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**A fast and efficient model for real-time anti-tumor drug efficacy test in the clinical setting
based on patient-derived tumor xenograft**

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Abstract

Drug response rates in cancer therapies are frequently unsatisfactory (first-line 30-40%, subsequent-line ~10% or less). The mouse model, patient-derived tumor xenograft (PDX), can help promote drug effective rate to over 80% by precisely electing efficacious agents. However, the low engraftment rates, extended testing cycle, and high cost limit its utilization in the clinical setting. A novel method, FastPDX, was developed, in which a substitutive engrafting procedure was applied, and the labor-intensive and time-consuming propagation of tumorgrafts was circumvented by pathological analysis. A comprehensive evaluation involving tumor biology and clinical performance was performed for this new model. The clinical evaluation engaged 431 PDX cases, 1050 FastPDX cases, 531 therapeutic entities, 6535 regimens, and a broad spectrum of solid tumors. Typical cases were also studied, covering various refractory malignant tumors. FastPDX kept biological architectures and genetic characteristics of the primary tumor and displayed an equivalent positive predictive value as PDX for the drug efficacy test. The testing cycle was reduced from 3-6 months to 3 weeks, and the cost was decreased by 85%. More importantly, the obstacle of low engraftment rate was circumvented, resulting in the percentage of applicable tests nearly 5-fold higher (94.8% vs. 16.7%) than PDX. FastPDX is particularly suitable in circumstances where standard care is not available and the therapeutic window is short, and for cases where tumors have indolent growth in mice. FastPDX preserved the advantages of classical PDX, overcame its major limitations, and showed great potential for real-time clinic drug selection in cancer therapy.

Keywords: patient-derived tumor xenograft, cancer therapy, clinical setting, engraftment rate

Background

Immune checkpoint inhibitors (ICIs), the current primary class for cancer immunotherapy, improved clinical outcomes significantly during the past decade. However, just a small subset (~10%) of patients could benefit from ICIs(1, 2), and ICIs as monotherapy have mixed results with modest efficacy if without further stratification(3), and generally, the response rate falls within 20% to 40%(4). To improve the efficacy of ICIs, combination with other therapeutic agents becomes the new direction(5). The rationale for combination therapy is turning the immune non-responsive tumor into responsive. Cytotoxic agents can induce cancer cell death, promote tumor antigen release, and stimulate immune effectors. Consequently, an efficacious chemotherapeutic drug will be a natural selection for the combination partner.

On the other hand, surgery, followed by adjuvant chemotherapy, targeted therapy, and radiotherapy, remains the mainstay for managing many solid tumors. Nevertheless, the patient response is generally unsatisfactory for current chemotherapy or targeted therapy, and particularly, the response rate usually is less than 10% after the front-line therapy(6). Furthermore, recommended agents for many rare tumors are usually very limited from guidelines and are not improved for many years (7-10); and personalized medicine is strongly demanded in those circumstances. Therefore, a robust drug screening system that can quickly identify efficacious drugs will significantly impact either ICIs combination therapy and standard cancer care(11, 12).

Patient-derived tumor xenograft (PDTX) is a type of animal model, which recapitulates various clinical features and captures the therapeutic outcomes effectively, established by directly implanting tumor tissues into an immunodeficient mouse. Applications of PDTX include evaluations of preclinical drugs, basic cancer research, identifications for the biomarkers, and assessments of drug efficacy for personalized therapy(13-19). Particularly, the effective rate of

anti-tumor drugs can be improved to over 80% under the guidance of PDTX(6, 16); therefore, it is a promising solution for precise drug selection in cancer therapies (14, 16, 20-22). However, there are still several obstacles for PDTX regarding the utilization for clinical decision making(23), with two essential issues that need to be solved. One difficulty is the low successful engraftment rate (usually around 50%, varies depending on multiple factors), which substantially reduces the test's applicability. The other challenge is generally a 3-6 months testing cycle that will make the test unsuitable for many cancer patients, especially those at an advanced stage. Other issues include high cost and the requirement of complex animal facilities.

In this study, we developed a new modeling protocol, named FastPDTX, that overcomes major disadvantages in PDTX and is more applicable in the clinical setting. In the new FastPDTX protocol, tumorgrafts are implanted in the gluteal region instead of subcutaneously or orthotopically because the enriched blood supply in this region helps a rapid vascularization, and therefore, facilitates drug delivery and the vital status of tumorgrafts. The fundamental alteration is that repetitive measurements of tumorgrafts during the efficacy test are replaced by pathological analysis, which is crucial for reducing time and overcoming the engraftment barrier. We determined key pathological indices and verified their validity through over 200 clinical cases. Our results show that FastPDTX features an equivalent positive predictive value as PDTX for efficacy test while significantly decreases the testing duration by 90% (from 3-6 months to 2-3 weeks) and the expenses by 85%. More importantly, the barrier of engraftment rates is circumvented, resulting in over 90% of applicable tests. FastPDTX is particularly suitable for the advanced malignant tumors where standard care is not available and the therapeutic window is limited, and for cases where tumors have indolent growth in mice, which is another obstacle in the traditional PDTX.

Methods

Cases and sample sources

1481 patient-derived tumor xenograft cases from four registered multi-center clinical studies (ChiCTR-OOC-17012731, ChiCTR-OOC-17013617, ChiCTR-POC-17013612, ChiCTR-1800018422) and researcher-initiated studies in China covering various cancers were enrolled, including gastric cancer ($N=190$), colorectal cancer ($N=146$), liver cancer ($N=497$), pancreatic cancer ($N=127$), sarcomas ($N=158$), gynecological cancer ($N=97$), cancer of the biliary duct ($N=98$), lung cancer ($N=33$), breast cancer ($N=25$), and others malignant tumors (110 rare type cancers). The primary tumoral specimens from surgery, biopsy, or endoscopic aspiration were used as the source for the either PDTX ($N=431$) or FastPDTX ($N=1050$) modeling. All protocols followed the guidelines of local institutional ethics regulations and were approved by the local institutional review board. Informed consent was obtained from all patients enrolled.

Animal cares

All recipients for xenograft were 6-8-week-old specific-pathogen-free (SPF) immunocompromised NCG mice (NOD-Prk^{dcem26Cd52} Il2rgem26Cd22, Nanjing Biomedical Research Institute, China) weighing 20 ± 2 g. The research was approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University following the National Institutes of Health guide for the care and use of Laboratory animals. All mice were housed in SPF animal facilities at a room temperature of 25 ± 2 °C and humidity of 40% -70%. Prior to the transplantation, all mice were housed for at least three days for acclimation. The sex of each selected mouse was same as the corresponding tumor donor.

Pretreatment of tumor specimen for PDTX or FastPDTX

Freshly collected tumor tissues were immediately preserved in the tissue preservation solution (Hyclone, Marlborough, U.S.A.) with the a supplemental of 100units/ml penicillin and 100 ug/ml streptomycin and were transported to the laboratory under refrigeration within 24 hours. The necrotic components, fats, and blood clots were removed before transplant.

PDTX modeling

The PDTX model was established as described (16). Briefly, 2 X 2X 2mm fresh tumor tissues were engrafted into NCG mice subcutaneously. Tumor volume was measured twice per week and was calculated according to the following formula: tumor volume = $[\text{length} * \text{width}^2] / 2$. When the volume reached 800-1000 mm³, the initial xenograft (passage 0, P0) was aseptically resected, and was re-partitioned and re-expanded in a similar way for subsequent generations. For the drug test, when the average tumor volume reached 100-150mm³, the mice were randomly assigned (5 mice per group) to control or treatment groups after the substandard mice (weight over Mean $\pm$ SEM/3, SEM, standard error of the mean) removed. The drug interventions were performed for 21 days in a similar manner to the clinical scheme and clinically relevant dose. Tumor volumes of control (Vc) and treatment (Vt) groups were measured to calculate tumor growth inhibition rate (TGI) using the following formula: $\text{TGI} = (\text{Vc} - \text{Vt}) / \text{Vc} * 100\%$. A regimen with $\text{TGI} \geq 80\%$ is designated as effective regimen (positive result).

FastPDTX modeling

The major engrafting procedures were similar as the PDTX modeling with some optimization as described(24). The key alteration in the FastPDTX was tumor tissues were engrafted into the gluteal region where the blood supply is abundant. After the vascularization was established (5 days after engrafting), the mice were exposed to candidate agents recommended by oncologists

for 7 days. Drug efficacy was assessed by histopathology and immunohistochemistry analysis of tumor cell necrosis rate (TCNR), and proliferative rate indexed by Ki-67. In addition, the percentage of tumor cells in the tumorgraft was also calculated to further improve accuracy. In the FastPDTX modeling, a therapeutic entity to be considered effective (positive result), it should meet the follow criteria: i.) containing at least 5% tumor cells in the specimen; ii.) at least one of indices, TCNR or Ki67, in both control and testing samples should be > 5%; iii.) the TCNR of experiment/control should > 10% or the ratio of Ki67 of experiment/control should < 80%. The one mouse per model per treatment scheme were adopted since the reproducibility of this scheme has been demonstrated.(25-27) For each tumor, at least 4 unique therapeutic entities were screened for both PDTX and FastPDTX. The schematic paradigm for PDTX and FastPDTX modeling were illustrated in Figure 1A.

Tumor growth rate calculation

Specific growth rate (SGR) was used to quantify tumor growth rate for all tumors by measuring tumor volume changes. The primary tumor tissue was implanted into the same mouse by PDTX and FastPDTX at the same starting time (t1) and with the same starting size (V1), and removed at the same time (t2) when the tumor sizes (V2) were measured. SGR was calculated according to the exponential growth equation as follows: $SGR = [\ln(V2 - V1)] / (t2 - t1)$.

Positive predictive value and concordant rate

Based on the classification of the response evaluation criteria in solid tumors (RECIST ver1.1), we designated the results as positive clinical responses if the patients' response were within one of the following categories: Complete Response (CR), Partial Response (PR), or Stable Disease (SD). The positive predictive value (PPV) was defined as the percentage of positive clinical responses in PDTX or FastPDTX predicted drug effective cases, i.e., $PPV = (\text{number of clinical$

positive response cases) / (number of PDTX or FastPDTX predicted positive cases). For the comparison between FastPDTX and classical PDTX, the concordant rate was used. When the predictive result for a drug regimen was same by FastPDTX and PDTX, i.e., both models predicted as effective or noneffective, the case was documented as a concordant case. The concordant rate = (number of concordant cases) / (total regimens tested by both FastPDTX and PDTX).

Whole Exome Sequencing (WES) analysis

Genomic DNA extraction and sequencing library construction were performed using standard protocols as described (28). 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. Whole-exome sequencing was performed on NovaSeq 6000 System 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 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(29) and converted into BAM files using SAMtools (version 0.1.18) (30). Duplicates were marked for filtering and INDELs were realigned using GATK v.3.4.46 IndelRealigner (31). 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 (32). 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. Z score was used to measure the significance of copy number variations. For each

specific gene, $Z \text{ score} = (\text{copy number of that gene in the tumorgraft} - \text{average copy number of that gene in original tumors}) / (\text{standard deviation of copy number of that gene in original tumors})$.
Z score > 3 indicates significant copy number variations.

Results

Optimized tumor growth in FastPDTX

In contrast to PDTX, where efficacy testing usually started at stage P3-P5 (tumorgraft passage 3-passege5), and the agent exposure was 21 days, the efficacy assessing in FastPDTX started directly at stage P0 and the during was seven days. The typical drug testing period of FastPDTX was only 15-20 days compared to 80-200 days in PDTX (Figure 1A). One precondition for successful drug testing is the well-established blood supply to the tumorgraft for effective drug delivery. Therefore, in the FastPDTX protocol, tumorgrafts were implanted in the gluteal region, where the blood supply is abundant, and presumptively vascularization could be established rapidly. To test this conjecture, a side-by-side comparison was performed, which was conducted by engrafting two same-sized tumor tissues into the same mice using the FastPDTX for one and the PDTX protocol for the other, and after a period of growth, the dimensions of the two tumorgrafts were compared at the same time. The test results clearly demonstrated a more rapid growth of tumorgraft when using the FastPDTX protocol (Figure 1B left). The tumorgraft from the FastPDTX is pinker overall than that from PDTX, indicating a better blood supply inside the graft (Figure 1B right). Clear blood vascular connections were observed from mouse tissue to the graft and inside the graft five days after the implantation (Figure 1C left). An immunohistochemistry inspection was performed using CD31 antibody to verify the establishment of blood supply inside the tumorgraft, and the results also showed a clear vascularization in the graft (Figure 1C right). In the interest of discriminating the difference between two protocols quantically, we further assessed the specific

growth rate (SGR) of tumorgrafts from various types of tumors under both modeling conditions. For all types of tumors, the general SGR under the FastPDTX condition was about 3-9 times higher than PDTX, indicating improved growth environments under the FastPDTX modeling conditions (Figure 1D).

FastPDTX preserves the characteristics of original tumors

Due to the significant internal differences between human beings and mice, transplanted tumors may alter to adapt to those environmental changes. These alterations may cause discrepancies between the modeling results and the patient's actual response. Therefore, we examined the resemblance between primary tumors and the xenografts from FastPDTX. We did not assess tumorgrafts from the traditional PDTX model since their identity to the primary tumors has already been shown.⁽¹⁶⁾ The first comparison was performed at the histopathologic level. A single-blind method was employed by comparing the concordance between the pathological diagnostic results of primary tumors (Pa) and the paired xenografts (P0). In total, 499 pairs of samples from different types of tumors were examined, and the overall concordance between Pa and P0 is 98%. Typical images from seven pairs of various tumors are shown (Figure 2A).

Copy number variations (CNVs) have been frequently observed in various tumors and are considered influencing drug resistance (33, 34). We also examined CNVs for major cancer driver genes in those seven pairs of samples using Whole Exome Sequencing (WES). Only sporadic CNVs between Pa and P0 were discovered, which indicated only minor differences between primary tumors and xenografts regarding the significant DNA fragment changes (Figure 2B).

Single nucleotide variation (SNV) or point mutation is the other major contributor to the alterations in drug pharmacokinetics (35). For those seven pairs of samples, we compared nonsynonymous SNVs as well, and a high degree of concordance between Pa and P0 was also observed (Figure

2C). Along with the rarity of CNVs, this result demonstrated that xenografts from FastPDTX faithfully preserved the primary tumors' genetic characteristics. Above mentioned results indicated that the xenografts from FastPDTX closely resemble the primary tumors.

Drug efficacy can be tested early using pathology

In traditional PDTX, the drug efficacy is assessed by measuring the tumorgraft's volume change 21 days after the drug treatment. We noticed that in our PDTX tests (Figure 2D) and many other previous studies(36, 37), tumorgrafts' volumes have already displayed significant divergence at day seven when treated with drugs with various efficacy. Thereby, we conjectured that the discrepancies would be more distinct if assessed by a microscopic examination inspecting the cellular morphological and pathological changes, and this examination could be performed at an early time point. To test this assumption, we examined necrotic and proliferative rates of tumor cells in the FastPDTX model seven days after the drug treatment. As expected, the drug-treated tumorgraft contained a substantial portion of necrotic cells (Figure 2E, top part, HE stain) and a significantly reduced amount of proliferative cells (Figure 2E, bottom part, Ki67 stain). These results support our opinion that drug efficacy can be assessed by pathological examinations and can be performed at an early time point.

Drug efficacy can be determined by pathological indices

Based on the results mentioned above, we decided to use tumor cell necrotic and proliferative rates as the drug efficacy assessment indices. We adapted one mouse per model per treatment method in FastPDTX since the reproducibility of this scheme has been demonstrated.(25-27) Because the cellular necrosis and the proliferating inhibition could be induced by other conditions such as previous neoadjuvant chemotherapy or an insufficient blood supply in tumorgrafts we used untreated tumorgrafts as a control to abate those potential disturbing effects. We quantified the

percentage of necrotic and proliferative cells and compared those two indices pairwise in control and treated samples. To determine appropriate thresholds that can correctly translate quantifications of these indices into the clinical responses, 12 therapeutic entities where the results of FastPDTX, PDTX, and clinical responses were all available were examined (Table 1 and Supplemental Table 1). We discovered that to make the efficacy assessment reliable, specimens (both control and tested tumorgrafts) should contain at least 5% tumor cells, and at least one index, either TCNR or Ki67, should be $> 5\%$. On the premise of these two conditions, the difference of TCNR between the tested sample and control was $>10\%$ (i.e., tested minus control $>10\%$), or the percentage of Ki67 positive cells of the tested sample over control was $<80\%$ would indicate a positive drug effect, which could be translated into clinical effective disease control (corresponding RECIST criteria CR, PR or SD). In this 12 regimens trial, the positive predictive value (PPV) of FastPDTX was 91.7%, and PDTX was 83.3% (Figure 3A, Table 1). Because the negative predictions could not be tested clinically, we did not calculate the sensitivity or specificity. We also compared the concordance between the predictions from FastPDTX and PDTX over 93 therapeutic entities, and the concordant rate between the two methods was 69.9% (Figure 3A, Supplemental Table 1).

FastPDTX predicts clinical outcomes

Comparing with 30-40% response rate of first-line drugs and 25%-10% of second-line and subsequent-line drugs in standard therapies, using guidance from PDTX, the drug-response rate can be over 80% (6, 17, 38). As a novel method, how well the FastPDTX can guide clinic needs to be fully assessed in the context of PDTX and real clinical outcomes being compared.

In the subsequent study, we examined the PPV of FastPDTX and PDTX for efficacious drugs in 218 cases, where the clinical responses were accessible. However, direct comparisons between PDTX and FastPDTX were not available. The PPV of FastPDTX was 82.3% (163/198), and PDTX was 81.8% (27/33); numbers were very close to each other and the previous other study(16) (Figure 3B, Supplemental Table 2,3). The PPV results suggested an equivalent performance of FastPDTX to PDTX in guiding efficacious drug selection.

FastPDTX is more applicable clinically

For real-time clinical drug selecting, a drug test should end in a high percentage of applicable test. To assess the performance of FastPDTX in this aspect, we compared two critical indices of FastPDTX and PDTX. The first index is the percentage of successful engraftment or engraftment rate, which is one decisive factor for whether the technique is clinical utilizable. However, due to the distinct technological divergence between the two models, the engraftment rate was defined differently. In our study, for the PDTX model, the successful engraftment was defined as an outgrowth of P0 tumorgrafts (volume reaches 60-80mm³) and at least three generations of successful subsequent propagation(39) (Figure 1A). For FastPDTX, the successful engraftment was designated as the living condition of the tumorgraft and established vesiculations before the drug testing started. In the PDTX cohort, 431 cases (from Jan. 2015 to Apr. 2018) were enrolled, covering nine major types of cancer as well as other malignant tumors, and the overall engraftment rate was 57.1% (246/431) (Figure 3C, D). For the FastPDTX cohort, 1050 cases were enrolled (from Apr. 2018 to Dec. 2019), spanning the same cancer spectrum as the PDTX with a 99.9% overall engraftment rate (1049/1050) (Figure 3C, D). The second index is the percentage of applicable tests, which we defined as the percentage of tests where the test results were employed successfully for patients. This index reflects the real benefit of FastPDTX under clinical conditions.

For the PDTX group, because of the extended testing period, many patients could not wait until the test finished; therefore, the percentage of applicable tests was low as 16.7% (72/431), while that of the FastPDTX group was 94.8% (995/1050) (Figure 3E, F).

FastPDTX allows personalized medicine for tumors hard to manage

As a consequence of the short testing cycle and high ratio of applicable tests (Figure 1A, 3F), FastPDTX is particularly useful for patients with refractory malignant tumor at advanced stage, and in circumstances where classical PDTX has difficulty to be applied. We present three typical cases illustrating how those patients can be benefited from FastPDTX.

Case 1(hard-to-grow tumor at advanced stage):

A 32-year-old triple-negative breast cancer patient who underwent several rounds of therapies, including surgery, radiotherapy, and several lines of chemotherapy, has developed lung and kidney multiple metastases. Before the testing, the patient already tried epirubicin, paclitaxel liposome, nimotuzumab, docetaxel, and capecitabine. Nine agents were screened with FastPDTX, and gemcitabine was recommended based on the Tumor Cell Necrosis Rate (TCNR) and Ki-67 protein positive staining (Ki67+) results. After 8 cycles of gemcitabine treatment, specific breast cancer markers showed a significant drop: CA153 from 42.21U/L to 30.39U/L, CA125 from 38.26 U/L to 11.06 U/L, and Cyfra21-1 from 16ng/ml to 3.81ng/ml. CT scan showed that the lung and kidney metastases were reduced significantly. The patient gained Partial Response (PR) for eight months (Figure 4A-E).

Case 2 (highly advanced malignant tumor):

A 49-year-old male with 40 years of hepatitis B positive history was diagnosed with hepatocellular carcinoma (HCC) that recurred in the liver, and he developed multiple metastases in both lungs

three years after the initial diagnosis. The patient has undergone surgery, transarterial chemoembolization (TACE), several lines of conventional treatments, and target therapy (sorafenib, 1 year). Because of the highly advanced tumor, the patient lost the chance for another surgery and was resistant to standard treatments. Six therapeutic entities were tested with FastPDTX, and cabozantinib was recommended. After two cycles of treatment, Alpha-Fetoprotein (AFP) dropped from 1285 ng/ml to 135 ng/ml, and Protein Induced by Vitamin K Absence/antagonist-II (PIVKA) decreased from 97691mAU/ml to 4686mAU/ml. CT scan showed lung and liver metastasized lesions were reduced significantly. The patient gained PR (Figure 4F-J) and gained the opportunity for debulking surgery.

Case 3 (refractory malignant tumor at advanced stage):

A 63-year-old male diagnosed with pancreatic duct adenocarcinoma (PDAC) and developed liver metastasis at stage IV. Screening results of FastPDTX showed that fluorouracil, albumin paclitaxel, and oxaliplatin were effective, and Irinotecan was moderately effective. Four weeks after distal pancreatectomy, five cycles of subtraction angiography guided arterial infusion chemotherapies were performed with the combination of FastPDTX guided drugs. The patient's CA-199 dropped from 1000U/ml to a normal level at the third cycle of chemotherapy. After five cycles of chemotherapies, the CT scan showed that pancreatic and liver metastatic lesions had disappeared. The clinical response was nearly CR (Figure 4K-O).

FastPDTX matches real clinical practice and increases cost-effectiveness

We reviewed the modeling conditions of FastPDTX and classical PDTX comprehensively. When comparing those conditions to real clinical procedures, we discovered that the modeling conditions of FastPDTX more closely matched the real clinical procedures (Table 2). Moreover, when the costs of FastPDTX and PDTX were compared (China market price), the cost of FastPDTX is only

about 15% of PDTX, and the cost-effectiveness ratio is almost 40 times higher than PDTX. These results strongly support the clinical utilizability of FastPDTX.

Discussion

Increasing amounts of evidence have shown that PDTX can faithfully preserve the characteristics of original tumors and predict patient response with high accuracy(19, 20, 40). So far, principal applications of PDTX are researches and preclinical drug screening(26). PDTX has also proven a valuable tool in customized therapy for refractory tumors or rare tumors that clinical guidelines for standard care are usually limited (15, 21, 41). However, procedures for PDTX modeling are complicated and time-consuming, resulting in low success rates and high costs. Those disadvantages seriously compromise the outstanding value of PDTX for efficacious drug selection in the clinical setting (14, 16, 23).

In this study, we tried to overcome those disadvantages by technical improvements. The pivotal technical alteration is the use of pathological inspections in FastPDTX to replace the measurement of tumor volume in PDTX, which results in two advantages. The first advantage is that the engraftment rate barrier is circumvented. In the traditional PDTX, successful engraftment requires tumorgrafts to be sequentially propagated, and this prerequisite often brings a substandard engraftment rate. In general, the engraftment rate depends on multiple variables, including mouse strains, types of tumors, anatomical locations for engrafting, and many others, which brought significant variances of engraftment rate across different studies (42). In most cases, the rate cannot meet the generally accepted standard of 60~70%(14) for personalized medicine. Due to the fast pathological analysis, the FastPDTX modeling simply needs the vital condition of tumorgrafts with vesicular connections for effective drug delivery and does not demand the growth and propagation of tumorgrafts. This alteration substantially improves the portion of successful tests

(Fig. 3D). Implanting tumors in the tissues where blood supply is abundant also helps improve engraftment rates and was reported previously(43). In the FastPDTX protocol development, we used the gluteal region as the primary implanting site and did observe optimized growth of tumorgrafts (Fig. 1B, C). This implanting site alteration is another contributor to the increased success rate.

The second advantage is reducing the entire testing cycle from 3-6 months to less than three weeks (Fig. 1A). Comparing with measuring tumor volumes in PDTX, which needs repetitive proliferation of tumorgrafts and thereby causes the extended testing period, the pathological inspections in FastPDTX require just a short growth time for pharmacological effects to take place. This timing reduction produces two significant clinical impacts. The first impact is the considerably improved applicability of FastPDTX (Fig. 3D), which furtherly was translated into the substantially increased number of clinically benefited patients (Fig. 3F). Second, the spectrum of applicable cancer types is broadened. The traditional PDTX modeling demands significant amounts of resources from the host for enough growth of tumorgraft. In particular, for hormone-driven cancer like breast cancer, the dominant type of female cancers, special requirements like long-term and adequate supplies of hormones are difficult for the mouse host to provide, resulting in a poor engraftment rate in PDTX modeling. In contrast, such a requirement was nonessential in FastPDTX modeling and resulted in superior applicability in the clinical setting. Additionally, indolent tumors in mice, such as neuroendocrine tumors, are also suitable for FastPDTX.

Comprehensive pathological indices are used in FastPDTX, including Ki67, TCNR, and percentage of tumor cells. Several factors were considered before those indices were chosen. Ki67 proliferative index is used commonly in clinical practice, especially for tumors with high proliferative activity(44-46). In breast cancer, Ki67 has already been listed in the National

Comprehensive Cancer Network (NCCN) guidelines as an essential basis for evaluating prognosis and malignancy(47). Also, TCNR has been used for evaluating tumor cell necrosis caused by therapeutic agents in tumors such as osteosarcoma(48, 49). The proportion of tumor cells in the graft is considered to minimize the variances caused by the tumor's heterogeneity. Indeed, pathological examination for efficacy evaluation in PDTX has been implicated in a previous study(50). However, the application of this method as a standard approach has never been reported. To validate the effectiveness of this new pathological approach, we performed an extensive comparison among FastPDTX, PDTX, and clinical outcomes covering >200 clinical cases. The results demonstrated that using this new pathological approach, FastPDTX has equivalent predictive power to PDTX regarding the efficacy evaluation (Fig. 3A, B). Therefore, the validity of this method is proven.

Our results showed a high degree of pathological and genetic consistency between grafts and primary tumors (Figure 2), indicating the high fidelity of FastPDTX. During the FastPDTX modeling, the tested graft is at the initial stage, and the test period is relatively short. These two conditions have two implications: First, the elimination of graft passaging can prevent makeup alterations of the whole graft. After a serial propagation, components of tumorgrafts such as stroma cells will eventually be replaced by murine type, and this change may generate unpredictable outcomes for the efficacy test. Multiple studies have shown various alterations in serially passed PDTX(51), but those alterations were barely found in primary tumorgrafts. Second, microenvironmental-change-induced epigenetic modifications and other genetic changes will be minimized. It has already been known that epigenetic modifications may be responsible for alterations in drug susceptibility (52), and consequently could produce biased test results in animal models. The more frequently grafts are passed and the longer the test period is, the higher the

possibility of epigenetic and genetic alterations and the greater the chance of biased test results. The tested tumorgrafts in FastPDTX are primary tissues with minimum growing time and no passage, which contributes to the reliability of tests.

Precision medicine is the future of cancer treatment and, ideally, should be available to the majority of cancer patients. By exclusion of labor-intensive and time-consuming serial passage of tumorgrafts, not only the testing time was significantly shortened in FastPDTX, but the testing cost was also reduced substantially to a feasible range for most families (Table 2).

Significant improvements have been made in the FastPDTX system comparing to PDTX. We illustrated three typical cases showing the advantage of FastPDTX in treating the hard-to-grow tumor (case 1, breast cancer), the highly advanced tumor (case 2, hepatocellular carcinoma), and the intractable tumor (pancreatic cancer). Meanwhile, additional issues in which FastPDTX need be improved further, such as cancer type-specific optimized criteria for efficacy evaluating, a pathological categorizing system that can be translated into RECIST equivalent, and multi-omics combined analysis. Regarding the clinical setting, other issues like sample delivery and animal facilities were not addressed in this study and need to be explored in the future. As for clinical decision making, FastPDTX reduces the testing cycle to an acceptable duration (2-3 weeks, comparable to the genetic testing cycle for targeted therapy) and yields over 90% applicable test while still keeps the same accuracy as PDTX. Therefore, FastPDTX has great potential for real-time clinical personalized drug selection in combination immunotherapy and traditional standard cancer care.

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Figure legends

Figure 1. General comparisons between FastPDTX and PDTX

(A) Schematic diagram showing the work flow and time frame of FastPDTX (top) and PDTX (bottom). (B) An example showing distinct growth rates of tumorgrafts using FastPDTX or PDTX protocol. (C) Rapid vascularization in FastPDTX tumorgrafts (showing four samples from different cases); arrows indicate either blood vascular (left) or CD31 positive staining (right, brown stained). (D) Specific growth rate (SGR) of various tumorgrafts under FastPDTX or PDTX conditions.

Figure 2. Establishment of pathological examination for efficacy assessment

(A) Histopathologic inspections (hematoxylin and eosin stain) of 7 pairs of original tumors (Patient) and their corresponding tumorgrafts (FastPDTX). (B) Copy number variations (CNVs) in the 7 tumorgrafts measured by Z score; Z score > 3 indicates significant copy number alteration. (C) Single nucleotide variations (SNVs) in tumorgrafts (P0) and original tumors (Pa). Minor allele frequency (MAF) was compared side by side (P0 vs. Pa). Each red circle indicates an allele. Blue straight line shows the ideal situation (no changes between P0 and Pa); red straight line shows the real results. (D) A typical growth curve of tumorgrafts in PDTX protocol. Red arrow indicates diverse tumor volumes at day 7 when treated with different drugs. (E) Distinct pathological patterns in drug treated tumorgrafts. Tumorgraft was examined 7 days after cabozantinib treatment. Red arrow and red dashed line circled area indicate necrotic cells; Blue arrows indicate live cells; Brown color shows Ki67 positive cells.

Figure 3. Comprehensive Comparison between FastPDTX and PDTX

(A) Pairwise comparison of positive predictive values for drug efficacy and the concordant rate between FastPDTX and PDTX. (B) Positive predictive values of FastPDTX and PDTX in independent cases. (C) Number of enrolled cases across various type of solid tumor in FastPDTX (blue) or PDTX (green). (D) Engraftment rate and percentage of applicable tests of FastPDTX and PDTX among various type of cancers. (E) Number of clinical benefited patients from FastPDTX or PDTX in this study since 2015. (F) The percentage of applicable test of FastPDTX or PDTX in this study since 2015.

Figure 4. Typical cases from FastPDTX

(A-E) Triple-negative breast cancer case: (A) testing regimens, agents, and tumor components. (B) histopathology staining (HE) and statistics of TCNR. (C) Ki67 staining (IHC) and statistics. (D) changes of breast cancer specific marker CA153, CA125 and Cyfra21-1. (E) CT scan images before and after treatment. (F-J) Corresponding regimens, statistics, biomarkers, or images from the advanced liver cancer case. (K-O) Corresponding regimens, statistics, biomarker, or images from the advanced pancreatic cancer case.

Table 1: Comparison of predictive results by FastPDTX and PDTX to real clinical outcomes

Case ID	Type	Regimen	FastPDTX				PDTX		Clinical response	Congruous between	
			Tumor cell (%)	TCNR	Ki67	Prediction	TGI	Prediction		Clinical/ FastPDTX	Clinical/ PDTX
050106	OS	Control	40%	0%	60%						
		Docetaxel+Gemcitabine	10%	10%	60%	P	77.3%	P	PR	Y	Y
		Etoposide+Ifosfamide	40%	20%	70%	P	86.7%	P	PR	Y	Y
050125	PC	Control	20%	5%	50%						
		Gemcitabine+Tegafur	30%	0%	10%	P	80.5%	P	CR	Y	Y
080093	CRC	Control	70%	0%	70%						
		Irinotecan+Cetuximab	10%	0%	20%	P	76.80%	P	SD	Y	Y
101007	OC	Control	20%	20%	20%						
		Bevacizumab+Paclitaxel+Lobaplatin	20%	30%	10%	P	47.30%	N	CR	Y	N
110150	GC	Control	70%	10%	90%						
		Albumin paclitaxel+Cisplatin	30%	5%	40%	P	91.60%	P	SD	Y	Y
130111	PC	Control	8%	0%	70%						
		Gemcitabine	2%	0%	<5%	P	86.70%	P	SD	Y	Y
200171	OS	Control	20%	0%	5%						
		Docetaxel+Gemcitabine	1%	10%	3%	P	61.5%	P	SD	Y	Y
212001	SS	Control	60%	0%	75%						
		lipo-doxorubicin	95%	0%	60%	P	68.2%	P	SD	Y	Y
240154	OS	Control	80%	5%	50%						
		Apatinib	70%	0%	80%	N	45.0%	N	PD	Y	Y
		Gemcitabine+Docetaxel	80%	20%	60%	P	97.9%	P	SD	Y	Y
		Etoposide+Ifosfamide	30%	0%	10%	P	96.2%	P	PD	N	N
Positive Predictive Value										11/12	10/12

Abbreviations: OS=osteosarcoma, PC=pancreatic cancer, CRC=colorectal cancer, OC=ovarian cancer, GC=gastric cancer, SS=synovial sarcoma, HCC=hepatocellular carcinoma. In the prediction column, P=a positive drug effect that equivalent to CR or PR or SD; N= a negative drug effect that equivalent to PD.

610 **Table 2. Comprehensive comparisons of PDX, FastPDX, and real clinical treatment (RCT)**

611

		FastPDX	PDX	RCT
Xenograft characteristics	Tumor microenvironment	Original microenvironment is kept at great extent	Stroma cells may be replaced by murine type	Original microenvironment
	Tumor architecture	Original stereoscopic structure	Original stereoscopic structure (P0)	Original stereoscopic structure
	Histopathological consistency	Almost full uniform (P0)	Highly uniform (decreasing with passages)	Original tissue
	Genetic consistency with original tumor	Almost full uniform(P0)	Highly uniform (decreasing with passages)	Original tissue
Drug administration	tumor tissue	P0	P1-P5	Primary tumor (Pa)
	Tumor size	8-12mm ³	100-150mm ³	Original size
	Administration Route	Gavage	Gavage	Oral
		Intraperitoneal injection	Intraperitoneal injection	Intravenous (IV)
	Starting point for patient	15-20 days (P0)	80-200 days (P1-P5)	20-30 days after surgery
	Drug regimen	Multiple regimens simultaneously	Multiple regimens simultaneously	One regimen
	Drug dose	Converted from clinical dosage	Converted from clinical dosage	Clinical guideline
	Evaluation criteria	Pathology, TCNR, Ki67	TGI	biomarkers, CT

Efficacy assessment	Test sensitivity	Sensitive	Moderately sensitive	Sensitive
	Testing cycle	About 20 days	About 120-180 days	-
Cost-efficiency	Average Price/patient*	~4500 USD	~30,000 USD	-
	Cost-effectiveness (USD/ patient)	~180,000	~4600	

612 *According to Chinese market price, test of four therapeutic entities.

Figure 1

B

C

D

Figure 2

Figure 3

Figure 4

Supplementary Files

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