FOCUSED COMMUNICATION

EUS-guided biopsies versus surgical specimens for establishing patient-derived pancreatic cancer organoids: a systematic review and meta-analysis

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Background and Aims: Patient-derived tumor organoids (PDTOs) are a promising new disease model in pancreatic cancer for use in personalized medicine. However, the overall success rate (SR) of establishing these cultures from EUS-guided biopsies is unknown.

Methods: We searched relevant database publications reporting SRs of PDTO establishment from pancreatic cancer. The primary outcome was SR stratified on tissue acquisition method (EUS-guided biopsies, percutaneous biopsies, and surgical specimens).

Results: Twenty-four studies were identified that included 1053 attempts at establishing PDTOs. Overall SR was 63% (95% confidence interval [CI], 54%-72%). Pooled SRs of PDTO establishment from EUS-guided biopsies, percutaneous biopsies, and surgical specimens were 60% (95% CI, 43%-76%), 36% (95% CI, 14%-61%), and 62% (95% CI, 48%-75%), respectively, and did not differ significantly ($P = .1975$).

Conclusion: The SR of PDTO establishment from EUS-guided biopsies is comparable to that from surgical specimens. Both techniques are suitable for tissue acquisition for PDTOs in clinical and research settings. (PROSPERO registration number: CRD42023425121.) (Gastrointest Endosc 2024;100:750-5.)

Pancreatic ductal adenocarcinoma (PDAC) is the fourth most common cause of cancer-related mortality and is projected to be the second leading cause of cancer-related death by 2040.[1] Patient-derived tumor organoids (PDTOs), 3-dimensional cell cultures derived from patient tumors and grown in a scaffold of basement membrane extract (BME) hydrogel, may present a breakthrough in personalized treatment of PDAC by customizing chemotherapeutic regimens based on their drug response. Tumor tissue for establishment of PDTOs has traditionally been acquired from surgical specimens, although establishment from percutaneous- or EUS-guided biopsies have also been shown. However, the exact success rate (SR) of establishment from either tissue acquisition method is unknown. Conventional comparison of reported SRs is hampered by differences in methodology and lack of consensus regarding what constitutes a successful culture but have been reported to be anywhere from <20%[2,3] to >80%.[4-6] Furthermore, accidental establishment of PDTOs

Abbreviations: BME, basement membrane extract; CI, confidence interval; PDAC, pancreatic ductal adenocarcinoma; PDTO, patient-derived tumor organoid; SoV, strength of validation; SR, success rate.

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consisting of healthy cells is a known issue.[7] Although studies have shown that 22% to 63% of PDTOs from tumor samples may consist primarily of nonmalignant cells,[8,9] thorough validation is not used consistently, making comparisons difficult.

The aim of the current systematic review and meta-analysis was to derive a pooled estimate of SRs of PDTO establishment from pancreatic cancer, stratified on a tissue acquisition method. The impact of using stringent validation methods and choice of success criteria for PDTOs on reported SRs was also investigated.

METHODS

Study selection and data extraction

Briefly, a study protocol was developed before initiation of the study (PROSPERO registration number: CRD4202342 5121) in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[10] A predefined search string (Organoid* AND Cancer AND Pancrea*) was used to search Medline, Embase, and Web of Science databases. Study selection and data extraction of SR was performed independently by at least 2 authors (S.E.G., P.K., or H.M.M.S.), and any conflicts were resolved by discussion or through consultation with a fourth author (B.K.).

Success criteria group and strength of validation

To address issues of heterogeneous definitions of success, studies were pooled according to their apparent success criteria. Studies were divided into 4 categories: establishment only/unspecified; cellular expansion; prolonged viability; and molecular validation.

Acknowledging that risk of misclassifying benign organoid cultures as successes would not only be influenced by choice of success criteria but also culture conditions, we designed a strength of validation (SoV) classification scheme that took both factors into account. If studies used culture medium derived from one or several key growth factors, limiting the expansion of nonmalignant cells, a "high" SoV score would be assigned if "prolonged viability," "cellular expansion," or "molecular validation" was used as success criteria. Otherwise, only studies that used "molecular validation" as success criteria received a high SoV classification. Remaining studies were classified as having a low SoV.

Statistical analyses

The double arcsine transformation of the proportions was used to obtain unbiased effect size estimates. The effect sizes were weighted by the inverse of the study variance. The results were pooled by using the DerSimonian-Laird method (random-effect model), as a high level of heterogeneity was expected. Heterogeneity was assessed by visual inspection of the plots and corresponding I^2 statistics, including subgroup analysis and leave-one-out sensitivity analysis.

Multivariate analysis using meta-regression was performed by using a mixed-effect linear model. All examined moderators of effect were chosen based on theoretical knowledge of the subject to avoid erroneously attributing heterogeneity to spurious moderators.

RESULTS

A literature search yielded 1524 unique articles and abstracts, of which 24 studies with attempted organoid establishment from 1053 procedures were included in the meta-analysis. These included 281 EUS-guided biopsies, 119 percutaneous biopsies (CT- or US-guided), and 477 surgical specimens; the remaining 178 were unspecified (Table 1).

Success rate for PDTO establishment and impact of tissue acquisition method

Deriving a pooled estimate SR across all studies using a meta-analysis approach yielded a weighted estimate of 63% (95% confidence interval [95% CI], 54%-72%) with high heterogeneity (I^2 = 90%), as expected. The robustness of the estimate was tested by using a leave-one-out analysis, but no influential studies were identified.

Subgroup analysis investigating the impact of the tissue acquisition method was performed, excluding studies not specifying this method (Fig. 1). Pooled SRs were 36% (95% CI, 14%-61%) from percutaneous biopsies (n = 4), 62% (95% CI, 48%-75%) from surgical specimens (n = 15), and 60% (95% CI, 43%-76%) from EUS-guided biopsies (n = 11). Differences between groups were nonsignificant (P = .1975), and significant heterogeneity persisted after adjusting for tissue acquisition method.

To further explore the impact of tissue acquisition, a comparative meta-analysis was performed on studies including both surgical specimens and EUS-guided biopsies (n = 6) or percutaneous biopsies (n = 2). The ratio between SRs of the EUS-guided biopsies and surgical specimens was .93 (95% CI, .79-1.10; P = .3899), slightly favoring surgical specimens but not significantly. Likewise, the SR ratio of percutaneous biopsies and surgical specimens was 1.58 (95% CI, .65-3.85; P = .3169), favoring establishment from percutaneous biopsies but not significantly.

Other sources of heterogeneity

Univariate subgroup analyses were performed to investigate possible sources of heterogeneity. Using SoV as a modifier resulted in an SR of 69% (95% CI, 57%-79%) and 52% (95% CI, 37%-66%) in the low and high SoV groups, respectively, but the difference was not significant (P = .0720).

Lower SR was reported in studies imposing a limit on number of passages that PDTOs should remain viable to be considered successful (55%; 95% CI, 25%-84%) or those

TABLE 1. Studies included in systematic review and meta-analysis

ID	Journal	TSM	Dome method	SoV classification	Criteria group	Successes/attempts (SR [%])			
						Total	EUS-guided biopsies	Surgical specimens	Percutaneous biopsy
Armstrong 2021[11]	Biomedicines	No	Yes	Low	Expansion	15/18 (83)	15/18 (83)	-	-
Beutel 2021[12]	Cancers	No	Yes	Low	Expansion	27/44 (64)	2/2* (100)	3/3* (100)	23/39 (59)
Demyan 2022[13]	Annals of Surgery	No	Yes	Low	Establishment	76/115 (67)	23/43 (53)	52/74 (75)	-
Driehuis 2019[14]	PNAS	Yes	Yes	High	Expansion	52/83 (63)	-	50/77 (63)	-
Grossman 2022[2]	Clinical Cancer Research	Yes	No	High	Expansion	13/79 (16)	3/28 (11)	1/10 (10)	7/37 (19)
Hennig 2019[15]	Stem Cells International	No	Yes	High	Molecular validation	22/31 (71)	5/6 (83)	17/25 (68)	-
Hirt 2022[16]	Cell Genomics	No	Yes	High	Molecular validation	11/17 (65)	-	11/17 (65)	-
Hogenson 2022[3]	JCI Insight	Mix†	No	Low/High†	Prolonged viability	15/91 (16)	2/16 (13)	8/53 (15)	5/22 (23)
Huang 2015[6]	Nature Medicine	Yes	No	Low	Unspecified	17/20 (85)	-	17/20 (85)	-
Ikezawa 2022[17]	Endoscopy International Open	Yes	Yes	High	Prolonged viability	4/5 (80)	4/5 (80)	-	-
Ishida 2022[18]	Anticancer Research	Yes	No	Low	Establishment	24/38 (63)	24/38 (63)	-	-
Lee 2022[19]	Gut and Liver	No	Yes	Low	Prolonged viability	12/20 (60)	12/20 (60)	-	-
Pauli 2017[20]	Cancer Discovery	Yes	Yes	High	Prolonged viability	5/7 (71)	-	-	-
Raghavan 2021[21]	Cell	No	Yes	High	Molecular validation	10/21 (48)	-	-	10/21 (48)
Seppälä 2020[22]	Annals of Surgery	No	Yes	Low	Establishment	59/77 (77)	35/45 (78)	24/32 (75)	-
Seppälä 2022[9]	Clinical Cancer Research	No	Yes	High	Molecular validation	12/20 (60)	-	12/20 (60)	-
Sharick 2020[23]	Frontiers in Oncology	Yes	Yes	High	Cellular Expansion	12/20 (60)	-	12/20 (60)	-
Shiihara 2021[24]	European Journal of Cancer	No	Yes	Low	Prolonged viability	8/20 (40)	-	8/20 (40)	-
Shi 2022[4]	Nature Communications	Yes	Yes	High	Cellular Expansion	80/99 (81)	-	-	-
Tiriac 2018[8]	Cancer Discovery	No	Yes	Low	Prolonged viability	104/138 (75)	43/60 (72)	61/78 (78)	-
Tsai 2018[25]	BMC Cancer	NA‡	Yes	NA	Cellular Expansion	28/37 (76)	-	-	-
Wang 2022[5]	Acta Pharmacologica Sinica	No	Yes	Low	Cellular Expansion	10/11 (91)	-	10/11 (91)	-
Watanabe 2022[26]	BMC Cancer	No	Yes	High	Molecular validation	8/19 (42)	-	8/19 (42)	-

TSM, Tumor-selective medium (growth medium deprived of one or several growth factors: Noggin, Epidermal Growth Factor, WNT, or R-spondin); *SoV*, strength of validation; *SR*, success rate; *NA*, not available.

*Excluded from meta-analysis because sample size was <5.

†Both complete and tumor selective medium used in the study, and both low and high SoV assigned to cultures depending on which was used.

‡Use of a proprietary organoid medium (IntestiCult Organoid Media, STEMCELL Technologies, Vancouver, BC, Canada) prevented determination of whether media was considered tumor selective.

including validation methods as part of their success criteria (58%; 95% CI, 47%-69%); these were compared with those defining success based on the ability of the PDTO to expand past a limit of cellularity (67%; 95% CI, 49%-83%) or those not specifying any criteria or solely based on appearance of organoid structures (72%; 95% CI, 64%-79%). No significant

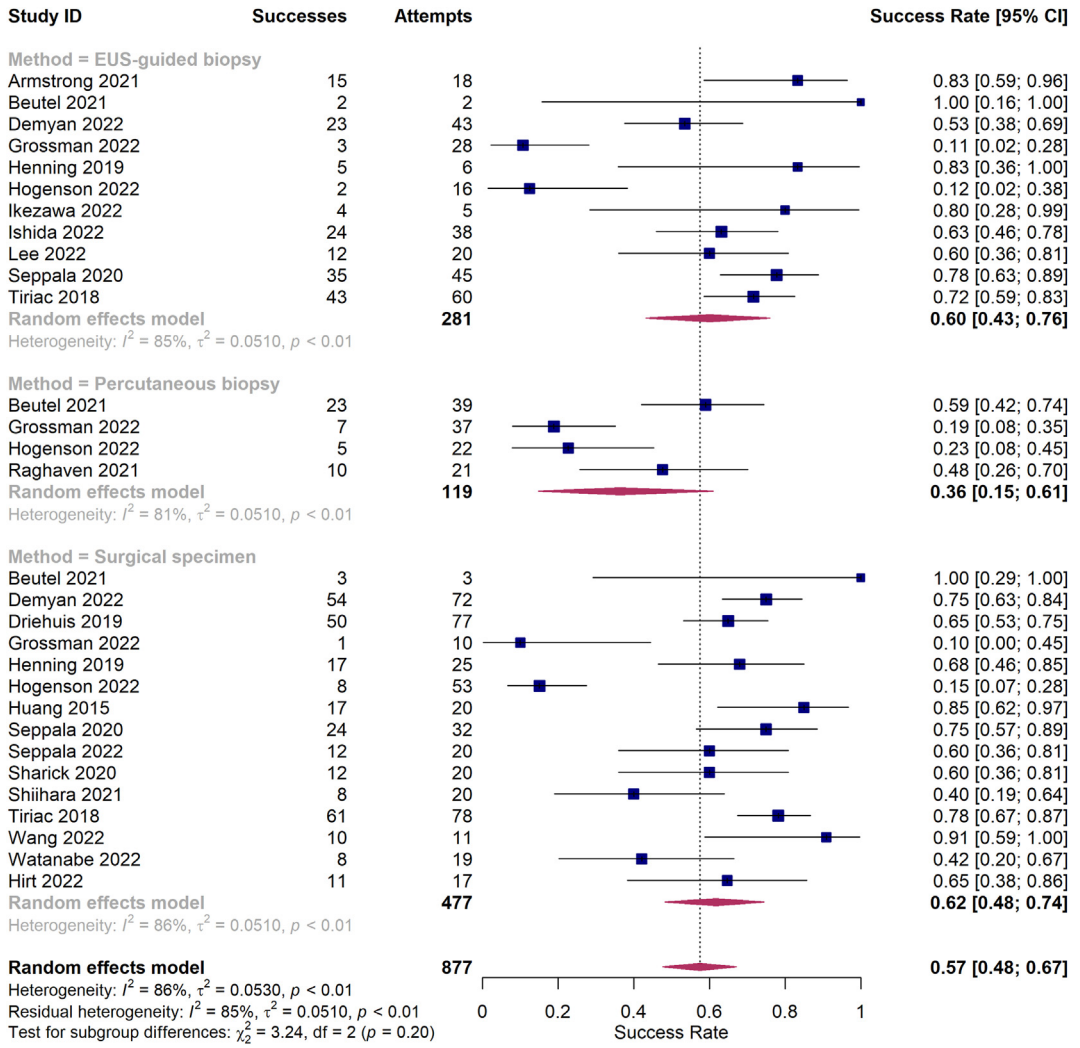

Figure 1. Forest plot and subgroup analyses of studies specifying tissue acquisition method. Studies not specifying tissue acquisition methods are not included here, and the pooled estimate from this subgroup analysis differs from the overall estimate. *CI*, Confidence interval.

difference was seen between studies in the different success criterion groups (P = .1957).

Cultivating PDTOs in "domes" in which cells are completely suspended in BME hydrogel (as opposed to growing them on low-attachment or BME hydrogel–coated surfaces overlaid with diluted BME hydrogel) was associated with an improved SR of 68% (95% CI, 50%-76%) compared with 41% (95% CI, 24%-58%) (P = .0068).

After performing subgroup analyses, we created a multivariate meta-regression model, including studies in which the tissue acquisition method was specified (n = 20). Tissue acquisition method, SoV classification, and culture were included as variables. The model found no association between tissue acquisition method and SR. However, higher SRs were predicted by use of the dome method (P < .0001) and low SoV classification (P = .0005). These results of the meta-regression showing no effect of tissue acquisition method supported our findings of the comparative meta-analysis and subgroup analysis.

DISCUSSION

To the best of our knowledge, this study provides the first pooled and weighted estimate of SR of PDTO establishment from pancreatic cancer of 63% (95% CI, 54%-72%). In addition, we found that SRs when using EUS-guided biopsies (60%; 95% CI, 43%-76%) and percutaneous biopsies (36%; 95% CI, 14%-61%) were comparable and noninferior to surgical specimens (62%; 95% CI, 48%-75%) (P = .1975). Although estimates of SRs from EUS-guided biopsies and surgical specimens were similar and based on >10 studies in each group, the estimate from percutaneous biopsies was noticeably lower and only based on 4 studies, resulting in wide CIs.

Comparison of SRs between studies has been difficult due to lack of consensus regarding success definitions. In this study, we stratified studies according to both success criteria and our SoV classification but found no significant difference between groups, supporting an approach using meta-

analysis. It is important to note that low SoV was a significant predictor of higher SR in our meta-regression analysis, hinting that lack of proper validation of cultures may cause overestimation of SR. However, even when taking SoV into account in this model, the tissue acquisition method was not a significant predictor, supporting that using EUS-guided biopsies is comparable to surgical specimens. Our findings were also supported by our comparative meta-analysis of studies using both methods, finding a nonsignificant difference between methods.

One technical factor of PDTO culturing was associated with better outcome. Use of dome-like cultures correlated with higher SR (68% vs 41%; $P = .0068$). We therefore recommend use of this type of culture technique to maximize SR when establishing new PDTOs.

This study has several limitations. Due to a high number of potential determinants of SR, we could not control for all possible confounders. Furthermore, only one study[2] reported that determination of SR was part of a predetermined outcome, and no studies were designed to directly compare tissue acquisition methods against each other. Data quality is therefore poor, and the results should be interpreted with caution.

Finally, it should be noted that the SR was rather high in the EUS-guided biopsy group, paving the way for using PDTOs established from these procedures in novel, personalized medicine protocols, including using drug screening, to search for off-label candidates for improved neoadjuvant and palliative treatment. This is an important finding considering that most patients are inoperable at the time of PDAC diagnosis, and reliable establishment of PDTOs from biopsy material is essential if this patient group is expected to yield any benefits from organoid-based personalized medicine.

CONCLUSION

EUS-guided biopsies are suitable for establishment of PDTOs with success rates comparable to using surgical specimens.

DISCLOSURE

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