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Abstract

This review assesses the possibility of utilizing malignant effusions (MEs) for generating patient-derived tumor organoids (PDTOs). Obtained through minimally invasive procedures MEs broaden the spectrum of organoid sources beyond resection specimens and tissue biopsies. A systematic search yielded 11 articles, detailing the successful generation of 190 ME-PDTOs (122 pleural effusions, 54 malignant ascites). Success rates ranged from 33% to 100%, with an average of 84% and median of 92%. A broad and easily applicable array of techniques can be employed, encompassing diverse collection methods, variable centrifugation speeds, and the inclusion of approaches like RBC lysis buffer or centrifuged ME supernatants supplementation, enhancing the versatility and accessibility of the methodology. ME-PDTOs were found to recapitulate primary tumor characteristics and were primarily used for drug screening applications. Thus, MEs are a reliable source for developing PDTOs, emphasizing the need for further research to maximize their potential, validate usage, and refine culturing processes.

Keywords: cancer, organoids, patient-derived organoids, malignant effusions, pleural effusion, ascites, functional precision medicine

Abbreviations: CRC, colorectal carcinoma; ECM, extracellular matrix; EGF, epidermal growth factor; EV, extracellular vesicles; FBS, fetal bovine serum; FGF, fibroblast growth factor; g, gravitational constant; HIPEC, hyperthermic intraperitoneal chemotherapy; HGF, hepatocyte growth factor; H&E, hematoxylin and eosin; IGF, insulin-like growth factor; ME, malignant effusion; ME-PDTO, malignant effusion-patient-derived tumor organoid; miRNA, microRNA; NAC, n-acetyl-l-cysteine; NGS, next generation sequencing; PDCC, patient-derived cancer cells; PDO, patient-derived organoid; PDTO, patient-derived tumor organoids; P/S, penicillin-streptomycin; RBC, red blood cell; rcf, relative centrifugal force; rpm, rounds per minute; TME, tumor microenvironment; TNM-classification, tumor node metastasis-classification.

1. Introduction

Cancer remains one of the most lethal diseases, with around ten million cancer deaths in 2020 (1). The cancer burden is expected to increase (1) and further oncologic research will be crucial in managing these growing numbers. Patient-derived tumor organoids (PDTOs) are emerging as a novel and high-fidelity *ex vivo* model for fundamental/translational cancer research, and as a predictive drug screening tool (2). PDTOs, generated from cancer tissues, are three-dimensional (3D), self-organizing multicellular constructs, exhibiting a remarkable capacity to closely replicate the morphology and heterogeneity of tumors (3). PDTO cultures have already been established for different tumor types such as pancreatic cancer (4-6), ovarian cancer (7-9), gastric cancer (10), colorectal cancer (11, 12), lung cancer (13, 14) and breast cancer (15). Recently, studies demonstrated that PDTOs (i) adequately retain tumor heterogeneity and the genomic landscape (9, 16, 17); (ii) are capable of long term storage and passaging (17, 18); (iii) can be established in a few weeks (13, 19); and (iv) correlate with clinical drug responses (4, 10, 11, 13, 20, 21).

Indeed, these PDTOs offer a new paradigm for functional precision medicine, an approach whereby living patient-derived cancer cells (PDCC) are directly treated with therapeutic agents to provide immediately translatable, personalized information to guide therapy (22). In addition to functional precision medicine, PDTOs can be of substantial use in cancer research (2). They can be applied to explore resistance mechanisms (23), the potential of novel therapeutic agents (19, 24) and repurposing of older (25), among others.

PDTOs are more cost-effective, more high-throughput and more ethical than patient-derived xenografts and far better at resembling the original tumor tissue than 2D cancer cell lines (2). However, there are various hurdles to overcome in regard to the implementation of PDTOs in the clinical setting. First, the success rates of PDTO establishment varies across different tumor types (2). Further, contamination and outgrowing of normal cells hamper implementation, especially in lung cancer (26). Third, it is more expensive and laborious then 2D cell lines (2). And finally, the methods to obtain tumoral tissue (e.g. tumor resection, biopsies) are highly invasive. Notwithstanding, recent studies managed to generate PDTOs from malignant effusions (MEs) instead of resection/biopsy specimens (6, 7, 19, 27-32).

MEs, such as malignant ascites and pleural effusion, are effusions characterized by the presence of tumor cells (33). The appearance of ME is considered an indication of metastatic

events due to peritoneal or pleural dissemination of the malignancy, suggesting a poor prognosis (34-36). An exception is malignant pleural mesothelioma where pleural effusions may be present for months before precise diagnosis is made, which mostly require pleural biopsies (37). However, the presence of ME is not only predictive of a worse outcome, it might also be severely debilitating for the patient (e.g. dyspnea, abdominal bloating and pain,…) (34, 36). A paracentesis/thoracentesis (respectively draining ascites or pleural fluid) is a method of removing fluid out of an abdominal or pleural cavity. It is mildly invasive and has a low risk of complications (38, 39). A paracentesis/thoracentesis is not solely carried out for diagnostic purposes, but more often for symptom relief. Usually, the drainage has to be performed multiple times, since the fluid has the tendency to reoccur. A paracentesis/thoracentesis is less invasive and less expensive than surgical resections and is considerably less prone to complications. This makes MEs an appealing source for the retrieval of PDCCs, which can be used for PDTO development.

This review aims to assemble the recent literature of the past five years regarding PDTOs originating out of MEs. Herein, we give an overview of the different techniques used, success rates, (dis)advantages and clinical applications of malignant effusion patient-derived tumor organoids (ME-PDTOs).

2. Materials and methods

2.1. Methods of search

A thorough literature search was conducted using two databases: PubMed and Thomson Reuters Web of Science. With regard to PubMed, following search query was used: (ascites[Text Word] OR carcinomatosis[Text Word] OR "malignant effusion*"[Text Word] OR "pleura* fluid*"[Text Word] OR paracentesis[Text Word] OR thoracentesis[Text Word] OR pleura*[Text Word]) AND (PDO[Text Word] OR PDTO[Text Word] OR organoid*[Text Word] OR "primary cell*"[Text Word]). A search restriction for publication date was applied: exclusively articles published in the last five years (1th of May 2018 and the 1th of May 2023) were included. Given the recent emergence of PDTOs and the innovative nature of ME-PDTOs, we restricted our literature search to the last five years to capture the most up-to-date information. By prioritizing recent research, our study aims to provide a concise and current overview of the state-of-the-art in organoid and ME-PDTO research. Additionally, some filters

were included, namely: “full text” (in text availability), “Humans” (Species) and “English” (Article language). Regarding Thomson Reuters Web of Science, following search query was used: “(ascites OR carcinomatosis OR “malignant effusion*” OR “pleura* fluid*” OR paracentesis OR thoracentesis OR pleura*) AND (PDO OR PDTO OR organoid* OR “primary cell*”)”. A search restriction for publication date was applied: exclusively articles published in the last five years (1th of May 2018 and the 1th of May 2023) were included. The search was refined by language (“English”) and document type (“Article”).

2.2. Screening for eligibility

After the exclusion of duplicates found in both search libraries, we screened the abstracts of the remaining articles. The following inclusion criteria were applied: (1) original study; (2) the article should be using human cancer cells; (3) cancer cells should be obtained from malignant effusions; (4) only full text English articles were included. The following exclusion criteria were applied: (1) reviews, lectures and book selections were excluded; (2) the number of successful and failed organoids is not indicated or cannot be derived from the provided data.

2.3. Data collection process and analysis

We developed a data extraction sheet in Microsoft® Excel®. Data was extracted in duplicate. Extracted data consisted of cancer type; type of ME (ascites, pleural effusion, both); overall sample size of study (including resection specimens, biopsies…); overall success rate; sample size of ME; success rate of ME-derived organoids; method of ME retrieval; volume of ME; sieving; usage of red blood cell (RBC) lysis; centrifugation; medium used; usage of supernatants; application(s); long vs short-term culturing. We defined long-term culture as surpassing more than five passages or in case of self-proclaimed long-term culturing practices. Prism version 9.1.2 (GraphPad) was used for graphical data representation.

2.4. Risk of bias in individual and across studies

This systematic review has several possible sources of bias. Foremost, this review will be influenced in a certain extent by publication bias, since not all trials lead to publications. The trials with successful results (e.g. high establishment rate of PDTOs of ME) will be more likely

to be published. Due to the small amount of literature concerning this topic, it is indeed a rather new field of investigation, not only randomized controlled trials but case-controlled and uncontrolled trials were included as well. These trials have a higher probability of having confounding factors and baseline difference. Moreover, this review is a pan-cancer review, with very small number of patients across most tumor types. In addition, a number of studies have been excluded, which did not adequately report the establishment rates of organoid development. While these exclusions were necessary to ensure data accuracy and reliability, it is possible that potentially useful information may have been inadvertently omitted.

3. Results

3.1. Literature search

Using the search criteria mentioned above, 124 articles were found, with 57 on PubMed and 67 on Web of Science. After removal of duplicates (n= 38), a total of 86 articles remained to be manually screened for inclusion. After a first screening, which was based on the abstract, 25 articles remained. These papers were read in full. Finally, 10 articles remained suitable for inclusion. Papers that did not meet our pre-defined inclusion criteria were discarded. One additional article was included, based on references. After this stepwise methodological search, 11 articles remained for analysis. The process of data selection using a PRISMA flow chart can be found in figure 1 (40). Table 1 provides a detailed overview of the included articles.

3.2. Characteristics of included studies

Most articles included the basic information about sample selection, sample processing, and culture conditions. However, information about the treatment status and tumor, node, metastasis (TNM)-classification was often missing and thus not analyzed in this review. In the included studies, a total of 190 PDTOs were established from MEs, of which 122 out of pleural effusion, 54 out of malignant ascites and 14 out of pericardial effusions (figure 2). Most organoid were established from lung cancer (n = 141) and ovarian cancer (n = 35). There were no cases of malignant pleural mesothelioma. The majority of studies reported on PDTOs derived from ME only (n = 8), while in three papers PDTOs were additionally established from other sources (e.g. tissue biopsies, resection specimens).

3.3. Efficiency in ME-derived PDTOs

The reported success rates for ME-PDTOs established varied between 33% and 100% (table 1). The median success rate was 92% and the mean was 84%. We combined data from different studies and found an overall success rate of 81%, with 190 PDTOs successfully generated out of a total of 234 ME samples. The tumoral origin was most often determined with hematoxylin and eosin (H&E) staining and immunohistochemistry (7, 19, 28, 29, 31, 41-44). Peng et al. (32) only used H&E staining. Some studies additionally carried out next generation sequencing (NGS) (30) or RNA sequencing for a part of the PDTOs (42), or all the PDTOs (19, 28). Four studies described successful long-term organoid cultures or cryopreservation of the organoids (7, 29, 31, 42) and three studies mainly focused on short-term culturing (7, 19, 44).

3.4. Methodology for the establishment of ME-derived organoids

The ME-PDTOs were established using different techniques (table 2), including different media (table 3).

3.5. Patient sample collection

Seven papers used drainages by thoracentesis or paracentesis, two articles collected the effusions via a surgical approach and two papers did not specify. The volume of ME used varied between 40 mL and 1000 mL. There appears to be no relationship between the collection method or the volume of the ME collected and the success ratio of the PDTO establishment.

3.5.1. Processing of malignant effusions

Almost all studies used centrifugation to establish a cell pellet, which was used for the generation of PDTOs. Only one study used a different technique and acquired a cell pellet through sedimentation in fetal bovine serum (42). Various centrifugation velocities, temperatures and times were used (see table 2). Sieving was used in two papers, while the other nine did not use this technique. Sieving of the cell pellet was carried out in one study through a 100 µm sieve to remove large aggregates and debris and a 38 µm sieve to remove

most mononuclear cells. All other studies proceeded with the full cell pellet (19). Only four studies did not lyse the red blood cells, while the other seven did this standardly.

3.5.2. Culturing conditions

All studies cultured the cell pellet in extra cellular matrix (ECM) domes varying between 10 to 75 µL, with a median of 30µL. Most studies (n=9) used Matrigel as ECM (7, 27-30, 32, 42, 44), two studies used Cultrex (19, 31) and one study developed their own ECM by adding three parts methacrylated type I collagen (6 mg/mL) to one part thiolated hyaluronan (1 mg/mL) and crosslinking the hydrogels with ultraviolet light (45).

It is well established that medium supplements have to be adjusted to the tumor types to efficiently culture PDTOs. An overview of the supplements included in this analysis can be found in table 3. Advanced DMEM/F12 was used in all but two studies as basic medium, often supplemented with Glutamax and/or HEPES. All media included a mixture of antibiotics and/or antimycotics. One study (28) used StemPro™ hESC SFM growth full medium and another (45) used RPMI 1640 supplemented with fetal bovine serum (FBS). Notably, there is considerable variability in components, even within the same tumor type. Basic compound, such as noggin, R-spondin, Wnt and B27, were not used in all studies. Noggin was used in six media, R-spondin in five and Wnt was added only once, namely in the gastric medium. B27 was supplemented most frequently to the media (n = 9), consistently present when advanced DMEM-F12 served as basic medium, followed by the frequent addition of the ROCK-inhibitor Y-27632 (n = 8), and EGF (n = 8). One study added heat-inactivated (56°C, 30 min) and 0.22 µm filtered supernatants, obtained after being centrifuged at 1200 rounds per minute (rpm) for 5 min, at varying concentrations (10%, 25%, 50%, 100%) into the final medium. This implementation resulted in a substantial increase in PDTO forming efficiency and organoid size across all concentrations except for the 100% concentration (29). However, as this technique was used in only one study, a universally standardized method could not be established.

The dissimilarity among the media for ovarian cancer is pronounced (7, 19, 27, 28). Despite three media incorporating R-spondin, Noggin, B27, Y-27632, NAC, nicotinamide, EGF and A8301, they still diverge significantly in other supplements such as SB203580, IGF1, HGF, forskolin, hydrocortisone, heregulinβ-1, β-estradiol, among others (7, 19, 27). Carvalho et al's

ovarian medium stands out with its unique composition - utilizing StemPro™ hESC SFM growth full medium as the base, lacking R-spondin and Noggin, B27, Y-27632, NAC, nicotinamide, EGF and A8310, with the sole addition of an unspecified FGF and 2-mercapthoethoethanol (28). Conversely, a striking uniformity emerges in the media for lung cancer organoids, with the exception of Mazzochi et al., who exclusively relies on RPMI 1640 and FBS as the growth medium (43). In each case, the medium comprises advanced DMEM/F12 supplemented with B-27, Y-27632, N2, EGF, and bFGF (32, 42, 44). An interesting observation occurred in the study by Wang et al. (44), where two breast cancer organoids unintentionally formed from pleural effusion and thrived in the medium originally intended for lung cancer. Unfortunately, a comparative analysis is not possible for media used in breast, colorectal, and gastric organoids, as these studies stand as singular representations within their specific tumor types.

3.6. Recapitulation of primary tumor characteristics

Different studies demonstrated that the PDTOs matched the PDCCs circulating in the ME and the parental tumors (29, 42, 44). Wang et al. (44) showed that ME-PDTOs maintain the morphologic and pathologic features of the parental tumor and reflect its individual characteristics. In this study, they analyzed the concordance of the somatic alterations between 20 matched ME and PDTOs, which was 71% (44). Moreover, they demonstrated that multiple ME-PDTOs from the same patient remained stable and adequately retained tumor heterogeneity. Li et al. (29) managed to demonstrate that malignant ascites-derived organoids retained the characteristics and mutated genes from the malignant ascites (87% average mutational overlap). Principal component analysis showed that PDTO organoids generated from pleural effusion are similar to the parental malignant cells (42).

3.7. Applications

Seven studies used the developed ME-PDTOs for drug screening. Wang et al. (44) performed a therapy prediction screening on 54 lung cancer organoids (chemotherapy, targeted therapy and combinations), which resulted in an overall sensitivity of 84%, specificity of 83% and accuracy of 83% when compared to the clinical response. Bi et al. (7) screened 2 ME-PDTOs of ovarian cancer for the most commonly employed antineoplastic drugs in gynecological

oncology, which mainly were chemotherapeutic drugs, but also a monoclonal antibody. They managed to have the results of the screening in 7-10 days after obtaining the initial sample. Li et al. (29) focused on chemotherapeutics only, and saw divergent responses to different chemotherapies. Ubink et al. (30) used PDTOs as a platform to test hyperthermic intraperitoneal chemotherapy (HIPEC) regimens on an individual patient level. Significant variation in responsiveness between mitomycin C and oxaliplatin were noted. Furthermore, applying HIPEC at typical clinical dosages resulted in minimal impact on the viability of multiple PDTOs lines (30). Chen et al. (19) mainly tested for targeted therapies with a focus on short term culturing. Their model can be expanded for at least six days and could be used for empiric drug sensitivity testing (19). Peng et al. (32) used two lung cancer organoids derived from malignant pleural effusion to successfully test whether to use targeted therapy combination strategies or monotherapy. Both the pretreated and treatment naïve patient achieved partial response.

Other studies focused more on fundamental and translational research. Bose et al. (27) used genetically encoded, fluorescent biosensors to investigate ovarian cancer metabolism. Extensive RNA-analysis was used by Surina et al. (42) to investigate the differences between patient-derived spheroids and organoids, their hypothesis is that the former mimics local cancer expansion, whereas the latter is a model for cancer metastasis. Two studies (28) managed to establish co-cultures. Carvalho et al. (28) discovered with these co-cultures of PDTO's and cancer associated fibroblasts (CAFs) critical signaling pathways, ligands and receptors, which have prognostic and therapeutic consequences.

4. Discussion

The success of ME-PDTOs is evident in the overall establishment rate of 81%, reflecting the generation of 190 organoids from a pool of 234 ME samples. This robust success rate positions ME-PDTOs as a promising and reliable source material. Notably, the mean and median success rates across the studies were 84% and 92%, respectively, surpassing those observed in PDTOs from resection specimens and biopsies, particularly in the context of lung cancer organoids (46). Moreover, ME-PDTOs demonstrate versatility by being successfully established from various malignancies, including ovarian, endometrial, gastric, breast, lung, and colorectal cancer. This diversity underscores the utility of MEs for the development of a broad variety of PDTOs. Thus, ME-PDTOs emerge as a valuable and flexible resource, providing

researchers with a range of organoid models for diverse cancer types. It is crucial to acknowledge the variation in success rates between studies, ranging from 33% to 100%, which is likely dependent on the number of samples included. Larger sample sizes are expected to more accurately reflect reality, as smaller sample sizes may be insufficient in representing the true success rates. No discernible link in high success rates is apparent with a specific methodology or tumor type. Nevertheless, we anticipate that the success ratio will vary based on the tumor type, mirroring the patterns observed in PDTO cultures generated from resection specimens (2).

However, it is crucial to acknowledge certain limitations within the reviewed studies. The majority of these investigations featured small sample sizes, and some were excluded due to inadequate reporting of establishment success rate (6, 9, 16, 47). The potential influence of publication bias should also be considered, as studies reporting positive outcomes may be more likely to be published. Additionally, a noteworthy aspect is the absence of clear criteria for defining a successful organoid across the studies included in this review. Alongside the lack of clear criteria, another notable issue is the absence of standardized terminology; for instance, the distinction between long-term and short-term organoids lacks consensus. Some research groups define long-term organoids as PDTOs surpassing one year in culture, 15 passages, or 5 passages (48-50). There is little consistency, and various criteria are employed. It is important to be mindful of the scarce evidence published about ME-PDTOs. Strong conclusions cannot be drawn according to the small sample sizes of above analyzed studies. The high rate of establishment (overall 81%) is mainly influenced by one study, however, different smaller proof of concept studies had similar rates (mean 84%, median 92%). Nonetheless, the evidence shows that generating PDTOs from ME is feasible and should be further investigated.

Various techniques are currently being explored for the development of ME-PDTOs, with no consensus emerging on an optimal method. Researcher are investigating diverse factors, such variations in centrifugation speed, utilization of RBC-lysis buffer, among others. Interestingly, high success rates can be obtained with both the addition and omission of RBC-lysis buffer. We therefore deduct that the use of RBC-lysis buffer is suitable in cases of a bloody sample or the presence of a red pellet, demonstrating its utility in this analysis without evident problems. Moreover, a substantial variation is observed in the volumes of the MEs used,

spanning from 40 mL to 1000 mL. This discrepancy can be attributed to the clinical context; diagnostic taps typically yield smaller volumes, whereas therapeutic taps involve larger quantities aimed at symptom relief. However, it is crucial to emphasize that, regardless of the volume collected, the paramount consideration lies in the number of viable cells obtained. Additionally, all the studies incorporated in the analysis exclusively employed natural extracellular matrices (ECMs) such as Matrigel and Cultrex. However, there is a growing interest in the utilization of synthetic scaffolds, primarily due to their enhanced controllability, marked by reduced batch-to-batch variability (51). Synthetic scaffolds offer increased customizability in terms of stiffness, porosity, and degradation rates, as well as a consistent composition (51). Notably, there is an interesting gap in research concerning the use of synthetic scaffolds in the context of ME-PDTOs, given the significant differences in stiffness between MEs and the solid tissue surrounding cancer cells. While some investigations have been conducted on the use of (semi-)synthetic scaffolds in spheroids derived from 2D cell lines (52, 53), such exploration has not extended to the domain of ME-PDTOs.

Regarding the choice of medium, there is an inconsistent use of growth factors and supplements, which is mainly due to the various tumor types included in this analysis. Despite these variations, certain tumor-specific culturing media exhibit significant similarities, particularly in the case of lung cancer medium as mentioned in the result section. Notably, one study has demonstrated a positive effect on organoid size and formation by supplementing the medium with ME supernatants. However, complete substitution of the medium with supernatants was found to hinder organoid growth (29). A similar favorable outcome with the incorporation of supernatants was observed in a study conducted by Velletri et al. (54). In this study, they introduced ascites supernatants to 2D cell lines, revealing that a concentration of 12.5% exhibited the highest efficacy. Their experiment underscored the possible benefit of supernatant supplementation in forming PDTOs from malignant effusions. The stimulation of organoid growth is attributable to malignant ascites extracellular vesicles (55). These extracellular vesicles (EVs) carry microRNAs (miRNA), proteins, lipids, etc., playing a crucial role in cell-to-cell interactions (56, 57), essential for organoid development. MiRNA within EVs regulates cancer proliferation, invasion, migration, chemoresistance immune response and reshaping the tumor microenvironment (TME) (57). To date, no studies have investigated the impact of supernatants on pleural effusion-derived organoids. On the other hand, Mazzochi et al. (43), adopted a different approach by using FBS as a supplement for

their organoid medium, a practice cautioned against in the literature (58). The use of animal-derived serums like FBS introduces challenges related to non-standardization, given the inherent heterogeneity and the unknown exact composition (58, 59). Moreover, concerns has been raised about the substantial and unknown effects on organoid culture and phenotype (60). In addition to malignant ascites and pleural effusions, pericardial effusions can be worthwhile developing organoids from (16, 44). Wang et al. managed to develop 14 PDTOs from pericardial effusions (44). This technique however, is more invasive than a paracentesis or thoracentesis. Another group successfully established bile-derived organoids from patients with biliary cancer with minimal invasiveness from nearly all patients, including inoperable cases (61). In conclusion, deriving organoids from varied malignant effusions and body fluids, including pericardial effusions and bile, presents a promising avenue in precision oncology in the metastatic setting.

Recent evidence suggests that ME-PDTOs can effectively recapitulate the genomic, transcriptomic and phenotypic characteristics of the malignant cells in the effusions and the parental tumors (16, 19, 29, 31, 42-44). However, one study found that PDTOs from ME and lymph node biopsies in the same patient sometimes exhibited both morphologic differences and varied sensitivities to drug screening, which suggests the existence of intermetastatic tumor heterogeneity (44). This observation underscores the capability of PDTOs to faithfully preserve the clonal heterogeneity inherent in individual patients, further emphasizing their relevance as a valuable model for studying tumor behavior and drug responses. However, solely using MEs might not give a completely accurate picture, due to intermetatastatic heterogeneity and clonal drift (62). It is noteworthy that no specific studies have yet been conducted to compare drug responses in the same patient for PDTO derived from biopsies/resections and ME-PDTOs.

The potential applications of ME-PDTOs extend beyond basic and translation research, and include the clinical setting as well, where they are used as a tool for precision medicine. ME-PDTOs offer a platform to screen for individual drug sensitivities. The feasibility was demonstrated by Li et al. (29) in malignant ascites-derived organoids (29). Other studies showed a correlation between the *ex vivo* drug response in ME-PDTOs and the *in vivo* therapeutic effect (7, 16, 31). Bi et al. managed to finalize the results of drug screenings in 7-10 days (7). The use of short-term cultures in drug screening has certain advantages over long-

term culturing. Not only does it allow drug testing within one week, but it might also be less prone to genetic drift or subclone selection than long-term culturing. Although promising, the use of ME-PDTOs as a tool for precision medicine is currently limited by the lack of standardization and mainly anecdotal evidence. Besides their use in clinical decision making, ME-PDTOs are amenable to different experimental techniques, such as testing novel therapeutic agents (16), researching the metabolic properties of cancer (27) and investigating resistance mechanisms and possibilities to overcome them (63). Recently, more complex methods are emerging such as co-culturing organoids with other important cell types (e.g. immune cells, stromal cells,….) to better recapitulate the TME and generate so called assembloids (6, 11, 64-66). This approach fills a crucial gap in current organoid cultures. Ongoing developments in co-culturing techniques now facilitate the exploration and prediction of immunotherapeutic effects, addressing a significant unmet clinical need (67). Other advanced methods, such as tumor-on-a-chip and microfluidics involve creating microscale devices that replicate the physiological conditions of tumors in the human body. They can be integrated with PDTO to offer a more accurate and dynamic exploration of disease biology, treatment development and toxicity screening, carefully summarized by Hwangbo et al. (68).

Generating PDTOs out of ME offers several advantages over developing organoids from biopsies or resection specimens: (i) procedures like paracentesis or thoracentesis are substantially less invasive compared to surgical tumor removal or a (endoscopic) biopsy, and only caries a low risk of complications (38, 39). (ii) They are more cost-effective due to requiring fewer materials and personnel, with no need for an operating room or general anesthesia. (iii) The recurrent nature of malignant effusions allows for sequential organoid culturing. This enables regular drug screening, facilitating adjustments to therapeutic regimens based on acquired drug resistance (29). However, it is important to note that after repeated drainages, there may be fewer viable cells present in the effusion, potentially impacting PDTO formation (44) and increasing procedural difficulty. (iv) Analysis reveals that the success rate of organoid generation from MEs surpasses the general establishment ratios for PDTOs derived from biopsies or resection specimens (26, 69). Moreover, the study of Wang et al. (44) underscores a significant disparity in establishment ratios, with non-ME approaches succeeding in only 58% of cases compared to the 82% success rate achieved with ME-PDTOs. (v) The substantially higher percentage of tumor cells within MEs, as compared to non-

malignant epithelial/mesothelial cells (70), facilitates the achievement of high purity PDTOs (14). This remains a persistent issue in the development of pure lung cancer organoids (26, 71), for which ME-PDTOs can prove advantageous. The limitations of lung cancer organoids were concisely reviewed by Ma et al. (71). (vi) MEs are more common in advanced stages of cancer (34-36), making the development of PDTOs from MEs increasingly important for precision oncology. This approach is especially crucial for patients with advanced or treatment-resistant cancers who have not responded to standard therapies. Additionally, MEs offer a valuable alternative source for creating PDTOs in advanced cancer cases, particularly when surgery is not a standard treatment option.

However, it is important to acknowledge certain limitations. (i) MEs are restricted to advanced malignancies and certain tumor types and (ii) some MEs exhibit low in cellularity (72), making it challenging to obtain sufficient tumor cells for the development of organoids. Cell counting before the processing of the sample could aid in adequate sample selection. (iii) The absence of the TME, attributed to the inherent non-adherence of malignant cells in MEs to the surrounding tissues. Importantly, this limitation is not unique to ME-PDTOs but extends to their solid-tissue counterparts, especially in long-term cultures. Mitigating this challenge involves co-culturing with immune cells, CAFs, endothelial cells, and related constituents (6, 11, 64-66), which is currently an active field of research. These limitations underscore the importance of carefully considering patient selection and the stage of cancer when opting for ME-PDTOs in precision oncology research.

5. Further research

PDTOs constitute a relatively new area of research, characterized by an increasing amount of data being gathered daily. However, the focus within the literature predominantly centers on PDTOs derived from solid tumor tissues derived from biopsies or surgical resections. As discussed above, MEs could be a robust source for tumor material and in some instances even better than biopsies or resection specimens (44). However, a significant gap exists in the availability of comprehensive large-scale data pertaining to MEs, constituting a primary limitation. Immediate future steps involve expanding studies with larger sample sizes. This will enable us to conduct a thorough comparative assessment of establishment rates and characteristics among ME-PDTOs derived from diverse cancer types. Moreover, this will guide us in establishing a standardized methodology for the generation of ME-PDTOs. This includes

researching considerations such as the incorporation of supernatants into the culture medium, especially in pleural effusions where this approach has not been previously explored. Furthermore, an exploration into the utilization of synthetic scaffolds is warranted, given the marked distinction in the surrounding environment of MEs compared to solid metastases and primary tumors. Additionally, a critical evaluation of organoid quality is mandated, focusing on the faithful recapitulation of parental tumor characteristics, organoid expandability, cryopreservation and relevant parameters. Elucidation and investigation of the predictive value of potential applications, including sequential measurements for therapy guidance and resistance prognostication, and the predictive efficacy of ME-PDTOs in drug screening, are of pivotal importance. Finally, the lack of uniform terminology and standardization, including criteria for distinguishing long-term from short-term culture, defining a successful patient-derived tumor organoid, and establishing clear definitions for various models such as organoids and spheroids, poses a significant obstacle to the systematic interpretation of studies. Urgent and collaborative efforts are imperative to formulate uniform definitions that can be universally adopted in the field of 3D-cell culturing, thereby enhancing clarity and comparability across research endeavors.

6. Conclusion

PDTOs provide a novel and powerful tool in the clinical setting and basic/translational research. Results of this literature search demonstrate that PDTOs can be generated out of MEs in a high percentage of the cases (overall 81%). There are various benefits to using ME-PDTOs: Firstly, the acquisition of PDCCs via drainages is (i) less invasive and (ii) more cost-effective. This technique facilitates (iii) sequential organoid formation and (iv) exhibits a higher success rate compared to organoids obtained from biopsies/solid tissues, particularly in the context of lung cancer. Moreover, (v) it increases the purity of the lung cancer PDTOs. Lastly, (vi) it presents a novel and valuable source for the implementation of precision oncology in the advanced cancer setting. Possible disadvantages are that (i) their use is limited to metastatic cancers and can thus not be used in early-stage cancers, (ii) the low cellularity in certain MEs and (iii) the absence of a TME. These organoids can be used for different applications, but publications mainly focus on drug screening and clinical decision making. Further research concerning the optimization of the culturing settings and the validation

whether ME-PDTOs recapitulate the heterogeneity and functional hierarchy of the parental tumor is crucial.

7. Tables and Figures

Table 1: Overview of the included studies and efficiency of ME-derived organoids

Author	Year	Cancer type	ME	Sample size			Success rate		
				Total°	Non-ME-DO	ME-DO	Total°	Non ME-DO	ME-DO
Bi (7)	2021	Ovarian/ endometrium	Ascites	52	45	7	83% (43/52)	82% (37/45)	85% (6/7)
Bose (27)	2022	Ovarian	Ascites	8	0	8	100% (8/8)	NA	100% (8/8)
Carvalho (28)	2022	Ovarian	Ascites, pleural fluid	8	0	8	100% (8/8)	NA	100% (8/8)
Chen (19)	2020	Ovarian	Ascites, pleural fluid	21	0	21	67% (14/21)	NA	67% (14/21)
Li (29)	2019	Gastric	Ascites	12	0	12	92% (11/12)	NA	92% (11/12)
Mazzocchi (43)	2019	Lung	Pleural fluid	2	0	2	100% (2/2)	NA	100% (2/2)
Pan (31)	2021	Breast	Pleural fluid	3	0	3	33% (1/3)	NA	33% (1/3)
Peng (32)	2022	Lung	Pleural fluid	2	0	2	100% (2/2)	NA	100% (2/2)
Surina (42)	2023	Lung	Pleural fluid	8	0	8	63% (5/8)	NA	63% (5/8)
Ubink (30)	2019	Colorectal	Ascites	14*	13	1	29% (4/14)	23% (3/13)	100% (1/1)

Wang (44)	2023	Lung/breast	Ascites, pleural fluid	214	52	162	76% (162/214)	58% (30/52)	82% (132/162)

ME-DO, malignant effusion-derived organoid; NA, not applicable

° Total of organoid culturing, including other source material.

**metastasis samples, 1 ascites sample; the already established organoid line (TOR10) is not included*

Table 2: methods of ME-derived organoid development

Author	Method of retrieval	Amount of ME	Centrifugation	RBC lysis	supernatants	ME-DO success rate
Bi (7)	surgical	50-100 mL	500xg, 10 min 4°C	yes	no	85% (6/7)
Bose (27)	not specified	not specified	1000 rpm, 5 min	yes	no	100% (8/8)
Carvalho (28)	paracentesis	not specified	1000 rpm, 5 min	yes	no	100% (8/8)
Chen (19)	paracentesis	not specified	365xg, 15 min	yes	no	67% (14/21)
Li (29)	paracentesis	not specified	1200 rpm, 5 min	no	yes (10%, 25%, 50%, 100%)	92% (11/12)
Mazzocchi (43)	paracentesis	500 mL-1L	not specified	yes	no	100% (2/2)
Pan (31)	paracentesis	50 mL	1300 rpm, 5 min	yes	no	33% (1/3)
Peng (32)	paracentesis	200-800 mL	112 rcf, 3 min	yes	no	100% (2/2)
Surina (42)	not specified	not specified	no	no	no	63% (5/8)
Ubink (30)	surgical	40 mL	400xg, 5 min	no	no	100% (1/1)
Wang (44)	paracentesis	200-1000 mL	300xg, 5 min 4°C	no	no	82% (132/162)

rpm, rounds per minute; g, gravitational constant; rcf, relative centrifugal force; ME, malignant effusion; RBC, red blood cell; ME-DO, malignant effusion-derived organoids

574 *Table 3: Overview of media used*
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	Bi (7)	Bose (27)	Carvalho (28)	Chen (19)	Peng (32)*	Mazzochi (43)	Surina (42)	Wang (44)	Pan (31)*	Ubink (30)	Li (29)
Cancer type	**Ovarian endometrial**	**Ovarian**	**Ovarian**	**Ovarian**	**Lung**	**Lung**	**Lung**	**Lung**	**Breast**	**CRC**	**Gastric**
Basic medium	Advanced DMEM/F12	Advanced DMEM/F12	StemPro™ hESC SFM growth full medium	Advanced DMEM/F12	Advanced DMEM/F12	RPMI 1640	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12	Advanced DMEM/F12
Glutamax	1x	1x	-	1%	-	-	-	1x	1x	400 µM	1x
Hepes	10 mM	-	-	10 mM	-	-	-	-	10 mM	10 mM	10 mM
Antbiotic-antimycotic	P/S (dose not specified); primocin 2%	P/S (100 U/mL)	P/S (10,000U/ 10 mg/mL), gentamicine (2,5 µg/mL), ampho-tericine B (2,5 µg/mL)	antibiotic-antimycotic (not specified), Primocin (100 µg/mL)	P/S (1%)	P/S (200U/mL)	P/S Ampho-tericine B (dose not specified)	P/S (1%)	P/S (100U/ml/ 100 mg/ml); primocin (50 mg/mL)	Penicilline (100 U/mL), Strepto-mycine (100µg/mL)	P/S (100U/ml/ 100 mg/ml); primocin (50 mg/mL)
Fetal Bovine Serum	-	-	-	-	-	5%	-	-	-	-	-
B27	1x	1x	-	1x	1x	-	1x	1x	1x	1x	1x
R-spondin1	250 ng/mL	50 ng/mL	-	10%	-	-	-	-	-	-	500 ng/mL
R-spondin 3	-	-	-	-	-	-	-	-	250 ng/mL	-	
Noggin	100 ng/mL	100 ng/mL	-	100 ng/mL	-	-	-	-	100 ng/mL	50 ng/mL	100 ng/mL
Wnt3a conditioned medium	-	-	-	-	-	-	-	-	-	-	50%
Y-27632	10 µM	10 µM	-	5 µM	10 µM	-	10 µM	10 µM	5 µM	-	10 µM
NAC	1,25 mM	5 mM	-	1,25 mM	-	-	-	-	1,25 mM	1 mM	1 mM
Nicotinamide	5 mM	5 mM	-	1 mM	-	-	-	-	5 mM	-	10 mM
N2	-	1x	-	-	1x	-	1x	1x	-	-	-
EGF	50 ng/mL (human)	50ng/mL	-	5 ng/mL	50 ng/mL (human)	-	50 ng/mL	50 ng/mL (human)	5 ng/mL	-	50 ng/mL
A8301	5 µM	250 nM	-	0,5 µM	-	-	-	-	500 nM	500 nM	2000 nM

bFGF (FGF-2)	-	-	-	-	20 ng/mL	-	20 ng/mL	20 ng/mL	-	-	-
FGF7	-	-	-	5 ng/mL	-	-	-	-	5 ng/mL	-	-
FGF10	100 ng/mL	-	-	20 ng/mL	-	-	-	-	-	-	10 ng/mL
FGF (not specified)	-	-	10 µg/mL	-	-	-	-	-	-	-	-
forskolin	10 µM	-	-	-	-	-	-	-	-	-	-
Hydro-cortisone	500 ng/mL	-	-	-	-	-	-	-	-	-	-
Heregulinβ-1	37,5 ng/mL	-	-	-	-	-	-	-	-	-	-
β-estradiol	100 M	10nM	-	-	-	-	-	-	-	-	-
HGF	-	10 ng/mL	-	-	-	-	-	-	-	-	-
IGF1	-	20 ng/mL	-	-	-	-	-	-	-	-	-
Neuroregulin I	-	10 ng/mL	-	5 nM	-	-	-	-	5 nM	-	-
SB203580	-	1 µM	-	-	-	-	-	-	-	-	-
Gastrin	-	-	-	-	-	-	-	-	-	-	1 nM
SB202190	-	-	-	-	-	-	-	-	500 nM	10 µM	-
2-Mercapto-ethanol	-	-	1x	-	-	-	-	-	-	-	-

* *referred to a previous publication for the methodology*

NAC, n-acetyl-l-cysteine; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insuline-like growth factor; P/S, penicillin-streptomycin.

582 *Graphical abstract*

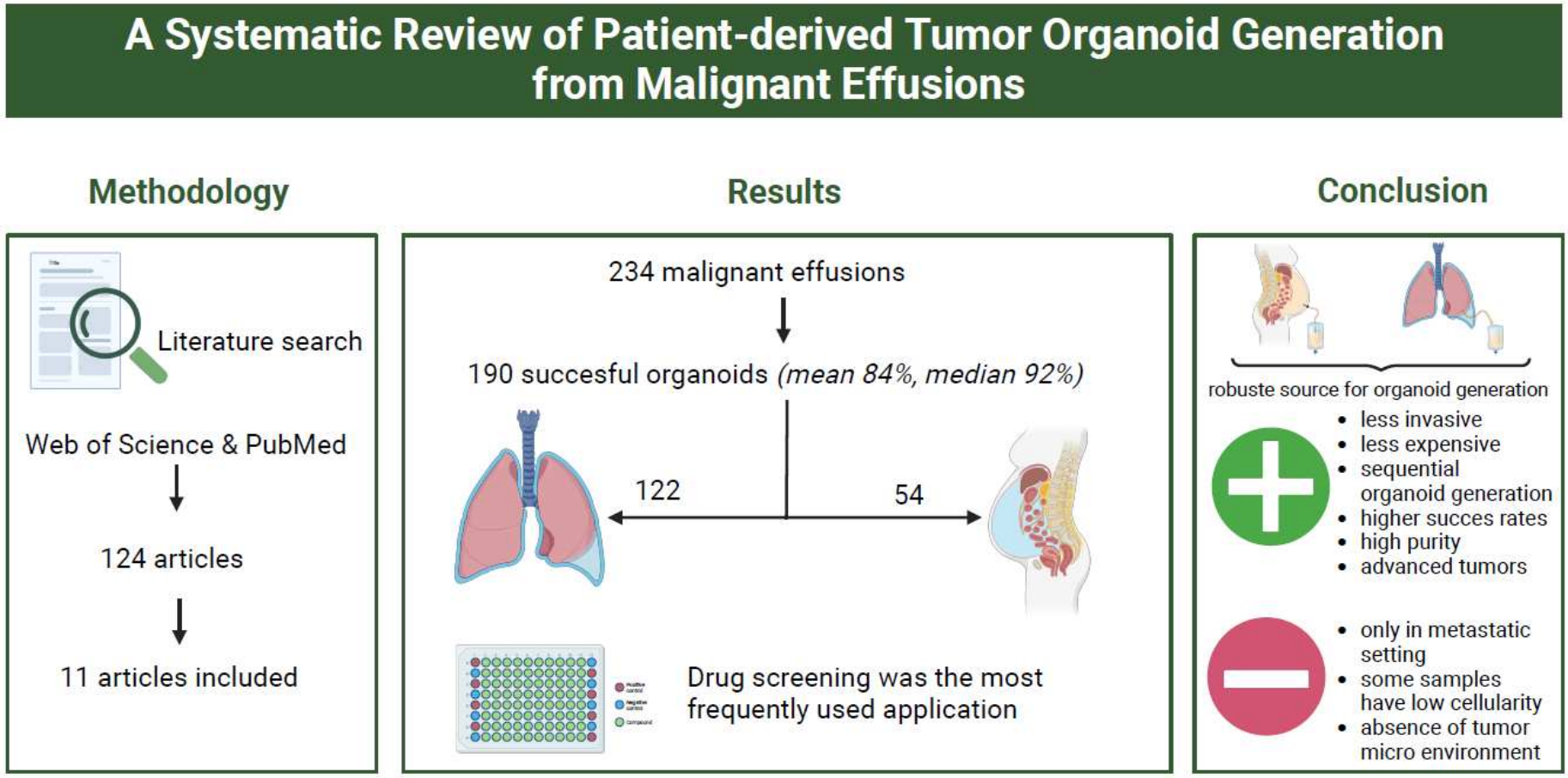
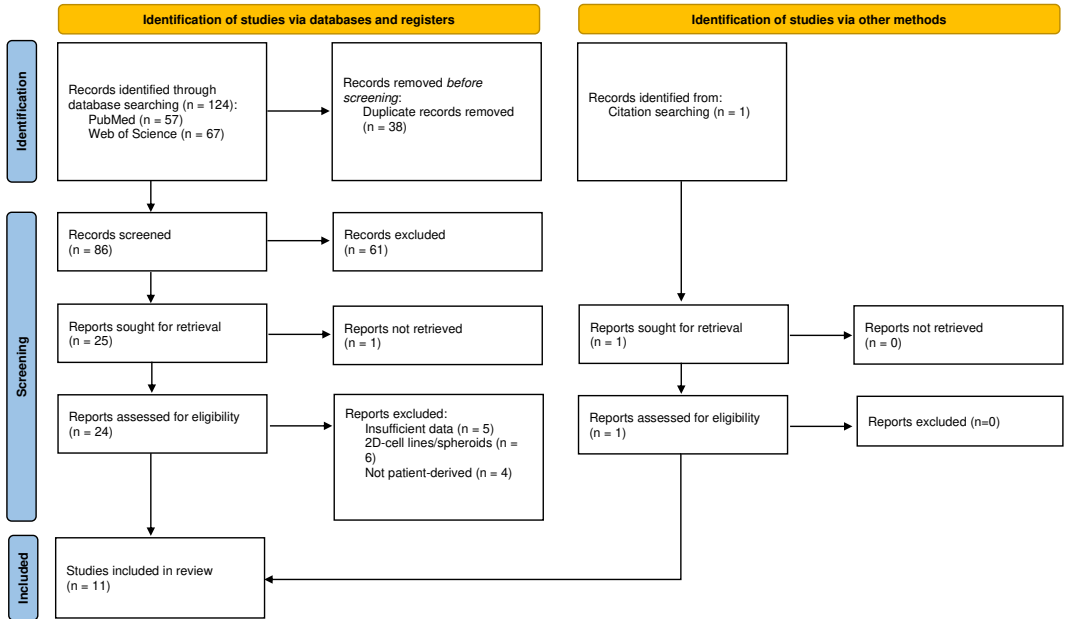
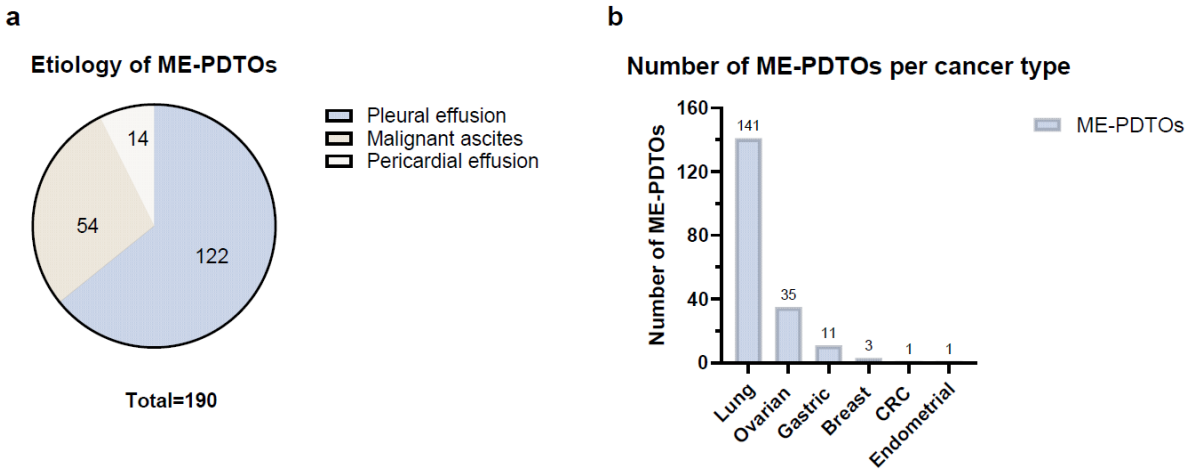


Figure 1: PRISMA 2020 flow diagram and the process of data selection



PRISMA flow diagram showing the study selection.

Figure 2: Etiology of ME-PDTOs



(a) Malignant pleural effusion was the most frequently used source in developing PDTOs. In total 122 PDTOs were generated using pleural effusions, 54 PDTOs were created using malignant ascites and 14 PDTOS using pericardial effusions. (b) Lung cancer organoids were most frequently created (n=141), followed by ovarian, gastric, breast, colorectal and endometrial carcinoma's. *CRC: colorectal carcinoma; ME: malignant effusion; PDTO: patient-derived tumor organoid.*

8. Additional information

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The graphical abstract was made with BioRender.

Authors' contribution

S.S. conceived the idea. S.S. wrote the review with supervision from C.D.. C.D., M.L.C., J.M.H.H., P.V.S., A.J., R.W., N.K., and H.P. provided critical feedback. S.S. made the figures. All authors contributed to the final manuscript. S.S. is the guarantor of the review.

Ethics approval and consent to participate: not relevant

Consent for publication: not relevant

Data availability

All data generated during this study are included in this published article.

Declaration of Competing Interests.

The authors declare no conflict of interest.

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