system compared with single-cell type culture.[20]

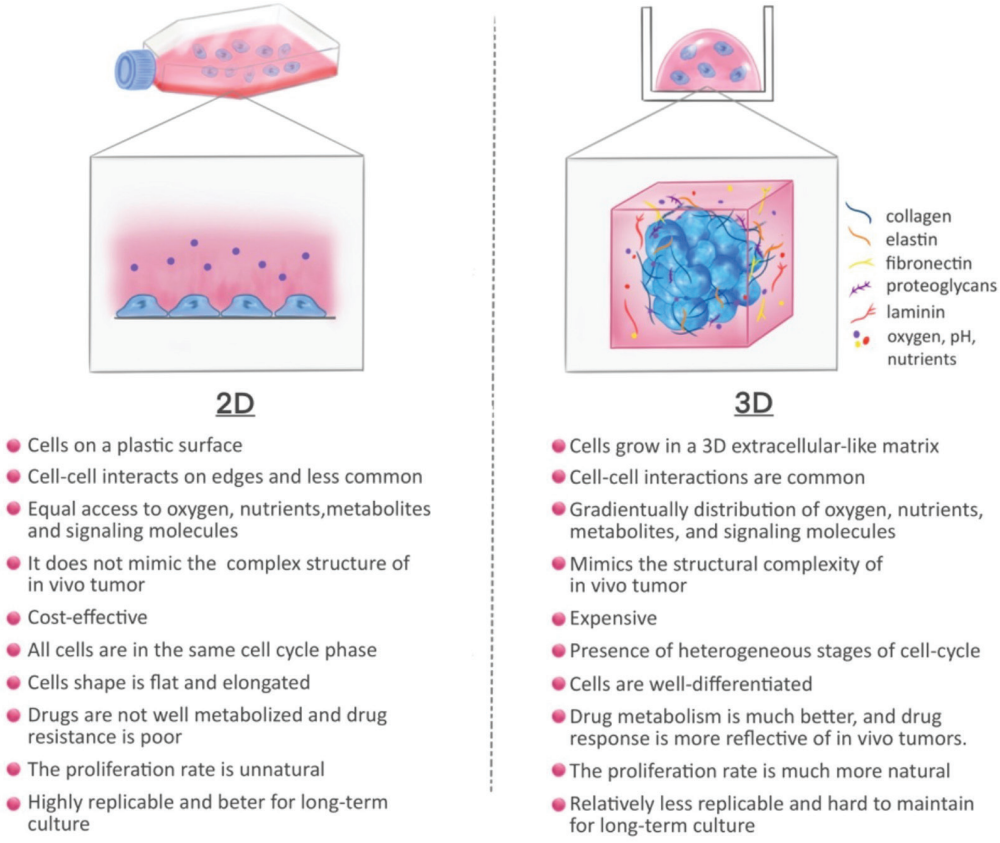

Figure 1. Identical features for 2D versus 3D culture systems
2D: Two-dimensional, 3D: Three-dimensional

Brooks et al.[21] devised an innovative approach utilizing 3D multicellular ovarian cancer spheroids within an omentum-mimicking hydrogel. The authors proposed incorporating patient-derived ascites in future studies to enhance the model's fidelity to the TME.[21] In similar vein, Malacrida et al.[22] generated a four-cell-culture model in plates to investigate the impact of platelets on omental metastases and to validate a robust, high-throughput model of ovarian cancer TME. However, despite these advancements, a 3D model capturing the complex ovarian TME and its relationship with ascites, including a functional vasculature, remains elusive. In a separate investigation, Ibrahim et al.[23] pioneered the creation of the initial vascularized model simulating the human peritoneum and ovarian cancer TME. The authors explored how the functions of mesothelial cells, endothelial cells, and adipocytes influenced tumor metastasis within this human 3D peritoneal model.[23]

Recently, a novel 3D disease modeling termed stem cell-based organoid modeling emerged.[24] The use of cancer organoids allows for the retention of the 3D structure of the TME, providing a physical context for molecular interactions.[25] Numerous studies on PC organoid modeling utilize hydrogels, Matrigel, and other materials to mimic the ECM in general.[26,27] The ECM has a unique structure and is a critical modulator of individual tumor behavior. Varinelli et al.[28] implemented a novel approach using decellularized ECM from the peritoneal cavity to support the cultivation of organoids originating from peritoneal metastasis (PM). This approach formed 3D nodules that closely resembled *in vivo* PC characteristics. The organoids preferred growing on ECM scaffolds obtained from neoplastic peritoneum, which were stiffer than standard scaffolds. Gene expression profiling of organoids cultured on various substrates faithfully mirrored clinical and biological characteristics. Moreover, the ECM appeared to influence the response to standard chemotherapy for PM. This 3D model, combining patient-derived decellularized ECM with organoids, provides a valuable platform for developing personalized therapeutic strategies in a biologically relevant context.[28]

All these models contributed novel insights into the molecular mechanism of PC and its treatment strategy. However, several limitations can be addressed using a tissue-based *in vitro* culture system known as an *ex vivo* model.

Ex vivo Models

Ex vivo models have become essential tools in cancer research, providing valuable insights into tumor biology, drug responses, and therapeutic advancements. These models, which involve cultivating and manipulating cancer cells or tissues outside the body, provide a controlled and reproducible environment for studying various cancer progression and treatment aspects. In addition to these advantages, the model offers several advantages for studying PC.[29]

Several studies focused on human tissue-based *ex vivo* models to mimic PM of different primary cancers, such as ovarian and colon cancer. A published article by Wong et al.[30] showed that utilizing human omentum to cultivate ovarian cancer cells in its adipose-rich environment allows for observing the factors influencing tumor growth and immune response regulation. The model is a valuable tool for studying the TME and offers a robust platform for developing and assessing new therapies targeting metastatic cancer cells within this niche. Importantly, this model is cost-effective, straightforward to generate, and applicable to translational research endeavors.[30] Mönch et al.[31] developed a human *ex vivo* peritoneal model using colorectal cancer (CRC) cell lines and patient-derived tumor organoids cultured with human peritoneum, maintaining peritoneal structures and revealing the presence of immune cells, fibroblasts, and ECM components. Co-culturing with CRC cells revealed cancer cell growth and migration into the peritoneum, mimicking CRC PM. This model provides a clinically relevant platform for studying PM mechanisms and exploring treatment options.[31] However, *ex vivo* modeling with human tissue has limitations, including variability between samples, challenges in reproducing experiments reliably, and limited ability to replicate therapeutic outcomes observed *in vivo* due to the absence of systemic factors and spatial constraints. The lack of a functional immune system in *ex vivo* models also limits their utility in studying the peritoneal immune response in carcinomatosis. Given the limitation of collecting human tissue samples for the PC *ex vivo* model, Schnell et al.[32] conducted unique research in which they created an *ex vivo* peritoneal model for evaluation of the efficacy of IP chemotherapy that is easy to use, reproducible, and cost-effective. The model resembles the human abdominal cavity in volume and shape, with an inner surface lined with serosa, allowing for pharmacological and biological analysis. The model uses a fresh urinary bladder from an adult bovine, which is inverted through an incision to expose the serosa on its inner side. It is regarded as an innovative and versatile *ex vivo* model for optimizing drug delivery of IP treatment strategies such as pressurized intraperitoneal aerosol chemotherapy (PIPAC), replacing the need for live animal experiments.[32]

To overcome the aforementioned limitations, novel approaches are needed to create *ex vivo* PC models.

In vivo Models

Cancer investigations have massively evolved through enlightening the complexities of the disease, and *ex vivo* models have played a crucial role in improving our knowledge. Although this model has many significant advantages, as with all experimental models, it also has several disadvantages. With regard to the advantages, *ex vivo* models mimic human cancers more realistically in terms of tumor structure, microenvironment, and physiology.[33] These advantages help scientists to comprehensively understand how carcinogenesis occurs and responds to treatments in living organisms. The most essential parameters for novel chemotherapeutic agents are efficacy and safety. Additionally, for the metastasis process, researchers can clarify the mechanism of spreading the cancer cells and create a potential treatment option to stop it. In addition, *ex vivo* models are convenient for investigating the interactions between immune and cancer cells.[34]

The literature identifies three primary *ex vivo* models: syngeneic, xenograft, and genetically engineered mouse models (GEMMs), with their unique characteristics shown in Figure 2.[35]

Syngeneic models utilize cells or tissue from donors with the same genetic background. This results in a more authentic TME, as the recipient animals have normal immunity. In studies involving immunocompetent mice, the CT26 cell line (syngeneic to BALB/c mice) and the MC38 cell line (syngeneic to C57BL/6 mice) are commonly used. While CT26 is a fast-growing grade IV carcinoma with similarities to aggressive, undifferentiated human CRC cells, MC-38 is a grade III adenocarcinoma. Both cell lines cause PC within 2-3 weeks of IP injection.[36,37]

In immunocompetent rats, the CC531 cell line (syngeneic to WAG or WAG/Rij rats) is commonly used. Widely used in metastasis research, CC531 is a 1, 2-dimethylhydrazine-induced adenocarcinoma with low immunogenicity. IP injection of CC531 causes widespread carcinomatosis and hemorrhagic ascites after 3 weeks.[38,39] However, the colon tumors in these models are chemically induced and do not fully mirror the genetic and molecular diversity seen in human cancers. Despite this limitation, syngeneic models are the preferred choice for studying cancer immunotherapy.[40,41]
Xenograft model generation using commercially available cell lines provides expedited tumor development, heightened engraftment rates, and reduced study durations, resulting in productive time and cost management. These cell lines boast comprehensive published data, well-defined genetic profiles, and established responsiveness to therapeutic interventions. Their proliferative capacity affords an inexhaustible cell reservoir for initiating xenografts, with facile integration of genetic modifications for diverse applications, including quantitative imaging methodologies.[42] PC xenograft models

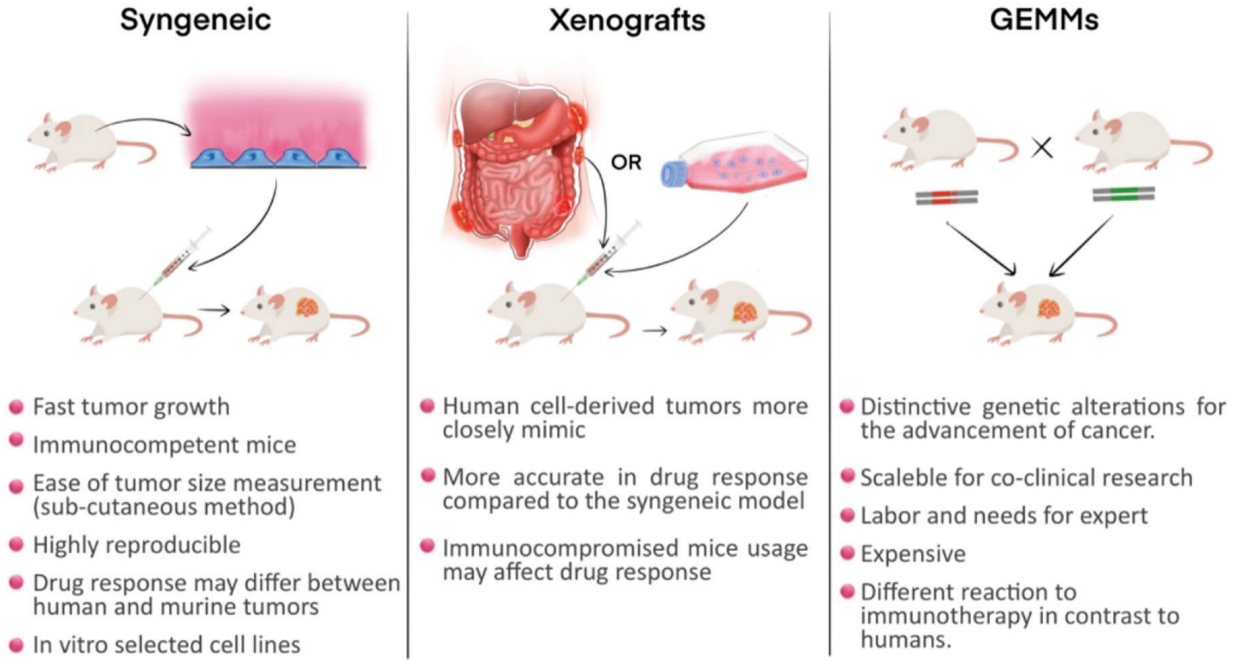

Figure 2. Main characteristics of different *in vivo* models
GEMMs: Genetically engineered mouse models

involve transplanting human cancer cells or tissue into the peritoneal cavity of immunodeficient mice, such as severe combined immunodeficiency (SCID), athymic nude, non-obese diabetic (NOD), or NOD SCID gamma mice; however, these models lack the ability to mount an immune response against human cells, which contributes to the promotion of tumor growth in the peritoneal cavity. Furthermore, the homogeneous nature of the source material raises concerns regarding the faithful representation of the original human cancer, and the absence of intratumoral heterogeneity in patient tumors during *in vitro* culture further underscores potential limitations.[43] PDXs may offer a more intricate portrayal of human cancers, albeit at the expense of prolonged latency periods and elevated financial commitments.

Although PDX models have been used to determine the efficacy and safety of chemotherapeutics, the main hindrance is the lack of an immunocompetent environment. To address this constraint, researchers employed GEMMs to study PC. This type of model has been used to study PM, including transgenic, knock-out, and knock-in mice, and can replicate various human cancers at a genetic level and demonstrate comparable phenotypes in the TME.[44,45] Numerous mouse models, including those expressing human tumor endogenous antigens such as carcinoembryonic antigen (CEA) as a transgene, have shown improved engraftment of tumor cells expressing this antigen.[46] However, more authentic and intricate models that closely mimic PM in humans have been developed by genetically modifying primary aggressive peritoneal tumors, such as those originating from the ovary, colon, stomach, or pancreas, to investigate early PM. In this regard, some studies have utilized triple-mutant mice ($p53^{LSL\text{-}R172H/+}$ $Dicer1^{flox/flox}$ $Pten^{flox/flox}$ $Amhr2^{cre/+}$).[47-49] This mouse model with $p53^{R172H}$ mutation, equivalent to human $p53^{R175H}$, common in ovarian high-grade serous carcinoma, develops tumors in the fallopian tube 1-2 months after birth, with all mice ultimately developing PC and severe hemorrhagic ascites causing mortality.

Tseng et al.[50] described a PC model where the histological morphology and immune microenvironment closely resemble PM high-grade serous carcinoma in humans. In immunocompetent mice, the combination of shRNA-p53 with overexpression of AKT and c-Myc oncogenes in the peritoneum led to the development of aggressive PC with visible implants within 21 days. This approach bypassed immunosurveillance and induced the formation of peritoneal tumors in the mice. Similarly, Iyer S et al.[51] developed cell lines combining loss of Trp53 and overexpression of CCNE1, AKT2, and Trp53R172H, driven by $Kras^{G12V}$ or Brd4 or Smarca4 overexpression. This model serves as a valuable platform for preclinical and translational research on PC, including testing immunotherapeutic agents, studying PC initiation and progression, identifying biomarkers, and predicting the origin of peritoneal cancer spreading.

Moreover, the xenograft model could be generated by patient-derived PC organoid engraftment in the mice that provide personalized PM modeling. A study by Fang et al.[52] successfully

established human malignant pleural mesothelioma organoids (MPMOs), providing a detailed description of the medium components necessary for MPMO culture. Examination and genomic analysis showed that MPMOs accurately represented the original tumors' histological characteristics and genomic diversity. These MPMOs effectively created subcutaneous and orthotopic xenograft models with high success rates. Drug sensitivity tests revealed varying medication responses among MPMOs, which correlated well with the clinical situations of the patients.[52]

Interpreting research results from animal models can be challenging due to differences in peritoneum physiology and function between rodents and humans. The highly vascularized omentum, which plays a key role in PC development in humans, has significantly lower vascularity in rodents. These differences highlight the importance of considering limitations in translating findings from rodents to humans.[53] The disadvantages of *in vivo* models are largely related to ethical problems. The ethical issues for animal models are highly critical. Scientists must adhere to strict ethical guidelines and reduce harm to the animal during the experimental process. A further disadvantage is the diversity of genetics and physiology of animals and humans, and a major hindrance to *in vivo* studies is that they are time-consuming and expensive.[54]

To conclude, experimental animals mimic the human PC model. Furthermore, due to the heterogeneity of cancer cell characteristics among patients, translating any treatment strategy to clinical practice has proven challenging. Therefore, it is imperative to develop personalized *in vivo* peritoneal cancer models to investigate individual cancer characteristics and predict the most effective treatment strategy for patients.

In silico Models

Improving our comprehension of cancer and other intricate diseases necessitates the integration of diverse datasets and algorithms. Combining *in vitro* and *in vivo* data with *in silico* models is crucial for addressing the inherent complexities of data. This integrated approach not only helps reveal underlying molecular mechanisms but also enhances our understanding of uncontrolled cell growth. Over time, a variety of biochemical and computational methods have been developed for studying diseases, with many initially relying on animal experiments. However, comparing cellular processes in both eukaryotic and prokaryotic organisms has proven valuable in elucidating specific aspects of disease progression, thereby enhancing the planning of future experiments. Adhering to principles of humane experimentation, advancements in alternative animal testing have focused on *in vitro* methods such as cell-based models and microfluidic chips, as well as clinical approaches such as microdosing and imaging.[55] The range of alternative methods has expanded to include computational approaches that draw on information from previous *in vitro* and *in vivo* experiments. *In silico* techniques, often overlooked, can play a critical role in understanding fundamental cancer processes, offering accuracy comparable to biological assays and providing crucial focus and direction to reduce experimental costs. Precision medicine aims to provide more personalized treatments, with digital twins representing a novel approach to achieving this goal. A clinical digital twin serves as a digital representation of an individual, offering tailored treatment recommendations, as illustrated in Figure 3. However, the centralized data gathering required to develop and enhance digital twin models is facing challenges related to patient privacy constraints.[56]

At present, no digital twin technique model design exists for any cancer type, including PC. Such a model could be beneficial in assessing personalized treatment strategies.

Treatment Strategies for Peritoneal Carcinomatosis

Intraperitoneal Treatment Approaches

The goal of therapy is to control the tumor for as long as possible and avoid or delay tumor-associated symptoms for most of the patients with PC. Quality of life (QoL) and survival time become determining factors in the therapy decision.[57]

In addition to "best-supportive care" and systemic treatment as standard therapy, locoregional therapy methods such as hyperthermic intraperitoneal chemotherapy (HIPEC) and PIPAC have also become established in recent years. Although HIPEC and PIPAC are procedures for the IP application of chemotherapy, fundamental differences must be considered when determining the indication.[58]

There are significant variations between protocols within the HIPEC framework. The diversities are based on chemotherapeutic drugs, temperature, carrier solution, volume, and duration of the treatment. The most frequently utilized drugs in preclinical animal studies are mitomycin C (MMC), cisplatin, oxaliplatin, paclitaxel, and doxorubicin. The temperatures applied varied widely for all these drugs, ranging from 39 °C to 44 °C.[59]

The carrier solution used in HIPEC significantly affects its pharmacokinetics. Park et al.[60] demonstrated this by combining oxaliplatin or MMC with different carrier solutions: a 1.5% Dianeal peritoneal dialysis solution, 5% dextrose solution, or 20% lipid solution. The choice of carrier solution in HIPEC affects drug pharmacokinetics. While peritoneal drug concentrations remain consistent across carriers, plasma concentrations vary significantly. Using a lipid carrier solution with MMC resulted in a threefold higher area under the curve ratio between peritoneum and plasma compared with a Dianeal solution. Oxaliplatin plasma concentrations were similar with lipid and Dianeal solutions but significantly higher with dextrose, potentially increasing systemic toxicity due to differences in membrane permeability.[60]

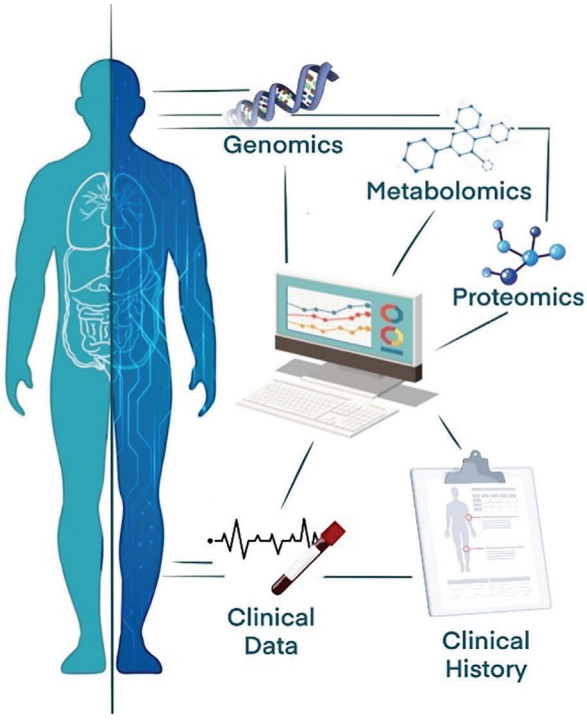

Figure 3. Digital twins for individualized treatments

The temperature is another critical factor in HIPEC treatment. Heat has been shown to have a positive impact on the 5-year survival rates of patients with PC.[61] The effectiveness of chemotherapy administered during HIPEC is boosted by a temperature-dependent factor called the thermal enhancement ratio.[62] Generally, three hyperthermic scales are recognized: mild (39 °C-41 °C), moderate (41 °C-43 °C), and severe (>43 °C) hyperthermia (34298644). Severe hyperthermia carries the risk of damaging healthy tissues and is not employed in HIPEC clinical practice. On the other hand, mild and moderate hyperthermia both increase tissue blood flow, stimulate the immune response, and enhance the cytotoxicity of chemotherapy in a temperature-dependent manner. Among the studies reviewed, moderate hyperthermia was the most commonly used type (71% vs. 29% for mild hyperthermia).[63] A study by Manoğlu et al.[64] successfully created an *in vivo* PM model by injecting a CC531 colon carcinoma cell line into the peritoneum to evaluate MMC and 5-fluorouracil efficacy in a HIPEC treatment system. The authors proved that HIPEC treatment is significantly more effective than normothermic MMC administrations.[64]

In vitro studies indicate that there is an ideal treatment duration where hyperthermia coupled with chemotherapy exhibits maximum efficacy. A recently published study by our team focused on improving the HIPEC treatment of PM originating from CRC. Due to the challenges in conducting randomized trials, the study proposes a novel *in vitro* 3D microfluidic PC model to test different HIPEC treatment parameters. The effects of current HIPEC protocols with oxaliplatin were tested on the developed 3D microfluidic PC model. The results showed that epithelial-mesenchymal transition-induced HCT116 colon carcinoma cells were less sensitive to oxaliplatin treatment and that increasing the temperature and duration of the treatment increased cytotoxicity. The study suggests that 200 mg/m^2 of oxaliplatin applied for 120 min is the more effective HIPEC treatment compared with 460 mg/m^2 for 30 and 60 min.[65] Studies highlight the importance of treatment duration in enhancing the efficacy of chemotherapy. Kirstein et al.[66] demonstrated that combining heat (42 °C) with oxaliplatin for 2 hours was more effective than using 30 min. Löffler et al.[67] found that a 30 min exposure to clinical oxaliplatin concentrations often fails to induce sufficient cell death, suggesting that longer application times are needed. Murata et al.[68] observed similar growth-inhibitory effects between 30 and 60 min treatments for most cell lines and chemotherapy combinations under hyperthermic conditions, but longer durations were more effective for specific cell lines, indicating a cell-line-dependent response to chemotherapeutics. These studies emphasize the significance of prolonging treatment duration to enhance drug efficacy.

Moreover, HIPEC treatment can be administered using either the conventional open abdominal technique (open HIPEC) or the closed technique. A novel approach, the Peritoneal Recirculation System [(PRS)-1.0 Combat] with CO_2 recirculation technology (PRS closed HIPEC), has been developed for closed HIPEC. Studies have shown that the

closed technique offers a superior homogeneous distribution of heat and anticancer agents. In a study by Diaz et al.,[69] 84 patients with curative CRC were treated using different HIPEC techniques. The closed HIPEC group demonstrated a significantly improved median overall survival of 67 months, compared with 43 months in the open HIPEC group (p<0.001). Median disease-free survival was also longer in the PRS closed HIPEC group (40 months) compared with the open HIPEC group (15 months, p<0.001). These results suggest that PRS closed HIPEC is a reliable and safe technique, offering a viable alternative for administering HIPEC.[69]

On the other hand, PIPAC exploits gas and pressure to overcome the limitations of IP chemotherapy, enhancing drug exposure and diffusion into tumor nodes. Evidence from *in vitro*, *in vivo*, *ex vivo*, and clinical studies suggests that PIPAC offers superior pharmacological properties to traditional fluid-based IP chemotherapy, leading to enhanced local efficacy and reduced systemic toxicity. Initial retrospective analyses in ovarian, gastric, and CRCs demonstrate promising results in palliative settings, with ongoing prospective trials assessing effectiveness and safety. Additionally, electrostatic precipitation PIPAC (ePIPAC) has been proposed to enhance pharmacological properties further. Preclinical evaluations show that ePIPAC is technically feasible, achieving improved tissue drug delivery compared with standard PIPAC.[70] In a study by Reymond et al.,[71] the ePIPAC procedure was technically feasible, with no intraoperative complications, was well-tolerated by patients, and had no adverse events exceeding CTCAE grade 2. Patient 1, diagnosed with PC of unknown origin, exhibited an objective histological and radiological response and survived for 11 months. Patient 2, diagnosed with ductal pancreatic cancer, underwent secondary resection following ePIPAC, resulting in no residual PM, but experienced tumor recurrence after 5 months. Patient 3, diagnosed with gallbladder adenocarcinoma, exhibited radiological improvement in liver infiltration and survived for 22 months without histological signs of PM.[71]

Clinical trials are needed to further evaluate the efficacy and application of PIPAC, but recent data on PIPAC with low-dose cisplatin and doxorubicin or oxaliplatin shows promising results. Studies on PC from various cancers have demonstrated the safety and tolerability of PIPAC, with a median survival rate of 15.7 months. The PIPAC method has been shown to induce histological regression and improve QoL in patients, with no change in QoL stabilization over 3 months of treatment.[72] These treatments are presented in Figure 4.

However, PIPAC may not be suitable for patients with recurrent disease following cytoreductive surgery (CRS) due to adhesions hindering aerosol diffusion.[73] Combining PIPAC with systemic chemotherapy has shown significant improvements in tumor response, clinical response, and QoL.[74]

Immunotherapy

Immunotherapy has become a hopeful strategy for PC treatment. The peritoneal cavity contains a diverse array of immune cells, which recent research highlights as pivotal in regulating tumor growth in this region. Nonetheless, peritoneal

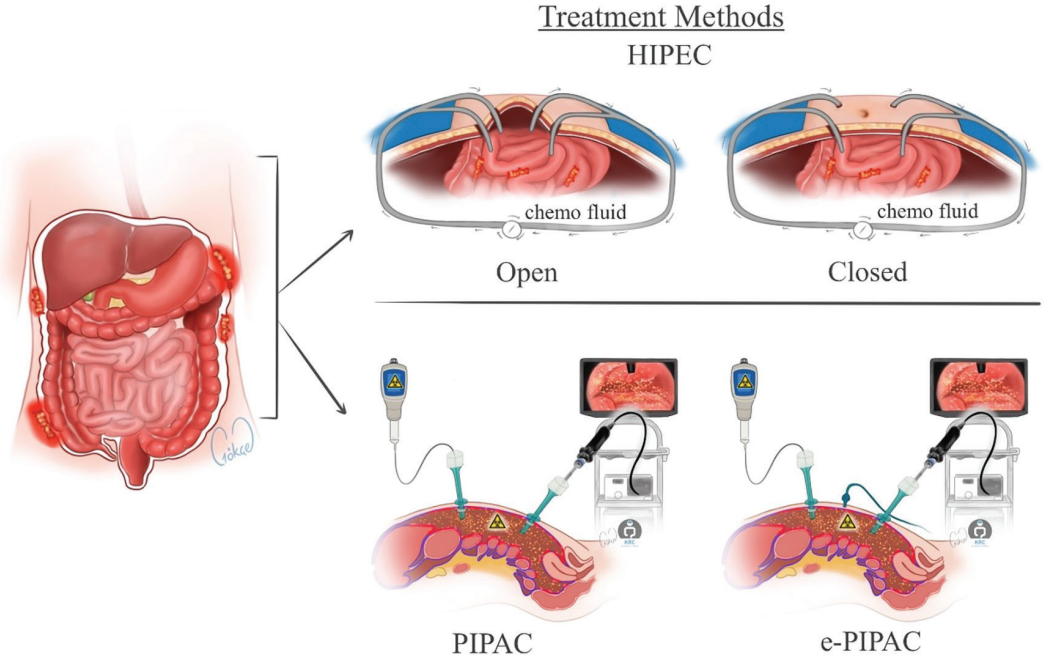

Figure 4. The open/closed HIPEC and PIPAC techniques
HIPEC: Hyperthermic intraperitoneal chemotherapy, PIPAC: Pressurized intraperitoneal aerosol chemotherapy, ePIPAC: Electrostatic precipitation PIPAC

tumors frequently evolve mechanisms to evade immune detection, resulting in disease advancement and unfavorable prognoses. To combat this challenge, substantial endeavors are underway to devise novel immunotherapeutic strategies that can augment immune cell migration into the peritoneum and enhance tumor immunogenicity.[75] Catumaxomab, a trifunctional antibody approved in Europe, is an example of IP immunotherapy that targets EpCAM, reducing malignant ascites. IP immunotherapy aims to break immunological tolerance to treat peritoneal diseases. Approaches such as boosting T-cell reactions and developing vaccines targeting tumor-specific antigens are under investigation. Potential therapies for PC encompass CAR-T cells, vaccines, dendritic cells with proinflammatory cytokines and natural killer cells, adoptive cell transfer, and immune checkpoint inhibitors. Here, CAR-T cells designed to target CEA-expressing tumors have demonstrated suppression, a response that was heightened with anti-PD-L1 or anti-Gr1 treatment. Additionally, CAR-T cells for folate receptor cancers, when paired with CD137 co-stimulatory signaling, facilitated T-cell infiltration and persistence within the body.[76] Studies such as Checkmate-649 have shown significantly improved overall survival in patients with advanced gastric cancer and PM with high PD-L1 expression (CPS ≥5) when treated with nivolumab and chemotherapy compared with chemotherapy alone.[77] Another study centered on individuals with solitary PC stemming from dMMR/MSI-H CRC reported a notable 46% response rate to immune checkpoint inhibitor therapy, a level of success challenging to attain with conventional chemotherapy.[78] Additionally, a study on claudin 18.2 targeting CAR-T therapy in patients with advanced gastric cancer showed promising responses.[79] Recent studies demonstrated that immune-enhanced patient tumor organoids (iPTOs) present a promising tool for predicting clinical outcomes in response to immunotherapies. A study by Votanopoulos et al.[80] reported an 85% agreement between iPTO models and actual patient responses, highlighting their potential for personalized treatment planning. These models facilitate the exploration of tumor-immune system interactions and can be utilized to screen the efficacy of immune checkpoint inhibitors.[80] Moreover, iPTOs can aid in the generation of tumor-reactive lymphocytes for use in adoptive cell transfer therapies.[81] Despite ongoing challenges in the standardization and scalability of these co-culture systems, they hold great promise in advancing precision oncology. By enabling patient-specific immunotherapy testing, iPTOs provide valuable insights into the TME. Their use could optimize the administration of expensive immunotherapies, leading to better patient outcomes and more efficient resource allocation.[82]

These studies suggest that immunotherapies could be effective and safe treatments for PC.

Intraperitoneal Photodynamic Diagnosis and Therapy

The CRS-HIPEC technique is recommended solely in cases where the peritoneal tumor burden is not extensive, as indicated by the Peritoneal Carcinomatosis Index (PCI) or other scoring systems.[83,84] The PCI is determined through intraoperative inspection and palpation, as conventional preoperative imaging methods such as computed tomography (CT) and 18F-positron emission tomography/CT often fail to accurately estimate the extent of the disease.[85,86] This leads to reported rates of futile laparotomy ranging from 5% to 15% in patients undergoing surgery for PC.[87-90] Although diagnostic laparoscopy may enhance PCI assessment, its additional predictive value is limited.[91] Thus, more precise imaging methods are necessary to identify suitable candidates for CRS-HIPEC among patients with low PCI and those with PM. Fluorescence labeling presents a novel approach for diagnosing and prognosing PC, with CEA being a prime target for CRC.[92,93] In fact, CEA is highly expressed in CRC cells, whereas its expression in healthy tissue is significantly lower.[94] Labetuzumab, a humanized monoclonal antibody targeting CEA, has been extensively studied as a radiotracer, therapeutic agent, and antibody-drug conjugate for various malignancies.[95,96] The dual-labeled form, [111In]In-DOTA-labetuzumab-IRDye800CW, has shown promise as a multimodal imaging agent for CRC in preclinical studies.[97,98] Clinical trials have evaluated the safety and feasibility of preoperative single-photon emission CT imaging, intraoperative radio detection, and near-infrared fluorescence-guided surgery following intravenous administration of different doses of [111In]In-DOTA-labetuzumab-800CW in patients with CRC PM. A conceptional image for IP photodynamic diagnosis/therapy is presented in Figure 5.

Moreover, IP photodynamic treatment (PDT) shows promise as a therapy for PC due to its superficial treatment effect. A Phase II trial using the photosensitizer, Photofrin®, demonstrated clinical tolerability but substantial toxicity, indicating a narrow therapeutic index. Despite this, responses were seen in heavily pre-treated patients, suggesting clinical effectiveness. However, Photofrin® showed little selectivity for tumors over normal tissues, contributing to its narrow therapeutic index. Newer, molecularly targeted photosensitizers and strategies to enhance PDT cytotoxicity offer the potential to improve the therapeutic index of the treatment. Nanotechnology and fractionated PDT administration are also being explored to enhance the treatment's effectiveness and tolerability. These advancements may lead to highly effective and well-tolerated IP PDT for treating carcinomatosis.[99]

Matts et al.[100] investigated whether fullerenes could enhance PDT efficacy against PC in a mouse model. Characterized by a thin layer of tumor nodules on abdominal organs, PC is known

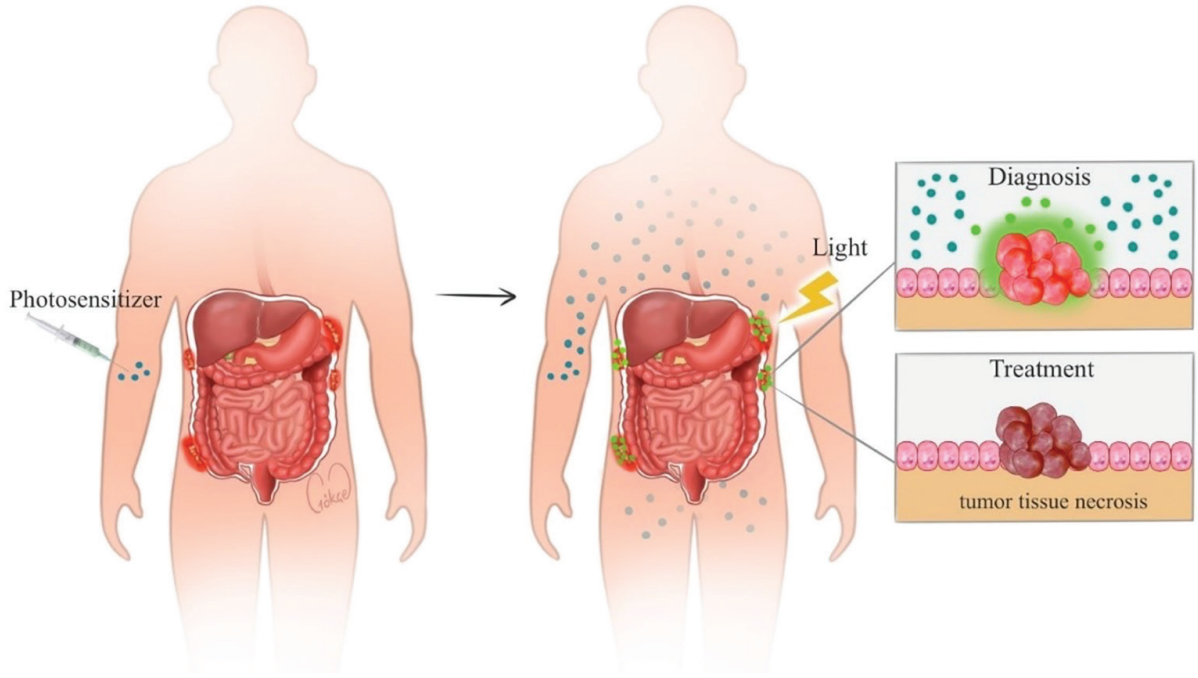

Figure 5. Photodynamic diagnosis and treatment for PC
PC: Peritoneal carcinomatosis

for its poor response to standard treatments in humans. The authors employed a colon adenocarcinoma cell line (CT26) modified to produce luciferase, allowing them to monitor IP tumor burden in BALB/c mice using real-time optical imaging with a sensitive low-light camera. After administering N-methylpyrrolidinium-fullerene in Cremophor®-EL micelles via IP injection, the mice were exposed to white-light illumination through a skin flap in the peritoneal wall. This treatment led to a notable decrease in bioluminescence and improved survival.[100]

Almerie et al.,[101] conducted a systematic review that included three human and 25 animal studies. Their analysis of phase I and II human trials using first-generation photosensitizers revealed the feasibility of applying PDT following surgical debulking in patients with PC, exhibiting some clinical benefits. However, the limited tumor selectivity of the photosensitizers resulted in notable toxicities, particularly capillary leak syndrome and bowel perforation. Animal studies indicated that PDT increased survival rates by 15-300% compared with control groups, with the treatment also leading to higher tumor necrosis values (PDT; 3.4±1.0 vs. control; 0.4±0.6, $p<0.05$) and reduced tumor size (residual tumor size =10% of untreated controls, $p<0.001$). Overall, the review indicates that PDT shows potential as a treatment option for PC.[101]

Samel et al.[102] focused on L293 cells that are genetically engineered to produce the CYP2B1 enzyme using a cytomegalovirus promoter, which activates ifosfamide, a cytotoxic drug. These modified cells were encapsulated in a cellulose sulfate formulation (Capcell). In an animal study involving BALB/c mice with green fluorescently labeled colon-26 cancer cells, early IP treatment combining ifosfamide with CYP2B1 cells led to complete tumor regression. In contrast, treatment beginning on day 5 or using ifosfamide alone resulted in partial responses. These findings highlight the potential of targeted IP chemotherapy, employing prodrug-enzyme combinations, as a practical approach for treating peritoneal spread from CRC.[102]

Recent advancements in tumor selectivity and light delivery systems show promise, but further refinement is needed before PDT can be widely used for PC.

Gene Therapy

Gene therapy delivers various types of genes to repair damaged genes causing disease. These gene therapy medicinal products are classified as advanced medicinal therapy products by the European Medicines Agency.[103] They repair tissue damage, replenish deficiencies, and prevent unwanted gene expression. Gene therapy can replace mutated genes with healthy copies, inhibit mutated gene expression, silence unwanted genes, replace deficient genes, or deliver therapeutic genes to target tissues for disease treatment.

Methods such as antisense RNA or nuclear phthalate can be employed to silence genes and inhibit oncogene expression, effectively slowing tumor cell proliferation. Suicide gene

therapy involves introducing a gene that converts an inactive prodrug into a toxic agent within the cells. This approach using inactive drugs is known as gene-directed enzyme prodrug therapy. Gene replacement therapy aims to correct specific gene mutations in cancer cells by introducing a functional gene copy using a vector. Vectors, which can be viral or non-viral, deliver genetic material for gene therapy. The goal of gene therapy is to deliver therapeutic genes to target cells using a reliable, safe, and effective carrier. Non-viral vectors are often favored over viral vectors due to their superior attributes.[104]

Gene therapy, categorized by cell type and treatment mode, modifies gene expression in living cells for therapeutic purposes. Its potential for fewer side effects sets it apart from traditional methods.[105]

Several studies focused on gene therapy for PC. In a study by Natatsuka et al.[106] the suppressor of cytokine signaling1 (SOCS1) was investigated for its potential as a therapeutic target in gastric cancer. Known for regulating cytokines, SOCS1 was found to suppress proliferation in four out of five gastric cancer cell lines by influencing cell cycle-associated molecules at the G2/M checkpoint. The study also showed promising results in a preclinical xenograft PC mouse model, suggesting that forced expression of SOCS1 could be a new therapeutic approach for treating PC in gastric cancer.[106] In another study by Wu et al.[107] antiangiogenic therapy targeting angiogenesis, a crucial process in tumor growth and metastasis, was investigated using pigment epithelium-derived factor (PEDF) as an angiogenesis inhibitor. Adeno-associated virus (AAV)-mediated human pigment epithelium-derived factor (hPEDF) was evaluated as a tumor suppressor for cancer gene therapy. Recombinant AAV2 encoding hPEDF (rAAV2-hPEDF) inhibited proliferation and tube formation of human umbilical vein endothelial cells *in vitro*. In a colorectal PC mouse model, rAAV2-hPEDF suppressed tumor growth and metastasis, prolonged survival, reduced microvessel density, and increased apoptosis in tumor tissues. Elevated hPEDF levels in the serum and ascites of treated mice indicate the potential of rAAV2-hPEDF as an antiangiogenic therapy agent. These investigations offer a novel treatment approach for PC.[107]

Conclusion

This review emphasizes the need for improved experimental models to accurately replicate the complexities of PC. Researchers can gain insights into the mechanisms of peritoneal dissemination by studying various animal models, cell cultures, and advanced technologies such as organoids and microfluidic platforms. While progress has been made, challenges remain, suggesting that future studies should integrate advanced imaging and molecular profiling to enhance translational relevance. Refinement of these models will advance our understanding of PC and aid in developing more effective therapies.

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Footnotes

Authorship Contributions

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