A multicenter clinical study: personalized medication for advanced gastrointestinal carcinomas with the guidance of Patient-Derived Tumor Xenograft (PDTX)

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Abstract

Background: Establish patient-derived tumor xenograft (PDTX) from advanced GICs and assess the clinical value and applicability of PDTX for the treatment of advanced GICs.

Method: Patients with advanced GICs were enrolled in a registered multi-center clinical study (ChiCTR-OOC-17012731). The performance of PDTX were evaluated includes: analyzing factors that affect the engraftment rate, comparing the histological consistency between primary tumors and tumorgrafts, examining the concordance between the drug effectiveness in PDTXs and clinical responses, and Identifying genetic variants and other factors associated with prognosis.

Results: Thirty-three patients were enrolled in the study with the engraftment rate of 75.8% (25/33). The successfulness of engraftment was independent of age, cancer types, pathological stages of tumors, and particularly sampling methods. Tumorgrafts kept same histopathological characteristics as primary tumors. Forty-nine regimens involving twenty-eight drugs were tested in seventeen tumorgrafts. The median time for drug testing was 134.5 day. The follow up information of 10 regimens from 9 patients were obtained.
The concordance of drug effectiveness in PDTXs and clinical responses was 100%. The tumor mutation burden (TMB) was correlated with the effectiveness of single drug regimens, while the outgrowth time of tumorgrafts was associated with the effectiveness of combined regimens.

**Conclusion:** The engraftment rate in advanced GICs is higher than other cancers and meets the general acceptable standard of applying personalized therapeutic strategies. Tumorgrafts from PDTX kept attributes of primary tumor. Predictions from PDTX modeling highly agree with the clinical drug responses. PDTX may already be clinical applicable for the personalized medication in advanced GICs.

**Keywords:** clinical study, patient-derived tumor xenograft, advanced gastrointestinal carcinomas, personalized medication

**Background**

Gastrointestinal cancers (GICs), the common malignant conditions that involve the gastrointestinal tract and organs, are responsible for a significant portion of malignant tumors. Globally, there were 4.7 million new GICs cases and 3.4 million related deaths in 2018, accounting for 26.1% of incidence and 35.2% of mortality in malignant tumors[1, 2]. In China, the prevalence is even worse, with 1.84 million new cases and 1.44 million deaths, and the corresponding incidence and mortality rates in malignant tumors are 43.1% and 50.4%, respectively[2].

In the past decade, the advancement of targeted therapy and immunotherapy has revolutionized many oncology fields, including GICs[3]. However, currently patients that can gain clinical benefit from targeted therapy or immunotherapy only account for a small portion in advanced GICs and chemotherapy is still the cornerstone for the patient care. For example, the HER2 overexpression accounts for 2–6% of patients with colorectal cancer[4], and the overall response rate (ORR) of anti-HER2 therapy was 30% and 38% in two Phase II trials, respectively[5, 6]. Patients with deficient mismatch repair (dMMR) were found to have a better response to the immunotherapy in several cancers[7], while the prevalence of dMMR in Stage III–IV GICs is around 10%[8], and with 40% of the overall response rate[9]. The choice of chemotherapy regimen is mainly based on practice guidelines and the experience of clinicians. So far, no targeted therapy or immunotherapy in the GICs can perform like that happened in non-small cell lung cancer, and chemotherapy is still the mainstay for the management of advanced GICs. However, regardless of therapeutic methods, chemotherapy alone or combined with other therapies, the low response rate is always an obstacle. Especially, after multiple-lines treatment, usually with less than 10% response rate[10], patients can barely get therapeutical
benefits from chemotherapy again from, which largely weaken the value of chemotherapy in advanced GICs. The main reason is the intertumoral heterogeneity and individualized biological and molecular characteristics, and therefore, personalized medication is required.

To improve the effectiveness of therapeutic drugs, the stratification of patients is necessary. Many approaches have been developed for this purpose, including genetic test, immunohistochemistry test, *in vitro* or *in vivo* preclinical models that may involve immortalized cells, organoid technology, patient-derived tumor xenograft (PDTX), and others. Among them, PDTX is a robust platform that recapitulates the extensive heterogeneity, retains the molecular diversity of original tumors, and effectively captures responses to therapies in patients[11-13]. PDTX model has been shown as the most effective, safe, and reliable clinical test platform. It has been extensively used in preclinical drug evaluation, biomarker identification, drug screening for personalized treatment[12, 14-18].

To assess the clinical value of PDTX in advanced GICs therapies, a prospective multi-centered exploratory study was conducted. Patients with various advanced GICs were recruited, and preclinical PDTX models for real time personalized medication were tried. The comprehensive factors, including engraftment rate, factors that affect the success rate of engraftment, congruence between tumorgrafts and original tumors at histopathological level, genomic variants, and accuracy of drug prediction, were examined.

**Materials and methods**

**Patient inclusion**

This study was carried out as a registered multi-center clinical collaborative study (ChiCTR-OOC-17012731) from January 2016 to January 2018. The primary inclusion criteria were: The patients with advanced GIC confirmed by histopathology and need anti-cancer treatment; fresh tumor tissue can be obtained by surgical resection or biopsy; at least one measurable lesion based on Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST 1.1) standard; Eastern Cooperative Oncology Group (ECOG) performance status score 0-2; the expected survival time must be ≥ 3 months; in case of postoperative subjects, the previous anti-tumor drug treatment (chemotherapy and / or targeted therapy) failed, the failure time from the study was ≥ 4 weeks, and the adverse reactions were essentially recovered. The main exclusion criteria were: participating in clinical studies of other drugs simultaneously; patients with liver, kidney, and bone marrow hematopoietic dysfunctions not meeting the requirements of chemotherapy; unwilling to follow the therapies recommended by the researcher based on the testing results after previous treatment failure. The main
exfoliation criteria: unable to carry out pharmacodynamic tests; serious violation of this study protocol, not comply with the therapeutic regimens; treatment plans altered due to significant changes in the conditions. All protocols followed the guild line of local institutional ethics regulations and were approved by the local institutional review board. Informed consent was obtained from all patients enrolled.

**Animal care**

All tumor transplant recipients were 6-8 weeks old, specific-pathogen-free (SPF) immunocompromised NCG mice (NOD-Prk^dcem26Cd52 il2rgem26Cd22, Nanjing Biomedical Research Institute) weighing 20 ± 2g. The research was approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University. All mice were housed in SPF animal facilities at room temperature of 25 ± 2 °C and humidity of 40% -70% for at least three days before the xenografting and at the same condition after. The gender of the selected mouse was consistent with the corresponding patient.

**Tissue preparation**

Fresh tumor tissues collected via surgery or biopsy were preserved within 15 minutes of sampling in the tissue preservation solution (Hyclone, Marlborough, U.S.A.) with the a supplemental of 100 units/ml penicillin and 100 ug/ml streptomycin and were transported to the laboratory under refrigeration. The necrotic components, fats, and blood were removed before the transplant. Xenografting was performed within 24 hours after sampling.

**PDTX model establishment**

The PDTX model was established similar as described[12]. Briefly, 2 mm X 2 mm X 2 mm fresh tumor tissues were engrafted into NCG mice subcutaneously. Tumor volume was measured twice weekly and was calculated according to the following formula: tumor volume = [length * width^2] / 2. The xenograft (P0) was aseptically resected when the volume reached 800-1000 mm^3, and extended generations P1-P5 were expanded in a similar way. When the average tumor volume reached 100-150mm^3, the mice were randomly assigned (5 mice per group) to control or treatment groups after removing substandard mice (Mean±SEM/3) (SEM, standard error of the mean). The drug administration interventions were performed for 3-4 weeks based on clinical regimens.

**Histopathological comparison between primary tumor and PDTX**

After the propagation (volume >60mm^2), fragments of the first passage of tumor grafts (P1) were fixed, embedded, sectioned, and stained with hematoxylin and eosin (HE) using standard procedure. The sections
of tumorgrafts were examined by an independent pathologist, and the histopathological classifications were compared with that of primary tumor counterparts. The concordance between the two results was recorded and analyzed.

**Tumor growth rates calculation**

Specific growth rate (SGR) was used to quantify tumor growth rate for all tumors based on measuring tumor volume changes. The primary tumor tissue was implanted into mice at the starting time (t1) and in a specific size (V1), and terminated at the time (t2) when the tumor sizes (V2) were measured. Tumor dimensions were weekly measured twice and recorded. SGR was calculated according to the exponential growth equation as follows: $\text{SGR} = \frac{\ln(V_2) - \ln(V_1)}{t_2 - t_1}$.

**Co-clinical drug screen**

In each PDTX model, at least 4 types of drugs or drug combinations (determined by physician) were tested to screen out the most effective regimen. Drugs were administrated either by gavage (for oral drugs) or intraperitoneal (IP) injection (for infusion or bolus). Doses were converted according to the weights of mice, and generally, a 10-folds dose of that used in clinical was applied. The therapeutical cycles were the same as that of clinical practice and were evenly distributed in 21 days. The tumor growth inhibition rate (TGI) was used to reflect drug efficacy and was calculated as follows: $\text{TGI} = \frac{(V_c - V_t)}{V_c} \times 100\%$.

Where $V_c$ is the average volume of tumorgrafts in the control group; $V_t$ is the average volume of tumorgrafts in the treatment group. The cut-off value of TGI was set to 60%, where $\text{TGI} > 60\%$ was classified as an effective regimen, otherwise ineffective. An effective regimen will be chosen for clinical treatment. If more than one effective regimen were identified, the regimen would be determined by the physician. Clinical responses were classified based on response evaluation criteria in solid tumors version 1.1(RECIST 1.1). Complete response (CR), partial response (PR), and stable disease (SD) were categorized as positive and progressive disease (PD) as negative. When the testing result of the PDTX was effective, and the clinical response was positive, the result was determined as consistent and otherwise inconsistent.

**Next-Generation Sequencing (NGS) analysis**

Genomic DNA extraction and sequencing library construction were performed using standard protocols as described. Whole-exome sequencing was performed on NovaSeq 6000 System (Illumina, San Diego, U.S.A.), and the primary base call files were converted into FASTQ sequence files using the bcl2fastq converter tool bcl2fastq-1.8.4 in the CASAVA 1.8 pipeline. The publicly available software FastQC was used...
to assess sequencing quality. For each lane, per-base quality scores across the length of the reads were examined. Lanes were deemed passing if the per-base quality score box plot indicated that > 75% of the reads had > Q20 for bases 1–80. In addition to the raw sequence quality, the alignment quality was also assessed using the Picard package. The FASTQ sequence files generated were then processed through an in-house pipeline constructed for whole-exome sequence analyses of paired cancer genomes. The sequencing reads were aligned to the reference genome build hg19, GRCh37 using BWA-mem v.0.7.5a[20], and converted into BAM files using SAMtools (version 0.1.18)[21]. Duplicates were marked for filtering, and INDELs were realigned using GATK v.3.4.46 IndelRealigner[22]. For somatic SNV and indel variant calling, GATK BQSR was applied to recalibrate base qualities. SNV and indel somatic variants were called using Strelka v.1.0.14[23]. Copy-number aberration was quantified and reported for each gene as the segmented normalized log2-transformed exon coverage ratios between each tumor sample and normal samples.

Statistical analysis

Student’s t-test was applied for the differences in tumor volumes between treated and control animals during drug screening. Associations between patient responses and in PD TX outcomes were analyzed using Fisher’s exact test. All statistical analyses were two-sided, and P<0.05 was considered significant.

Results

Characteristics of primary cohort

Thirty-three patients, aged from 31 to 71 years old, 24 males and 9 females, median at 55 including colorectal cancer (10), pancreatic cancer (10), gastric cancer (5), liver cancer (3), esophageal cancer (2), duodenal cancer (2), and gallbladder cancer (1) (Table 1 and Supplemental table 1).
Table 1. Summary of Patient Information

| Histology/Stage | Patients (n) | Median | Percentage/Range |
|----------------|-------------|--------|------------------|
| Male           | 24          | 72.7%  |
| Female         | 9           | 27.3%  |
| Age (Y)        |             | 55     | 28-71            |
| Stage          |             |        |                  |
| III            | 4           | 12.1%  |
| IV             | 29          | 87.9%  |
| Histology      |             |        |                  |
| adeno          | 27          | 90.9%  |
| squamous       | 1           | 3.0%   |
| HCC            | 2           | 6.1%   |
| CRC            | 10          | 30.3%  |
| DC             | 2           | 6.1%   |
| ESCC           | 2           | 6.1%   |
| GBC            | 1           | 3.0%   |
| GCA            | 5           | 15.2%  |
| HCC            | 3           | 9.1%   |
| PC             | 10          | 30.3%  |

CRC=colorectal carcinoma; DC=duodenal carcinoma; ESCC=esophageal cancer; GBC=Gallbladder carcinoma; GCA= gastric cancer; HCC= hepatocellular carcinoma; PC= pancreatic cancer.

Engraftment rate and Influential factors

The schematic diagram of PDTX establishment and other analytical experiments is illustrated in Figure 1A. Among 33 primary tumourgrafts, 25 (75.8%) were propagated successfully, including 17 cases with accomplished drug testing, 9 cases with follow-up results, and 23 cases with Whole Exome Sequencing (WES) analysis. We analyzed clinical factors that may affect the successfulness of engraftment, and discovered that the engraftment rate was independent of age, gender, tumor location, sampling methods, and pathological stage of tumors (Table 2).
Figure 1. the establishment of PDTX in advanced gastrointestinal cancers. A. schematic illustration of PDTX modeling. B. Pathological comparison between the primary tumor (Pa) and the first passage of tumorgrafts (P0) from various types of cancers. Abbreviations: WES: Whole Exon Sequencing; SNV: Single Nucleotide Variation; PA: Pancreatic Cancer; Ade: Adenocarcinoma; WD: Well Differentiated; MD: Moderate Differentiated.
Table 2. Mono factor analysis of engraftment rate (n/%)

| Factors/Type (n) | Successful engraftment | Unsuccessful engraftment | P (Fisher’s test) |
|-----------------|-------------------------|--------------------------|------------------|
| Gender          |                         |                          |                  |
| Male (24)       | 20 (83.3%)              | 4 (16.7%)                | 0.1695           |
| Female (9)      | 5 (55.6%)               | 4 (44.4%)                |                  |
| Age             |                         |                          | 1.000            |
| <60 (19)        | 14 (73.7%)              | 5 (26.3%)                |                  |
| ≥60 (14)        | 11 (78.6%)              | 3 (21.4%)                |                  |
| Sampling methods|                         |                          | 0.6728           |
| Surgery (10)    | 7 (70%)                 | 3 (30%)                  |                  |
| Biopsy (23)     | 18 (78.3%)              | 5 (21.6%)                |                  |
| Tumor location  |                         |                          | 0.2379           |
| Digestive tract (19) | 16 (84.2%)  | 3 (15.8%)                |                  |
| Digestive gland (14) | 9 (64.3%)           | 5 (35.7%)                |                  |
| Stage           |                         |                          | 0.5503           |
| Stage II–III    | 4 (100%)                | 0 (0%)                   |                  |
| Stage IV        | 21 (72.4%)              | 8 (27.6%)                |                  |
| Types           |                         |                          |                  |
| Colorectal      | 8 (80%)                 | 2 (20%)                  |                  |
| Pancreatic      | 7 (70%)                 | 3 (30%)                  |                  |
| Liver           | 2 (66.7%)               | 1 (33.3%)                |                  |
| Gastric         | 5 (100%)                | 0 (0%)                   |                  |
| Duodenum        | 2 (100%)                | 0 (0%)                   |                  |
| Esophageal      | 1 (50%)                 | 1 (50%)                  |                  |
| Gallbladder     | 0 (0%)                  | 1 (100%)                 |                  |
| Total (33)      | 25 (75.8%)              | 8 (24.2%)                |                  |

Histopathology of tumorgrafts

We also analyzed histology of tumorgrafts (P0) by comparing to matched primary tumors (Pa). All paired sections displayed significant morphological similarity, and classifications by two independent pathologists also demonstrated a 100% consistency between Pa and P1. Five pairs of typical images from various types of tumors were shown (Figure 1B).

Drug efficacy test and comparison to the clinical response

Drug efficacy were tested after tumorgrafts were propagated (Figure 1A). Among all enrolled cases, 51.5% of them (17 cases) accomplished full drug tests, involving 49 single drug or combined regimens with 28 types of chemotherapeutic regents engaged. The testing period (from sampling to the end of efficacy test) ranged from 67-202 days with median time of 134.5 days. Follow up information from 9 cases involving 10
regimens were obtained. Among them, eight regimens in 7 cases displayed a positive result in PDTX models and corresponding clinical response were two PR cases and six SD cases. In the other two cases where patient responses were PD, the PDTX testing result were also negative (Table 3, Supplemental tale 2). The consistency between patients’ response and PDTX testing is 100%. Three patients (13011,110150, 110160) who continued the guidance of PDTX gained SD with PFS of 7, 5 and 6 months.
| Case ID  | Drug regimens in PDTX | TGI (%) | Clinical medication                  | Patient response | PDTX agree with clinic |
|----------|-----------------------|---------|--------------------------------------|------------------|-----------------------|
| DS-dg-5  | Oxaliplatin           | 0.8     | Folinic acid Fluorouracil Oxaliplatin| PD               | Yes                   |
| DS-dt-14 | Cetuximab             |         | Cetuximab                            |                  |                       |
|          | Folinic acid Fluorouracil Oxaliplatin |         | Folinic acid Fluorouracil Oxaliplatin |                  |                       |
| DS-dt-16 | Bevacizumab           | 50.8    | Bevacizumab                          | PD               | Yes                   |
|          | Irinotecan Raltitrexed |         | Irinotecan Raltitrexed               |                  |                       |
| DS-dg-6  | Gemcitabine           | 86.7    | Gemcitabine Bevacizumab              | SD               | Yes                   |
| DS-dt-4  | Gemcitabine TS-1      | 80.5    | Gemcitabine TS-1r                    | PR               | Yes                   |
| DS-dt-6  | Albumin-bound paclitaxel Cisplatin | 91.6    | Albumin-bound paclitaxel Cisplatin   | SD               | Yes                   |
|          | Albumin-bound paclitaxel Tegafur | 64.2    | Albumin-bound paclitaxel Tegafur     | PR               | Yes                   |
| DS-dg-9  | Gemcitabine Cisplatin | 70.1    | Gemcitabine Cisplatin                | SD               | Yes                   |
| DS-dt-15 | Oxaliplatin Bevacizumab Capecitabine | 79.7    | Oxaliplatin Bevacizumab Capecitabine | SD               | Yes                   |
| DS-dt-19 | Bevacizumab           | 88.6    | Bevacizumab Calcium Folinate Irinotecan | SD               | Yes                   |
|          | Irinotecan            |         | 5-Fluorouracil                       |                  |                       |

TGI=tumor growth inhibition rate, TGI<60%=non-effective, TGI≥60%=effective

**Genetic mutations and patients’ prognosis**

Somatic mutations in tumor grafts from 23 cases were examined by whole exon sequencing (WES) and nonsynonymous substitution in major cancer driver genes were shown (Figure 2). The most frequently mutated gene discovered were those also previously found as hot spots in colorectal cancers and gastric
cancers[24-26], such as TP53, KRAS, APC, DLC1, MSH6, PMS2, and others. (Figure 2A). The overall tumor mutation burden (TMB) of all examined tumorgrafts is 6.0±1.5 (n=25), and for responsive (TGI≥60%) tumorgrafts is 5.8±1.1 (n=13) and non-responsive ones (TGI<60%) is 6.8±1.5 (n=12). In the monotherapy group, the TMB of non-responsive tumorgrafts was significantly higher than responsive ones (7.5±1.2 vs. 5.8±1.2, p=0.026), whereas for combined therapies, the TMBs were similar in responsive and non-responsive tumorgrafts (5.7±0.9 vs. 6.2±1.6, p=0.6025) (Figure 2B).
Figure 2. Mutational landscape of tumorgrafts and drug efficacy. A. Major driver mutations discovered in tumorgrafts and corresponding drug responses. B. Correlation of tumor mutation burden (TMB) with the
drug effects. C. Correlation of tumor formation time with the drug effects. D. The comparison of effective rate between single-drug and combined-drug regimens.

Outgrowth time and prognosis

The first-round propagation of tumorgrafts, which specified as from the engraftment of primary tumor until the tumorgraft’s volume reaches 60 mm$^3$ (P0, Figure 1A), was defined as the outgrowth of tumorgraft in our study. The outgrowth time usually reflects the potency of tumor’s malignancy$^{[27, 28]}$. The overall outgrowth time was 7~76 days. In the combined therapy group, the average outgrowth time of responsive tumorgrafts (38± 14 days) was shorter than that of non-responsive ones (52± 10 days), however, not statistically significant ($p=0.1297$). No differences of average outgrowth times were discovered between responsive (36± 19 days) and non-responsive (36± 14 days) in monotherapy group (Figure 2C). Generally, the positive response rate in combined chemotherapy group was about two-times high to that in the monotherapy group (64.6% vs. 30.2%, Figure 2D).

Typical cases

Here we illustrate the performance of PDTX in guiding clinical medications with two typical cases.

Case1:

A 63-year-old male was diagnosed with colon adenocarcinoma at stage IV with liver metastasis. PDTX was established using the specimen from metastatic lesion via biopsy, and four regimens involving seven drugs were screened. Two regimens including the one (Cetuximab, Oxaliplatin, 5-Fluorouracil and Calcium Folate) concurrently used for the patient treatment showed strong TGI at day 9 (Figure 3A-D). Consequently, the concurrent regimen, which was the combination of folinic acid, fluorouracil, oxaliplatin, and cetuximab, was extended for 6 cycles. The liver lesions were reduced after 3 cycles of treatment (Figure 3E, F), and carbohydrate antigen 19-9 (CA19-9) decreased from 11019.0U/ml to 3918.0U/ml and carcinoembryonic antigen (CEA) decreased from 11366ug/L to 4146ug/L after 5 cycles of treatment (Figure 3G, H). The clinical evaluation of patient’s response was SD, consistent with the PDTX predicting.

Case2:

A 54-year-old female was diagnosed with moderately differentiated pancreatic adenocarcinoma at stage IV with liver metastasis. Specimen from the primary lesion via surgical resection was used for PDTX, and four regimens involving seven drugs were screened. The combination of gemcitabine with TS-1 (Tegafur, Gimeracil, and Oteracil Potassium Capsules) showed a TGI of 80.5% (Figure 3I-L), and was chosen for the medication. The therapy continued for 3 cycles, and the metastatic lesion reduced significantly after 2 cycles.
of treatment. CA19-9 decreased from 1945U/mL to 63.1U/mL and CEA decreased from 80μg/L to 9.6μg/L. The clinical evaluation was PR (Figure 3M-P).

Figure 3

Figure 3. Typical cases of PDTX in advanced GICs. A-H, Advance CRC; I-P, Advance PDAC. A and I, Schematic time frame of modeling in each case. B and J, Drug regimens tested in each case. C, D and K, L, Growth inhibition of tumorgrafts for each drug regimen. E, F and M, N, Biomarker changes before and aftertreatment. G, H and O, P, CT scans of tumors before and aftertreatment. Red circles indicate the locations of tumors. CEA= Carcinoembryonic antigen; CA19-9= carbohydrate antigen 19-9.

Discussion

The incidence of GICs continuously increases world widely. Many patients are already at advanced stage when diagnosed, and thereby, the prognosis for those patients generally is not favorable. Patients’ responses to chemotherapy could diverge remarkably due to the heterogeneity of disease and individual differences.
Unified regimens for all patients based on guidelines may lead some first-line drug insensitive patients miss the best therapeutic window, especially patients with advanced disease.

PDTX is an idea model for testing the drug susceptibility that the has been reported in a broad spectrum of solid tumors, including common cancers such as colorectal cancer, lung cancer, breast cancer and rare cancers like adenoid cystic carcinoma and cholangiocarcinoma. The engraftment rate of PDTX differs depending on the tumor types with general success rate around 50%. Among all GICs, the engraftment rate of colorectal cancer is usually higher than others at about 64–89%[29]. In this study, we tried to use a common method to establish the PDTX model with the specimens from 33 patients who were diagnosed with advanced GICs. The overall engraftment rate is 75.8%, which already meet the basic requirement of 60–70% engraftment rate for personalized medication[14]. Statistical analysis showed that the success rate was independent of age, gender, sampling method, and stage, indicating the common PDTX technique might be universally applicable in advanced GICs. Particularly, small amount of specimen sampled by endoscopic biopsy, puncture and aspiration showed no difference in engraftment rate comparing to large amount of specimen obtained by surgical resection. This has significant implication for advanced GICs because many such kind of patients have lost the opportunity of surgery.

Many factors could affect the engraftment rate including: conditions for sampling and transportation, mouse stain used for PDTX, tumor types, amount of specimen, and technology used for modeling. There were 8 cases failed to establish PDTX in this study. Since the sample size is small we just performed a simple analysis instead of statistics. Among 8 cases, 4 were male and 4 were female, 4 were surgical specimens and 4 were puncture specimens. All patients were at stage IV showing no differences. Three unsuccess cases were from pancreatic cancer, accounting 30% of all pancreatic cancer cases. The rest cases were from esophageal cancer, colon cancer, liver cancer, rectal cancer, and gallbladder, one case for each type. Preliminary analysis suggest tumor type was not the cause of unsuccessful engraftment. It is presumably the technique flaw yet need validation by more samples.

PDTX model can highly preserve the histological characteristics, molecular diversity, heterogeneity and microenvironment of primary tumor making it the closest to patients among all tumor models. Zhu et al[29] successfully established 63 PDTX models in NOD / SCID mice using gastroscopic biopsy tissues from 185 patients with gastric cancer. The results showed that the histopathological characteristics of PDTX model were highly consistent with primary tumor. The consistency between PDTX and primary tumor of Lauren classification was 88.9% (56 / 63), the cell differentiation degree was 90.5% (57 / 63), and the HER2
mutation was 95.2% (60 / 63). The mice used in this study is NCG (nod-prkdcem26cd52 il2regm26cd22) strain, which is deficient in T cells, B cells and NK cells. It is the most immune-comprised commercialized strain and is the most suitable strain for human cell or tissue transplantation. All the 25 PDTX models were confirmed as tumor tissue by pathology, and were consistent with the primary tumor at pathological and morphological level.

PDTX model can accurately reflect the patient response, and therefore predicts the patients’ susceptibility to certain chemo-drugs, which can be used to optimize the clinical regimens for personalized midication. Izumchenko[12] and others established 578 PDTXs from multiple types of tumors, of which 237 cases were subjected whole genome sequencing and confirming the genetic consistency between the primary tumor and tumorgraft. They also compared the clinical efficacy of 129 regimens in 92 patients with the prediction of PDTX and the result showed a consistency of 87% (112/129). In this study, the clinical follow-up results of 10 regimens in 9 patients were obtained. The clinical responses and PDTX predictions of 8 regimens were all positive, and the other 2 regimens were negative. The clinical responses and PDTX predictions results were completely consistent. However, only 4 of the 10 regimens in PDTX were completely same as the clinical regimen. Among the other six regimens, five (a11016, 130111, 110160 and 0101006) of them have additional drugs in the clinical regimens comparing to the PDTX model, and the remaining one has one drug removed in the clinical regimen. Although the final clinical regimens are often adjusted depending on the physician’s decision and the predictions from PDTX were consistent with the patient response, such kind of circumstances still should be avoided when scheming in future.

PDTX model can screen multiple drug regimens simultaneously and thereby can give important reference for formulating individualized medication plan. Especially, when recurrence and metastasis occur, the results of previously tested drugs will provide an important reference for guiding the subsequent-line therapy. Hidalgo M et al[30] carried out a clinical study in 2011 on PDTX for guiding the chemotherapy for advanced refractory cancer. PDTX model were successfully established in 12 cases, including 7 cases from GICs. 232 regimens involving 63 drugs were tested, and 17 treatment plans suggested by PDTX modeling were engaged on the patients in 11 cases with 15 plans achieved long-term PR. Subsequently, multiple studies[31-35] reported PDTX guided personalized medication that cover both chemotherapy and targeted therapy illustrating the advantage of PDTX for therapy optimization. In this study, 3 patients (130111, 110150 and 110160) also continued the medication following the guidance of PDTX and obtained SD.
Clinically, the effectiveness rate of single drug usually is low and it is a challenge to choose single drug regimen. In this study, we found a statistically significant negative correlation between the effectiveness of monotherapy and tumor mutation burden (TMB). As a biomarker developed in recent years, TMB has become one of the most important biomarkers in immuno-therapy. For example, in June 2020, U.S. FDA approved a TMB associated pan-cancer indication for pembrolizumab. However, whether TMB can be used as a biomarker for other kinds of drug treatment is still extremely lack of relevant research, and could be the future direction.

In this study, we also found that some unconventional drugs showed high drug efficacy in PDTX model. For example, mitomycin C showed effective inhibition of tumorgrafts in 4 of the 5 pdtx models (supplemental figure 1), although the drug was not really applied on patients. As an antineoplastic drug, mitomycin C was originally used for upper gastrointestinal cancer (such as esophageal cancer), anal cancer, breast cancer, and superficial bladder cancer, but its indication is still expanding. For example, in April 2020, FDA approved the application of mitomycin C in low-grade urothelial carcinoma. For patients with advanced cancer, the regimens recommended in the guidelines are usually limited. In the case of no standard treatment, doctors usually carry out individualized treatment according to literature or personal experience. Through parallelly testing multiple drug PDTX provides the basis for selecting drugs properly.

Invasiveness and malignant proliferation are often associated with poor prognosis. The growth rate of tumorgraft can reflect the proliferative ability of the primary tumor. The growth rate of tumorgrafts were also examined and were correlated to drug effect. The results showed that the faster the growth rate of the transplanted tumor, the lower the possibility of single drug inhibition, and more need to combine drug regimens to inhibit. However, the results have not yet reached statistical significance, and more research is needed to support the exploration.

The PDTX modeling is a significant development in translational medicine. It creates new concepts methodologically for cancer research. However, there are still many challenges in clinical practice. First, PDTX modeling requires fresh tumor tissue, and the tissues need be properly transported and transplanted in time. If the patient cannot perform surgery or biopsy, or no facilities available for transportation and transplantation, the model cannot be established. Second, the PDTX model is unapplicable for evaluating the efficacy of immunosuppressants and immunomodulators. Finally, the overall engraftment rate is still unsatisfactory, and the timeframe for the whole process is still too long. For patients with rapid progressing disease will have lost the therapeutic opportunity before the testing results come out.
The limitation of this study lies in the small sample size, and a large-sample-sized study to validate conclusions in this research will be our next direction. For the consistency between primary tumor and tumorgrafts in PDTX model, additional comparison such as immunohistochemistry and/or molecular biomarkers for specific types of tumors could be also be applied. New biomarkers and/or targets could also be explored. Communication with clinicians should be further addressed to ensure the consistency between laboratory and clinical protocols in future studies.

**Conclusion**

To summarize the above points, PDTX modeling may already be applicable for the personalized medication in advanced GICs. PDTX faithfully preserve the heterogeneity of primary tumors, accurately predict drug response, rapidly stratify patients, and directionally guide the optimization of regimens. The PDTX modeling can reduce the ineffective clinical medications, and assist the real time personalized medication.

**Abbreviations**

CA19-9: Carbohydrate antigen 19-9  
CEA: Carcinoembryonic antigen  
CR: Complete response  
dMMR: deficient mismatch repair  
GICs: Gastrointestinal cancers  
HE: Hematoxylin and eosin  
IP: Intraperitoneal  
NGS: Next-Generation Sequencing  
ORR: Overall response rate  
PD: Progressive disease  
PDTX: Patient-Derived Tumor Xenograft  
PR: Partial response  
RECIST: Response Evaluation Criteria in Solid Tumors  
SD: Stable disease  
SGR: Specific growth rate  
SPF: Specific-pathogen-free
TGI: Tumor growth inhibition rate
TMB: Tumor mutation burden
WES: Whole Exome Sequencing

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YC, SQ, JL developed the main idea and YC wrote the manuscript; YZ and his team performed the experiments; GD, BS, JY, YB, XW, YX, LZ, KD, YQ, SY participated the experimental design and provided samples.

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Ethics approval and consent to participate
This study was reviewed and approved by the ethics committee of Bayi Hospital Affiliated to Nanjing University of Chinese Medicine, patients with advanced GICs were enrolled in a registered multi-center clinical study (ChiCTR-OC-17012731). All experiments involving laboratory animals followed the Guidelines for Animal Experiments.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interests.

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