Incorporating tumor genomic profiling and drug screening through circulating tumor cell-derived organoids into clinical practice for the management of Gastrointestinal Cancers

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Abstract

Purpose

Precision medicine aims to revolutionize healthcare by tailoring treatment regimens. The study aimed to integrate comprehensive tumor genomic profiling (CTGP) by targeted-gene panel sequencing and drug screening by circulating tumor cell-derived organoids (CTOs) into clinical practice for the treatment of gastrointestinal (GI) cancers.

Methods

Nine patients with various GI cancers underwent CTGP and CTO drug sensitivity testing. CTGP results guided targeted therapy and immunotherapy, while CTO drug sensitivity predicted response to chemotherapy and targeted agents. The drug recommendations from two platforms were correlated with the treatment response from the suggested medications retrospectively.

Results

Five patients received therapies aligned with CTGP, including HER2-targeted treatment, immunotherapy, and BRAF/MEK dual inhibition, showing positive responses. CTO drug sensitivity predicted progression under regorafenib (low potential benefit) and good response to chemotherapy with high potential benefit. The combination of CTGP and CTO drug sensitivity may exhibit significant correlation with clinical outcomes during treatment with candidate drugs, demonstrating a sensitivity of 79% and an accuracy of 75%. This encompasses various treatment modalities such as chemotherapy, targeted therapy, and immunotherapy.

Conclusion

The present investigation elucidated the integration of CTGP and CTO drug sensitivity screening into clinical practice in a complementary manner, showcasing a significant correlation between testing results and treatment response. Additional prospective evaluation of these two testing modalities in a large cohort is warranted to confirm whether the inclusion of CTO drug sensitivity screening confers enhanced survival benefits compared to utilizing CTGP alone.

Introduction

Precision medicine is transforming oncology by enabling individualized prevention, diagnosis, management, and patient care[1]. Understanding the genetic and molecular underpinnings of diseases is critical for delivering personalized care while accounting for tumor heterogeneity and immune microenvironment[2]. The goal of precision medicine is to develop and provide effective, tailored treatments that can improve outcomes and reduce healthcare costs[3].

Comprehensive tumor genomic profiling (CTGP) is among the most essential tools in precision medicine[3]. CTGPs, including next generation sequencing (NGS) as time- and cost-efficient technology that utilizes massively parallel, high-throughput systems to sequence an individual’s entire genome or specific DNA/RNA regions[4, 5]. Using patients’ genomic profiles, physicians can tailor treatment plans based on gene alternations and pathogenic pathways. By identifying shared driver mutations across different tumor types, clinicians can potentially treat these cancers with the same targeted therapies, regardless of their tissue of origin. For instance, high microsatellite instability (MSI), high tumor mutation burden (TMB), and BRAFV600E mutations are tumor-agnostic biomarkers indicating favorable responses to corresponding targeted agents or immunotherapy across various cancers[6].

Despite its utility, CTGP has limitations regarding clinical applications. According to OncoKB therapeutic level of evidence, only 41% of all samples exhibit alternations that can potentially be targeted by drugs and only 7.5% of all samples exhibit predictable responses to a specific treatment regimen[7]. Moreover, NGS provides little information on the therapeutic prediction of cytotoxic chemotherapy or candidate drug prioritization. Thus, complementary tools are needed to address limitations of NGS.
Circulating tumor cell (CTC)-derived organoids (CTOs), on the other hand, are three-dimensional in vitro cancer models. CTCs isolated from patient blood can generate CTOs recapitulating primary tumor architecture, functions, and features[8]. As surrogates for original tumors, CTOs can predict treatment responses and guide selection of targeted therapy, and chemotherapy[9-11]. Compared to traditional tissue biopsies, liquid biopsies to obtain CTCs enable minimal, continuous sampling, overcoming bias from tumor heterogeneity. CTOs from gastric and colon cancer patients exhibit high success culture rates (over 60%) while preserving primary tumor heterogeneity and characteristics, making them suitable for broad-spectrum drug screening[8, 12, 13].

Here, we investigated the associations between CTGP, CTO drug sensitivity, and outcomes in patient with advanced gastrointestinal cancers in real-world settings.

**Methods**

**Patient enrollment**

We conducted a retrospective analysis of CTGP and CTO drug sensitivity data for patients with advanced or metastatic gastrointestinal (GI) cancers who underwent both tests at Taipei Veterans General Hospital between June 2019 and December 2022. Our study aimed to investigate the correlations between the treatment suggestions guided by these tests and the corresponding clinical responses observed in these patients during the same time period.

**Comprehensive tumor genomic profiling**

CTGP was evaluated by NGS. NGS of biopsy samples or formalin-fixed, paraffin-embedded blocks utilized OncoDeep®, FoundationOne® CDx, or ACTOnco® platforms. These platforms detect alterations in 313-440 cancer-related genes, including single nucleotide variants, indels, copy number changes, fusions, MSI, and TMB. OncoDeep® also incorporates PD-L1 immunohistochemistry (clone 22C3).

**Circulating tumor cell enrichment and organoids expansion**

We utilized the EVA Select system for CTC enrichment and organoid expansion. Blood samples were collected using K2EDTA tubes (BD Bioscience), and peripheral blood mononuclear cells (PBMCs) containing CTCs were isolated by Ficoll-Paque centrifugation. The PBMCs were then subjected to further enrichment using a CTC enrichment cocktail (StemCell Technologies) as previously described[14, 15]. The enriched CTCs were seeded on binary colloidal crystal substrates coated with silica and polymethyl methacrylate in a platelet lysate-based medium (DMEM/F12 supplemented with platelet lysate and B27; ThermoFisher). The culture medium was further supplemented with EGF, FGF, and PDGF[16] and replenished every 4 days. Organoid growth was closely monitored using microscopy, and the presence of CTCs within the organoids was confirmed by immunofluorescence staining for pan-cytokeratin and CD45. Detailed information regarding the CTO drug screening results for each patient can be found in Supplementary Table 1.

**CTO Drug screening**

During the study period, the selection of drugs for testing in each patient’s CTOs was determined based on the treating physician’s decisions, which were informed by the patient’s CTGP report results and the physician’s clinical experience at the time of treatment. The physicians had selected targeted therapies considered potentially effective based on the genomic findings, as well as chemotherapies supported by literature evidence, even if they were not included in the standard National Comprehensive Cancer Network guidelines.

CTOs were resuspended in culture medium and aliquoted into 96-well plates. Physician-selected drugs were tested. Viable cells were quantified by luminometric measurement of cytosolic ATP (Promega). Each aliquot had 2,000 relative light units for drug sensitivity assays, performed in triplicate. After 6 days of drugs exposure, viable cells were quantified by ATP levels.
Results were normalized to untreated controls and analyzed against the EVA Select database containing drug response distributions and outcome data to automatically determine the thresholds for potential clinical benefit of each drug. The results were categorized into three levels of potential benefit: low, moderate, and high, which were represented by red, yellow, and green colors, respectively.

Assessments of treatment response

Treatment responses were evaluated every 2-3 months by CT or MRI and classified by RECIST 1.1 as complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). Progression-free survival was time from diagnosis/treatment initiation/recurrence to progression or end of follow-up. Overall survival was the date from diagnosis to death or the end of follow-up (December 31, 2022).

The timeline from diagnosis to treatment guidance and assessment of correlation

Fig. 1 delineates an intricate process for determining cancer treatment within our study, commencing with tumor diagnosis. Following the procurement of a pathological specimen, the preliminary phase involves executing comprehensive genomic profiling, which is accomplished within a span of 2-3 weeks, alongside the cultivation of CTCs over a duration of 3 weeks.

The knowledge acquired from CTGP is subsequently employed to determine the next line clinical treatment and the drugs used to test the CTCs. The first-line treatment response was also used to guide the drug chosen to test on the CTCs. Drugs sensitivity assay on CTOs, which took about another 2 to 3 weeks after the CTGPs results, guided the next-line treatment. CTGP and treatment responses of the first 2 lines of treatments also influence the next line treatment.

Within the scope of our investigation, we conducted a retrospective analysis to assess the concordance between the chosen clinical regimens and the results derived from both CTGP and the CTO drug sensitivity tests (Supplement table 2). Prediction accuracy was classified in the following situations: if a drug suggested by genomic profiling or tested to have high potential benefit was used clinically and the patient had a positive response, or if a drug was shown to have low potential benefit, was used clinically, and the patient experienced tumor progression.

Results

Patient characteristics, genomic data, and circulating tumor cell drug sensitivity

The cohort included 5 men and 4 women with median age 54 years. There were 4 gastric cancers, 4 colorectal cancers, and 1 duodenal cancer. The cornerstone of the treatment strategy was the first-line therapy after enrollment, which included a combination of targeted agents like trastuzumab and immunotherapies such as nivolumab, alongside chemotherapeutic regimens comprising 5-fluorouracil (5-FU), oxaliplatin, and the like. For 7 patients, there is a transition to second-line treatments, which are tailored based on their disease's response to initial therapies. These treatments range from the immunotherapy nivolumab to kinase inhibitors and chemotherapy drugs such as docetaxel and gemcitabine. Table 1 shows detailed characteristics, cancer types, stages, prior treatments, and first and second-line treatments after enrollment.

Table 1. Patients characteristics, cancer types, stages, prior treatments, and first and second line treatments and enrollment
<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Gender</th>
<th>Cancer Type</th>
<th>Stage</th>
<th>Metastasis</th>
<th>Prior Treatment/Surgery</th>
<th>1st-line Treatment</th>
<th>2nd-line Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39/M</td>
<td>Gastric adenoCA</td>
<td>IV</td>
<td>cT3N3M1</td>
<td>N/A</td>
<td>Trastuzumab, Pembrolizumab</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>63/M</td>
<td>Gastric adenoCA</td>
<td>IV</td>
<td>pT4N3M1</td>
<td>Total gastrectomy, lymph node dissection, Roux-en-Y esophagojejun al anastomosis, seeding tumor excision</td>
<td>Oxaliplatin, TS-1</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>70/M</td>
<td>Gastric adenosquamous CA</td>
<td>IV</td>
<td>cT4bM3M1</td>
<td>N/A</td>
<td>Nivolumab</td>
<td>Docetaxel</td>
</tr>
<tr>
<td>4</td>
<td>76/M</td>
<td>Gastric adenoCA</td>
<td>IV</td>
<td>cT4bN1M1</td>
<td>N/A</td>
<td>5-FU, Leucovorin, Oxaliplatin</td>
<td>Ramucirumab, 5-FU, Leucovorin, Irinotecan</td>
</tr>
<tr>
<td>5</td>
<td>69/F</td>
<td>Duodenal adenoCA</td>
<td>IV</td>
<td>pT3N0M1</td>
<td>Gastrojejunostomy and cholejejunostomy</td>
<td>Bevacizumab, 5FU, Leucovorin, Oxaliplatin</td>
<td>Gemcitabine, Cisplatin</td>
</tr>
<tr>
<td>6</td>
<td>47/F</td>
<td>Colon adenoCA</td>
<td>IV</td>
<td>cT4N2M1</td>
<td>N/A</td>
<td>Bevacizumab, 5-FU, Leucovorin, Oxaliplatin, Irinotecan</td>
<td>Cetuximab, Dabrafenib, Trametinib</td>
</tr>
<tr>
<td>7</td>
<td>54/F</td>
<td>Colon adenoCA</td>
<td>IV</td>
<td>cT3N1M1</td>
<td>N/A</td>
<td>Bevacizumab, 5-FU, Leucovorin, Oxaliplatin, Irinotecan</td>
<td>Cetuximab, Dabrafenib, Trametinib</td>
</tr>
<tr>
<td>8</td>
<td>36/M</td>
<td>Colon adenoCA</td>
<td>IV</td>
<td>pT4aN2bM1</td>
<td>Etoposide, cisplatin, cetuximab, FOLFOX, FOLFIRI, nivolumab, ipilimumab</td>
<td>Pembrolizumab, Lenvatinib, Paclitaxel</td>
<td>Nivolumab, Regorafenib</td>
</tr>
<tr>
<td>9</td>
<td>43/F</td>
<td>Rectal adenoCA</td>
<td>I</td>
<td>pT2N0M0</td>
<td>Laparoscopic low-anterior resection, laparotomy with pelvic lymphadenectomy, radiotherapy, FOLFOX, cetuximab, FOLFIRI</td>
<td>Bevacizumab, Trifluoridine, Tipiracil</td>
<td>Nivolumab, Regorafenib</td>
</tr>
</tbody>
</table>

CA: carcinoma; N/A: not available; FOLFOX: oxaliplatin, 5-FU and folina; FOLFIRI: irinotecan, 5-FU and folina; FOLFOXIRI: oxaliplatin, irinotecan, 5-FU and folina.

CTGP results and drug suggestions for each patient was displayed in Table 2. Patients with high TMB, a combined positive score (CPS) combined positive score > 1, or MSI-H were suggested to use immune checkpoint inhibitors, for instance nivolumab and pembrolizumab. A high copy number of HER2 may be recommended trastuzumab, while BRAF mutations may be advised to consider treatment like BRAF inhibitors such as dabrafenib. We also notes cases where no specific drugs are suggested, underscoring the challenges in finding suitable targeted therapies for certain genetic profiles.
Table 2. Comprehensive tumor genomic profiling results and drug suggestions for each patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>Platform</th>
<th>CTGP</th>
<th>Drug suggestions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OncoDeep</td>
<td>MSS, TMB-L, HER2 amp (CN: 4), TP53 mut, PDL1 CPS 10</td>
<td>Trastuzumab, Immune checkpoint inhibitor</td>
</tr>
<tr>
<td>2</td>
<td>OncoDeep</td>
<td>MSS, TMB-L, ARID1A mut, KMT2D mut, PDL1 CPS 8</td>
<td>Docetaxel, Paclitaxel</td>
</tr>
<tr>
<td>3</td>
<td>ACTOnco</td>
<td>MSI-H, TMB-H (286 muts/Mb), ARID1A, FAT mut, TSC2 heterozygous del</td>
<td>Dasatinib, Immune checkpoint inhibitor, PARP inhibitor</td>
</tr>
<tr>
<td>4</td>
<td>Foundation</td>
<td>MSS, TMB-L, CDKN2A, KRAS G12D, SMAD4, TP53 mut, PDL1 CPS 0</td>
<td>CDK4/6 inhibitor</td>
</tr>
<tr>
<td>5</td>
<td>ACTOnco</td>
<td>MSS, TMB-L, KRAS G12V, TP53 mut, MLH1 heterozygous del</td>
<td>Binimetinib, Cobimetinib, Sorafenib, Trametinib</td>
</tr>
<tr>
<td>6</td>
<td>ACTOnco</td>
<td>MSS, TMB-L, TP53 mut, BRAF V600E mut, CHEK2, FBXW7, NF2, PTCH1, PTEN, TSC1 heterogenous del</td>
<td>BRAF inhibitor, Cetuximab, MEK inhibitor</td>
</tr>
<tr>
<td>7</td>
<td>ACTOnco</td>
<td>MSS, BRAF V600E mut</td>
<td>BRAF inhibitor, Cetuximab, MEK inhibitor</td>
</tr>
<tr>
<td>8</td>
<td>ACTOnco</td>
<td>MSS, TMB-L, CDKN2A heterozygous del, ATM, BRCA1, BRCA2, CDKN2A, MRE11, NF2, RAD50, RAD51C, TSC2 heterozygous del, KRAS amp (CN 35)</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>Oncomine</td>
<td>MSS, TMB-L, FGFR4, FBXW7 mut</td>
<td>N/A</td>
</tr>
</tbody>
</table>

CTGP: comprehensive tumor genomic profiling; CN: copy number; MSS: microsatellite stable; TMB-H: high tumor mutation burden; TMB-L: low tumor mutation burden; N/A: not available; PDL1: programmed death-ligand 1

CTC expansion

CTC presence was confirmed by positive pan-cytokeratin and negative CD45 staining. DAPI showed viable gastrointestinal adenocarcinoma cells in organoids. CTO expansion was achieved within 4 weeks on average (100% success). Fig. 2 shows immunostaining results for selected gastric, duodenal and colorectal cancer patients.

Association between genomic data, drug sensitivity, and treatment response

Retrospective analysis showed that treatment response in six patients aligned with CTGp outcomes (Table 2 and 3). Of them, two achieved responses to immune checkpoint inhibitors linked to PD-L1 expression, TMB, or MSI; trastuzumab was effective in a patient with tumors harboring HER2 amplification; and two with the \textit{BRAF V600E} mutation benefited from dual anti-BRAF and MEK inhibitors. Two colorectal cancer patients with \textit{BRAF V600E} mutation achieved disease control with cetuximab, dabrafenib, and trametinib combination, supported by CTO drug screening (Table 3). The detailed CTO drug screening results and the correlation between applied chemotherapy with response and CTO drug sensitivity screening results are provided in Supplementary Table 1 and Supplementary Table 2, respectively.

Table 3. The correlation between applied treatment (targeted therapy or immunotherapy) with response and testing results.
<table>
<thead>
<tr>
<th>Patient</th>
<th>Targeted Therapy/immunotherapy</th>
<th>CTGP matched</th>
<th>CTO Drug Sensitivity Screening Results</th>
<th>Treatment response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Trastuzumab</td>
<td>Yes</td>
<td>LPB</td>
<td>PR</td>
</tr>
<tr>
<td>3</td>
<td>Nivolumab*</td>
<td>Yes</td>
<td>-</td>
<td>CR</td>
</tr>
<tr>
<td>6</td>
<td>Dabrafenib</td>
<td>Yes</td>
<td>HPB</td>
<td>PR</td>
</tr>
<tr>
<td></td>
<td>Trametinib</td>
<td>Yes</td>
<td>HPB</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Dabrafenib, Trametinib</td>
<td>Yes</td>
<td>LPB</td>
<td>SD</td>
</tr>
<tr>
<td>8</td>
<td>Regorafenib</td>
<td>-</td>
<td>LPB</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>Nivolumab</td>
<td>No</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Regorafenib</td>
<td>-</td>
<td>LPB</td>
<td>PD</td>
</tr>
<tr>
<td></td>
<td>Nivolumab</td>
<td>No</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*: not applicable in CTO Drug Sensitivity Screening

*: no specific genetic markers from

CTGP: comprehensive tumor genomic profiling; CTO: circulating tumor cell-derived organoids; LPB: low potential benefit; HPB: high potential benefit; CR: complete response; PR: partial response; SD: stable disease; PD: progressive disease.

For CTC-drug screening, the overall prediction combines both targeted and chemotherapy drug predictions, with an accuracy of 75%, a sensitivity of 78%, and an F1 score of 0.78 (Table 4). The second-line of 5-FU treatment for one patient was omitted from the analysis because the adaptive efficacy was observed.

Table 4. The overall prediction of testing results from CTGP plus CTO drug sensitivity screening to clinical response

<table>
<thead>
<tr>
<th>overall prediction (CTGP plus CTO drug sensitivity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Response (+)</td>
</tr>
<tr>
<td>effectiveness (+)</td>
</tr>
<tr>
<td>effectiveness (-)</td>
</tr>
<tr>
<td>Accuracy</td>
</tr>
<tr>
<td>Sensitivity</td>
</tr>
<tr>
<td>F1 score</td>
</tr>
</tbody>
</table>

Clinical response (+): partial response or stable disease

Clinical response (-): disease progression

Case description:

Below we present illustrative case histories describing our approach in all nine patients with gastrointestinal cancers undergoing both genomic profiling and CTO drug sensitivity analysis. The correlation between actual patient treatments used and the CTGP and CTO drug sensitivity results is comprehensively outlined in Supplementary Table 3.

Case 1
A 35-year-old male presented with stage IV esophagogastric adenocarcinoma with lymph node metastases (cT3N3M1). Tumor staining showed borderline human epidermal growth factor receptor 2 (HER2) scores (2+) with positive fluorescence in situ hybridization results. OncoDeep® profiling revealed microsatellite stability (MSS), low TMB, TP53 mutation, and a PDL1 CPS of 10. CTO drug sensitivity screening indicated moderate potential benefit from 5-FU and irinotecan, but low potential benefit from trastuzumab and oxaliplatin. Before obtaining these results, we implemented combination therapy with pembrolizumab, trastuzumab, oxaliplatin and TS-1. A near complete response was achieved after 12 courses of treatment, prompting salvage subtotal gastrectomy. Maintenance therapy continued with pembrolizumab, trastuzumab and TS-1.

Case 2

A 63-year-old man presented with stage IV gastroesophageal junction adenocarcinoma with peritoneal seeding (pT4N3M1). Prior to systemic treatment, he underwent total gastrectomy, lymph node dissection, Roux-en-Y esophagojejunal anastomosis, and excision of seeded tumors. OncoDeep® profiling revealed MSS, low TMB, and ARID1A and KMT2D mutations, with a PD-L1 CPS of 8. CTO drug sensitivity indicated high potential benefit from 5-fluorouracil, cabozantinib, crizotinib, docetaxel, irinotecan, oxaliplatin, and regorafenib. We prescribed 6 months of TS-1 and oxaliplatin, followed by maintenance with TS-1, leading to a PR.

Case 3

A 70-year-old man presented with stage IV gastric adenosquamous carcinoma with mesocolon, pancreatic and para-aortic lymph node involvement (cT4bM3M1). ACTOnco® profiling revealed MSI-H, a high TMB of 28.6 mutations/megabase (muts/Mb), and mutations in ARID1A, FAT1, and heterozygous deletion of TSC2. Immunotherapy, poly ADP ribose polymerase (PARP) inhibitors, dasatinib, and everolimus were suggested. CTO drug sensitivity indicated potential benefits from docetaxel, everolimus, gemcitabine, and regorafenib. Due to his age, the patient received biweekly nivolumab monotherapy, achieving near CR for 20.4 months before progression with new peritoneal seeding. Subsequent docetaxel treatment led to a response of PR.

Case 4

A 76-year-old male presented with stage IV gastric adenocarcinoma with pancreatic invasion and mesenteric seeding (cT4bN1M1). First-line treatment with 5-FU, leucovorin and oxaliplatin (FOLFOX) initially achieved PR but progressed after 3 months. Second-line ramucirumab, 5-FU, leucovorin and irinotecan (FOLFIRI) was given after PD. FoundationOneCDx® profiling revealed MSS, low TMB, mutations in KRAS G12D, SMAD4, TP53, and CDKN2A deletion. CTO drug sensitivity indicated high potential benefit from 5-FU, cabozantinib, docetaxel, regorafenib, and everolimus, but only moderate benefit from irinotecan, paclitaxel, lenvatinib, and palbociclib. The patient did benefit from 5-FU and had a PR after FOLFOX. He eventually died of peritonitis.

Case 5

A 69-year-old woman with stage IV duodenal adenocarcinoma (pT3N0M0, peritoneal seeding) underwent surgeries and initial bevacizumab and FOLFOX treatment, which led to PD after four months. ACTOnco® findings of MSS, low TMB, KRAS G12V and TP53 mutation, and MLH1 heterozygous deletion prompted recommendations for sorafenib, BRAF, and PARP inhibitors. Her drug sensitivity profile suggested high benefits from cobimetinib and gemcitabine, with moderate benefits from other drugs. Subsequent gemcitabine and cisplatin treatment achieved SD. However, treatment was halted due to severe side effects, including neutropenic fever and septic shock.

Case 6

A 47-year-old female patient presented with stage IV poorly differentiated transverse colon adenocarcinoma with liver, bone, and lung metastases (cT4N2M1). IHC staining revealed BRAF V600E mutation, wild-type RAS genes, and epidermal growth factor receptor mutation. First-line bevacizumab, 5-FU, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) achieved PR for 3
months before progression with new lung metastases. However, the patient subsequently had disease progression with lung metastases. ACTOnco® profiling showed MSS; a low TMB; mutations in **BRAF V600E, CHEK2, FBXW7, NF2, PTCH1, PTEN,** and **TSC1** heterozygous deletions, indicating potential response to combined cetuximab, BRAF inhibitors, MEK inhibitors, and mTOR inhibitors. CTO drug sensitivity suggested high potential benefits from everolimus, regorafenib, trametinib, and vemurafenib, and moderate benefits from cabozantinib, cobimetinib, crizotinib, lenvatinib, olaparib, and palbociclib. Based on genomic and drug sensitivity data, cetuximab, dabrafenib, and trametinib were prescribed, initially achieving PR but progressing aggressively after 3 months with bowel obstruction, massive malignant ascites, and pleural effusion.

Case 7

A 54-year-old female patient presented with stage IV sigmoid adenocarcinoma with ascites, peritoneal seeding, and lung metastases (cT3N1M1). She received 7 cycles of bevacizumab and FOLFOXIRI, but disease worsened. The regimen was changed to cetuximab, dabrafenib, and trametinib, achieving SD. Further IHC revealed MSS, **BRAF V600E** mutation, wild-type **RAS**, and epidermal growth factor receptor mutation. CTO drug sensitivity profile revealed high potential benefits from erdafitinib and trametinib; moderate benefits from cobimetinib, dabrafenib, everolimus, lenvatinib, olaparib, regorafenib, and vemurafenib; and low potential benefits from palbociclib. However, lung and peritoneal metastases progressed after 7 months. Regorafenib was prescribed but the patient refused further treatment.

Case 8

A 34-year-old male patient presented with stage IV sigmoid adenoneuroendocrine carcinoma with liver, spleen, bone, and lung metastases (pT4aN2bM1). He received etoposide/cisplatin, cetuximab/FOLFOXIRI, and nivolumab/ipilimumab, but disease worsened. ACTOnco® revealed MSS, low TMB, **SMAD4** homozygous deletion, and heterozygous deletions in **ATM, BRCA1, BRCA2, CDKN2A, MRE11, NF2, RAD50, RAD51C,** and **TSC2** heterozygous deletion, with **KRAS** amplification (copy number 35), suggesting potential response to CDK4/6 inhibitors, mTOR inhibitors, PARP inhibitors, and sorafenib. CTO drug sensitivity indicated no high benefit drugs, moderate benefit from cobimetinib, dacarbazine, everolimus, niraparib, olaparib, paclitaxel, palbociclib, regorafenib, and vemurafenib, low potential benefit from topotecan. Pembrolizumab, lenvatinib, and paclitaxel were prescribed, achieving PR before progressing with increased peritoneal seeding and bowel obstruction. Nivolumab and regorafenib were given but withdrawn after gastric ulcer perforation, requiring emergency laparotomy. With persisting sepsis, the patient opted for palliative care thereafter.

Case 9

A 43-year-old female presented with stage I rectal adenocarcinoma (pT2N0M0) and underwent laparoscopic low-anterior resection. Two years later, she developed lymph node recurrence and peritoneal seeding. She had received radiotherapy, FOLFOX, cetuximab/FOLFIRI and laparotomy with pelvic lymphadenectomy at another hospital. Second and subsequent lines of bevacizumab/FOLFOX, bevacizumab/lonsurf, and nivolumab/regorafenib were prescribed but disease progressed. Oncomine® revealed MSS, low TMB, and mutations in **FGFR4** and **FBXW7**. CTO drug sensitivity indicated no high benefit drugs, moderate benefit from alpelisib, erdafitinib, everolimus, lenvatinib, doxorubicin, and gemcitabine, and low benefit from abemaciclib, lapatinib, palbociclib, palbociclib, regorafenib, ribociclib, trastuzumab, and T-DM1, suggesting potential resistance to previous therapies.

**Discussion**

In our study, five out of nine patients receiving CTGP-guided immunotherapy or targeted therapy achieved disease control. Six patients benefited from chemotherapy or targeted therapy based on CTO drug screenings. However, three patients subjected to chemotherapies, later found to have low potential benefits via CTC screening, and experienced poor disease control. Two individuals, for whom CTGP and CTC screenings suggested no effective treatments, experienced disease progression. The integration of CTGP and CTO drug screening yielded clinical benefits for all nine patients undergoing both evaluations. This study provided the insights of how to interpret these testing results and correlate their predictive drugs with treatment response...
in real-world practice. To combine NGS and CTC screening may overcome individual limitations of each test, enhancing treatment precision and further improving patients’ outcomes.

Patients receiving regimens supported by both NGS and CTO screening exhibited positive clinical responses, whereas those prescribed ineffective regimens per both platforms progressed. Thus, integrating genomic profiles and CTO drug sensitivity complements limitations of each platform. NGS effectively predicts targeted agents and immunotherapy but has limited chemotherapy guidance or drug prioritization. CTO screening assesses chemotherapy/targeted therapy responses yet cannot predict immunotherapy outcomes. Concurrent use of both tools shows promise in optimizing treatment effectiveness and survival.

However, CTOs have limitations. They are more expensive and labor-intensive than 2D cultures. High-throughput screening is still an emerging technique. Although CTOs preserve genomic features of original tumors, they cannot replicate the tumor microenvironment comprising extracellular matrix and stromal cells that influence treatment sensitivity [17-19]. Co-culturing CTOs with tumor-associated fibroblasts may enable more accurate representation of in vivo microenvironments and immune responses[20].

In case 1, genomic profiling revealed a PD-L1 CPS of 10 and HER2 amplification, strongly suggesting efficacy of anti-PD-1 inhibitors and HER2-targeted agents. However, circulating tumor cell drug sensitivity test showed resistance to trastuzumab and T-DM1. Clinically, near complete response was achieved with combination pembrolizumab, trastuzumab, oxaliplatin, and TS-1. The patient remains stable on maintenance pembrolizumab/trastuzumab/TS-1. Trastuzumab is a humanized monoclonal antibody targeting HER2, inducing antibody-dependent cell-mediated cytotoxicity[21]. The absence of immune cell infiltration in organoid models could significantly hinder their ability to forecast the efficacy of monoclonal antibodies and immune checkpoint inhibitors. This limitation may impact the precision of treatment predictions within these experimental frameworks.

In case 9, CTO drug sensitivity profile predicted lenvatinib inefficacy, while the genomic data indicated FGFR4 alternation. Although no successful FGFR4-specific targeted therapies exist, several multi-kinase inhibitors can target FGFR pathways, including regorafenib, lenvatinib, and pazopanib. Lenvatinib inhibits FGFR 1–4, vascular endothelial growth factor receptor 1–3, platelet-derived growth factor receptor-alpha, RET, and KIT, suppressing angiogenesis and tumor growth[22]. The discrepancy of lenvatinib prediction suggested the organoid model cannot replicate angiogenesis, hindering its predictive ability with respect to antiangiogenic drugs.

Several emerging technologies have been developed to address the shortage of microenvironments for organoid platforms. One such technology is the “organ-on-a-chip” microengineering platform, which involves coculturing endothelial cells with fibroblasts in a hydrogel. Compared with traditional organoid models, this method leads to the formation of blood vessels in the central chamber and allows for accurate representations of cell–cell and cell–environment interactions, functional features, and gene expression in original tissues[23-25]. Neal et al. developed an air–liquid surface method in which original tumor cells are cocultured with tumor epithelia, tumor-infiltrating T and B lymphocytes, NK cells, and macrophages. Single droplet analysis revealed that this model preserves the gene expression and immune spectrum of the original tumor, thereby enhancing the accuracy of predictions regarding patient response to personalized immune checkpoint therapy[26]. The previous methods attempted to simulate and optimize the tumor immune microenvironment through various modifications.

Combining automated and high-throughput organoid culture with drug sensitivity assays may be a promising strategy for enhancing drug screening, clinical decision-making, and treatment response assessment. Khoo et al. introduced a novel microfluidic method for generating CTC clusters from the blood samples of patients; this method does not require the use of growth factor supplements or previous CTC enhancement, and it is associated with a considerably short derivation time and a high success rate[27]. Boussaad et al. evaluated an integrated and comprehensive platform that automates all steps required for a three-dimensional midbrain organoid culture[28]. Boehnke et al. successfully developed a 384-well three-dimensional automated liquid-handling organoid culture platform that embeds colon cancer cells in specific extracellular matrices; the researchers further validated its accuracy by testing samples from patients with colorectal cancer[29]. Building upon the discussions, the introduction of refined drug screening methods utilizing CTC-derived organoids, in conjunction with a
combined analysis of CTO drug screening and CTGP, may modestly contribute to the enhancement of treatment decision-
making and potentially better prognoses in patients facing complex and advanced-stage cancers..

Our study has several limitations that should be acknowledged. Firstly, the retrospective nature of the study may introduce potential biases in patient selection and data analysis. However, we have attempted to mitigate this by carefully reviewing and verifying all relevant data to ensure accuracy and reliability. Second, the small cohort size might limit the generalizability of our findings. Nevertheless, our study serves as a valuable proof-of-concept that highlights the potential of integrating CTGP and CTO drug sensitivity profiling in guiding treatment decisions for advanced GI cancers. Thirdly, the absence of sequential pre- and post-treatment comparative analysis of CTGP and CTO drug sensitivity changes may hinder our ability to fully capture the dynamic nature of tumor evolution and treatment response. However, our study provides a snapshot of the correlation between genomic profiles, CTO drug sensitivity, and clinical outcomes at a specific time point, which still offers valuable insights into the potential utility of this integrated approach. Additionally, while deviations from genomic/CTC-informed regimens may have occurred in some cases, we have thoroughly documented and analyzed these instances to understand their impact on treatment outcomes. Lastly, although our study focuses exclusively on GI malignancies from a single institution, the principles and methodologies employed in our research can be readily adapted and applied to other cancer types and healthcare settings.

Conclusion

Our study is among the first to explore the efficacy and identification of potential therapeutic agents by integrating the analysis of CTGP and CTOs drug sensitivity. The concurrent use of CTOs and CTGP demonstrates a significant correlation with clinical response, underscoring the promising role of this combined approach. Further prospective evaluation of these two testing modalities in a larger patient cohort is warranted to validate their clinical utility.

Declarations

Ethical approval

The Taipei Veterans General Hospital’s institutional review board granted approval, with the study conducted in full alignment with the Declaration of Helsinki’s ethical principles.

Patient consent

All participating patients provided signed consent for the use of their clinical data and images in this manuscript, ensuring adherence to ethical guidelines.

Availability of data and materials

Data supporting this study’s findings can be requested from the corresponding author, Nai-Jung, Chiang. To protect our participants’ privacy, the data are not openly available, ensuring confidentiality and adherence to ethical standards.

Competing interests

The authors declare no conflicts of interest.

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Authors’ contribution
Conception and study design: NJC, YPH, and MHC. Patient enrollment: YPH, MHC, and SCC. Data collection and manuscript preparation: WCW, YPH and NJC. Patient care, treatment administration and multidisciplinary discussion: WCW, CFH, MHC, SCC, NHC and YPH.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author, NJC. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

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References


Figures
Figure 1

Workflow from enrollment, tumor diagnosis, to comprehensive tumor genetic profiling, circulating tumor cell derived organoid drug sensitivity test, treatment recommendations, and confirmation of clinical utility and correlation.
Figure 2

Results of immunofluorescence staining performed to confirm the presence of circulating tumor cells and viable gastrointestinal adenocarcinoma cells in organoids. These organoids were established using specimens collected from patients (one with gastric cancer, one with duodenal cancer, and one with colorectal cancer). Scale bar: 20 μm. Abbreviations: GC, gastric cancer; DC, duodenal cancer; CRC, colorectal cancer; PanCK, pan-cytokeratin; DAPI, 4′,6-diamidino-2-phenylindole.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementTableCTGPandCTCandGIcancers20240422.docx