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Case Report

Functional Precision Medicine Successfully Guides Therapeutic Regimen of 'Cancer of Unknown Primary' Later Classified as Triple-Negative Breast Cancer: A Case Report

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Keywords
Patient-derived organoids · Drug sensitivity test · Drug screening · Unknown primary · Triple-negative breast cancer

Abstract
Introduction: Controlled randomized trials, molecular analytics, and guideline recommendations have so far been irreplaceable tools to ensure appropriate treatment and decision-making for physicians and patients. Individual patient models are increasingly complementing these methods, particularly in the case of advanced cancers, rare cancers, and cancers of unknown primary (CUP), as in these cases comprehensive clinical evidence is unavailable, often resulting in poor treatment success, even after stratification. ***Case Presentation:*** Here we report a 53-year-old patient with CUP with axillary lymph node metastases for whom patient-derived 3D (PD3D®) tumor organoids successfully guided personalized treatment. PD3D tumor models were used to screen drugs that are effective at the suspected primary tumor site. The screen

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revealed sensitivity to doxorubicin, which is not indicated for CUP treatment but hinted toward breast cancer that was subsequently confirmed as triple-negative breast cancer (TNBC). The patient showed partial remission to first-line doxorubicin and cyclophosphamide, which were followed by docetaxel. Subsequent radiotherapy eventually led to a complete remission, which is still ongoing. ***Conclusion:*** We conclude that pre-therapeutic drug sensitivity screening with PD3D tumor models can be essential in guiding and enabling an effective personalized treatment for patients with hard-to-treat cancers, like CUP or TNBC.

Introduction

Cancers of unknown primary (CUP) are defined as confirmed malignant metastatic disease, for which standardized diagnostics were unable to identify the primary tumor site. They account for less than 5% of cancers and are associated with a median survival of ~3 months [1]. In most patients, no primary tumor is identified during the course of the disease, nor is an obvious analogy to a known cancer entity detected. This complicates the choice of therapy for these so-called unfavorable CUP patients, who constitute ~80% of all CUP cases. In general, platinum-based doublet chemotherapy with either a taxane or gemcitabine is recommended as the standard of care (ESMO level of evidence III, grade of recommendation B) [1]. However, to date, no randomized trial has demonstrated statistically significant superior efficacy for any of the protocols studied [1].

Currently used treatment regimens have not been altered by technological advances. Next-generation sequencing (NGS) studies were unable to reveal general molecular targeted therapy strategies, as these studies showed a highly heterogeneous mutational landscape with TP53 as the most common but not actionable genetic alteration [1]. Since there is currently no high-level evidence that gene expression profiling-directed therapies improve patient outcomes, such approaches are not recommended outside clinical trials (ESMO: II, D) [1]. Due to the poor prognosis and the lack of comprehensive clinical evidence, new approaches are warranted to identify effective therapies for CUP patients.

Patient-derived 3D (PD3D®) tumor cell cultures, so-called tumor organoids, are an emerging tool to predict individual drug sensitivity even before treatment is initiated. PD3D models are generated from vital tumor tissue samples of cancer patients and recapitulate more features of the disease than genetics alone. In fact, PD3D models maintain cellular and molecular characteristics of the original tumor including physiological properties and, importantly, drug metabolism [2–5]. Hence, PD3D-based drug screens can be used to identify the individual treatment strategy with the best chance of therapeutic success. Likewise, ineffective treatments can be identified and avoided, along with their potentially harmful side effects.

We report a case of a patient with CUP as an initial diagnosis. Despite extensive primary diagnostics, including positron emission tomography-computed tomography (PET-CT), no primary tumor was detected. Axillary lymph node metastases from a poorly differentiated adenocarcinoma showed a TP53 mutation and moderate PD-L1 expression (combined positive score (CPS) 35), but no targetable genetic modification. To guide therapeutic strategy, PD3D models were generated. The results showed unexpected sensitivity to doxorubicin, which is not recommended for the treatment of CUP, but were consistent with subsequent identification of primary breast carcinoma. Treatment with 4 cycles of doxorubicin and cyclophosphamide and 4 cycles of docetaxel, a standard treatment regimen for breast cancer,

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followed by local and regional radiotherapy, led to complete remission. Due to the involvement of infraclavicular, supraclavicular, and cervical lymph nodes, curative surgery was not possible. The CARE Checklist has been completed by the authors for this case report, attached as online supplementary material (for all online suppl. material, see https://doi.org/10.1159/000538137).

Case Presentation

A 53-year-old white woman presented to our clinic with enlarged cervical lymph nodes (timeline of relevant interventions and outcomes shown in Fig. 1). Clinical staging evaluation with sono-mammography, conventional CT, and PET-CT showed pathologic lymph nodes in the cervical, supraclavicular, infraclavicular, and predominantly in the left axilla (shown in Fig. 2a). No primary tumor could be detected. One axillary lymph node was surgically removed. After histopathologic examination, the disease was classified as CUP with lymph node metastases from a poorly differentiated grade 3 adenocarcinoma.

Immunohistochemistry analyses revealed that tumor cells were negative for bcl2, vimentin, S100, CK5/6, p40, Gata3, estrogen receptor, chromogranin, synaptophysin, CK20, CDx2, and TTF-1. Positive staining reactions were detected for p53, C-Kit, CK7, SOX10, and partially for alpha-Actin. Ki67 proliferation marker was high at about 90%. Immunohistochemical assays for PD-L1 showed a weak and unspecific reaction in only a few tumor cells, corresponding to a tumor proportion score (TPS) < 1%, whereas a larger proportion of surrounding immune cells showed a positive reaction, corresponding to a CPS of 35. Additionally, NGS was carried out, covering a panel of 161 cancer genes. A non-targetable TP53 mutation was detected, while no genetic alterations were found in any of the other genes examined.

At the patient's request, chemosensitivity testing on PD3D tumor models was performed at ASC Oncology. A tissue fragment of the metastatic lymph node was used to generate an individual PD3D model for drug screening. Recommended drugs for the most likely suspected primary tumor sites were used for the sensitivity assay. Test results were available 42 days after sample collection, and a report summarizing the findings was provided to the treating physicians. Of the eight therapy options tested, only doxorubicin showed considerable effects on the tumor cells by reducing cell growth by more than 50% at clinically relevant concentrations (shown in Fig. 3). Because the individual IC_{50} value was below the maximum achievable plasma concentration ($IC_{50} < c_{max}$), doxorubicin had the potential to lead to tumor regression in the patient (shown in Fig. 3b). Gemcitabine and 5-fluoruracil (5-FU) also showed some activity, reducing cell growth by approximately 50%. However, growth reduction never fell below 50%, or growth reduction was only reached at the documented maximum plasma concentration of the drug. Both make it unlikely that there would be a significant reduction of the tumor in the patient but rather stable disease.

Initiation of therapy with doxorubicin was arranged with the patient, a rather uncommon first-line treatment for a CUP diagnosis, but the decision was patient driven. On the first day of doxorubicin monotherapy (60 mg/m^2), a CT scan was performed, as the previous baseline scan had been performed 3 months previously. This CT scan showed a lesion in the left breast, which was then histologically confirmed as the primary tumor site as well as TNBC without PD-L1 expression (immune cell (IC) score = 0). In parallel with the diagnostic procedures, a total of two cycles of doxorubicin were administered. After further discussion of the case at the local tumor board, the patient could be convinced to expand treatment with cyclophosphamide (600 mg/m^2) for two more cycles and then switch to docetaxel (100 mg/m^2) for 4 cycles, followed by extended

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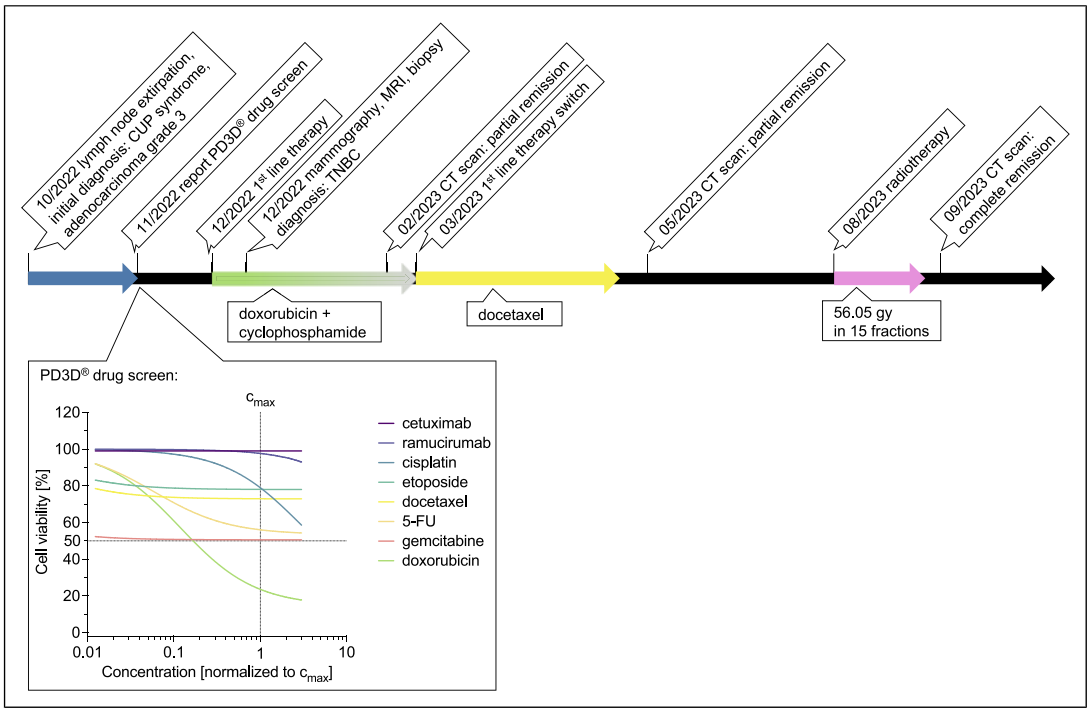

Fig. 1. Timeline of main interventions and outcomes in the patient's medical history.

radiotherapy with a potential chance of long-term remission. The use of immunotherapy was also discussed, but in a formally metastatic situation (positive cervical lymph nodes) with an IC 0, no indication was seen.

CT scans before and after docetaxel treatment showed a very good response (partial remission shown in Fig. 2c, d), so that subsequently, the consolidating radiotherapy of the mamma and the affected lymph nodes was arranged. Due to the involvement of cervical lymph nodes, curative surgery was not possible. About 7 weeks after the last dose of first-line chemotherapy, radiation therapy was given at a cumulative total dose of 56.05 Gy according to the START-B protocol of 40 Gy in 15 fractions over 3 weeks in the breast, plus an extended boost in eight fractions in the originally PET-positive lymphatic drainage extending to the cervical region. A subsequent CT scan showed a complete remission (shown in Fig. 2e). The patient is still alive >12 months after an initial prognosis of 3 months median survival and is planning to re-enter the workforce.

Discussion

The field of functional precision oncology is becoming increasingly important for optimal patient care. This case report demonstrates how personalized in vitro drug screens using individual PD3D tumor models successfully guide first-line therapy in a patient with poor prognosis. In the presented case, screening results provided a rationale for the use of an atypical therapy option, which was later supported by additional diagnostics including imaging, established guidelines and – above all – maximized clinical benefit to the patient. As such, the clinical utility and added value to patients and physicians have been exemplified, especially for those patients for whom neither

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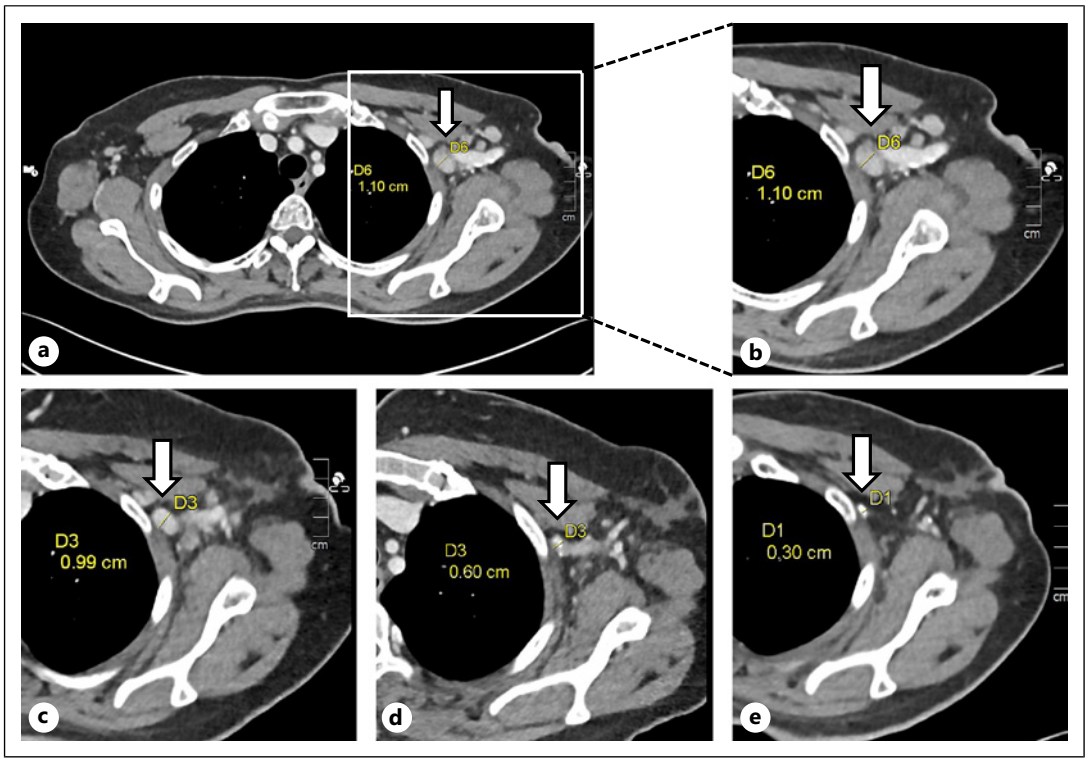

Fig. 2. Computed tomography (CT) scans of an axillary lymph node metastasis in the course of treatment. **a** CT scan at the initial diagnosis. **b** Zoomed section from **a**. **c** CT scan after treatment with doxorubicin and cyclophosphamide, the first half of chemotherapy, showing partial remission. **d** CT scan after treatment with docetaxel, the second half of chemotherapy, showing continued remission. **e** CT scan after radiotherapy showing complete remission.

actionable mutations nor other treatment-related biomarkers are available, as well as for patients with a rare cancer where clinical evidence for a treatment rationale is low or non-existent.

The data which we present are consistent with data collected across numerous co-clinical trials studying patient-derived tumor organoids as predictors of drug response to facilitate clinical decision-making and improve personalized therapy [6–8]. Organoids can be generated from basically all kinds of solid tumors at any time within the course of the disease. In principle, the test poses an n-of-1 clinical trial to determine individual sensitivity to selected treatments and can be integrated into clinical practice before initiating systemic therapy. In combination with other clinical data, results enable the selection of the optimal personalized treatment strategy with the greatest potential for treatment success.

Like any model system, PD3D-based drug tests also have limitations. One challenge we faced in this case was the duration of the testing procedure. Generation and expansion of patient-specific 3D models usually take three to five weeks, depending on the quantity and quality of the available starting material as well as the biology of the tumor. Results may be delayed after the diagnostic evaluation, so therapy may need to be initiated and possibly switched later. At present, testing is not yet available for lymphomas and leukemia.

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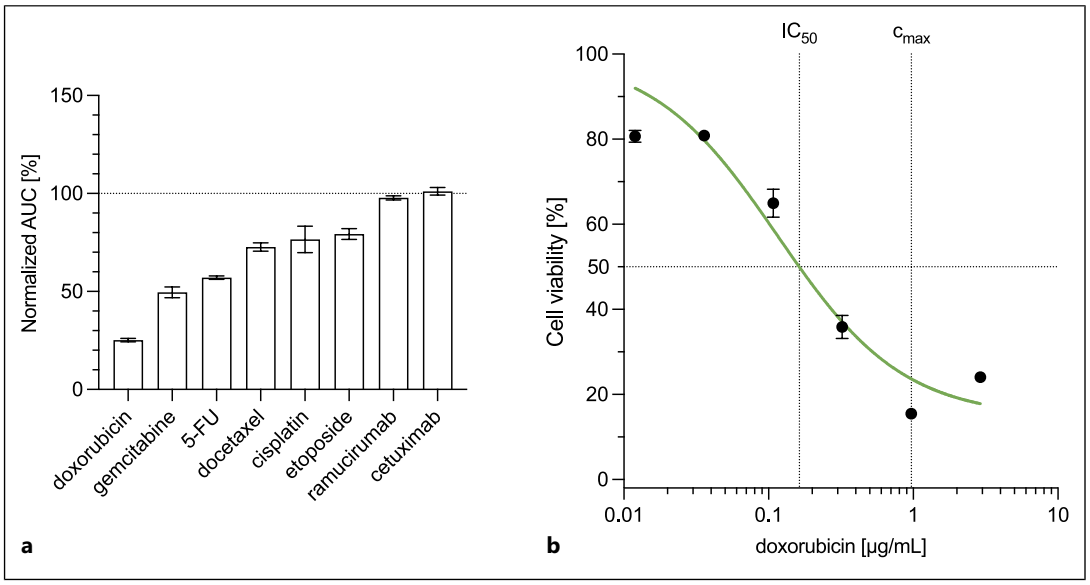

Fig. 3. Results of drug sensitivity testing. **a** Sensitivity profile of PD3D tumor organoids shown as normalized area under the dose-response curves (AUC) of tested drugs. **b** Dose-response curve for doxorubicin. 5-FU, 5-fluoruracil.

Conclusion

We describe a case of a patient with an initial CUP diagnosis that was later reclassified as TNBC. In vitro PD3D drug testing guided personalized treatment as well as the detection of the primary tumor site in this case, enabling optimal individual therapy from the outset. Poor prognosis of both CUP and metastatic TNBC could be exceeded and could result in a complete remission which is so far still ongoing.

Acknowledgments

Foremost, we thank the patient for supporting the scientific progress by releasing her clinical data for this publication. We also thank the staff at the Klinikum Wels-Grieskirchen for providing and caring for this patient and all the other patients. We give a big shoutout to the teams from CELLphenomics GmbH and ASC Oncology GmbH for their dedication to successful screening and ideas. Further, we thank Joris Buiks for providing support in visualization and graphic design. Last, but not least, we thank Marcus Vetter for always sharing ideas and having fruitful discussions.

Statement of Ethics

Ethical approval is not required for this study in accordance with local or national guidelines. The patient provided written consent to the procedure, and all study procedures were performed in accordance with relevant guidelines, such as the Declaration of Helsinki, as well as local regulations. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. No identifiable images or data are included in this report.

Conflict of Interest Statement

C.R.A.R. is the founder and shareholder of ASC Oncology GmbH and CELLphenomics GmbH. M.J.R. is an employee of ASC Oncology GmbH and CELLphenomics GmbH. J.L. and L.W. are employees of CELLphenomics GmbH. L.R. is an employee of ASC Oncology GmbH. All other authors have no conflicts of interest to declare.

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Author Contributions

All authors contributed to the work and have read and approved the final manuscript. Conceptualization and resources: J.H., C.R.A.R., and J.T. Investigation: M.C., S.H., J.L., and J.T. Supervision: J.H., C.R.A.R., M.J.R., J.T., and L.W. Project administration: C.R.A.R., L.R., M.J.R., and L.W. Manuscript preparation: L.R. Manuscript review and editing: J.H., S.H., J.L., C.R.A.R., M.J.R., L.R., and L.W.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author. Sonja Heibl, sonja.heibl@klinikum-wegr.at for clinical enquiries or Christian René Alexander Regenbrecht, christian.-regenbrecht@asc-oncology.com for scientific enquiries.

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