Cancer of unknown primary and BRAF V600E meeting the BEACON combination: A case report

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Abstract. The diagnostic work-up of cancer of unknown primary (CUP) is a challenging task; in addition, only a little data on BRAF targeting in CUP are currently available. Traditionally, the identification of favourable and unfavourable CUP subsets directs the choice of treatment. The present article reports the case of a 50-year-old male patient presenting with a BRAF-mutated CUP, a rare and generally unfavourable subset. Based on imaging, immunohistochemistry and a high value of carbohydrate antigen 19-9, an upper gastrointestinal profile was initially presumed. After disease progression on treatment with a first-line platinum-based doublet chemotherapy, a significant response was documented after treatment with the BEACON combination. The present case report highlighted the paradigm shift in diagnosis and treatment of CUP from a histology-based approach to molecular profiling with the introduction of precision medicine.

Introduction

Cancers of unknown primary (CUP) are a heterogeneous group of metastatic tumours in which no primary tumour site can be identified at time of diagnosis, despite extensive clinical and pathological investigations. The incidence of CUP has decreased from around 3-5% in the 1990's to 1-2% in the current era, as a consequence of technological developments in the diagnostic field. They most commonly present with metastasis in lymph nodes, lung, liver or bone. The majority of CUP patients (80%) do not respond well to chemotherapy and therefore have a poor prognosis, with a median overall survival of 6-10 months. The origin of CUP and their biological characteristics remain poorly understood in the current clinical practice (1-4).

In the diagnostic work-up of a potential CUP, clinical practice guidelines suggest thorough investigations which include clinical evaluation, physical examination, biochemical analyses and radiological tests. Specific further analysis can be warranted based on metastatic pattern, clinical and biochemical information. Lastly, immunohistochemical (IHC) testing should be performed, as it is the most important tool in search for the tissue of origin. If after all these investigations the primary tumour remains unknown, a diagnosis of CUP is confirmed (2,4).

Since no site-specific therapies are appropriate in the treatment of CUP, management is mainly based on clinical and immunohistochemical characteristics. Unfortunately, these treatments often result in a modest response rate. Either an indication of a site of origin or access to personalized medicine may assist in the choice of treatment, potentially improving the prognosis of these patients. More recently, molecular diagnostics have been proposed to guide treatment, based on the assumption that CUP respond similarly to treatment as their predicted primary tumours. However, up until now, no differences in outcome between the empirical and molecular-guided treatments have been reported in CUP (1-4).

With this case report, we want to point out the importance of molecular profiling on top of the use of tumour markers and immunohistochemistry in the management of CUP.

Case presentation

A 50-year-old Caucasian male presented in February 2022 to the department of gastroenterology of the AZ Sint-Jan hospital in Bruges (Belgium) with a vague upper abdominal discomfort for several months, recently evolving to postprandial stabbing pain and night sweats. A minor loss of body weight of 1.5 kilograms (87.5 kilograms for a body length of 172 centimetres) was noted without anorexia. Four months earlier a small tubular adenoma of the sigmoid colon was resected during an otherwise normal colonoscopy. The patient had no significant medical history except symptomatic hemorrhoidal disease treated with bandligation a few years earlier, furthermore no allergies, no prior hospitalizations and a negative familial cancer history.

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Clinical examination revealed no abnormalities except for hepatomegaly and a prominent right axillary lymph node. Blood analysis showed mildly raised values of C-reactive protein (CRP 40 mg/l, reference <5.0 mg/l), lactate dehydrogenase (LDH 300 U/L, reference ≤250 U/L) and liver enzymes. Carcino-embryogenic antigen (CEA) was normal, but a remarkably high cancer antigen 19-9 level (CA19-9 14,968 kU/L, reference ≤34 kU/L) was noted. Computed tomography (CT) scan identified diffuse liver and lung lesions beside enlarged axillary, infradiaphragmatic and hilar lymph nodes (Fig. 1). Additional metabolic imaging with ^{18}FDG PET-CT revealed a diffuse metastatic spread with involvement of the kidneys, adrenal glands, striated muscle and bone but no primary tumour site was revealed (Fig. 2). The pathological examination of a biopsied liver lesion (Fig. 3) and enlarged right axillary lymph node (Fig. 3) revealed the same histologic morphological image, both suggestive for an adenocarcinoma. Therefore IHC staining was only performed on the liver biopsy specimen to reserve enough tissue of the axillary lymph node to perform the next generation sequencing (NGS). Based on the IHC of the liver biopsy (CK7+ / CK19+ / CK20-/ TTF1-/ SATB2 +/-) a gastrointestinal or hepatobiliary origin, MMR proficient, was suspected. Molecular profiling by DNA NGS on the axillary lymph node revealed BRAF V600E mutation (p.Val600Glu/c.1799T>A) as driver mutation, furthermore RAS and PIK3CA wild type. RNA NGS was not performed by our pathologists, because a driver mutation was found by DNA NGS. Upper endoscopy and endoscopic ultrasound of the upper abdomen (including oesophagus, stomach, proximal duodenum, pancreas and biliary tract) did not reveal a primary tumour. Bronchoscopy was not performed as no primary tumour of the lungs was suspected.

A Discover MI 15 cm axial field-of-view PET/CT (GE Healthcare, Milwaukee, USA) was used for imaging. Standard protocols are followed: patients fasted for at least 4 h before 18F-FDG injection, blood glucose below 200 mg/dl before injection, amount of tracer was based on body mass index and the weight of the patient and was administered intravenously under standard conditions. PET imaging was started 60 min after injection, patients were positioned in the scanner with their arms raised. PET consisted of 7 to 9 bed positions of 1.5 min duration each, from the skull vertex to the mid-thigh. Diagnostic CT-scan was used for attenuation correction (120 keV, smart mA, intravenous contrast, metal artefact reduction). The reconstruction of PET images was made by VPFX-S (GE Healthcare; time-of-flight, point spread function correction, OSEM).

For immunohistochemistry analysis (IHC), the BenchMark ULTRA IHC/ISH system (Roche Diagnostics) is used for CK7/CK19/CK20/SATB2/TTF1 analysis, making use of the UltraView DAB procedures (v1.02.0018). The procedure for HE colouring is executed on the Tissue-Tek Prisma Plus machine (Inventory number/SOP: AP03.03.36/T03.03.13). Details of the corresponding protocols are listed in appendix.

The NGS analysis was performed in our patient using the TSO500 panel (Illumina), following the BALLETT-Belgian Approach for Local Laboratory Extensive Tumour Testing-study protocol (https://clinicaltrials.gov/study/NCT05058937). The targeted NGS panel of 523 genes allows for the detection of single nucleotide variants, small indels, copy number variations and fusions, as well as for the determination of the 'tumour mutational burden' (TMB) and the 'microsatellite-instability' status (MSI). The DNA NGS data were made available in a publicly curated EGA-database (European Genome-Phenome Archive), reference code ID EGA50000000689 (URL: https://ega-archive.org/datasets/EGAD50000000689).

After discussion in the multidisciplinary team (MDT), a first-line combination chemotherapy with gemcitabine and cisplatinum three-weekly (gemcitabine 1,000 mg/m^2 and cisplatin 20 mg/m^2, both on day 1 and day 8 of the treatment cycle) was proposed based on the serum markers, immunohistochemical characteristics and no primary tumour site was found despite extensive diagnostic work-up. The treatment was generally well tolerated. The first cycle was complicated by a thrombosis of the right axillary and subclavian vein, treated with low molecular weight heparins. Evaluation after 3 treatment cycles (June 2022) showed mixed response on ^{18}FDG PET-CT with progression of the liver and bone lesions and regression of the metastases in muscle, lung and lymph nodes. The CA19-9 level had risen to 99,366 kU/L.

In regard of the molecular profile, a second-line treatment with combination of encorafenib, a BRAF inhibitor (300 mg daily oral dose) and cetuximab, an anti-EGFR (400 mg/m^2 at the start, followed by 8 times 250 mg/m^2 weekly and ultimately by 400 mg/m^2 every two weeks for another 7 times) was proposed. The therapy was very well tolerated by the patient, with a clear improvement of the general condition and fatigue. CA 19-9 level decreased to 16,594 kU/L after 3 weeks (July 2022) and further to 4,153 kU/L another 3 weeks later (July 2022). Moreover, a significant response in all lesions was documented on ^{18}FDG PET-CT seven weeks after the start of the second-line therapy, in August 2022 (Fig. 4).

Eight weeks later (November 2022) an asymptomatic rise of CA 19-9 occurred to 21,785kU/L, more than doubling after another 6 weeks (December 2022). A new ^{18}FDG PET-CT revealed a dramatic tumour progression with growth of the existing lesions as well as the appearance of some new metastases (Fig. 5). Therapy was switched to third-line mFOLFOX and bevacizumab (oxaliplatin 85 mg/m^2 day 1, folinic acid 400 mg/m^2 day 1, fluorouracil 400 mg/m^2 day 1 + 2400 mg/m^2/48 h, bevacizumab 5 mg/kg day 1). Only ten days later (December 2022) the patient was hospitalized with acute respiratory distress caused by a combination of bilateral pulmonary infiltrates, pulmonary embolism and bilateral pleural effusion. In the meantime the CA 19-9 level had doubled again. Ultimately the patient died three days later, on his request supported by palliative sedation.

Discussion

Cancer of unknown primary (CUP) is defined as a group of metastatic tumours without an apparent primary site despite extensive diagnostic effort. It is characterized by early metastatic spread and an aggressive course of the disease (2,4).

There are two known subsets of CUP: a favourable one, with similar treatment and prognosis as those metastatic cancers with a known primary tumour site, and an unfavourable one, mostly empirically treated with combination regimens, resulting in a generally poor response and patient survival.

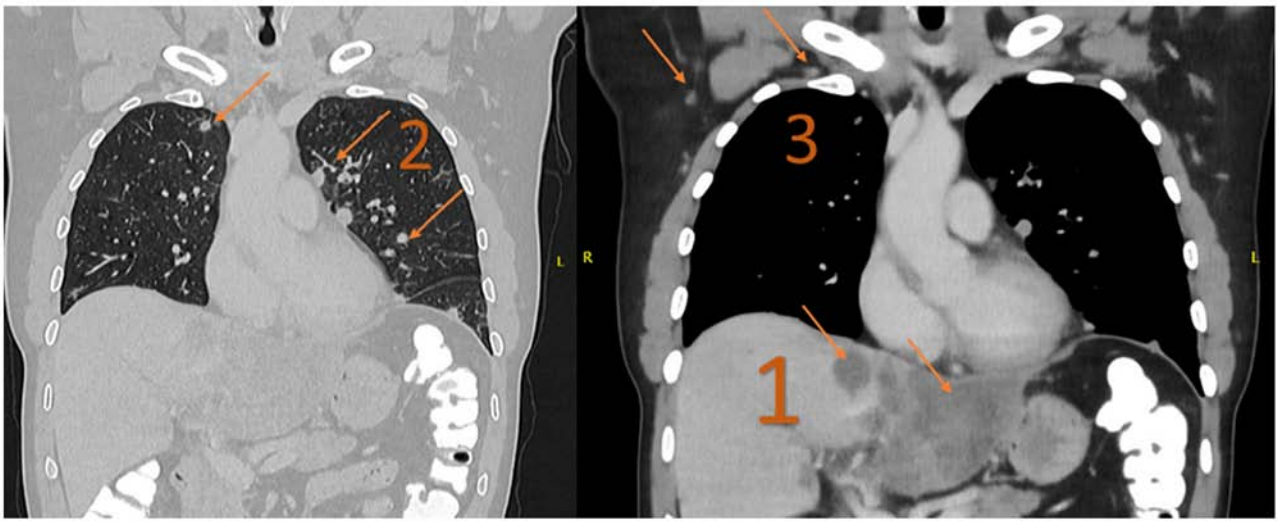

Figure 1. Initial CT images showing diffuse liver (1) and lung (2) lesions beside enlarged axillary, infradiaphragmatic and hilar lymph nodes (3).

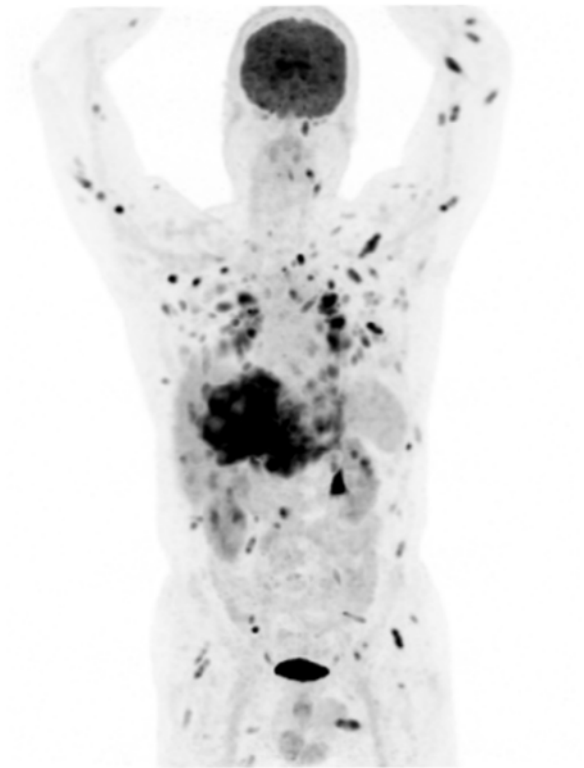

Figure 2. 18FDG positron emission tomography-computed tomography at diagnosis unveiling metastatic spread to the kidneys, adrenal glands, striated muscle and bone besides no suggestion of a primary tumour.

Some particular subsets such as colorectal, lung and renal CUP profiles have been identified (1,2). It has been hypothesized that CUP also possess two distinct genetic signatures: a first one for its primary site and a second primary-independent one with usually pro-metastatic characteristics, responsible for the different natural history. Moreover, a unique pattern of molecular abnormalities has been reported in almost 90% of patients (5,6). Potential characteristics of CUP are chromosomal alterations, self-sufficiency in growth signals, resistance to growth-inhibitory signals, reprogramming of energy metabolism, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion, metastasis and evasion of immune destruction (5).

In the diagnostic process, an accurate work-up is needed which consists of imaging followed by immunohistochemistry (IHC), serum tumour markers and molecular profiling with the focus on identification of specific subsets to direct the choice of treatment (6-10).

In patients with CUP, serum epithelial tumour markers are often overexpressed in a non-specific and non-sensitive way. On the other hand they are readily available and may help to narrow down the differential diagnosis (9,11-14). Especially CA19-9, a cell surface glycoprotein complex, is overexpressed in benign as well as malignant disorders including cancers of the pancreas, the biliary tree, the liver, the gastrointestinal tract, the lungs, the urogynaecological system, the thyroid and the salivary glands (9). CA19-9 expression requires a Lewis gene product, found only in patients with Le (a-b+) or Le (a+b-) blood groups. During the carcinogenesis, hematogenous metastasis is facilitated through progressive predominance of sialyl Lewis-a, being the result of an epigenetic process (9). In 2020 an intriguing high CA19-9/CEA ratio, as in our patient, was described in patients with BRAF V600E mutated MSS colorectal cancer (15).

The immunohistochemistry (IHC) testing goes through a systematic and stepwise approach. The basic IHC panel defines the cancer cell lineage. The second step identifies the subtype of carcinoma. The third step, as in our patient, predicts the primary site of adenocarcinoma based on a combination of markers such as CK7, CK20, CDX2, TTF1 and PAX8 amongst others. In particular, the differential expression of cytokeratin 7 and 20 (CK7, CK20) is extremely useful but the patterns are not absolute in the characterization of epithelial neoplasms (7). Moreover, basing the treatment regimen on the non-specific overexpression of CA19-9 can be misleading, as was the case in our patient. Nevertheless, intrahepatic cholangiocarcinomas are often misdiagnosed as CUP which emphasizes the importance of an accurate diagnostic work-up (16).

Molecular profiling has led to an individualized approach based on oncogenic drivers, thereby gradually replacing

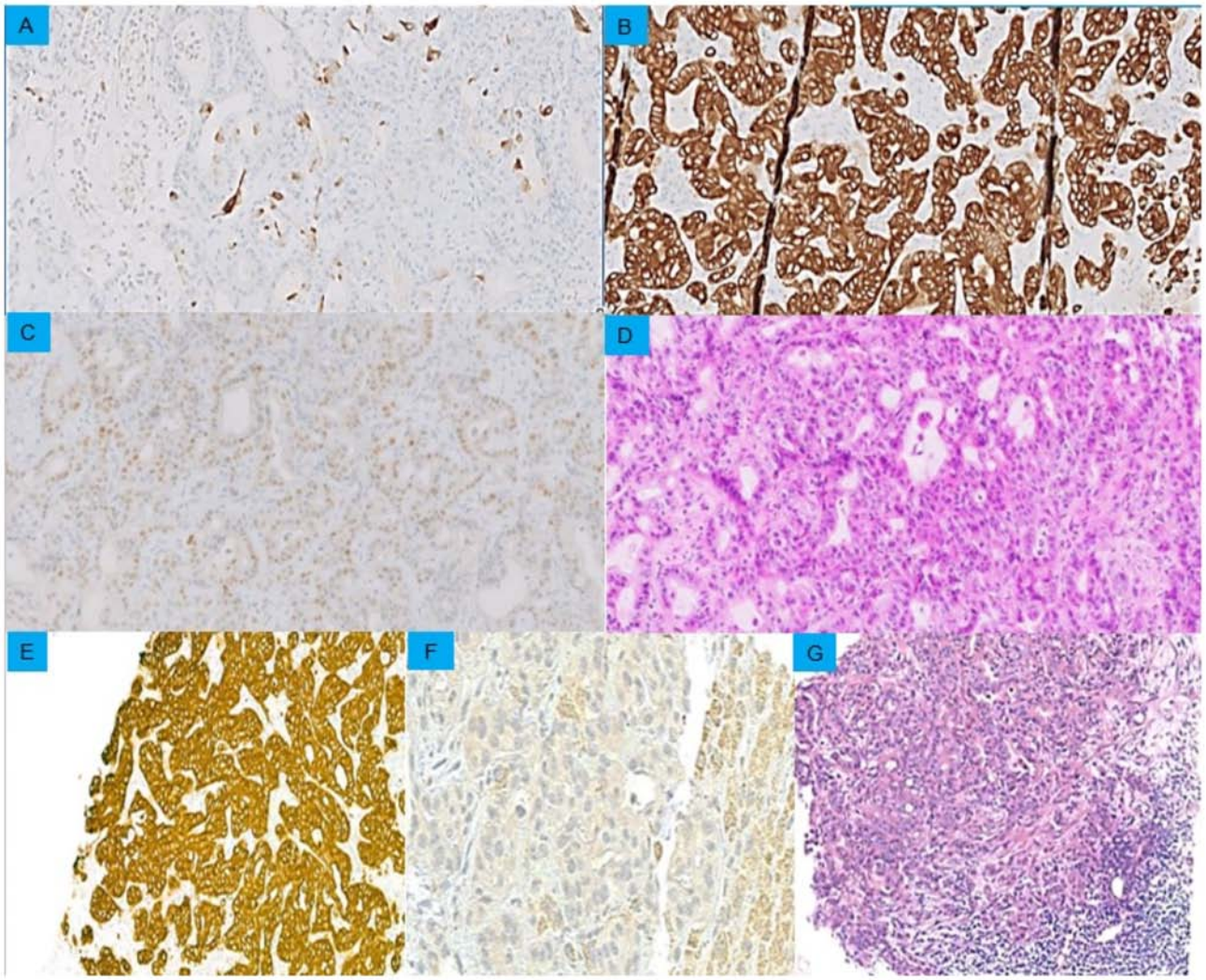

Figure 3. (A) CK20 negative stain on liver biopsy specimen. The gland ducts are negative; some individual cells stain positively but the bulk of the tumour is negative (magnification, x10; 0.473 mm²; 320˚). (B) CK7 positive stain on liver biopsy specimen (magnification, x10; 0.473 mm²; 30˚). (C) SATB2 shows weakly positive staining on liver biopsy specimen (magnification, x10; 0.473 mm²; 320˚). (D) Liver biopsy specimen indicating infiltration by an adenocarcinoma G2 on HE (magnification, x10; 0.473 mm²; 30˚). (E) CK19 positive stain on liver biopsy specimen (magnification, x1; 0.200 mm²). (F) TTF1-negative tumour on liver biopsy specimen, with liver mucosa as background. Right side: The liver has a non-specific brown granulation; left side: The tumour is negative, because the nuclei do not stain (magnification, x10; 0.050 mm²). (G) Axillary node biopsy specimen shows infiltration by an adenocarcinoma G2 on HE (magnification, 5x; 0.200 mm²). HE, haematoxylin and eosin; CK, creatine kinase; SATB2, special AT-rich sequence-binding protein 2; TTF1, Thyroid Transcription Factor 1.

standard combination chemotherapies. The oncogenic driver BRAF V600E (p.Val600Glu/c.1799T>A), accounting for 90% of all activating BRAF mutations, is responsible for the activation of endogenous kinase activity which results in cell proliferation, invasion and spreading. This particular mutation is generally associated with a poor patient prognosis. To date, the predictive role of BRAF mutations on anti-EGFR agents remains unclear, based on differing conclusions from two separate meta-analyses (17-20).

In our patient, both histological features and IHC profile (CK7+ / CK19+ / CK20-/ TTF1-/SATB2 +/-) were suggestive for an adenocarcinoma, without determining a specific primary tumour location (Fig. 3). HE showed infiltration by atypical cells with pleiomorphism and mitoses, forming trabeculae and acinar structures with focal necrosis. CK19 positivity lead to suspicion of a gastro-intestinal or hepatobiliary origin. Based on the undetermined SATB2 and negative CK20, a tumour origin in the lower gastrointestinal tract was less likely. The high serum CA19.9 was more in favour of a hepatobiliary origin. Unfortunately based on the histological, morphological and immunohistochemical findings alone, no clear differentiation between an adenocarcinoma of the higher intestinal tract, the pancreas or cholangiocarcinoma was possible.

Differentiation with an intrahepatic cholangiocarcinomas is difficult, because these tumours have no specific markers. Histologically, they are classically characterized by infiltrating well-formed cribriform glandular tubes and fibrous stroma. The glandular tubes (like any adenocarcinoma) are bordered by cells with atypia of the nuclei and pleomorphism of the nuclei (21).

Also extensive clinical and radiological information did not help to find the primary tumour in our patient. As a consequence, no clear primary tumour origin but also no favourable CUP subset (e.g. colon-like CUP) could be determined despite elaborate work-up. Moreover, the presence of a poor prognostic BRAF mutation lead to the decision of the

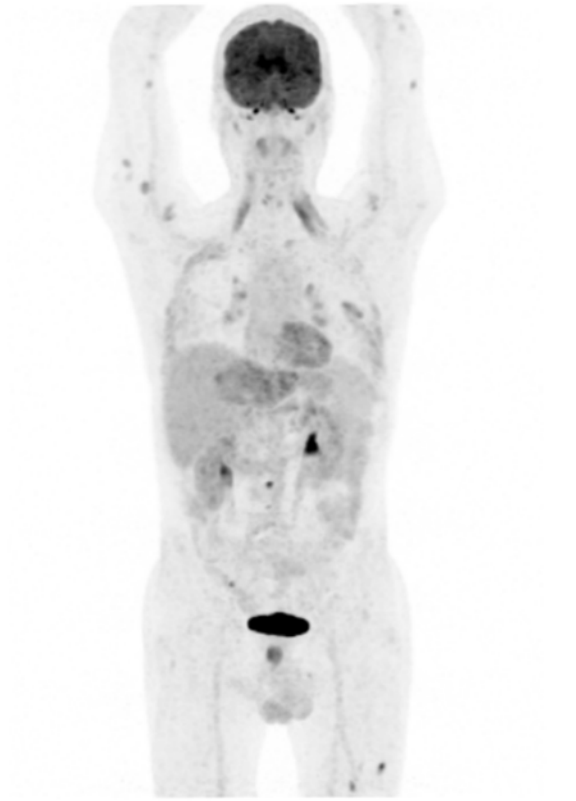

Figure 4. Significant response in all lesions on 18FDG positron emission tomography-computed tomography after 7 weeks of treatment with the BEACON combination encorafenib-cetuximab.

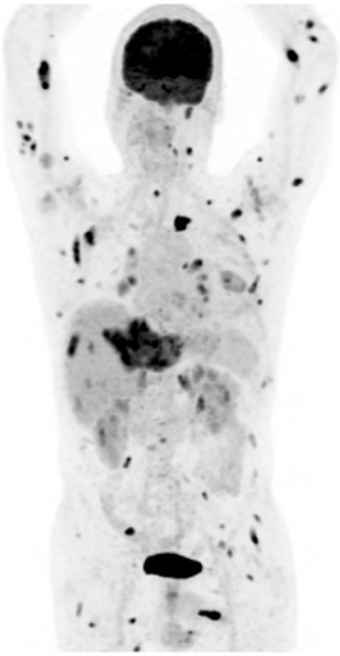

Figure 5. Progressive disease on 18FDG positron emission tomography-computed tomography with new metastases in liver, muscle, ribs and pleural invasion with pleural effusion after 21 weeks of treatment with the BEACON combination encorafenib-cetuximab.

MDT to treat the tumour as an unfavourable CUP. Along the ESMO guidelines, a platinum-based doublet chemotherapy is accepted as the gold standard, thus combination therapy with cisplatinum and gemcitabine was chosen as first-line treatment regimen (4).

After disease progression, second-line treatment with a BRAF inhibitor (encorafenib) together with an anti-EGFR monoclonal antibody (cetuximab) was chosen: the so-called BEACON combination. BEACON was a randomized phase 3 trial investigating the use of a third-generation RAF inhibitor, encorafenib, combined with an EGFR inhibitor, cetuximab, with or without a MEK inhibitor, binimetinib, in comparison to standard chemotherapy plus cetuximab in 665 patients with BRAF V600E-mutant metastatic colorectal cancer whose disease had progressed following one or two prior regimens. The median overall survival was 9.0 months in the triplet-therapy group with a response rate of 26%, compared to respectively 5.4 months and 2% in the control group (17,18). Research of the literature did not reveal other indications beside of CRC, in which this combination therapy was used. The treatment choice in our case report got sealed by a prompt and significant, though only temporarily, treatment response. The rapid progression is most suitably explained by the secondary overexpression of the PI3K/Akt/mTOR pathway, which is stated in the literature as an unfavourable prognosticator in CUP (6,19).

There is a strong current evidence that different tumours with BRAF mutations, such as intrahepatic cholangiocarcinomas, are good targets for BRAF/MEK pathway inhibitors. Together with the strong positive response to a tumour-type agnostic 'targeted' therapy in this patient, it highlights the importance of molecular profiling in the management of CUP. In parallel with the growing insight into the molecular mechanisms of cancer, the replacement of the histological cancer classification by a molecular one is to be expected. More emphasis on a tissue-agnostic approach in medical oncology may be the ultimate consequence (19,22).

In conclusion, this case report is the first - to our knowledge - to implement treatment with the BEACON regimen for CUP, therefore emphasizing the importance of using all the current available tools in the diagnostic work-up of CUP: anatomic and metabolic imaging, serum tumour markers, immunohistochemistry and in particular molecular profiling via next-generation sequencing. NGS forms the base for a tailored and individualized therapy, skipping empirical first-line chemotherapy in favour of a tumour-type agnostic treatment. Further investigation of the underlying hallmarks of CUP may lead to the identification of new treatment targets. In parallel with a progressive dismantling of the unfavourable subsets and a reduction of the percentage of real CUP by technological evolution, a paradigm shift is to be expected from a tissue-gnostic to a tissue-agnostic and customized approach, based on a molecular rather than a histological cancer classification.

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Availability of data and materials

DNA NGS results used in this case report were made available in a publicly curated European Genome-Phenome Archive (reference code ID, EGA50000000689; https://ega-archive.org/datasets/EGAD50000000689). The NGS data mentioned in this case report are part of the database of the Belgian Approach for Local Laboratory Extensive Tumor Testing study, a nation-wide study measuring the impact of comprehensive genomic profiling on access to - and uptake of - personalized medicines and on clinical outcomes for patients across Belgium. Both the wet lab execution of the comprehensive genomic profiling and the biological-clinical classification of the variants were performed in a fully standardized way among the 9 participating Belgian local NGS laboratories (ClinicalTrials.gov identifier, NCT05058937; study ID no., BSMO2020-2; https://classic.clinicaltrials.gov/ct2/show/NCT05058937).

Authors' contributions

All authors contributed to the article. VDW and MM designed the study. LV was responsible for data collection and analysis with a focus on histopathology. AVDE was responsible for data collection and analysis, with a focus on radiological imaging. VDW and MM were responsible for data collection, analysed the literature and drafted, edited and reviewed the manuscript. VDW and MM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Written informed consent for publication of the data and accompanying images was given by the patient.

Competing interests

The authors declare that they have no competing interests.

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