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Case Report

A Case Report of Next-generation Sequencing in a Patient with Carcinoma of Unknown Primary: The First Step in a Tumor Agnostic Approach

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Abstract

Cancer treatment has been revolutionized in the last 10 years. Previously, highly toxic chemotherapy regimens that attack both healthy cells and cancer cells as well as induce significant side effects were used. Nowadays, a more targeted approach is employed. Cancer cells are being treated at the molecular level. Patients with carcinoma of unknown primary who previously only had "broad spectrum" combination therapy as a treatment option can now have their cancer's genome sequenced with next-generation sequencing in a matter of hours and be offered a more targeted approach. Here, we report a case of a patient with metastatic cancer of an unknown primary origin who was progressing on multiple lines of treatment. Next-generation sequencing showed that the patient had a high tumor mutational burden; therefore, he was able to access immunotherapy through a compassionate access scheme, which resulted in a near complete and sustained clinical and radiological response.

Introduction

The treatment of cancer has been revolutionized in the last decade. We have seen a paradigm shift from treating cancer based on the histology and tumor site to a tumor agnostic approach, where we treat cancer based on its genetic makeup. Within the last five years, the Food and Drug Administration in the United States has approved two drugs with tumor agnostic indications. Pembrolizumab has been approved for tumors with DNA mismatch repair deficiency, while Larotrectinib has been approved for all solid tumors with neurotrophic receptor tyrosine kinase gene fusion. A tumor agnostic approach invariably involves looking at the entire genome of the cancer. Comprehensive genomic profiling (CGP) is a next-generation sequencing (NGS) approach that uses a single assay to assess the cancer genome. This approach is still in its infancy as NGS may reveal many targets whose significance is unknown, or we may not have a suitable targeted therapy available for the mutations. Nonetheless, NGS remains an attractive option for those patients who have exhausted standard lines of treatment but still have a good performance status and are keen for more treatment.

A tumor agnostic approach becomes even more relevant in patients who have carcinoma of unknown primary (CUP). Historically, patients with CUP have had poor outcomes. According to statistics provided by the National Cancer Registration and Analysis Service in England between 2012 and 2016, patients diagnosed with CUP had a one-year overall survival of approximately 16%. CUP patients presenting as an emergency with extensive metastatic disease and a poor performance status are more than likely to be untreatable. However, some CUP patients with a good performance status who present with oligometastatic disease and preserved organ function have been noted. This category of patients may be potentially treatable. However, as 20–25% of them have a poorly differentiated carcinoma on histology, they do not always respond well to standard chemotherapy. CGP can potentially provide another avenue of treatment for these patients by highlighting targetable mutations in the cancer genome or giving a tumor mutational burden (TMB), which is the number of non-inherited mutations per million bases of the investigated genomic sequence. A high TMB can predict a higher response rate to immunotherapy and improve survival in this group of patients.[1]

Case presentation

A man in his 50s presented to his general practitioner with an

Keywords: Next-generation sequencing; Tumor agnostic approach; Immunotherapy; Lung cancer; Cancer of unknown primary; Genomic medicine.

Abbreviations: CGP, comprehensive genomic profiling; CT, computed tomography; CUP, cancer of unknown primary; HER2, human epidermal growth factor receptor 2; NGS, Next-generation sequencing; TMB, tumor mutational burden.

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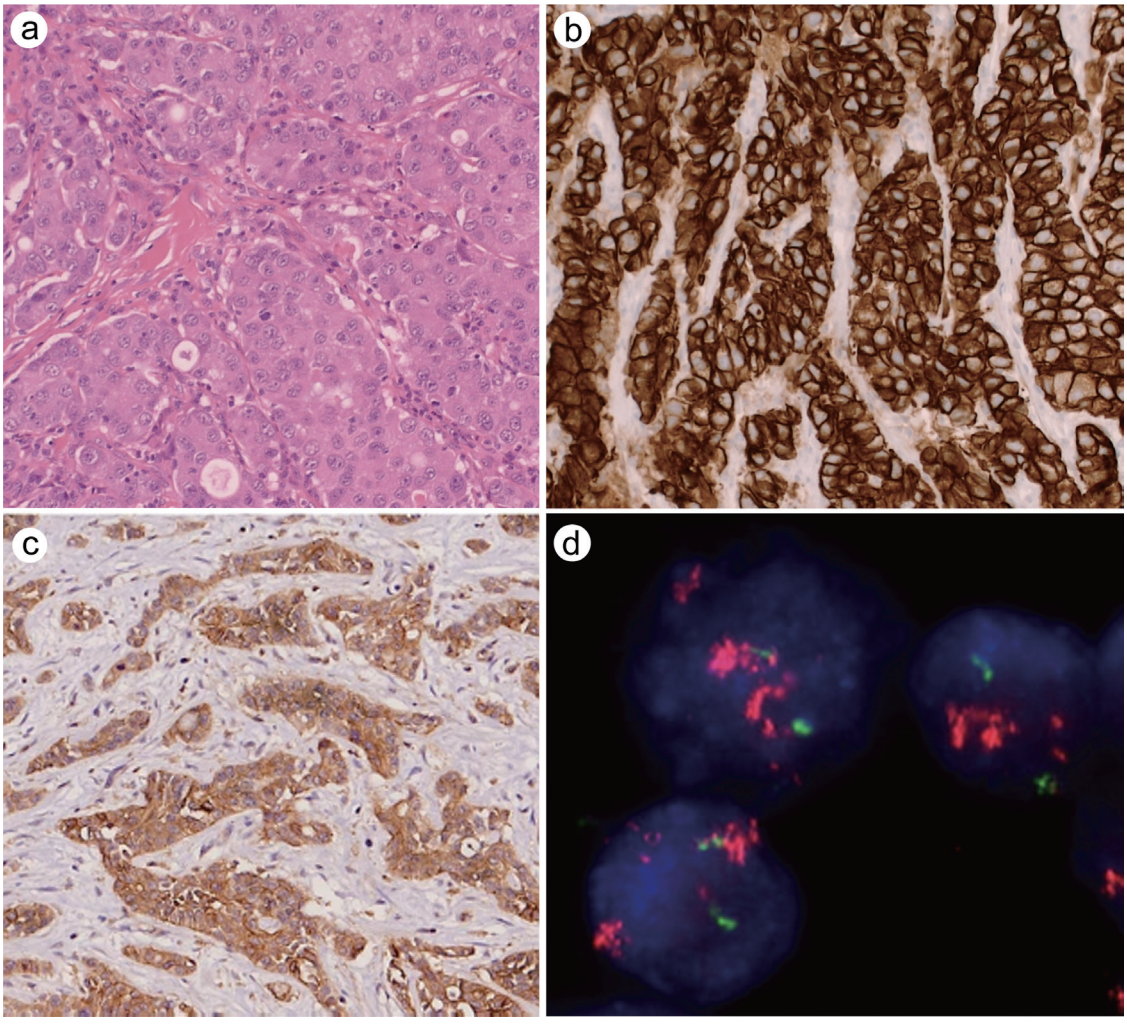

Fig. 1. Histopathology of slides. (a) Hematoxylin and eosin (4×); (b) cytokeratin 7 (10×); (c) cluster of differentiation 58 (10×); (d) human epidermal growth factor receptor 2.

8-month history of a dry cough and an unintentional weight loss of around 5 kg. His Eastern Cooperative Oncology Group performance status was 0. He reported no change in his bowel habits or urinary dysfunction. There was no history of night sweats or fevers, and there was no palpable lymphadenopathy on physical examination. His chest was clear to auscultation, and his abdominal, genital, and skin examination did not reveal any abnormality. He also had a digital rectal examination, which showed a normal-sized prostate and no other abnormality. He was a nonsmoker and a social drinker of alcohol. He lived with his wife and had no significant past medical history or drug allergies. There was no relevant family history of cancer.

His general practitioner referred him to the local hospital for a chest X-ray, which showed some widening of the paratracheal stripe. This in itself was nonspecific but given the patient's symptoms, he was referred for a computed tomography (CT) scan of the chest. This showed prevascular and right peritracheal lymphadenopathy, with a 26-mm right pretracheal lymph node. The mediastinal, hilar, and infrahilar lymph nodes were not enlarged. There was no evidence of a primary tumor seen on the scan. Tumor markers assayed at the time, including carcinoembryonic antigen and carbohydrate antigen 19–9, were within the normal range. His case was discussed at the local lung multidisciplinary team meeting. It was suggested that he undergo an endobronchial ultrasound to biopsy his enlarged lymph nodes. The lymph node biopsy showed a poorly differentiated adenocarcinoma. The immunohistochemistry results revealed positive staining for cytokeratin 7 and cluster of differentiation 58 as well as negative staining for thyroid transcription factor 1, cytokeratin 20, and caudal type homeobox 2. In addition, positive human epidermal growth factor receptor 2 (HER2) was observed by fluorescence *in-situ* hybridization (Fig. 1). The immunohistochemistry findings suggested an adenocarcinoma of upper gastrointestinal origin.

The patient underwent a gastroscopy, which did not reveal any abnormality. His case was then referred to the CUP multidisciplinary team, who advised a positron emission tomography scan as part of the CUP work-up. The positron emission tomography scan showed an avid right supraclavicular lymph node (maximum standardized uptake value of 16.2) and foci of mediastinal nodes consistent with malignancy, but no primary tumor was identified. He was commenced on chemotherapy with Carboplatin (area under the curve of 5) and Paclitaxel (175 mg/m^2) every three weeks as per the standard regimen for CUP adenocarcinomas. Unfortu-

nately, the patient had a severe anaphylactic reaction to Paclitaxel (hypotension, hypoxia tachycardia, and bronchospasm) during his third cycle, and the decision was made not to rechallenge. A CT scan done after three cycles of chemotherapy showed a partial response to his mediastinal lymphadenopathy, with no evidence of distant metastatic disease. He was referred to a radiation oncologist for consideration of radiotherapy for his thoracic disease. He received 55 Gy in 20 fractions over four weeks. Unfortunately, a restaging scan performed at the end of treatment showed new mesenteric lymph nodes and a subcutaneous mass in the posterior left flank measuring 1.5 cm × 2 cm. The thoracic lymphadenopathy did show a partial response to radiotherapy.

Although fatigued after his radiotherapy, the patient still had a good performance status and was keen on further treatment. As the original biopsy was consistent with an upper gastrointestinal malignancy that was HER2 positive, he was started on a new line of chemotherapy with a bolus of 5-fluorouracil (400 mg/m^2), an infusion of 5-flurouracil (2,400 mg/m^2), folinic acid (400 mg/m^2), and oxaliplatin (85 mg/m^2), and intravenous trastuzumab (6 mg/kg loading followed by 4 mg/kg) every two weeks. His pretreatment echocardiogram showed a normal left ventricular function with an ejection fraction of over 60%. He tolerated this regimen well and received five cycles. A CT scan performed after five cycles, however, showed progressive lymphadenopathy on both sides of the diaphragm.

This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

Investigations

The patient had now progressed on two lines of systemic chemotherapy as well as radiotherapy. His performance status remained quite good, and he was keen to explore more treatment options. At that time, we could access a Foundation One® CGP panel on compassionate access. The results of the Foundation One ® panel were as follows: high TMB (28 mutations/megabase), indicating that atezolizumab, nivolumab, pembrolizumab, avelumab, and durvalumab were possible treatments; HER2 positive, indicating that lapatinib, trastuzumab, pertuzumab, trastuzumab emtansine and trastuzumab deruxtecan were possible treatments; loss of the serine/threonine kinase 11 gene, indicating that everolimus and temsirolimus were possible treatments; and amplification of rapamycin-insensitive companion of mammalian target of rapamycin, which did not have any approved therapies and clinical trials are ongoing.

At that time, there were no suitable clinical trials open in Ireland, and the patient did not want to travel abroad. The patient had already progressed on HER2-directed therapy. Immunotherapy was also not licensed for cancers showing a high TMB. Atezolizumab had recently been approved as a second-line therapy in patients with metastatic non-small cell lung cancer in the OAK trial. We were fortunate enough to access this drug from the company on a compassionate access scheme.

Treatment

The patient was commenced on atezolizumab immunotherapy (1,200 mg every three weeks). He tolerated the treatment, without any major side effects. His fatigue started to improve, and he started putting on weight again. A CT scan done after three months of treatment showed stable appearances. It has now been 20 months since the patient started atezolizumab, and he has had stable scans since then.

Outcome and follow-up

The patient will continue on atezolizumab, with restaging scans performed every six months to monitor any progression. There are no data to suggest how long immunotherapy should be continued in these patients as they are responding to treatment. The current consensus is to continue treatment until disease progression or unacceptable side effects.

Discussion

Worldwide efforts to catalog mutations in multiple cancer types are underway, and this is likely to lead to discoveries that will be translated into new diagnostic, prognostic, and therapeutic targets.[2] We are in an age of precision oncology. “Broad spectrum” combination chemotherapies are being used less frequently as we are now in a position to target cancer cells at the molecular level.

According to the National Human Genome Research Institute, the original Human Genome Project took 13 years to complete at an estimated cost of USD 2.7 billion. Now, Genome sequencing costs £6,841 per cancer case (comprising matched tumor and germline samples) and £7,050 per rare disease case (three samples).[3] The sequencing of genes at an affordable cost and the ability to look for targetable mutations has compelled us to use a bespoke cancer management strategy rather than a one-size-fits-all strategy.

At the end of 2019, there were 64 anticancer therapies that targeted a molecular alteration, and there were 24 targetable molecular alterations. It is very relevant that in 19 of these, the detection of the alteration was required to effectively indicate a particular prescription.[4] CGP may likely become part of the cancer workup protocols. Liquid biopsies will become indispensable for the further development and use of these targeted therapies. This will allow for constant monitoring, pretreatments, and post-treatments; perhaps, they will indicate the prognosis.[5]

CUP accounts for approximately 3% of all malignant neoplasms; therefore, it is one of the ten most frequent cancer diagnoses.[6] CUP can be defined as an epithelial or neuroendocrine malignancy based on histology, with no primary site identified despite initial investigations, specialist review, and selected specialist investigations as appropriate. It can be useful to divide CUP into prognostic groups based on the clinical and pathological features on presentation.

The prognosis is good if any of the following conditions are met: single operable metastatic site; axillary nodes in females (adenocarcinoma), peritoneal disease in females (serous or papillary histology), or bone disease in men (high prostate-specific antigen level); extragonadal germ cell syndrome (midline/retroperitoneal disease with raised alpha-fetoprotein or human chorionic gonadotropin levels. Meanwhile, the prognosis is intermediate if there is metastatic disease but preserved organ function or a good performance status for systemic anticancer treatment. Furthermore, the prognosis is poor if there is metastatic disease with compromised organ function; a poor performance status with premorbid frailty; or emergency admission with cancer symptoms as the first presentation.

Metastatic CUP has limited effective therapeutic options, given its phenotypic and genotypic diversity.[7] However, these patients are now seeing some hope with CGP. In a study of 200 tumors from patients with CUP, analysis using the FoundationOne® NGS assay revealed that 169/200 had potentially actionable mutations.[8]

Patients with a high TMB are predicted to be sensitive to immunotherapies.[1] Therefore, the TMB may be a biomarker for sensitivity to immunotherapy, irrespective of the tumor type (tumor

agnostic approach). The TMB has not been explored widely for tumors of unknown primary sites, but it may reveal additional treatment options.[9] A recent phase IIa multiple basket study looked at atezolizumab for the treatment of patients with a high TMB. Atezolizumab monotherapy had promising, durable clinical activity across a variety of advanced solid tumor types in patients with a TMB of ≥16 mutations/megabase, tumors lacking other suitable treatment options, and who were immunotherapy-naive at enrolment, regardless of their microsatellite instability status. Limited activity was observed in tumors with TMB ≥10 and <16 mutations/megabase.[10]

A tumor agnostic approach has shifted the focus from identifying the primary site of cancer to detecting cancer biomarkers and targetable mutations. As we learn more about the genomics of cancer, NGS will soon become an indispensable tool not just for CUP but for all malignancies.

Conclusion

NGS is rapidly becoming the standard of care in patients with CUP. It can also provide new avenues of treatment for patients who have exhausted standard lines of treatment. It should be considered early in the diagnostic pathway for patients with CUP or when patients are nearing the end of their standard lines of treatment.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Lead author responsible for writing the manuscript (TK), obtained consent (GC), performed the literature review (GC, AA), and overall review of the manuscript (AY).

Ethical statement

This study was performed in accordance with the Declaration of Helsinki (as revised in 2013). Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

Data sharing statement

All data related to this article are published together with the article.

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