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Patient-derived organoids for prediction of treatment response in oesophageal adenocarcinoma

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Introduction

Oesophageal cancer, comprising adenocarcinoma (OAC) and squamous cell carcinoma subtypes, accounts for approximately 450,000 deaths annually worldwide (1,2). For locally-advanced OAC, the current standard of care is neoadjuvant chemoradiation (CROSS) or perioperative chemotherapy (FLOT) (3,4). Although both confer a survival benefit, 40% of patients undergoing FLOT and 25% of patients undergoing CROSS demonstrate minimal pathological response, suggesting alternative regimens could be more effective (2-4). The superiority of either regimen is not clear, with a recent randomized controlled trial demonstrating clinical equipoise between perioperative chemotherapy and CROSS (5).

Next-generation sequencing of OAC revealed significant inter-tumour heterogeneity and few common mutations, without identifying mutations predicting susceptibility to neoadjuvant treatment (6). Predicting neoadjuvant response remains challenging in the absence of relevant biomarkers.

Patient-derived organoids (PDOs) are three-dimensional cultures derived from patient tumour cells that recapitulate the genetic and morphological characteristics of the primary tumour (7-10). The feasibility of establishing OAC PDOs from endoscopic biopsies has been demonstrated (6,7,10). PDOs are inexpensive, have a high success rate in establishing models, and allow efficient, high-throughput drug screening (8). PDOs have been evaluated for drug-screening in the post-induction and metastatic settings in other gastrointestinal malignancies (9-11). The use of PDOs in the treatment-naïve setting has not been studied.

As neoadjuvant therapy is fundamental to treating OAC, we investigated whether OAC PDOs reflect response to drugs used as neoadjuvant, perioperative, or palliative agents in corresponding patients, and whether these may form the basis for personalising therapies on both curative and palliative pathways.

Methods

The study was approved by the UHN Research Ethics Board (REB#36616 and CAPCR#14-8514.5). For consenting patients, tissue and blood samples were taken at initial endoscopy. Organoid generation and drug treatment protocols have been described previously and in supplemental methods, with treatment at passage 4 or greater (10).

All patients were discussed at a multidisciplinary tumour board. Neoadjuvant therapy included CROSS or FLOT regimens, with an institutional preference for CROSS for oesophageal and Siewert I/II cancers, and FLOT for Siewert III cancers (3,4). Pathological assessment was undertaken by specialized GI pathologists, with standardized synoptic reporting. Tumour regression grade (TRG) was per the College of American Pathologists guidelines (19). Neoadjuvant 'responder' phenotypes were patients with TRG 0-1. Patients with metastatic disease were treated with combination chemotherapy, as per the treating oncologist. Therapeutic response in the metastatic setting was based on imaging, using the RECIST criteria (12).

Analysis was conducted using the 'drc: Analysis of Dose-Response Curves' package for R and *jamovi* (version 1.6, retrieved from <https://www.jamovi.org>), with 'deathwatch' and 'jsurvival' modules. Separating organoids into 'responder' and 'non-responder' phenotypes was based on observed IC50 values referenced back to the fold-change in IC50 for organoids with corresponding *in vivo* outcomes. A minimum 3-fold difference in mean IC50 values was used to separate the cohorts.

Results

Twenty-three PDOs from patients undergoing neoadjuvant CROSS or FLOT were treated with an 11-point cisplatin dose protocol (Supplementary table 1, Figure 1a). There was a significant correlation between TRG and IC50 ($R_s=0.56$; $p=0.005$) and EC50 ($R_s=0.54$; $p=0.009$), with a significant difference in mean IC50 between 'responders' and 'non-responders' ($p=0.02$, Figure 1b). Twenty-four PDOs from patients undergoing neoadjuvant therapy were treated with the 11-point paclitaxel dose protocol (Figure 1c). There were no significant correlations between

TRG and IC50 or EC50 ($R_s=-0.35$ and -0.21 , $p=0.09$ and 0.35). There was no difference in mean IC50 when comparing 'responders' and 'non-responders' ($p=0.23$, Figure 1d). Mean IC50 and EC50 values (Figure 1e), show significant differences in concentration by TRG for cisplatin, but not paclitaxel.

PDOs from patients with synchronous metastases ($n=8$) receiving platinum-based or taxane-based chemotherapy were assessed (Supplemental Table 2). PDOs were generated from treatment-naïve tissue, allowing assessment of *in vitro* tumour response without the pressures of clonal selection, and acquired chemotherapy resistance. For cisplatin-treated PDOs, there was a correlation between IC50, EC50 and AUC and clinical response ($R_s=0.76$, $p=0.03$ in each case). Similarly, there was a difference in IC50 cisplatin concentration for 'responders' and 'non-responders' ($p=0.04$, figure 2a). For paclitaxel-treated organoids, there were correlations between IC50 and AUC and clinical response ($R_s=0.79$ and $p=0.017$). IC50 concentration differed between 'responders' and 'non-responders' for paclitaxel ($p=0.05$, Figure 2b).

The study was driven by a desire to better predict neoadjuvant treatment response in OAC to avoid unnecessary toxicity, facilitate early surgery or, ideally, personalise induction regimens. To identify potential alternative induction regimens, PDOs from patients on curative pathways were also treated with irinotecan and epirubicin ($n=18$ and $n=13$ respectively). As patients were not treated with these drugs, we split PDOs into 'responder' and 'non-responder' subsets based on mean IC50 difference between groups. For irinotecan, there was a 4.4-fold difference in IC50 between 'responder' and 'non-responder' PDOs (Figure 2c). Of these PDOs, there were 4 'responders' to irinotecan where the corresponding patients were unresponsive to CROSS (TRG 3). For the PDO 'non-responders' to irinotecan, one patient had TRG1 following CROSS. For epirubicin, there was a 3.1-fold difference in IC50 between 'responders' and 'non-responders' (Figure 2d). Of the 'responders', one patient had TRG3 following CROSS, while another had TRG3 following FLOT. For the 'non-responders', two patients had TRG1 following CROSS.

Discussion

Establishing reliable methods for predicting response to neoadjuvant therapy is a 'holy grail' of oesophageal cancer. The study showed a clear correlation between drug responses *in vitro* in OAC PDOs generated from naïve cancer tissue and tumour response in patients undergoing neoadjuvant therapy or first-line palliative chemotherapy. Although in other GI malignancies there may be a correlation between metastatic-derived PDO response to chemotherapy and patient response, these are typically recurrent cancers pre-treated with chemotherapy (8,9,11,13,14). Of interest, the differing responses to different agents suggest that the CROSS-responder cohort may not necessarily be the same as FLOT-responders and raise the possibility that high-throughput screening of PDOs may predict non-responders and suggest alternative chemotherapeutic regimens.

This study has limitations. The correlation between treatment and response in platinum-based chemotherapy is clear, but the relationship for taxanes less so. Although a well-established chemotherapeutic, the mechanism of action of taxanes is less well understood, and tumour microenvironment may play a greater role than previously thought, impacting *in vitro* response (15). Ooft et al. saw similar outcomes in metastatic colorectal cancer PDOs (11).

Although PDOs are grown in a three-dimensional matrix, it cannot fully mirror growth *in vivo*. Certain growth factors, fibroblasts and inflammatory cells are absent. Furthermore, there are no data on immunotherapy and PDO function. While T-cell co-culture has been established in other models (16), no functioning OAC PDO/T-cell co-culture has been reported. Neoadjuvant regimens consist of multiple drug agents acting synergistically. The present study examined single agents, but this may allow selection of bespoke regimens based on organoid response. There are suggestions that culture medium and environment may influence PDO responses to chemotherapies (17). The exact impact of culture conditions on therapeutic response is unclear for oesophageal PDOs and given disease heterogeneity, may be difficult to quantify. Indeed, OAC can also demonstrate significant intra-patient heterogeneity and metastatic sites or even regions

within the tumor may not respond identically to PDOs. Further study will be required to assess this. Nevertheless, PDOs may offer the ability to personalise treatments in OAC.

Author contributions

Jarlath C Bolger (Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing), Jonathan Allen (Data curation, Formal Analysis, Methodology, Software, Writing – review and editing), Nikolina Radulovich (Data curation, Formal analysis, Methodology, Resources, Writing – review and editing), Christine Ng (Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – review and editing), Mathieu Derouet (Conceptualization, Investigation, Methodology, Writing - review and editing), Premalatha Shathasivam (Data curation, Investigation, Methodology, Project Administration, Writing – review and editing), Gavin W Wilson (Data Curation, Formal Analysis, Methodology, Software, Supervision, Writing – editing and review), Ming-Sound Tsao (Investigation, Methodology, Resources, Writing – editing and review), Elena Elimova (Investigation, Methodology, Resources, Writing – editing and review), Gail E Darling (Conceptualization, Funding acquisition, Investigation, Supervision, Writing – editing and review), Jonathan C Yeung (Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Supervision, Validation, Writing – original draft, Writing – editing and review).

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Figure legends

Figure 1: A) normalized concentration curves for 11-point drug response curves for cisplatin-treated organoids from i) 'responder' and ii) 'non-responder' subsets based on TRG. Each line represents a single organoid. B) Box-plot demonstrating differences in mean IC50 between 'responder' and 'non-responder' subsets for cisplatin treated organoids. Median, 1st and 3rd quartile, and the maximum and minimum values are presented. C) normalized concentration curves for 11-point drug response curves for paclitaxel-treated organoids from i) 'responder' and ii) 'non-responder' subsets based on TRG. D) Box-plot demonstrating differences in mean IC50 between 'responder' and 'non-responder' subsets for paclitaxel treated organoids. E) Comparison of IC50 and EC50 for cisplatin and paclitaxel treated organoids. Values for IC50 and EC50 for platinum treated organoids are μM concentration of cisplatin (standard deviation). Values for IC50 and EC50 for taxane treated organoids are mM concentration of paclitaxel (standard deviation). Values are compared using one-way ANOVA (Kruskal-Wallis).

Figure 2: A) Boxplot demonstrating differences in mean IC50 for platinum-based chemotherapy between 'responder' and 'non-responder' subsets. B) Boxplot demonstrating differences in mean IC50 for taxane chemotherapy between 'responder' and 'non-responder' subsets. C i and ii) IC50 and EC50 of organoids treated with irinotecan, split into 'responders' and 'non-responders' based on a 4.4-fold difference in mean IC50 ($n=18$). IC50 and EC50 concentrations on y-axis are μM concentration. 4 'responders' had TRG3 following CROSS, and 1 'non-responder' had TRG1 following CROSS. D i and ii) IC50 and EC50 of organoids treated with epirubicin split into 'responders' and 'non-responders' based on a 3.1-fold difference in mean IC50 ($n=13$). IC50 and EC50 concentrations on y-axis are μM concentration. 2 'responders' had TRG3 following neoadjuvant therapy (1 CROSS and 1 FLOT) and 2 'non-responder' had TRG1 following CROSS.