Medium-throughput Drug Screening of Patient-derived Organoids from Colorectal Peritoneal Metastases to Direct Personalized Therapy


ABSTRACT

Purpose: Patients with colorectal cancer with peritoneal metastases (CRPMs) have limited treatment options and the lowest colorectal cancer survival rates. We aimed to determine whether organoid testing could help guide precision treatment for patients with CRPMs, as the clinical utility of prospective, functional drug screening including nonstandard agents is unknown.

Experimental Design: CRPM organoids (peritonoids) isolated from patients underwent parallel next-generation sequencing and medium-throughput drug panel testing to identify specific drug sensitivities for each patient. We measured the utility of such a testing including: success of peritonoid generation, time to cultivate peritonoids, reproducibility of the medium-throughput drug testing, and documented changes to clinical therapy as a result of the testing.

Results: Peritonoids were successfully generated and validated from 68% (19/28) of patients undergoing standard care. Genomic and drug profiling was completed within 8 weeks and a formal report ranking drug sensitivities was provided to the medical oncology team upon failure of standard care treatment. This resulted in a treatment change for two patients, one of whom had a partial response despite previously progressing on multiple rounds of standard care chemotherapy. The barrier to implementing this technology in Australia is the need for drug access and funding for off-label indications.

Conclusions: Our approach is feasible, reproducible, and can guide novel therapeutic choices in this poor prognosis cohort, where new treatment options are urgently needed. This platform is relevant to many solid organ malignancies.

Introduction

Colorectal cancer is the second leading cause of cancer-related mortality worldwide (1). The peritoneum is a common site for metastases (2), but confers the worst survival rates among patients with metastatic colorectal cancer (mCRC). Patients with unresectable colorectal peritoneal metastases (CRPMs) have a median survival of 12–16 months and 5-year survival of less than 5% (3, 4). CRPMs respond poorly to modern chemotherapy regimens compared with other sites of colorectal cancer metastasis (5). While approximately 60% of colorectal cancer liver metastases respond to modern systemic therapy, less than one-third of CRPMs demonstrate any response (6, 7). With only a handful of systemic therapy options available, patients with CRPMs rapidly exhaust treatment options.

The advent of cytoreductive surgery with hyperthermic intraperitoneal chemotherapy (HIPEC) has offered carefully selected patients with CRPMs a favorable 27–41 months median survival, with a 23%–42% 5-year survival (8, 9). However, despite successful cytoreductive surgery and HIPEC, up to 80% of patients recur within 2 years (10–12). Treatment after disease recurrence centers on systemic chemotherapy, but has shown limited efficacy; Novel drug delivery methods such as pressurized aerosolized chemotherapy show promise as a feasible palliative surgical option in patients with recurrent or unresectable CRPMs, but need further evaluation in clinical trials (13, 14). There is an urgent need to explore new modalities and means of selecting treatment for patients with CRPMs.

Precision medicine is both a current challenge and opportunity facing the oncology community. The fundamental question becomes how to rationally assign drug treatments, not by cancer site, or even pathologic subtype, but based on the unique molecular biology of each cancer and each patient. Genomics has successfully guided choice of targeted therapies across multiple cancer types (15–17), but clinical outcomes led purely by cancer genomics have been disappointing (18). Previous analyses by targeted next-generation sequencing (NSG)
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Translational Relevance

Recent studies have indicated that patient-derived organoid cultures can retrospectively predict treatment responses to standard care chemotherapies for various solid tumors such as gastric, colorectal, bladder, ovarian, and pancreatic cancers. Here, we show that genomics and medium-throughput drug screening of patient-derived organoids from colorectal peritoneal metastases (CRPMs) can be used to prospectively guide innovative (off-label) therapy choices for this poor prognosis cohort. This study paves the way for a future phase II clinical trial to evaluate the utility of this organoid-based platform to deliver personalized therapy in patients with CRPMs, particularly in situations where standard care has been exhausted.

Materials and Methods

Study design

APOLLO is a multicenter Australian study designed to measure the utility of an organoid-based platform integrating genomics and functional testing to guide treatment choice for patients with CRPMs that have exhausted standard care. The objectives were to assess success rate of peritonoid generation; time to cultivate peritonoids; reproducibility of high-throughput drug testing; and document changes to, and utility of an organoid-based platform integrating genomics and functional testing to guide treatment choice for patients with CRPMs that have exhausted standard care.

All participants gave informed written consent and research was conducted in accordance with the Declaration of Helsinki, the NHMRC Statement on Ethical Conduct in Human Research, and institutional approvals (PMCC 15/76 and HREC/16/SAC/344 SSA/17/TQE/291). Patients with microsatellite stable (MSS) CRPMs under-}

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NextSeq 500 (Illumina). Analysis of NGS data can be found in Supplementary Materials and Methods.

SEngine Precision Medicine drug testing

Peritontoids were assessed for purity and viability following ex vivo expansion in media containing DMEM/F12, 50 ng/ml rhEGF (both from Corning CellGro) with 100 μg/ml Primocin (InvivoGen), 10 mmol/L HEPES, 1× B27, 1× Glutamax (all from Gibco), 1× N2 (Thermo Fisher Scientific), 100 ng/ml Noggin, 100 ng/ml Wnt-3a (both from R&D Systems), 500 ng/ml R-Spondin-1 (PeproTech), 1 mmol/L Nicotinamide (Sigma Aldrich), and 10 μmol/L Y-27632 (Selleckchem). Drug tests were conducted on peritontoids from passage 4 to 8. Eight-hundred cells per well were seeded into 384-well assay plates containing 50-μl media supplemented with 5% Matrigel (Corning) for high-throughput screening as described. A broadly targeted 87 drug pan-cancer focused small-molecule library (first five samples, Supplementary Table S1A) was acoustically administered (Labcyte Echo) as single agents using contactless, nanovolume liquid transfers to create a 3-log, 6-dose drug curve; drug concentrations ranged from 33 pmol/L to 200 μmol/L, depending on individual drug properties. Dose ranges for targeted agents were designed to capture previously reported Cmax values (serum level) and the asymptotic response range. Dose ranges for chemotherapies were derived from clinical dosage guidelines, to encompass one logarithmic unit above and two logarithmic units below the dose suggested for a person of average size (1.8M2 body surface area). This library was further refined to create a 35-drug colorectal cancer-focused panel (Supplementary Table S1B) to better reflect clinically available options for the subsequent patient samples. Peritontoids and tumors were challenged with drugs for 6 days, following which, relative viability was determined by whole-well ATP quantification using Cell-Titer-Glo 2.0 (Promega) and normalized to vehicle-only controls (maximal DMSO concentration used was 0.2%). Additional drug testing methods’ details including formulas for generation of AUC data and low-throughput testing at second laboratory site are provided in Supplementary Materials and Methods. We use the AUC for drug response as this metric combines information about the efficacy (how much cell viability is decreased by each drug) and potency (the amount of drug needed to reduce viability; EC50, IC50) of each drug.

The SEngine Precision Medicine internal pan-cancer database contained 57 samples from 16 tumor types, with n = 8 (14%) derived from colorectal cancer. All tumor organoids were cultured using media containing standard core organoid components, with slight tissue-specific variations, and screened in an identical manner using the SEngine Clinical Laboratory Improvement Amendments (CLIA)-approved standard protocol, as described for peritontoids above. The drug library used was validated for activity and consistent for peritontoids. AUC data from drug response curves was subjected to hierarchical clustering using the WGCNA R package version 1.68, R version 3.6.0 on Windows. The topological overlap matrix was calculated (Pearson correlation) with pairwise complete observations. The resulting dendrogram was superimposed on to the heatmap for visualization (pheatmap version 1.0.12). Z-score transformation of AUC data for each drug was performed using R version 3.6.0 on Windows and displayed using violin plots (Supplementary Fig. S3A).

Data and materials availability

NGS data are available in dbGaP (phs002023.v1.p1) and GEO (GSE147971). All data needed to evaluate the conclusions are present in the article and/or the Supplementary Materials and Methods. Distribution of peritontoid lines is limited to existing approved sites through the signed informed consent of study participants. Approval for distribution may be granted by application to the HREC.

Results

Characterization of patients with CRPMs

Twenty-eight patients (ages 43–81 years) with MSS colorectal cancer and CRPMs were recruited over 18 months and prospectively studied. Supplementary Table S2 provides a clinical summary. With the exception of five patients who had unresectable CRPMs, all underwent cytoreductive surgery and HIPEC. Five patients had synchronous primary colorectal cancer and CRPMs at diagnosis and the remaining patients had metachronous peritoneal metastases. At the time tissue samples were obtained, two patients were treatment naïve, however, the majority had received multiple cycles of standard chemotherapy treatment including FOLFIRI, FOLFOX, and/or CAPOX, the biologics cetuximab or bevacizumab, and/or chemoradiotherapy for those with rectal cancer (Supplementary Table S2).

The majority of CRPMs are stroma-rich, poor prognosis, consensus molecular subtype four

Colorectal cancer can be stratified by RNA expression into four consensus molecular subtypes (CMS; ref. 32). While this has yet to result in subtype-specific interventions, the characterization provides information about the underlying molecular pathways that are dysregulated in each tumor type and may provide rationale for drug sensitivities above existing biomarkers, such as RAS and BRAF mutation and microsatellite instability (MSI) status. We undertook mRNA-seq of 14 of the CRPMs tissue samples from this cohort. CMS analysis revealed the majority (71%, 10/14) to be CMS4 (Supplementary Fig. S1A), a subtype known to confer worse overall and relapse-free survival (32). Three matched synchronous primary and CRPM samples were examined. Of these, the primary tumors studied were CMS2, CMS3, and CMS4; however all three matching CRPM samples were classified as stromal-rich CMS4, suggesting evolution of the transcriptional signatures from the primary to the metastatic site (Supplementary Fig. S1A). As would be expected for MSS disease, no samples were assigned to CMS1, which is associated with MSI and immune infiltration and activation (32). Gene set enrichment analysis confirmed activation of TGFβ pathway and epithelial-to-mesenchymal transition genes in the CMS4 samples, an epithelial differentiation signature in CMS3, and WNT signature in CMS2.
These findings are consistent with previously identified expression signatures for these CMS (ref. 32; Supplementary Fig. S1B).

**Characterization of organoids derived from CRPM (peritonoids)**

Using an optimized culture technique, peritonoids were successfully generated and validated from 19 of 28 patients (68%; Supplementary Fig. S1C). This success rate is consistent with prior studies (26). In addition, we isolated matched organoids (here tumoroids) from synchronously resected primary colorectal cancer for two patients. Peritonoids were validated with a combination of STR analysis and IHC (Cytokeratin-20, CK20 and Caudal Type homeobox2, CDX2; Fig. 1; Supplementary Fig. S1D–S1G). Tumorigenicity was confirmed by in vivo tumor growth following subcutaneous administration of peritonoids into immunocompromised mice (Supplementary Fig. S1D–S1G). WES was undertaken on peritonoids (from 13 patients) and germline DNA from blood was used to evaluate DNA alterations that may predict sensitivity or resistance to targeted therapies. High-confidence calls of genomic variants and copy-number alterations in 551 cancer-associated or actionable genes are summarized in Supplementary Table S1C and Supplementary Fig. S2A, and were 100% concordant with KRAS, NRAS, and BRAF testing undertaken in independent pathology laboratories using FFPE tumor tissue (n = 9 CRPM tissue samples; Supplementary Table S2).

The peritonoids recapitulated genetic alterations commonly associated with colorectal cancer (refs. 33, 34; e.g., APC, TP53, and KRAS; Supplementary Table S1C–S1D). Furthermore, the samples had a mean somatic mutation rate of 1.8/MB (Supplementary Table S1E), in-line with their pathologic characterization as MSS disease (19). The Catalogue of Somatic Mutations in Cancer DNA mutation signature, which can suggest the molecular etiology of DNA alterations in each sample, was also evaluated in the context of therapeutics that can target specific mechanisms of DNA alterations (e.g., PARP inhibitors for BRCA-mutant cancers; Supplementary Fig. S2B). We concurrently undertook these genomic analyses with drug sensitivity testing.

**Medium-throughput peritonoid drug sensitivity testing**

To evaluate novel therapeutic options, peritonoids from 15 patients were subjected to medium-throughput drug–dose response screening using the CLIA-certified PARIS platform. Peritonoids from the first five patients were challenged with an 87 pan-cancer drug panel consisting of both chemotherapies and targeted agents. A curated panel of 35 targeted agents was selected for subsequent patients based on availability in Australia as well as predicted efficacy in CRPMs (Supplementary Table S1A and S1B). Compounds that target the most common alterations found in mCRC, that is, the “druggable” landscape of mCRC (33) were well-represented in this smaller drug library (Supplementary Table S1D).

A heatmap depicting unsupervised clustering of patient-derived organoid drug sensitivity for the 35 drugs, along with limited clinical and genomic characteristics for the 15 patients, is shown in Fig. 2. Drugs with similar mechanisms of action generate similar responses across the peritonoids tested (e.g., the two first-generation EGFR inhibitors gefitinib and erlotinib, MEK inhibitors cotinib and trametinib, ALK inhibitors ceritinib and crizotinib, and PARP inhibitors olaparib and rucaparib). In contrast, the majority of peritonoids also had unique and specific responses (Fig. 2; Supplementary Fig. S3A). Unique patient-specific findings along with common drug sensitivities across the cohort underpin the value of functional drug testing. Of note, replicate plating of peritonoids from patient 5 on two separate test dates 2 weeks apart generated very similar results against the drug panel (Pearson r = 0.89, Fig. 2; Supplementary Fig. S3B).

Testing with selected drugs from the library at a second laboratory site, using the same peritonoid lines, also significantly correlated with results from the medium-throughput platform (Pearson r = 0.86; P < 0.05; Supplementary Fig. S3C). These results underscore the
accuracy of the CLIA-accredited testing platform. Paired primary colorectal cancer tumoroids and peritonioids clustered near each other for patient 6 but were very distantly related for patient 7, suggesting that there may be shared drug sensitivity between the primary and peritoneal disease but this may also be patient and sample dependent. Peritonioids from patient 15 were broadly insensitive to all panel drugs ex vivo. This patient had aggressive disease, as assessed by early-peritoneal recurrence despite initial low volume disease and successful cytoreductive surgery/HIPEC. We were unable to determine the accuracy of the peritonioid testing platform for this patient, as no promising drug candidates were uncovered through the screen. As such the patient was offered standard care FOLFOX treatment rather than organoid-directed care.

**Ex vivo, functional drug sensitivities are consistent with genomically predicted targets**

Medium-throughput screening of targeted small-molecule inhibitors identified several drug sensitivities that were unique to individual patients and consistent with their genetic biomarkers. For example, the presence of a *PTEN* mutation in patient 5 was significantly associated with sensitivity to PARP inhibition \( (P < 0.0005; \text{Fig. 3A}) \) in line with preclinical data (35, 36). Alongside the truncating mutation in *PTEN*, peritonioids from patient 5 also harbored a *PIK3CA* frameshift mutation (N1068fs), and responded exceptionally well to both PI3K p110a catalytic subunit inhibitors present in the 87-drug panel (alpelisib and taselisib) and inhibitors of downstream targets AKT and MTOR (Supplementary Fig. S3D). Peritonioids from patient 1 contained a
pathogenic PIK3CA N1044K mutation and were also exceptionally sensitive to both p110a inhibitors as well as the AKT inhibitor MK2206 (Fig. 3B; Supplementary Fig. S3E). Patient 1’s peritonoids harbored EGFR, ERBB2, and ERBB4 copy gain and demonstrated sensitivities to all seven EGFR inhibitors tested with exceptional responses (>95% growth inhibition at lowest concentrations tested, 33 nmol/L) seen for the two patients with ERBB4 activity, afatinib and poziotinib (Fig. 3C). Many peritonoids demonstrated partial responses (PR) to one or more EGFR family inhibitors, consistent with the established dependency on EGFR signaling in colorectal cancer (37). However, activating mutations in Kras were significantly associated with decreased sensitivity to five of seven EGFR inhibitors, as would be expected from clinical trial data (refs. 37, 38; Fig. 3D). While these specific genetic alterations have been previously shown to influence response to targeted agents, genomic alterations could explain some, but not all, observed drug sensitivities (Fig. 2; Supplementary Table S1C), a finding that underscores the need for functional testing in precision medicine.

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Patient-derived peritonoid sensitivity to standard care chemotherapeutic regimes

Most patients were treated with standard care chemo(radio)therapy prior to tissue sampling, with many undergoing further chemotherapy as per standard practice following sample collection (Supplementary Table S2). An assessment of peritonoid sensitivity to standard FOLFOX and FOLFIRI regimes \textit{ex vivo} was undertaken for nine patients (Supplementary Fig. S4). Consistent with a recent study using organoids cultured from mCRC (primarily liver; ref. 26), \textit{ex vivo} peritonoid FOLFOX sensitivity failed to clearly separate samples from patients who had \textit{in vivo} PR or stable disease (SD) versus progressive disease following FOLFOX treatment (Supplementary Fig. S4A–S4E). However, the two patients with clinically responsive disease had not received oxaliplatin therapies previously in the clinic and gave rise to two of the most sensitive peritonoid lines to FOLFOX \textit{ex vivo}. Our patient cohort did not contain any patients with PR/SD following FOLFIRI treatment hence we were unable to assess the predictive value of this testing platform for the FOLFIRI regimen (Supplementary Fig. S4F–S4M). In two patients with synchronously resected primary colorectal and metastatic tumors, both patients’ tumoroids derived from the primary tumor were more sensitive to FOLFIRI than their metastatic counterpart. For one patient, the tumoroid culture was also more sensitive to the FOLFIRI regimen than the related peritonoid culture (Supplementary Fig. S4N and S4O).

Specific patient results and outcomes: using peritonoids to inform novel therapies for treatment-refractory CRPMs

During the study period, 13 of the 19 patients with CRPMs for whom peritonoids were successfully cultured had disease progression despite standard care chemotherapeutic treatment. Outside of our study, patient 3 was started on regorafenib, a multikinase inhibitor, recently approved in Australia for treatment refractory mCRC. Peritonoid testing demonstrated insensitivity to regorafenib with viability measurements similar to vehicle alone (AUC 1.07; Fig. 2). Clinically, this patient failed to respond to regorafenib, consistent with \textit{ex vivo} findings. However, all peritonoid lines in our study were also insensitive to regorafenib, possibly due to an inherent resistance of CRPM to this drug or because this drug targets stromal and angiogenic tumor properties that are not well-modeled by the epithelial organoid cultures. Two patients exhibited specific sensitivities to agents in the functional screen (Supplementary Fig. S5A) and started off-label drug treatment based on peritonoid test results. Peritonoids from patient 1 were KRASG12D mutant (Supplementary Table S1C) and broadly insensitive to most monotherapy chemotherapeutics in the library (Supplementary Fig. S5A). Nevertheless, treatment with particular targeted agents, the MEK inhibitors (MEKi) trametinib and cobimetinib and multi-tyrosine kinase inhibitor, vandetanib, dramatically reduced organoid viability (Supplementary Fig. S5B–S5D). However, the inability to obtain compassionate access to MEKi, coupled with drug funding limitations prohibited us from offering a MEKi to patient 1. Vandetanib is approved for use as a single agent in the treatment of advanced medullary thyroid cancer and was obtained under compassionate access for this patient. Treatment was changed to vandetanib when the patient already had widespread, extensive disease progression on standard care chemotherapy. Unfortunately no disease response with 4 weeks of vandetanib therapy was noted before the patient passed away.

Peritonoids from patient 2 were sensitive to multiple therapeutic, including chemotherapies that the patient had not been exposed to, despite this patient undergoing multiple rounds of standard care chemo(radio)therapy prior to CRPM sampling for organoid culture.
Peritonoid-guided drug choice for chemo-refractory disease. Disease in 43-year-old patient 2 worsened despite standard care surgery and five rounds of chemotherapy, including EGFRi treatment. The medical oncology team considered candidate alternate the therapies based on peritonoid testing results. Peritonoid dose-response curves (in blue) for Wee1 inhibitor adavo-sertib (A), osimertinib (B), vorinostat (C), and gemcitabine (D). In gray is average cell viability from prior SEngine testing of cancer cell and organoid lines. Error bars, SD. FDG PET-CT scan of patient immediately prior to treatment (A) and frontal images (B–H). Sagittal (E and F) and frontal images (G and H). Position of the transverse axial slice (bottom) is indicated by open arrowhead in top image. Pelvic hotspot marked in blue (E and F) has reduced from 15 to 9.7 and right abdomen lesion (closed arrow, serosal deposit on bowel) is no longer visible.

(Supplementary Table S2; Supplementary Fig. S5A). Of note, these peritonoids displayed a striking sensitivity to an inhibitor of the WEE1-M-phase cell-cycle checkpoint kinase, adavo-sertib, especially when compared with the average dose response across a reference set of all samples within the SEngine Precision Medicine internal database (Fig. 4A). The database is used to highlight outstanding responses to a specific drug for a given sample to refine drugs of interest. By comparing results for a specific patient to this broader set of cancer organoid and cell viability data, we are able to identify drugs that are particularly effective for a specific sample, thereby allowing for a precision medicine approach. Adavo-sertib is currently in phase II clinical trial for advanced colorectal cancer in the United Kingdom-based FOCUS4-C trial for patients with RAS and TP53 mutation or loss of histone marks (39). We were unable to obtain access to adavo-sertib for our patient. Further therapeutic options based on peritonoid drug sensitivity for this patient, such as the EGFR inhibitor osimertinib (Fig. 4B) and the HDAC inhibitor vorinostat (Fig. 4C) were explored, but access to off-label use was restricted by funding. Finally the antimetabolite, gemcitabine, was offered to the patient on the basis of peritonoid testing (Fig. 4D) combined with drug access, cost, and toxicity considerations. Peritonoid sensitivity to gemcitabine was also validated at a second laboratory site (Supplementary Fig. S5E–S5G) and the patient was treated with a gemcitabine–capcitabine combination. After 3 months of therapy there was a PR demonstrated by FDG PET-CT scans, followed by disease progression following a further 2 months of treatment (Fig. 4E–H). This was despite this patient showing prior, continual disease progression on standard care chemotherapy. These results illustrate the power of functional testing to identify effective chemotherapies outside of standard of care. We continue to monitor patients in this cohort and will provide peritonoid-directed therapy options should standard care be exhausted for the remainder of surviving patients.

Discussion

Historically, the inability to replicate tumor heterogeneity is pos-tulated to be one of the key reasons that have limited the use of conventional cell lines to guide precision medicine. Here, we generated ex vivo tumor models that more faithfully recapitulate the cellular and genomic heterogeneity present in the original tumor, by generating peritonoids from at least two different CRPM sites in operative specimens. Multiple retrospective studies have now linked ex vivo drug responses of patient-derived organoids to clinical outcomes across multiple solid cancers (24–26, 28, 29), albeit with fairly small patient cohorts to date. A major aim of this body of work was to determine whether patient-derived peritonoids could be cultured and genomic and drug sensitivity analyses reported, within a time-frame that enabled alternate therapy options to be acted on by the treating physician for patients. To this end, peritonoid cultures were successfully grown in 68% (19/28) of cases, taking between 3–6 weeks to generate. Within 2 months of tissue sampling, a combined genomics and drug sensitivity report was ready for consideration by the medical oncologist.

The majority of patients in this cohort were recruited while still having standard care options available, allowing time for organoid testing well within the clinically actionable window for these patients. A shortfall is the time-lag between tissue sampling and actioning the results. For patient 1, peritonoids were derived from CRPM tissue sampled 16 months prior to treatment change to the peritonoid-guided therapy. For patient 2, the time between tissue sampling and treatment change to peritonoid-guided therapy was reduced to 6 months and resulted in a PR in the patient. The decision to change therapy is not only made on the availability of the data, but also the clinical circumstances of the patient. However, with this platform it is entirely feasible to rebiopsy accessible tumor deposit(s) in the event of disease progression to reevaluate genomic changes and drug sensitivities. Furthermore, as we attempted here, multiple sampling of disease should be included when possible to better guide selection of therapies that are efficacious across potentially heterogeneous disease sites.
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Highlighting the utility of functional testing, the response of peritonoids to specific drugs with known genomic biomarkers was predictable in some, but not all samples. The MSK-Impact study of 1,134 matched primary and mCRC (including ~50 CRPMs) revealed that CRPMs were enriched for alterations to the PI3K and MAPK pathways compared with other metastatic sites (33). These common genomic alterations in MAPK and PI3K pathway genes are also reflected in our CRPM cohort (Supplementary Table S4). Concordantly, peritonoids with PIK3CA mutations (n = 2) exhibited exceptional responses to PI3K inhibitors, while the cohort was broadly sensitive to MEK inhibitors (62% have mutation and/or copy gain to KRAS, NRAS, or BRAF) and EGFR inhibitors (54% had EGFR copy gain), highlighting again the critical importance of EGFR/MAPK signaling for colorectal cancer. Mimicking the clinical situation, KRAS-mutant peritonoids were, however, less sensitive to EGFR inhibition. Acting on these peritonoid drug sensitivity data for MAPK targets with currently nonstandard, combination treatments to constrain feedback loops that reactivate MAPK signaling in colorectal cancer, such as that trialed for BRAF-mutant colorectal cancer (40) or in combination with immune checkpoint modulation in the future. We also uncovered unpredicted sensitivity to agents without validated biomarkers (such as gemcitabine) that are not normally used for the treatment of colorectal cancer.

In summary, this study addresses a clinically unmet need to explore and evaluate novel treatment options for patients with CRPMs. We have successfully established a patient-derived, peritonioid-based platform to direct personalized therapy in this poor prognosis cohort of patients. Our platform delivers functional testing and genomic data in a form and time-frame that is clinically relevant for our current care pathways. We were limited in our impact by the anticipated difficulties in drug funding and access for off-label indications. Because of this limitation we were not able to change patient treatment to the most efficacious drugs identified from ex vivo peritonioid screening. As approaches such as ours mature, and are backed by larger randomized controlled clinical trials, there will be the need to modernize drug approvals to include more tumor agnostic indications and one-off, personalized approvals. The tools and techniques exist, as we have shown here, to grow, propagate, transport, and analyze living tumor samples. We now have the very exciting opportunity to search for new ways to better define individual drug sensitivities, linked to understanding tumor genomics and biology, to inform practice, and help our patients.

Disclosure of Potential Conflicts of Interest

M. Churchill, R. Rosati, and A.B. Richardson are employees/paid consultants for SEngine Precision Medicine. S. Pereira, G.A. Whitney, C. Grandori are employees/paid consultants for and hold ownership interest (including patents) in SEngine Precision Medicine. J. Tie is an employee/paid consultant for MedImmune, and reports receiving speakers bureau honoraria from Merck Serono, Amgen, Servier, Sirtex, Roche, and Eisai. R.G. Ramsay reports receiving commercial research grants from Invirion and Merck Serono. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions


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