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ORIGINAL ARTICLE

Patient-Derived Xenografts Can Be Reliably Generated from Patient Clinical Biopsy Specimens

Matthew C. Hernandez[1] · John R. Bergquist[1] · Jennifer L. Leiting[1] · Tommy Ivanics[2] · Lin Yang[3] · Rory L. Smoot[1] · David M. Nagorney[1] · Mark J. Truty[1]

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Abstract

Background Patient-derived xenografts (PDX) are clinically relevant human cancer models that can be used to guide individualized medicine. We aimed to generate PDX models from clinically obtained biopsy specimens (surgical or image-guided) hypothesizing that low volume biopsy specimens could provide sufficient viable tissue to successfully engraft PDX models from patients with unresectable or metastatic disease.

Materials and Methods We maintain a prospective high volume gastrointestinal malignancy PDX program. With informed consent and institutional approval, biopsy specimens (surgical or image-guided) were obtained from patients with unresectable or metastatic tumors: pancreatic adenocarcinoma (PDAC), cholangiocarcinoma, gastric and gallbladder carcinoma. Biopsies were implanted into immunodeficient mice. Tumor growth was monitored, viable tumor was passed into subsequent generations, and histopathology was confirmed.

Results In this study, biopsy specimens from 29 patients were used for PDX engraftment. Successful PDX engraftment was variable with highest engraftment rates in gastric and gallbladder carcinoma specimens (100%) compared to engraftment rates of 33% and 29% in PDAC and cholangiocarcinoma respectively. PDX models created from metastasis biopsies compared to unresectable primary tumor tissue demonstrated higher engraftment rates (69% versus 15.4%, $p = 0.001$). PDX models demonstrated higher engraftment rates when biopsies were obtained during surgical operations ($n = 15$) compared to image-guided ($n = 14$) (73% versus 14%, $p = 0.003$). Patient age, pretreatment status, or ischemic time was not different between biopsy methods.

Conclusions PDX models can be successfully created from clinical biopsy specimens in patients with metastatic or unresectable GI cancers. The use of clinical biopsy specimens for PDX engraftment can expand the repertoire of stage-specific PDX models for downstream basic/translational research.

Keywords PDX · Biopsy, patient derived xenograft · Unresectable · FNA

✉ Mark J. Truty
truty.mark@mayo.edu

Matthew C. Hernandez
Hernandez.matthew@mayo.edu

John R. Bergquist
Bergquist.john@mayo.edu

Jennifer L. Leiting
leiting.jennifer@mayo.edu

Tommy Ivanics
tommy.ivanics@gmail.com

Lin Yang
yang.lin@mayo.edu

Rory L. Smoot
smoot.rory@mayo.edu

David M. Nagorney
nagorney.david@mayo.edu

[1] Section of Hepatobiliary and Pancreatic Surgery, Division of Subspecialty General Surgery, Department of Surgery, Mayo Clinic, 200 First St. Southwest, Rochester, MN 55905, USA

[2] Department of Surgery, Henry Ford Medical Center, Detroit, MI, USA

[3] Center for Individualized Medicine, Mayo Clinic, Rochester, MN, USA

Introduction

Reliable and humanized test systems are needed to characterize tumor biology in order to assess novel and targeted medical therapies in the preclinical setting.[1,2] Two and three dimensional cell cultures, organotypic assays, and transgenic mice are translational platforms that can be used for distinct experimental and mechanistic approaches. However, cancer research and precision medicine require test systems that incorporate patient-specific tumor histopathologic and biologic phenotype.[3,4] Patient-derived xenografts (PDX) are clinically relevant platforms that allow for amplification of precious patient cancer tissue.[5] PDX models have been shown to recapitulate original patient tumor architecture with high fidelity.[6] These sophisticated cancer models bridge translational research gaps that other models cannot.[7,8] Since both patient's cancer and PDX tumor can be molecularly characterized and compared, a profile of actionable targets for novel and precise therapy can be created but only if a PDX is successfully generated.[9]

Biopsy specimens have been used to generate genomic analyses to guide personalized therapy as well as PDX model generation.[10,11] Roife et al. demonstrated that fine needle aspirate biopsies acquired from resected patient tissue could be successfully engrafted into immunodeficient mice with engraftment rates similar to traditional techniques.[10] In the future, GI malignancy diagnoses are expected to increase and PDX generation from patients in all stages of disease will be important.[12–15] Since a considerable proportion of patients present with metastatic or unresectable disease, these patients are often excluded from PDX generation and the benefits of preclinical therapeutic assessment using PDX are lost.[16,17] More importantly, PDX models generated solely from patients with resectable cancers limit the oncologic practicality of PDX models given the vast molecular and behavioral heterogeneity between localized primary tumors in comparison to locally advanced unresectable or metastatic types. To address this clinical and translational gap, we aimed to create malignant gastrointestinal tumor PDX models derived from clinical biopsy specimens in patients with unresectable or metastatic GI malignancies. We hypothesized that low volume surgical or radiological core biopsies without tissue dissociation could be utilized to reliably generate PDX models that preserve patient tumor and stromal histopathological architecture.

Methods

Informed Consent and Biopsy Procedure

Prior to any surgical or radiologic procedure and tumor implantation, informed consent was obtained through a protocol approved by the Mayo Clinic Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC). Research informed consent was obtained for patients who present for resection or for biopsy, and this includes a discussion that tissue obtained for research is surplus tissue obtained after clinical needs are satisfied. The indication for biopsy in these patients included histologic confirmation in known metastatic disease or for suspicious appearing masses evaluated during diagnostic laparoscopy prior to planned resection.

Tumor tissue was obtained exclusively during patient biopsy. All biopsies were obtained either during image-guided (computed tomography, CT or ultrasonography, US) procedures or at time of diagnostic laparoscopy. For image-guided biopsies, multiple passes using an 18 gauge core needle biopsy tool were performed and cores (1, 2) were obtained. During surgical biopsies, either an 18 gauge core needle biopsy was performed using direct visual/intraoperative ultrasound guidance or peritoneal metastatic disease was biopsied using a laparoscopic grasper and cautery. After clinical needs were satisfied, surplus fresh tissue fragments/cores were placed into chilled cell culture media composed of Roswell Park Memorial Institute 1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 2% penicillin/streptomycin (Sigma Aldrich, St. Louis, MO).

PDX Engraftment

Fresh biopsied tissues were sharply sectioned with a sterile scalpel into several 1–2 mm fragments. Fragments were suspended in 400 μL of MatriGel (Corning, Corning, NY). Next, NOD/SCID (Non-obese Severely Combined Immunodeficiency, Department of Comparative Medicine, Mayo Clinic, Rochester, MN) mice were placed in a mouse anesthesia induction chamber and anesthetized using 1–3% isofluorane. Mice were sanitized with 70% ethanol, bilateral subcutaneous pockets were created using sharp/blunt dissection, and Matrigel-soaked tumor fragments were inserted. Wounds were closed using Vetbond (3M, Maplewood, MN). Mice were monitored for tumor growth biweekly using manual palpation. All mice were bred and housed at the Department of Comparative Medicine, Mayo Clinic Rochester, MN, in accordance with Institutional Animal Care and Use Committee guidelines. NOD/SCID (Non-obese Severely Combined Immunodeficiency) mice were obtained. Mice (8–10 weeks old) were housed in a 12-h light/dark cycle with access to food and acidified water.

PDX Metrics, Data Collection, and Analysis

Prospectively collected data were reviewed and demographic, clinical, procedural, pathologic, and follow-up data were abstracted. Mayo Clinic GI pathologists reviewed all available pathology specimens and any xenograft tumor that was generated after PDX engraftment. The current American Joint

Committee on Cancer (AJCC) pathologic tumor stage was determined from pathology slide review as well as from the primary pathology report.[18] Details of patient neoadjuvant therapy were collected. Table 1 outlines metrics obtained for PDX tumor growth. The ischemic time (IT), time to tumor formation (TTF), time to tumor harvest (TTH), overall patient engraftment efficiency (OPEE), and individual patient engraftment efficiency (IPEE) were recorded. IPEE is a measure of tumor-specific efficiency and subsequently is biologically relevant. It is the number of individually unique patient tumors that are successfully engrafted divided by the number implanted. For example, a single patient may have PDX generation from a primary and metastatic site (lung or liver). Despite tumors growing from the same patient, engraftment rates may differ. Tumors are harvested at approximately 15 mm via manual caliper measurement as defined in our protocol. Histologically validated derived PDX tumors obtained during harvest of first generation (F1) PDX mice were subsequently passed into the second generation (F2) to amplify PDX tumor tissues.

Both male and female mice were utilized in the study. Biopsy specimens were inserted into female or male mice to correspond with the patients' sex. Normal values were reported using means ± SD and no normal values were reported using medians in interquartile range (IQR).Student's t test was utilized to compare groups. Non-parametric tests were used where parametric were inappropriate. All data were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla CA).

Table 1 PDX metrics utilized to characterize the cohort

Metric	Description	Purpose
Overall patient engraftment efficiency (OPEE)	Number of unique patient tumors successfully engrafted divided by total number of unique patient tumor xenograft attempts	Most critical metric as this describes the overall PDX efficiency and success for any given patient tumor
Individual patient engraftment efficiency (IPEE)	Number of individual unique patient tumors successfully engrafted divided by number implanted mice for that given patient tumor	Biologically relevant individual tumor-specific engraftment efficiency—each engraftment is a unique experiment
Time to tumor formation (TTF)	Time at which initial tumor growth is palpable	Initial kinetic growth metric
Time to tumor harvest (TTH)	Time at which tumor is harvested and histologically proven	Overall kinetic growth metric
Ischemic time (IT)	Duration of time from resection to completion of xenograft implantation	Most rate-limited technical aspect of PDX generation

Results

During 2013–2017, 29 patients underwent PDX engraftment using primary patient biopsy tissue from either surgical ($n = 15$) or image-guided biopsy ($n = 14$). The histologic types included gastric cancer ($n = 4$), gallbladder carcinoma ($n = 2$), cholangiocarcinoma—hilar or intrahepatic ($n = 17$), and pancreatic ductal adenocarcinoma ($n = 6$). All patients demonstrated unresectable or metastatic disease at the time of biopsy. The overall ischemic time was 32 min [23–42]. Overall patient engraftment efficiency (OPEE) ranged from 0 to 100% dependent on tumor type and this was not affected by ischemic time. All engrafted PDX models were successfully passed into the F3 generations.

The most likely to engraft tumor types using biopsy-derived tissue were gallbladder and gastric carcinomas. The OPEE for these types was 100%, Table 2. Pancreatic adenocarcinoma and cholangiocarcinoma demonstrated OPEE variability with 33% and 29% respectively. There was variability in the median TTF duration. For pancreatic adenocarcinoma ($n = 6$), the median [IQR] TTF was 67 days [42–93]. In cases of gallbladder carcinoma ($n = 2$), the median TTF was 32 and 28 days and in all types of cholangiocarcinoma ($n = 17$) the median TTF was 35 days [21–50]. In cases of gastric carcinoma ($n = 4$), the median TTF was 136 days [55–186].

The IPEE differed in each tumor type. In cases of cholangiocarcinoma, there was a median IPEE of 60% [30–88%], reflecting high engraftment within individual tumor types. Patients with gallbladder carcinoma also demonstrated high IPEE, 100 and 67% respectively. Similarly, in patients with pancreatic adenocarcinoma, the IPEE was elevated at 80% [60–100%]. Finally, while gastric biopsy–derived xenografts demonstrated high OPEE, the tumors demonstrated low IPEE (33% [23–38%]) which reflects lower engraftment in multiple implanted mice. PDX tissue generated in the F1 generation was passed into the F2 and subsequent generations for successfully engrafted individual tumors ($n = 16$). Accurate recapitulation of tumor histopathological features (F1 to F3 generations) was seen in all generated PDX compared to original patient tissue and demonstrated in two PDX models (cholangiocarcinoma and gastric carcinoma) in Fig. 1.

The biopsied tissue was most frequently primary tumor tissue 18 (62%) compared to a metastatic nodule 11 (38%). Increased OPEE was displayed in models engrafted from biopsies of metastatic tissue compared to unresectable primary tumor tissue (69% versus 15.4%, $p = 0.001$), Fig. 2. In Table 2, the number of xenografts derived from peritoneal metastases and biopsies of primary cancerous tissue for each cancer type is presented. The method by which the tissue was obtained (surgical versus image-guided) also affected the OPEE. Surgically obtained biopsies ($n = 15$) exhibited increased PDX engraftment compared to image-guided methods ($n = 14$) (73% versus 14%, $p = 0.003$). Additionally, the mean

Table 2 Characteristics of PDX and tumor types

	Gastric carcinoma $N = 4$	Gallbladder carcinoma $N = 2$	Cholangiocarcinoma $N = 17$	Pancreatic ductal adenocarcinoma $N = 6$
Surgical biopsy (%)	100	100	76	83
Image-guided biopsy (%)	0	0	24	17
Ischemic time (min)	38 [32–42]	46 [40–52]	27 [18–71]	31 [26–36]
Peritoneal metastasis (*n*, %)	4, 100	1, 50	3, 18	3, 50
Biopsy of primary cancerous tissue (*n*, %)	0, 0	1, 50	14, 82	3, 50
OPEE	100	100	29.4	33.3
IPEE	0.33 [0.23–0.38]	0.84 [0.66–1.0]	0.6 [0.3–0.88]	0.8 [0.6–1.0]
TTF	136 [55–186]	32 [28–36]	35 [21–50]	67 [42–93]
TTH	187 [132–232]	120 [50–189]	72 [50–135]	110 [101–118]
Preoperative CA 19-19 (mg/dL)	94 [57126]	342 [7–678]	152 [41–1550]	160 [68–244]
Neoadjuvant treatment (%)	25	0	6	50

OPEE overall patient engraftment efficiency, *IPEE* individual patient engraftment efficiency, *TTF* time to tumor formation, *TTH* time to tumor harvest

(± SD) IPEE was increased in surgical versus image-guided biopsies (80% [60–100%] versus 52% [40–75%], $p = 0.026$).

Discussion

This study demonstrates that PDX models can be successfully created from clinically derived patient biopsy specimens. Biopsy-derived xenografts can be implanted from various different gastrointestinal malignancies and accurately recapitulated primary tumor histomorphology. Either surgical or image-guided techniques can be used with higher rates of engraftment demonstrated from surgical biopsies. Since the prevalence of GI tumors is increasing, the benefits of PDX modeling might be expanded to a cohort of patients with unresectable or metastatic disease who are typically not considered for traditional surgical resection and PDX generation. PDX models derived from patients with these advanced biologic phenotypes are critically needed in order to assess and optimize current therapeutic strategies.[15,19]

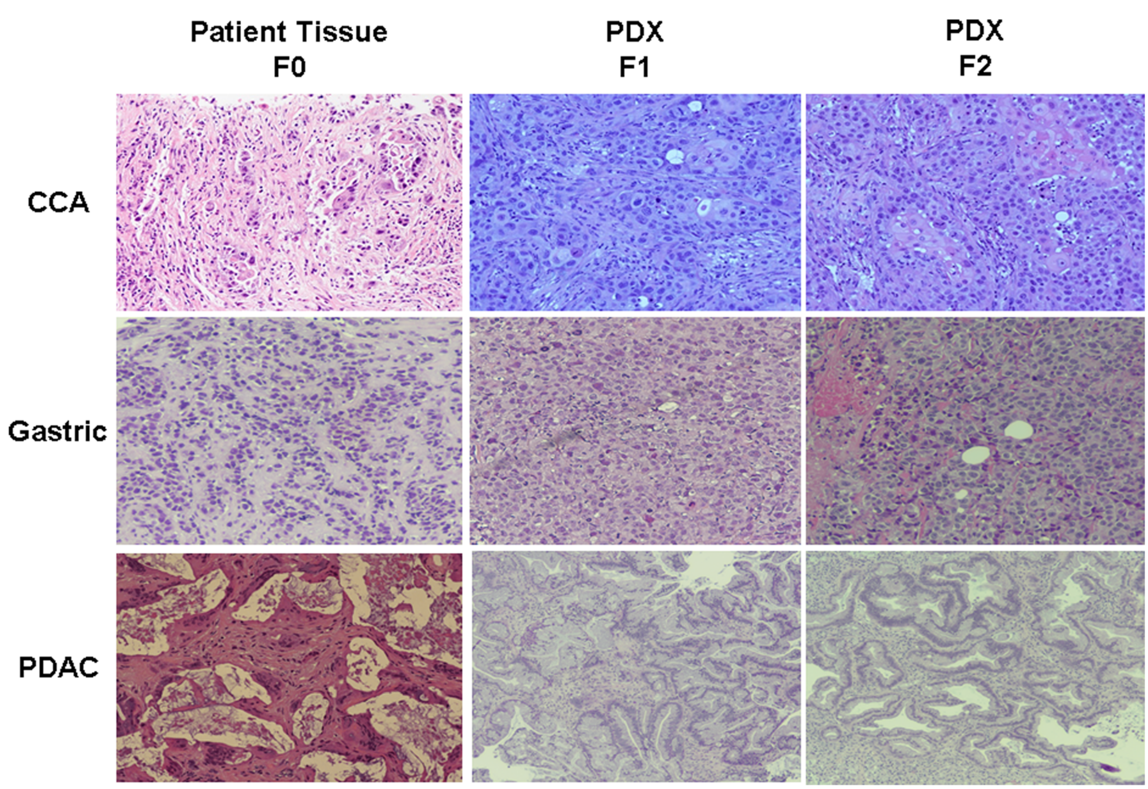

Fig. 1 Histologic verification of biopsy derived xenografts

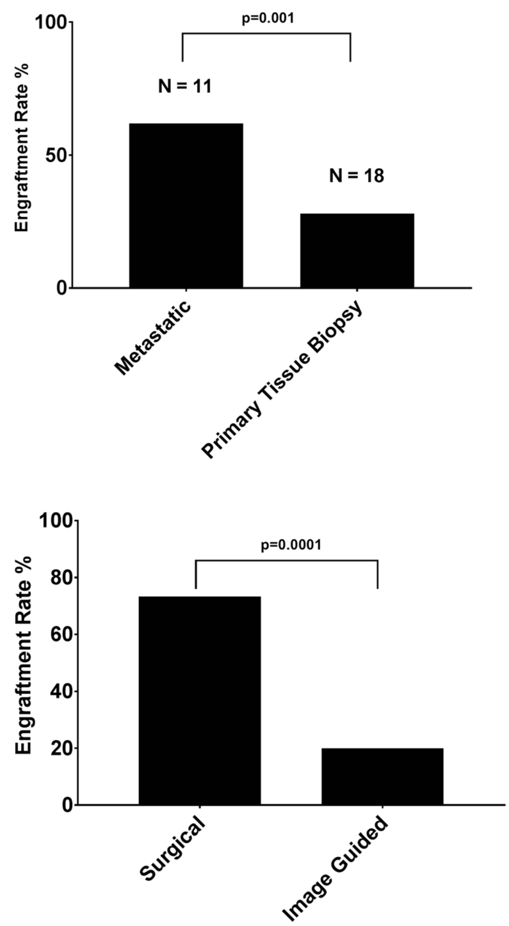

Fig. 2 Engraftment rates stratified by biopsy site and method

Current xenotransplantation methods predominantly rely on implantation of surgically resected tumor specimens.[20] As biopsy technique continues to improve, access to malignant tissue becomes increasingly available.[21,22] Biopsy specimens may then be utilized for a variety of translational and preclinical platforms. PDX, whether generated from resected or biopsied tissue, can be used to evaluate patient chemotherapeutic response for unresectable or recurrent disease.[10,11,23,24] In this study, the overall engraftment efficiency was 44.8% which is comparable to previous studies that utilized biopsy specimens to generate PDX.[10,25,26] Biopsy-derived PDX models represent a cohort of unresectable or metastatic tumor phenotypes that would otherwise have not been a candidate for conventional PDX generation.

In order to confirm this technique, Roife et al. demonstrated that fine needle aspirates (FNA) provided sufficient viable tissue for PDX generation.[10] Traditional xenograft techniques were directly compared to FNA biopsy-derived xenotransplantation with comparable engraftment rates. The authors also highlighted the generation of a pancreatic adenocarcinoma xenograft obtained from an outpatient image-guided biopsy specimen, suggesting that patients with advanced stage diseases may benefit from progress in biopsy-derived xenograft techniques. The present study's cohort comprised wholly of clinically obtained patient biopsy specimens (directly from the operating or interventional radiology rooms). This underscores the feasibility of generating biopsy-derived PDX from a spectrum of gastrointestinal malignancies routinely. Utilizing patient biopsy specimens is of increasing translational importance and developing biopsy-derived PDX models deserves further consideration to advance personalized medicine approaches.

To better study the clonal and genetic changes in pancreatic cancer—from diagnosis, neoadjuvant therapy, surgical resection, and recurrence—biopsied tissue is crucial for analysis. Improving management of aggressive solid tumor malignancies depends on earlier diagnosis and predictive methods to guide treatment as well as assess potential response to novel therapies at the time of recurrence.[27] Recent work in the field of breast cancer has established the utility of developing pretreatment and posttreatment xenografts.[28] This work evaluated differences in drug resistance and demonstrated genomic differences between xenografts derived from biopsy specimens pre and postneoadjuvant treatment. Routine provision of both treatment-naïve and treatment-resistant biopsy-derived xenografts will shift the PDX paradigm. In conjunction with next generation sequencing data, the comparison of multiple therapies will help shape precision oncology further.[29] Moreover, for patients with chemoresistant recurrent disease, options are limited.[30–32] Routine generation of PDX using biopsy specimens will expand our understanding on the locally advanced or metastatic phenotype.

Adequate biopsy volume may have impacted PDX engraftment rates. PDX derived from image-guided biopsy specimens demonstrated lower OPEE and IPEE rates which may have been due to inadequately sampled tissue or biopsy of non-viable tumor. Allaway et al. suggested improved sampling by utilizing multiple biopsies during the same endoscopic procedure in order to improve viable tumor sampling.[11] In another study, PDX were successfully engrafted using patient CT biopsy specimens.[33] These studies in conjunction with the present study suggest that a variety of techniques can be utilized to obtain viable tissue and that a thorough assessment of metastatic disease or lymph node involvement may aid in identifying readily accessible lesions and increase the change for sampling viable tumor. These approaches are important when considering biopsy PDX generation.

There are several barriers to the successful generation of xenografts. We did not demonstrate a significant difference with respect to overall engraftment and patient pretreatment status although we did see trends in pretreated xenografts demonstrating longer TTF and decreased engraftment ratios. This is likely due to our small and variable tumor type cohort. Xenograft technique is operator-dependent. In order to minimize other confounders, we attempt to xenograft all tissue as rapidly as possible (< 60 min) to minimize the duration of ischemia time and as such this did not appear to affect the engraftment ratio in this cohort. Image-guided biopsy methods may not provide adequate tissue samples to reliably create xenografts which are an expensive and resource exhaustive platform as biopsy techniques may not sample the most viable areas. We did not process these tumors using trypsinization or cellular dissociation. Due to the low volume of tumor cores available and our understanding of the impact that ischemic time has on engraftment success, we directly implant and biopsied tissue into immunodeficient mice using our technique. Trypsinization is an important step to liberate malignant cells; however, we aimed to generate PDX models with an intact stroma in early generations so as to closely recapitulate the patient's biologic and architectural phenotype. Using a large and variable cohort of patient tumors, recent work characterizing the genomic copy number alterations of multiply passaged PDX tumors were compared to matched patient tumors demonstrated distinct differences.[34] This has significant implications on the utility of PDX models to act as surrogates for patient clinical response to tested therapies as the clonal selection of a patient tumor may not demonstrate similar therapeutic responses. Nevertheless, we stress the importance of meticulous cryopreservation of low passage PDX-derived tissue given this limitation. Despite this potential limitation, generation of PDX tissue from advanced malignancies will substantiate efforts to better understand the biologic differences from metastatic tumors and assess functional sensitivities.

Conclusion

Biopsy-derived PDX can be achieved with low volume tissue samples. Routine generation of PDX models from biopsy specimens of patients with unresectable or metastatic disease is feasible in the clinical setting. Surgical biopsy specimens appear superior in comparison to image-guided biopsies and there is variable engraftment dependent on histologic tumor type and stage of disease, unresectable primary vs metastatic tumor. Future work to establish PDX models for a variety of malignancies in multiple stages of disease and treatment status will significantly improve our understanding of these cancers and offer insights into potential individualized treatment.

Author Contribution Study design was developed by MCH, LY, JRB, and MJT. Data generation was performed by MCH, TI, LY, MJT, MLK, RLS, SPC, and DMN. Data analysis and interpretation were performed by MCH, LY, JLL, JRB, and MJT. Manuscript writing was performed by MCH, TI, LY, MJT, JRB, JLL, MLK, RLS, SPC, and DMN.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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