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ORIGINAL ARTICLE

Chemotherapy for Pancreatic Cancer can be Optimized with the Use of Drug Resistance Testing Using Tissue Organoids

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ABSTRACT

Objective: The purpose of the present investigation was to ascertain how drug resistance in pancreatic cancer organoids affects the efficacy of chemotherapy.

Study Design: Retrospective study

Place and Duration: Jinnah International hospital, Abbottabad, 15th March- 15th September, 2022

Methods: A total of 72 patients of both genders with pancreatic cancer were presented in this study. After obtaining written consent from participants, we recorded detailed demographic information about them, including their age, sex, and body mass index. Pathological evaluation of tumour regression grade (TRG) was compared to data on the dose and schedule of neoCTx treatment. SPSS 22.0 was used to analyze all data.

Results: This study had 43 (59.7%) males and 29 (40.3%) females. 22 (30.6%) patients were aged between 18-30 years, 25 (34.7%) patients had aged between 31-40 years, 15 (20.8%) patients had aged 41-50 years, and 10 (13.9%) patients had aged> 50 years. In a study with 15 CTx-naive PDO lines, 8 showed a distinct response to FOLFIRINOX or Gem/Pac. As much as 34.7 per cent of patients with NeoCTx PDOs experienced an unfavorable reaction to their neoadjuvant treatment. Modified treatments in which the lowest successful individual medicine was eliminated from the full regimen showed no meaningful change in PDO response.

Conclusion: It is possible that drug testing on CTx-nave PDAC PDOs and neoCTx PDOs will help determine the optimal neoadjuvant and adjuvant treatment regimens. In order to increase the proportion of patients completing the course of neoadjuvant treatment, it may be beneficial to individualize poly-chemotherapy regimens by removing chemicals with low effectiveness.

Keywords: Pancreatic Cancer, Chemotherapy, Drug Resistance, Tissue Organoids

INTRODUCTION

Substantial cancer detection and therapy advancements have been made in recent years [1, 2]. Despite these advances, cancer remains a major global health concern, and there are still many problems to be overcome before cancer patients' lives may be improved and their deaths prevented. One of the biggest challenges is coming up with effective treatment protocols. Traditional cancer models fail to accurately represent actual tumours, eliminating many promising therapies in clinical trials.

Cancer research has benefited greatly from conventional tumour models, such as two-dimensional (2D) cell line cultures and patient-derived tumour xenografts (PDTXs). However, the therapeutic utility of these two models is severely constrained by several limitations. Some crucial aspects, including the immune response, microenvironment, stroma compartments, and organ-specific activities, are not well-simulated in 2D cell line cultures. In addition, PDTXs are expensive, time-consuming, and resource-intensive [7], cancer cell lines lose the genetic heterogeneity of the original tumours after several passages [5]. Malignancies evolve in a way that is unique to mice [6]. The use of organoids in research has emerged from the shadows. Organoids are 3D constructions that may be grown in a petri dish from various stem cell types, including embryonic, induced pluripotent, somatic, and cancerous ones.

Stem cells are an undifferentiated cell subset with the ability to self-renew and the capability to repair and replace damaged tissues and organs. Stem cells and adult stems are two types of stem cells distinguished by the stage of development at which they were first found. Isolated from embryos, embryonic stem cells may divide indefinitely, self-renew, and differentiate into various cell types. Adult stem cells include undeveloped pluripotent or multipotent progenitor cells. Adult organisms rely on progenitor cells, which may be found in various tissues, to repair and regenerate damaged areas.

Advances in solitary sequencing, a powerful approach for defining varied cell types, have allowed the accurate investigation of several cells in pancreatic cancer. This technique has been used to investigate a wide range of malignancies.[8,9] We can also learn how pancreatic cancer cells differentiate and how the immune system responds to the disease by using cell grouping and annotation (Hwang et al., 2021). [10]

One major reason tumour cells are resistant to treatment is that they have problems carrying out cell death[11]. Copper-dependent death is programmed cell death triggered by copper's direct binding to fatty acylation elements of the tricarboxylic acid cycle. Copper plays a dual role in carcinogenesis, fostering tumour growth and inducing redox stress in cancerous cells. Copper is used in cancer treatment as a direct pharmaceutical and regulator of antibiotic susceptibility and absorption. Yu et al.'s work provided more evidence that copper deficiency could bel as an alternative therapy for pancreatic cancer. [12]

Patient-derived organoids (PDOs) are a model system that might steer optimum therapy through individualized therapies since they are a three-dimensional cultured cell system. PDOs are easily and quickly made from tissue samples obtained through surgical excision or ultrasonic fine needle aspirations (EUS-FNAs). Most of the genetic changes found in the original tumours are preserved in the PDOs, making them useful for defining treatment response and resistance [13]. PDO response was initially studied about treatment success in gastrointestinal cancer; subsequent studies have provided further evidence that patient reaction may be anticipated by ex vivo experiments [14,15].

Here, we looked back at the effect that a change in neoCTx administration had on pathological response and other prognostic indicators in patients with LA-PDAC. Next, we pharmacotyped PDAC Assets (e.g. from chemotherapy-naive and -pretreated patients utilizing standard-of-care regimens and assessed the efficacy of customized treatment.

MATERIAL AND METHODS

This retrospective study was conducted at Jinnah International Hospital, Abbottabad and comprised 72 patients. After obtaining written consent from participants, we recorded detailed demographic information about them, including their age, sex, and

body mass index. Patients who received systemic treatment besides FOLFIRINOX or Gem/nab-Pac after cycle two or who switched neoCTx were not included.

NeoCTx was considered 'complete course' when FOLFIRINOX was administered for at least four 14-day cycles and Jewel was administered for at least 3 28-day periods without dose reduction. PDAC organoid generation following surgical resection specimens has been documented [17]. When erythrocytes were discovered, the protocol for processing FNA biopsies was altered by reducing the digestion time to 45 min and employing ACK lysate (Thermo Fisher, Boston, MA, USA). PDAC organoids were raised in DMEM/F12+++ media adding 1 Glutamax (Invitrogen), 1 Iptg (Invitrogen), and 1 Pen/Strep (Qiagen) supplied to WNT3a-conditioned medium (50percent) of the respondents v/v), RSPO1- and Noggin middle (10% v/v each), 1 B27 (Qiagen), and 1 N2 (Invitrogen). Stable growth (> passage Primary tissue that had been cryopreserved had its DNA retrieved by macrodissection. Each sample's total DNA concentration was 120 ng for targeted sequencing. Following the manufacturer's instructions, libraries were prepared using TruSight Oncology 500 Kits from Illumina in San Diego, California, USA. A platform called Illumina NextSeq 500 was used to pool the barcoded samples and read them (2 x 150 bp paired-end). Utilizing Illumina's TSO500 methodology, raw sequence data were processed (ruo-2.2.0.12). Through the use of gnomAD exomes, the small variants were noted. r2.1. On paraffin-embedded material, CK19 (ab20210, pH 6.0, 1:100; Abcam, Cambridge, UK) or TP53 (#2524, pH 6.0, 1:200; Cell Signaling Technology, Danvers, MA, USA) immunohistochemistry (IHC) staining was done. Before being cut into sections and stained, allografts were first frozen in 4% formalin solution for 30 minutes. They were then dried and paraffin-embedded. employing an, pictures were captured. Prior to sectioning and staining, allografts were frozen in 4% formalin solution for 30 minutes, dried, and embedded in paraffin. An EVOS FL Automated microscope was used to capture the images.

As mentioned, modified medication combination trials were carried out. The same PDO pellet was used to create PDOs for the FOLFIRINOX left changed assay (irinotecan + cisplatin, irinotecan + 5-fluorouracil, and oxaliplatin + 5-FU) and alternative treatment (GemIri). Single-agent testing were matched with the Gem/Pac therapy. To calculate AUC, Jewel overlapping regions and single-agent testing were used.

Chi-squared test in GraphPad Prism 8.4 was used to analyse the impact of full-course or tailored neoCTx on LA-PDAC patient treatment outcomes.

After removing the blank, pharmacotyping tests were assessed by adjusting to the mean of a negative controls. GraphPad Prism 8.4 was used to plot the drug's relative survivability curves and calculate AUC. Pharmacotyped PDO lines were separated into FOLFIRINOX- or Gem/Pac-pretreated and CTx-naive groups. By dividing AUC even by the greatest area for the specific drug dilution range, comparative AUC (relAUC) was calculated. The data from the examined PDO lines' single- and paired assay relaunches were averaged for every group and analyzed using Mann-Whitney testing.

The formula for AUC z-score normalisation is z = (x)/, where x represents the mean AUC from PDO line analysed in three different tests, the mean AUC from all PDO lines investigated, and the standard deviation from any and all PDO lines reviewed.

Following was the analysis of the modified FOLFIRINOX and Gem/Pac tests: Similarly to unmodified combination testing, the order to acquire confidence for each medication dilution was calculated, as well as the AUC. Three-way ANOVA was used for the statistical analysis the leave-one-out drug mixtures with multiple comparisons to the FOLFIRINOX treble drug data. A one-way ANOVA test was used to analyse modified Jewel (*p 0.05, **p 0.01, ***p 0.001).

Single-agent drug reactions were fitted in a linear regression with Gem, Pac, or their interaction Gem Pac as the variable to ascertain if Gem/Pac combination drugs operate additively, synergistically, or antagonistically.

RESULTS

This study had 43 (59.7%) males and 29 (40.3%) females. 22 (30.6%) patients were aged between 18-30 years, 25 (34.7%) patients had aged between 31-40 years, 15 (20.8%) patients had aged 41-50 years, and 10 (13.9%) patients had age > 50 years.(Table-1)

Table-1: Baseline details of enrolled cases

Variables	Frequency	Percentage
Gender		
Male	43	59.7
Female	29	40.3
Age		
18-30 years	22	30.6
31-40 years	25	34.7
41-50 years	15	20.8
>50 years	10	13.9

CTx was administered without any modification (a "full course") in 74.5 percent of patients who were treated with the FOLFIRINOX (n = 47) regimen, and in 72 percent of patients who were treated with the Gem/nab-Pac (n = 25) regimen. (Figure 1)

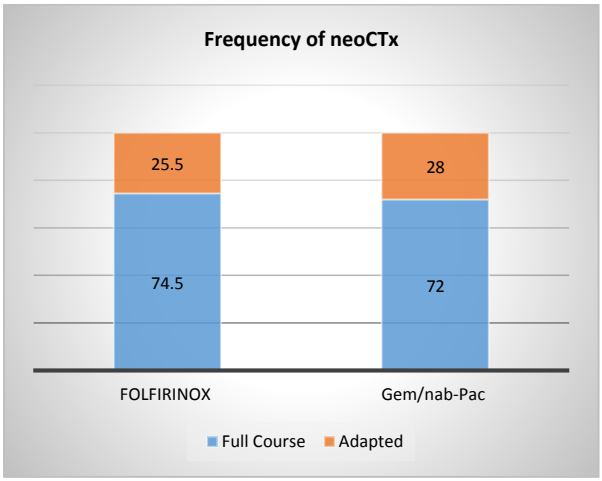

Figure-1: Full-course versus adapted neoCTx with FOLFIRINOX or gemcitabine + nab-paclitaxel (Gem/nab-Pac)

In a study with 15 CTx-naive PDO lines, 8 showed a distinct response to FOLFIRINOX or Gem/Pac. As much as 34.7 percent of patients with NeoCTx PDOs experienced an unfavourable reaction to their neoadjuvant treatment. Modified treatments in which the lowest successful individual medicine was eliminated from the full regimen showed no meaningful change in PDO response.(Table-2)

Table-2: Response of NeoCTx with outcomes

Variables	FOLFIRINOX (47)	Gem/Pac (25)
Distinct Response		
Yes	5 (10.6%)	3 (12%)
No	42 (89.4%)	22 (88%)
Outcomes		
Favorable	13 (27.7%)	12 (48%)
Un-favorable	34 (72.3%)	13 (52%)

DISCUSSION

Our PDAC PDO obtained for the study included samples from individuals who had never received chemotherapy and those who had had prior treatment for PDAC. Patients with a significant response had a lower number of viable tumour cells in their collections, which may explain why the success rate of organoid

creation from pretreated patients linked with TRG (TRG3). Despite the small sample size, it was possible to identify a correlation between the previous combination therapy that had been delivered to the patients and the development of drug resistance in the neoCTx PDOs. Our findings might be attributed to the selection for resistance or the concentration of already resistant clones inside the tumour that occurs after systemic chemotherapy. It is not interesting to note that not all neoCTx PDO lines exhibited resistance to the corresponding regime, nor were they all more vulnerable to the alternate regimen. Because of this, post-treatment PDOs may be one day factor in choosing the best adjuvant therapy.

Therefore, the absence of indicators showing greater effectiveness of the FOLFIRINOX or Jewel regimens for the particular patient is now hindering the selection of the proper neoCTX. Both regimens were effective in the neoadjuvant context, according to the most recent data from the SWOG-S1505 study. We demonstrated that a distinct reaction to simply the combination against Jewel could be documented in almost half of PDOs. Accordingly, PDOs may serve as biomarkers to aid doctors in selecting the most effective regimen for neoadjuvant therapy of LA-PDAC. Since it has been established that even patients with largely resectable PDAC benefit from neoCTx [16], the group of PDAC patients for whom pharmacotyping could play a role will likely rise shortly.

In the past, good outcomes have been achieved when cytotoxic medicines are used as a common strategy to boost treatment efficiency in late malignancies [17]. Despite this, it has been demonstrated that independent pharmacological activity accounts for a considerable portion of the benefits of combination therapy [18]. Specifically, we found that additive effects were estimated in two of the three PDOs. In contrast, synergistic effects were only seen in one PDO line when analysing the interaction between the medications constituting the Gem/Pac combination regimen. When dealing with genetically diverse cancers, administering many drugs simultaneously improves the likelihood of overcoming drug resistance. The increased risk of toxicities necessitating medication dose modifications, treatment switches, or chemotherapy discontinuation is a drawback of multi-drug regimens. Our PDAC cohort also saw a worse reaction to neoCTx after modifying the conventional therapy to reduce side effects. It is consistent with previous research in quasi-lung, breast, and liver malignancies [19, 20]. TRG distribution was different between individuals who completed their initial course of treatment and those who did not. The likelihood of achieving a R0 resection and the extent to which the tumour had spread into regional lymph nodes were both improved by a positive response to neoCTx.

Based on our retrospective study, 20 patients (27.8%) were given an adapted treatment regardless of whatever neoCTx they were given. Patients who did not have surgical resection following neoCTx or who moved between FOLFIRINOX or Gem/Pac to the corresponding other regimen were not included in the study. Therefore, the number of patients who adapted to their treatment plan is likely larger. Finding the components of multi-drug chemotherapy regimens responsible for cytotoxicity might lead to further therapeutic advancements. We have demonstrated that a dosage response curve is maintained when ineffective medications are removed from a poly-drug regime using PDOs as a surrogate for the patient's tumour. This was the case for both FOLFIRINOX and Gem/Pac, two clinically significant combination regimens. By removing one medication from a chemotherapy combination, the regimen may become less drug-specific while reducing the treatment's systemic adverse effects. The overall survival (OS) of patients with LA-PDAC may improve if CTx is optimised to enhance the proportion of patients who receive a full course of neoCTx, which has been shown to raise the rate of medium and excellent pathological response.

Due to a lower ECOG status following surgery, only half of the patients can get adjuvant treatment [21]. However, independent of nodal and resection margin status, survival is enhanced when PDAC patients can obtain adjuvant CTx [22,23]. Patients in the adjuvant context might benefit from the same strategy used to improve neoadjuvant poly-drug CTx by using fewer, more efficacious medications. Moreover, a larger dose of each may be administered by using a smaller number of medications, which might boost the response rate.

CONCLUSION

It is possible that drug testing on both CTx-nave PDAC PDOs and neoCTx PDOs will help in determining the optimal neoadjuvant and adjuvant treatment regimens. In order to increase the proportion of patients completing the course of neoadjuvant treatment, it may be beneficial to individualize poly-chemotherapy regimens by removing chemicals with low effectiveness.

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