Predicting response to cytotoxic chemotherapy

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Abstract

Cytotoxic chemotherapies have been a mainstay of cancer treatment for over 40 years. As they are typically administered without the use of precision biomarkers, some patients can suffer severe side effects without any benefit. The development of novel biomarkers to enable precision use of these therapies could reduce toxic side effects, improve overall response rates and reduce unnecessary healthcare expenditures. In this study we used chromosomal instability (CIN) signatures to predict a patient's response to platinum-based and doxorubicin chemotherapy. We retrospectively validated our predictor of platinum sensitivity across 41 high grade serous ovarian cancer patients. Additionally, we discovered a new biomarker for doxorubicin sensitivity based on a CIN signature related to focal DNA amplification, which we retrospectively validated across 26 patients treated with doxorubicin following platinum. We also assessed the performance of these predictors using circulating tumour DNA. As multiple CIN signature biomarkers can be quantified using a single genomic test, this represents a unified approach to guide multiple therapy choices for cytotoxic chemotherapies with the potential to shift current one-size-fits all chemotherapy treatment towards precision medicine.

Introduction

Cytotoxic chemotherapies exploit the defective properties of a cancer cell, such as impaired DNA repair mechanisms, to preferentially drive cancer cells to programmed cell death[1]. However, most chemotherapies also have detrimental effects on healthy cells, potentially causing severe side effects despite administration alongside modern day supportive care[2]. Many of these agents were approved for clinical use before the adoption of therapy selection biomarkers, in contrast to new targeted therapies, which increasingly require the presence of a companion diagnostic test to determine whether a patient is eligible for treatment[3].

Therefore, a large number of patients treated with cytotoxics will experience extreme side effects with no benefit from the therapy. Enabling precision use of these agents could allow patients resistant to a therapy to avoid unnecessary side effects. This may also enable them to receive an alternative therapy, ultimately improving overall health outcomes. Furthermore, precision use of cytotoxics can reduce healthcare costs by lowering expenditure on ineffective cancer therapies and additional medical interventions for treatment complications.

High-grade serous ovarian cancer (HGSOC) is a striking example where precision use of cytotoxics could improve patient benefit. Compared to other cancers (e.g. breast), ovarian cancer has barely benefited from precision medicine. It is one of the deadliest gynaecological cancers worldwide, accounting for ~184,000 deaths per annum[4,5] with a poor survival rate that has not changed in more than 20 years[6]. Current ovarian cancer treatment relies heavily on cytotoxic chemotherapy with standard of care typically being a combination of carboplatin/paclitaxel and primary or interval debulking surgery (first-line of treatment), and combinations of carboplatin, paclitaxel, and doxorubicin at second and subsequent lines of treatment. The recent introduction of PARP inhibitors as a maintenance therapy following first-line treatment has improved patient outcomes[7], however, first and second-line treatments remain unchanged.

We have recently developed a new type of biomarker that has the potential to address the challenge of precision use of cytotoxic chemotherapy. This technology encodes the genome-wide imprints of distinct types of chromosomal instability (CIN) operating in a tumour genome using CIN signatures[8,9]. These signatures represent the activity of different defective pathways in a tumour that cause different patterns of DNA copy number change. In this way, CIN signatures give us the opportunity to identify potential biological mechanisms to target therapeutically. Initially prototyped in ovarian cancer[9] and then extended pan-cancer[8], these signatures have shown potential to predict response to platinum-based chemotherapy in patients across multiple cancers[8] and other cytotoxic chemotherapies in a preclinical setting[8,10]. The full spectrum of signatures can be quantified in a tumour using standard genomic gene panels tests or shallow whole genome sequencing. We therefore hypothesise that CIN signatures can be used in a unified genomic test to predict response to multiple cytotoxic chemotherapies at diagnosis.

In this study we retrospectively assessed the potential of CIN signatures to predict response to both first-line treatment with platinum-based chemotherapy and subsequent second-line treatment with doxorubicin in HGSOC.

Results

Patient samples, organoids and spheroids

To test the ability of our CIN signature biomarkers to retrospectively predict response to cytotoxic chemotherapies in the clinic, we used samples from patients enrolled in the OV04 clinical study (Cambridge Translational Cancer Research Ovarian Study 04, approved by the Institutional Ethics Committee - REC08/H0306/61). OV04 is an ongoing observational study that records patient clinical data and collects patient material for the purpose of biomarker

and scientific discovery. To compile a real-world cohort for this study, we assessed patients enrolled in OV04 from 2010-2019 and identified 54 patients with available tumour samples who received platinum-based chemotherapy as a first-line therapy and/or doxorubicin as a subsequent treatment following approved standards of care (see Methods). Each patient underwent a histopathology review and was confirmed to have high-grade serous ovarian cancer (HGSOC), with the exception of 3 patients. Samples collected from these patients totaled 51 tumour samples and 29 cell free DNA (cfDNA) samples extracted from plasma. Selected tumour tissue samples and cfDNA samples were shallow whole-genome sequenced, DNA copy number profiles derived, CIN signatures computed[8,9], and quality control filtered (see Figure 1 for sample workflow). Following quality control, a final cohort of 41 ovarian cancer patients was identified as suitable for retrospective assessment of treatment response prediction (Figure 1). Progression-free survival (PFS) intervals were calculated following platinum-based treatment and doxorubicin treatment using the serum marker CA-125 as recommended by the Gynecological Cancer InterGroup (GCIG)[11] (see Methods).

In addition to the 41 patient cohort, additional patient samples from the OV04 study were used to harvest spheroids from ascitic fluid and derive organoids for the purpose of CIN signature biomarker discovery for doxorubicin prediction. These samples were subjected to low-pass whole-genome sequencing and *in vitro* treatment with doxorubicin (see Methods and Figure 1b for sample workflow). Further characterisation of these collections of patient derived spheroids and patient derived organoids can be found in parallel studies[10,12].

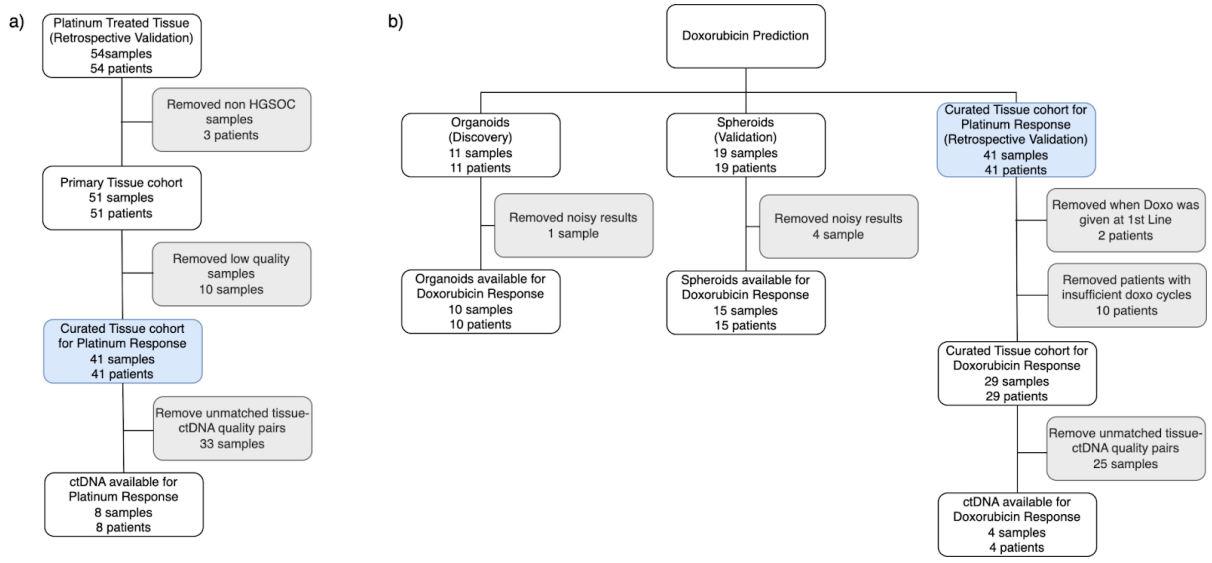


Figure 1. REMARK diagram. a) Flow diagram summarising the quality control filtering of samples and patients to get the curated cohort for the retrospective validation of platinum response prediction. **b)** Flow diagram detailing organoids and spheroids filtered for the phases of biomarker discovery and validation for Doxorubicin response, as well as samples and patients filtered to get the curated cohort for the retrospective validation of Doxorubicin response prediction.

Predicting platinum-based chemotherapy response

Previously, we demonstrated that our CIN signatures CX2 and CX3, representing patterns of DNA copy number change as a result of two different types of impaired homologous recombination, were able to predict sensitivity to platinum-based chemotherapy across ovarian and esophageal cancers[8]. This was performed using overall survival as a surrogate measure of sensitivity as all patients received platinum-based chemotherapy first-line. Here, we aimed to validate this predictor using a more direct measurement of sensitivity in terms of PFS following first-line treatment with platinum-based chemotherapy.

Classification of the 41 patients into sensitive and resistant groups using the CX2/CX3 classifier yielded a significant difference in median PFS (hazard ratio=0.4, p=0.02 wald test, Figure 2a). Cox proportional hazards model analysis, taking into account age at diagnosis and tumour stage covariates, also yielded significant separation between sensitive and resistant groups (hazard ratio=0.39, p=0.013 wald test, 2 observations removed due to missing tumour stage data, Figure 2b). These results validate the ability of CX2 and CX3 to predict sensitivity to platinum-based chemotherapy treatment in an independent cohort with a more direct measure of response than our previous study[8].

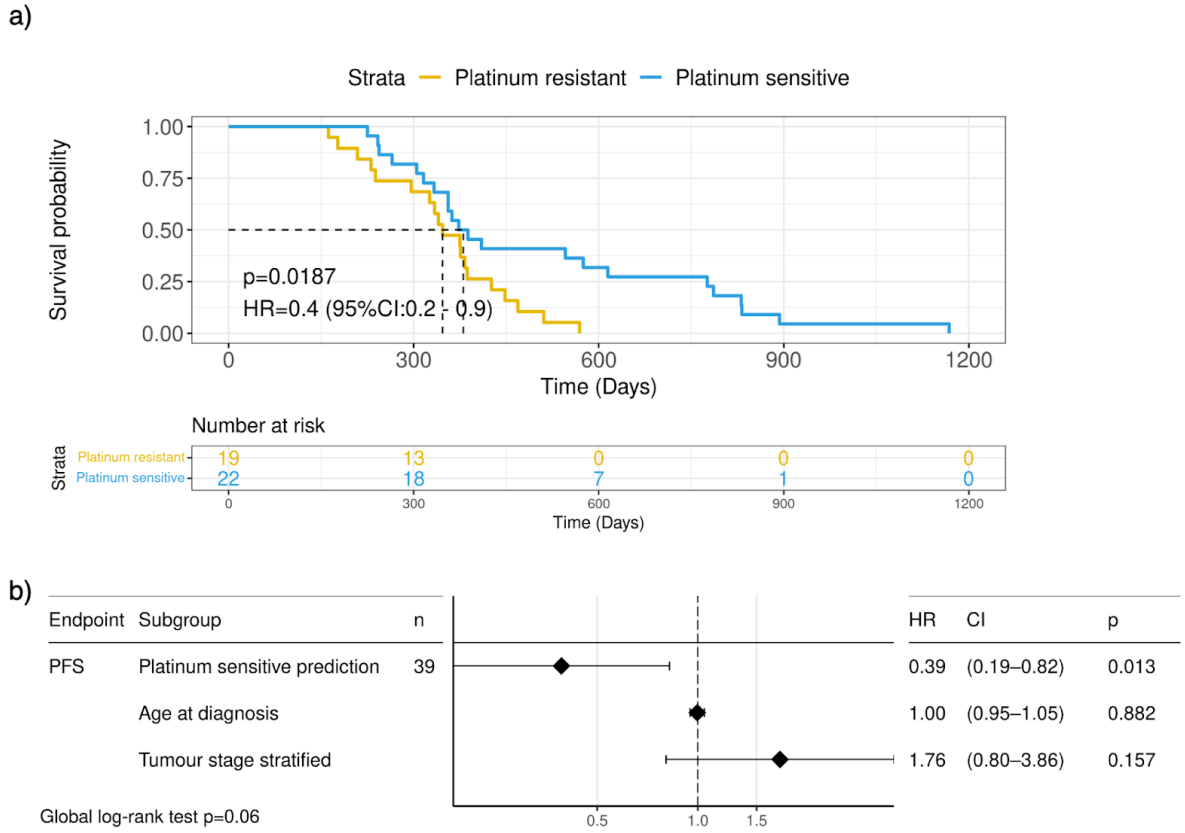


Figure 2. Performance assessment of platinum-based chemotherapy response prediction using progression free survival across 41 ovarian cancer patients. a) Kaplan Meier curves for patients predicted to be sensitive (blue) and resistant (yellow) **b)** Cox proportional hazards model accounting for age at diagnosis and tumour stage.

Identifying a doxorubicin response predictor

We next sought a predictor of response to doxorubicin treatment as it is one of the most commonly used drugs in ovarian cancer at second-line. Doxorubicin is a widely used topoisomerase-II poison, utilised in the treatment of ovarian cancer, breast cancer, bladder cancer, sarcomas, lymphomas and leukaemia. It is available in liposomal (i.e. liposome-encapsulated) and non-liposomal forms, and like many other genotoxic chemotherapies, causes DNA damage. This damage commonly results in extrachromosomal DNA, which in many cases is encapsulated in micronuclei. As such, treatment with doxorubicin is known to induce micronuclei formation[13]. Micronuclei can accumulate in high numbers in a tumour via uneven segregation during cell division and/or can have independent replication machinery giving rise to high numbers of copies of the DNA contained within[14]. As micronuclei rupture can elicit an immune response[15], it is possible that some of the benefit of doxorubicin treatment comes via activation of the immune system. However, if a tumour has already passed through an evolutionary bottleneck where it can tolerate micronuclei formation and avoid the subsequent immune response[15], then treatment with doxorubicin is unlikely to have an effect. Therefore, we hypothesised that tumours with evidence of micronuclei prior to treatment may be resistant, whereas those without evidence of micronuclei may be sensitive. A common indicator of micronuclei formation is the presence of focal amplification events[14], which we have previously shown can be measured via ovarian CIN signature 6[9]. As such, we sought to determine whether ovarian CIN signature 6 was predictive of doxorubicin response.

To test this, we treated 10 ovarian cancer organoids with doxorubicin *in vitro*, measured response via IC50 and observed signature 6 activity computed from low-pass WGS of the organoids prior to treatment (Figure 3a). Supporting our hypothesis was the observation that two most sensitive organoids had 0 signature activity whereas the remainder had >0 activity. To further refine this observation, we attempted to estimate the fraction of the organoids that could be considered sensitive to doxorubicin based on the observed sensitivity to platinum first-line in the originating patients, as patients resistant to platinum chemotherapy are expected to have an 18% response rate to doxorubicin monotherapy[16–22], whereas sensitive patients a 28% response rate[23]. Using the observed platinum sensitivity in the originating patients (Supplementary Table 1), we estimated that 2 of the 10 organoids could be considered sensitive to doxorubicin and 8 resistant (Methods, Figure 3a). Therefore, we considered the two organoids with the lowest IC50 to be sensitive, thus a threshold of >0 in signature 6 activity perfectly predicted the sensitivity status of the organoids to doxorubicin treatment ($p=0.022$, permutation test).

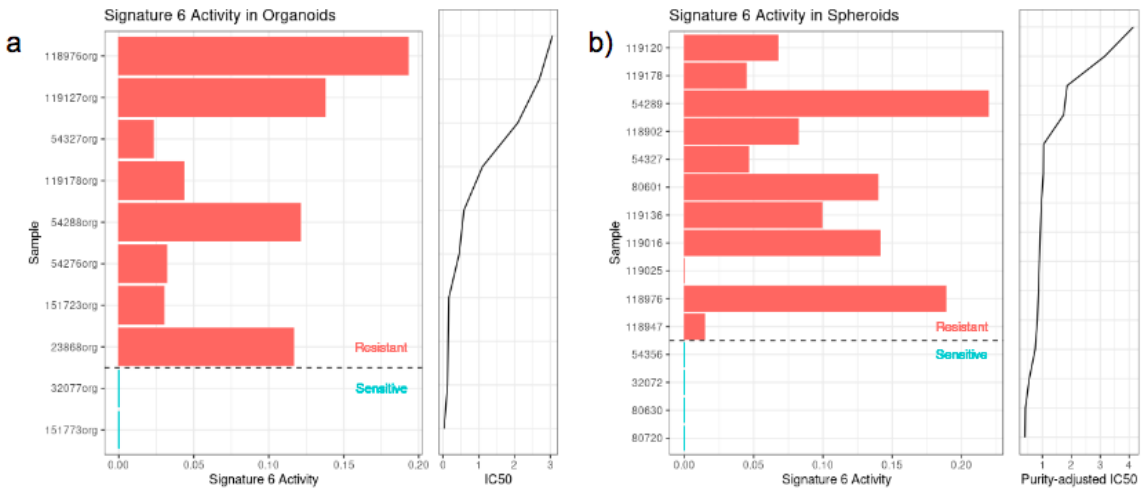


Figure 3. Doxorubicin response prediction using copy number signature 6 activity across organoids and spheroids. a) Ovarian copy number signature 6 exposures (x-axis) across 10 patient derived organoids (y-axis). Organoids are colour coded based on their sensitivity (blue) or resistance (red) to *in vitro* doxorubicin treatment. **b)** Ovarian copy number signature 6 exposures (x-axis) across 15 patient derived spheroids harvested from ascitic fluid (y-axis). Spheroids are colour coded based on their sensitivity (blue) or resistance (red) to *in vitro* doxorubicin treatment.

Validating the doxorubicin response predictor *in vitro*

To validate this doxorubicin response predictor we used an alternative *in vitro* treatment system. We harvested tumour spheroids from 15 patient's ascitic fluid and performed short term culture, treating them with doxorubicin and observing response via IC50. Similar to the organoids, the expected number of sensitive spheroids was estimated using clinical response data from the originating patients (see Methods). After ranking spheroids by IC50, 4 spheroids were considered sensitive and 11 resistant (Figure 3b). In this cohort, all sensitive spheroids showed 0 signature 6 activity, indicating 100% sensitivity to identify doxorubicin responders. However, 1 of the resistant spheroids also showed 0 signature 6 activity, suggesting 90% specificity for identifying responsive spheroids (p=0.004, permutation test).

Validating the doxorubicin response predictor in patients

Given the promising performance of the doxorubicin response predictor *in vitro*, we proceeded to validate the predictor across a cohort of ovarian cancer patients treated with doxorubicin. Here, a retrospective analysis was performed using 29 patients assembled from the OV04 clinical study.

Signature 6 activity was computed for the 29 tumour tissue samples, patients classified into sensitive and resistant groups, and PFS compared between the groups. A cox-proportional hazards model, controlling for first-line platinum PFS, showed that patients predicted as sensitive had significantly longer PFS intervals than those predicted as resistant (hazard

ratio=0.0004, p=0.004 wald test, Figure 4a). This was also true after correcting for age at diagnosis and tumour stage (hazard ratio=0.0001, p=0.004 wald test, 1 observation removed due to missing tumour stage data, Figure 4b).

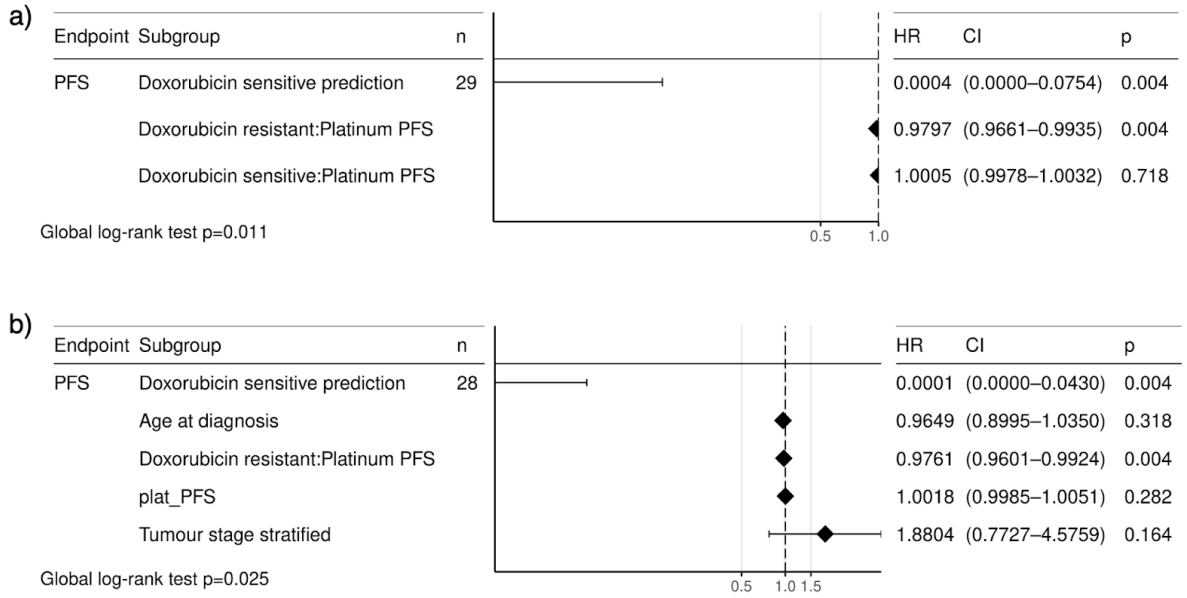


Figure 4. Performance assessment of doxorubicin response prediction. a) Cox proportional hazards model (Cox PH) accounting for platinum PFS. **b)** Cox PH model accounting for platinum PFS, age at diagnosis and tumour stage.

Predicting treatment response using liquid biopsy

Next we assessed the feasibility of applying our classifier to liquid biopsies. Where possible, cell-free DNA (cfDNA) was extracted from plasma collected at diagnosis and subjected to low-pass whole genome sequencing. DNA copy number profiles were derived and samples were classified as having either low ctDNA fraction or high ctDNA fraction. Out of the 41 patient cohort, 29 had plasma samples available. Of these 29 samples, 8 - representing 27.6% of the cohort - were considered as high ctDNA fraction samples. These high-fraction ctDNA samples were analysed for CIN signature analysis in the same way as solid tumours samples. The remaining plasma samples had insufficient overall tumour DNA to assess chromosomal instability utilising the CIN signature methods prepared to date.

In general, high-concordance of copy number profiles was observed between ctDNA and tumour tissue (Figure 5a) for the matched pairs. Similarly, CIN signatures also showed high levels of concordance (Figure 5b, median cosine similarity for pan-cancer signatures was 0.74, median cosine similarity for ovarian signatures was 0.88). 87.5% (7 patients) showed agreement between the platinum response predictions obtained from the tumour tissue sample and the ctDNA sample.

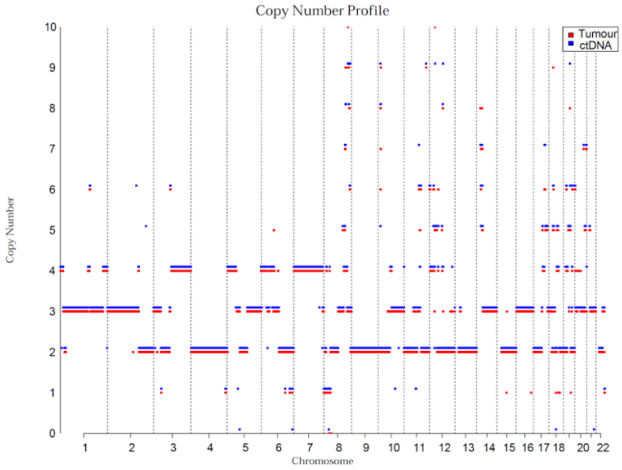
Within the 29 patients in the doxorubicin prediction cohort, 13.8% (4 patients) had matching high ctDNA fraction samples available for comparison. Of this group, there was 100%

agreement between the doxorubicin response predictions obtained from the tumour tissue sample with the cfDNA sample (Figure 5c).

Patient 713 was the only case where the platinum prediction derived from tissue and blood samples differed. This patient was predicted to be resistant based on the tumour tissue sample collected at diagnosis and sensitive based on the plasma sample (ctDNA) collected at diagnosis. Using the ctDNA prediction instead of the tissue improved the p-value of the log-rank survival test from 0.0187 to 0.0158, maintaining the same hazard ratio of 0.4, suggesting that the ctDNA based prediction may be superior to the tumour tissue sample (Supplementary Figure 1).

These results suggest that for approximately 14-27% of patients, our cytotoxic chemotherapy response predictor may be applied using a simple blood test, without the need for a tumour biopsy or a surgical specimen.

a)



b)

Figure 5. **Comparison of response prediction using tissue or liquid biopsy a)** Matched tumour tissue sample and ctDNA sample from Patient 139 showing concordant copy number profiles **b)** Pan-cancer CIN signature and ovarian cancer CIN signatures distribution derived from tumour tissue and ctDNA/plasma sample pairs with associated prediction concordance.

Discussion

In this study we used HGSOC as a test case to demonstrate the potential for CIN signature biomarkers to predict response to multiple chemotherapies including platinum-based chemotherapy and doxorubicin. This is particularly promising as these cytotoxic chemotherapies are commonly used in multiple cancers as one-size-fits all treatments, without any biomarker-driven stratification in the clinic. Furthermore, as the CIN signature biomarkers are defined pan-cancer, it is possible that these results will replicate across other tumour types.

Identifying patients who will not respond to doxorubicin or platinum could allow patients to avoid the toxic side effects of chemotherapies that are unlikely to benefit them. Developing a test to provide this information as clinical decision support would allow treating physicians to more quickly stratify non-responding patients to other approved therapy choices that may result in better therapeutic outcomes. In the absence of suitable approved alternatives, this may also have utility for identifying clinical trial patient populations with an unmet medical need. Such an intervention also has the potential to save healthcare payers from unnecessary healthcare costs that do not necessarily result in patient benefit, and in fact several government initiatives have been established to encourage such stratified medicine approaches[24–27]. This current study has also shown that it may be feasible to build such a test on both tumour tissue biopsies as well as liquid biopsies of patient ascites or blood samples. The latter of which are more easily accessed in the clinic, potentially reducing future barriers to clinical adoption.

Previous approaches attempting to predict response to platinum-based chemotherapy[28,29] and doxorubicin[30,31] have been developed based on gene expression signatures for specific tumour types. Gene expression-based classifiers are challenging to implement in the clinic as clinical samples are often a mix of tumour, immune and stromal cells with variable gene expression programs. Due to this variability, these tests are often hard to apply to general patient populations and as such have not yet been developed into clinical diagnostics. Furthermore, given the strong tissue-specific effects of gene expression programs, these tests are generally not adaptable to different tumour types. In contrast, our CIN signature biomarkers are derived from the tumour genome and can be accurately measured across cancer types[8].

Despite the samples for this study being selected from a cohort spanning a 10 year collection period at a major tertiary referral centre, only 41 patient samples were considered suitable for analysis. Scoping our analysis to HGSOC meant that only 29 of the 41 patient samples could be utilised for doxorubicin response biomarker discovery. This precipitated the need to utilise the 7 ovarian specific signatures instead of the 17 generalised pan-cancer signatures due to the number of biomarker combinations possible. In addition, our analysis

was conducted on patient tumours that were collected prior to the approval of PARP inhibitors as a maintenance therapy, which has since been implemented in the clinic for ovarian cancer. It is currently unclear what effect PARP inhibitor maintenance therapy may have on the prediction of doxorubicin and platinum response.

Within ovarian cancer, future work is needed to increase the sample numbers for our analysis and further validate the robustness of our predictions on clinical trial quality data, which could be obtained as part of a prospective observational trial. This work would also need to assess the potential impact of PARP inhibitor maintenance therapy in predicting response to subsequent second-line therapies. Our analysis was limited to two common chemotherapies, platinum and doxorubicin, however there are other chemotherapies therapies approved for the treatment of ovarian cancer including paclitaxel, which could be considered. Preclinical evidence for the CIN signatures to be able to predict response to paclitaxel treatment[8] holds promise that other predictors could also be built into a single unified genomic test. Outside of ovarian cancer, response predictions for doxorubicin and other chemotherapies can also be explored. In order to transition this work into patient benefit, further work is necessary to understand the regulatory pathway of a clinical decision support test for already approved chemotherapies. One of the main challenges that will need to be overcome is the heterogeneity of genomic testing in a clinical environment. Currently, different hospital systems employ different genomic assays including gene panel sequencing and whole genome sequencing. Thus, the utilisation of CIN signature biomarkers for chemotherapy response prediction will need to be explored across a variety of sequencing technologies.

This study represents an advance in the field, as to our knowledge, it is one of the first examples of using novel genomic analysis techniques for patient stratification for multiple medicines that were not originally developed as targeted therapies. Further, the study demonstrates the potential to extract predictive insights from both tumour tissue samples and ctDNA samples, which may increase accessibility and utility in a clinical environment. In this study we have focused on HGSOC, however we have previously demonstrated the potential to extend CIN signature analysis from ovarian cancer into other cancer types[8]. Given the CIN signatures are constructed using pan-cancer patient cohorts, our observations have potential future implications on research into clinical biomarkers for patient stratification and precision medicine approaches in cancer.

Methods

See Supplementary Materials.

Acknowledgements

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References

1. Huang, R. & Zhou, P.-K. DNA damage repair: historical perspectives, mechanistic pathways and clinical translation for targeted cancer therapy. *Signal Transduct Target Ther* **6**, 254 (2021).
2. Kuderer, N. M., Desai, A., Lustberg, M. B. & Lyman, G. H. Mitigating acute chemotherapy-associated adverse events in patients with cancer. *Nat. Rev. Clin. Oncol.* **19**, 681–697 (2022).
3. Jørgensen, J. T. Companion and Complementary Diagnostics: Clinical and Regulatory Perspectives. *Trends Cancer Res.* **2**, 706–712 (2016).
4. McMullen, M., Karakasis, K., Rottapel, R. & Oza, A. M. Advances in ovarian cancer, from biology to treatment. *Nat Cancer* **2**, 6–8 (2021).
5. Bray, F. *et al.* Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **68**, 394–424 (2018).
6. Vaughan, S. *et al.* Rethinking ovarian cancer: recommendations for improving outcomes. *Nat. Rev. Cancer* **11**, 719–725 (2011).
7. Nag, S., Aggarwal, S., Rauthan, A. & Warrier, N. Maintenance therapy for newly diagnosed epithelial ovarian cancer- a review. *J. Ovarian Res.* **15**, 88 (2022).
8. Drews, R. M. *et al.* A pan-cancer compendium of chromosomal instability. *Nature* **606**, 976–983 (2022).
9. Macintyre, G. *et al.* Copy number signatures and mutational processes in ovarian carcinoma. *Nat. Genet.* (2018) doi:10.1038/s41588-018-0179-8.
10. Vias, M. *et al.* High-grade serous ovarian carcinoma organoids as models of chromosomal instability. *bioRxiv* 2022.09.01.506155 (2022) doi:10.1101/2022.09.01.506155.
11. Rustin, G. J. S. *et al.* Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed by the Gynecological Cancer Intergroup (GCIG). *Int. J. Gynecol. Cancer* **21**, 419–423 (2011).
12. Martins, F. C. *et al.* Clonal somatic copy number altered driver events inform drug sensitivity in high-grade serous ovarian cancer. *Nat. Commun.* **13**, 6360 (2022).
13. Bhuyan, B. K. *et al.* Comparative genotoxicity of adriamycin and menogarol, two anthracycline antitumor agents. *Cancer Res.* **43**, 5293–5297 (1983).
14. Yi, E., Chamorro González, R., Henssen, A. G. & Verhaak, R. G. W. Extrachromosomal DNA amplifications in cancer. *Nat. Rev. Genet.* **23**, 760–771 (2022).
15. Bakhoum, S. F. *et al.* Chromosomal instability drives metastasis through a cytosolic DNA response. *Nature* **553**, 467–472 (2018).
16. Mutch, D. G. *et al.* Randomized phase III trial of gemcitabine compared with pegylated liposomal doxorubicin in patients with platinum-resistant ovarian cancer. *J. Clin. Oncol.* **25**, 2811–2818 (2007).
17. O’Byrne, K. J., Bliss, T. V. P. & Graham, J. D. A Phase III study of Doxil/Caylex versus paclitaxel in platinum treated taxane naive relapsed ovarian cancer. (2002).
18. Kaye, S. B. *et al.* Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent

ovarian cancer. *J. Clin. Oncol.* **30**, 372–379 (2012).
19. Ferrandina, G. *et al.* Phase III trial of gemcitabine compared with pegylated liposomal doxorubicin in progressive or recurrent ovarian cancer. *J. Clin. Oncol.* **26**, 890–896 (2008).
20. Gordon, A. N., Tonda, M., Sun, S., Rackoff, W. & Doxil Study 30-49 Investigators. Long-term survival advantage for women treated with pegylated liposomal doxorubicin compared with topotecan in a phase 3 randomized study of recurrent and refractory epithelial ovarian cancer. *Gynecol. Oncol.* **95**, 1–8 (2004).
21. Pujade-Lauraine, E. *et al.* AURELIA: A randomized phase III trial evaluating bevacizumab (BEV) plus chemotherapy (CT) for platinum (PT)-resistant recurrent ovarian cancer (OC). *J. Clin. Orthod.* **30**, LBA5002–LBA5002 (2012).
22. Rose, P. *et al.* Phase 3 Study: Canfosfamide (C, TLK286) plus carboplatin (P) vs liposomal doxorubicin (D) as 2nd line therapy of platinum (P) resistant ovarian cancer (OC). *J. Clin. Orthod.* **25**, LBA5529–LBA5529 (2007).
23. Gordon, A. N. *et al.* Recurrent epithelial ovarian carcinoma: a randomized phase III study of pegylated liposomal doxorubicin versus topotecan. *J. Clin. Oncol.* **19**, 3312–3322 (2001).
24. Genome UK: shared commitments for UK-wide implementation 2022 to 2025. *GOV.UK* https://www.gov.uk/government/publications/genome-uk-shared-commitments-for-uk-wide-implementation-2022-to-2025/genome-uk-shared-commitments-for-uk-wide-implementation-2022-to-2025.
25. The Grand Challenge missions. *GOV.UK* https://www.gov.uk/government/publications/industrial-strategy-the-grand-challenges/missions.
26. Data to early diagnosis and precision medicine. https://www.ukri.org/what-we-offer/browse-our-areas-of-investment-and-support/data-to-early-diagnosis-and-precision-medicine/.
27. Our research mission. *Our Future Health* https://ourfuturehealth.org.uk/our-research-mission/.
28. Chen, S. *et al.* A risk model of gene signatures for predicting platinum response and survival in ovarian cancer. *J. Ovarian Res.* **15**, 39 (2022).
29. Weir, B. A. *et al.* Characterizing the cancer genome in lung adenocarcinoma. *Nature* **450**, 893–898 (2007).
30. Buhl, A. S. K., Christensen, I. J., Knudsen, S. & Jensen, P. B. Doxorubicin response prediction in neoadjuvant breast cancer therapy. *J. Clin. Orthod.* **37**, e12119–e12119 (2019).
31. Cleator, S. *et al.* Gene expression patterns for doxorubicin (Adriamycin) and cyclophosphamide (cytoxan) (AC) response and resistance. *Breast Cancer Res. Treat.* **95**, 229–233 (2006).