Pure testicular choriocarcinoma, a rare and highly malignant subtype with challenging treatment: A case report and review of the literature

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Abstract. Testicular choriocarcinoma (CC) is the rarest subtype of germ cell tumours (GCTs) of the testis, with a high malignant potential and early haematogenous metastasis. Radical surgical resection should be performed primarily for histological diagnosis, while chemotherapy remains the mainstay of therapy for advanced disease. In the present study, the case of a 65-year-old male patient diagnosed with metastatic testicular CC, who did not fully respond to chemotherapy is reported. This patient underwent surgical removal of the testicular tumour, chemotherapy with etoposide and cisplatin, and radiotherapy of the intracranial lesions. Although the serum human chorionic gonadotropin (HCG) levels of the patient and most of the metastases continued decreasing during chemotherapy, complete response was not achieved after six cycles of chemotherapy. The patient refused high-dose chemotherapy and autologous stem cell transplantation due to severe side effects, and eventually developed respiratory failure on maintenance therapy with oral etoposide. A literature review was then performed, aiming to summarize the characteristics and therapeutic principles of testicular CC. In addition, the emerging therapeutic agents that could be used in maintenance therapy for GCTs, particularly for testicular CC, were also discussed. The limited clinical trials of targeted treatments showed potential benefit for long survival of patients with selected GCTs with fewer side effects. In particular, immunotherapy showed unique potential for testicular CC in preclinical studies, offering new approaches of maintenance therapy for advanced disease. Further studies should shed light on the identification of prognostic factors that predict the response to immune-based therapy in GCTs.

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Introduction

Testicular cancer (TC) is a rare disease, accounting for only ~1% of all male neoplasms, and 3.5% of male genital system tumours (1). The incidence of TC has steadily risen in recent years, resulting in an increase in mortality (1). Germ cell tumours (GCTs) account for ~95% of TC, including seminomas and non-seminomatous germ cell tumours (NSGCTs). NSGCTs consist of diverse histological subtypes including embryonal carcinoma, yolk sac carcinoma, teratoma, choriocarcinoma (CC) and mixed testicular CC (2,3). Of all NSGCTs, testicular CC is the rarest subtype and is characterized as highly aggressive with early haematogenous metastasis to the lungs, liver, brain and other organs (4). As CC is usually detected as a component of mixed testicular GCTs, pure CC is extremely rare in TC (0.2-0.6%) (5,6).

As a unique malignancy consisting of mononuclear cytotrophoblasts (cytotrophoblasts, intermediate trophoblasts) and multinucleated syncytiotrophobalsts, CC can commonly arise in three potential scenarios that are characterized by the production of human chorionic gonadotrophin (HCG). These scenarios include: i) Gestational CC occurring within or outside of the uterus followed by pregnancy; ii) non-gestational CC originating from germ cells in the gonads or midline locations outside of the gonads (such as the mediastinum, retroperitoneum, and pineal gland) (7); and iii) infrequently, non-gestational CC primarily presenting in parenchymal organs, such as the lungs and gastrointestinal tract (8,9).

In cases of testicular CC, tumours originate from germ cells in the testicular gonads of males with abnormally high levels of HCG. It predominantly affects children and young adults, accompanied by varying proportions of seminoma, embryonal carcinoma, yolk sac tumour, and teratoma (4). Hence, pure testicular CC is extremely rare and distinguished by unique clinical features which set it apart from other types of NSGCTs. As established, CC tends to rapidly proliferate and invade, and is prone to vascularity that leads to subsequent necrosis (10). Although CC is rare in males, the tendency for haemorrhaging persists in a similar way to female patients.

Furthermore, patients with CC often experience early metastases, with hematogenous spread to organs beyond regional lymph nodes, such as the lungs, liver, and brain (11). All of these factors contribute to a poorer prognosis of testicular CC in comparison with other types of TCs (2).

Therefore, although TC in general is highly curable, treating testicular CC, a unique type of testicular NSGCT, can be challenging. Radical orchiectomy should be performed promptly after clinical diagnosis, for the purpose of proper diagnosis and primary tumour control. Subsequently, chemotherapy plays a crucial role in cases at either early or advanced stages (12). However, treatment options for refractory or relapse cases are limited. Alternative chemotherapy protocols and high-dose chemotherapy (HDCT) with autologous stem cell transplantation (ASCT) should be considered in such circumstances. Nevertheless, new agents targeting these patients have yet to be extensively explored (13).

In the present study, a case of pure testicular CC of a male patient aged 65 years with lung and brain metastases is reported. The patient survived for 8 months after chemotherapy and radiotherapy, but eventually succumbed due to failure of maintenance of chemotherapy. Considering the severe side effects of chemotherapy, promising targeted therapies that have shown potential benefit in prolonging the survival of patients with GCTs are summarized. All of these new targeted therapies offer new approaches to maintenance therapy for patients with advanced GCTs, with fewer side effects.

Case report

A 65-year-old male patient from Tibet was referred to Hospital of Chengdu Office of People's Government of Tibetan Autonomous Region (Chengdu, China), on 11 September 2021, for cough, haemoptysis and thoracic back pain experienced in the previous four weeks. The patient also complained of an enlarging right testicular mass detected in the previous two weeks. The patient denied any history of fever, abdominal pain, headache and vomiting and had no relevant medical or surgical history.

Upon physical examination, the patient was conscious and vitally stable. Diffuse enlargement of the right testicle was observed, which was firm and tender to palpation. The left testicle was normal.

Basic laboratory evaluation revealed severe anaemia with a haemoglobin concentration of 5.8 g/dl. Initial laboratory results of the tumour markers showed high HCG (35,586 mIU/ml; normal range, <2 mIU/ml), lactate dehydrogenase (LDH) was elevated to 339 U/l (normal range, 125-250 U/l), and alpha-fetoprotein (AFP) was <2.5 ng/ml.

Magnetic resonance imaging (MRI) of the pelvis showed a right scrotal mass with malignant features (6x4.8x3.4 cm) (Fig. 1A and B). A computed tomography (CT) scan of the chest revealed multiple metastases to the bilateral lungs. A brain MRI was then performed for further evaluation and showed no malignant lesions in the brain.

After 4 units of red blood cell transfusion, the patient underwent orchiectomy and the specimen underwent pathological examination. Removed tumour tissue was immediately fixed with 4% neutral formalin for histopathological diagnosis and immunohistochemical (IHC) staining for 24 h. After being dehydrated through alcohol, the samples were embedded by paraffin.

For the microscopic histochemical analysis, 4-μm slices were cut from the paraffin blocks, deparaffinized in xylene and hydrated through the application of a series of alcohol. Sections were stained with haematoxylin (3%; 2 min) and eosin (0.5%; 1 min), and then the sections were observed by two independent pathologists under a light microscope (BX43; Olympus Corporation).

For the IHC analysis, serial 4-μm sections obtained from the paraffin block were stained for HCG, pan-cytokeratin (pan-CK), Ki67, AFP, vimentin (Vim), CD117, OCT3/4 and CD30. Sections were incubated overnight at 4˚C with the primary antibodies including: Rabbit anti-human HCG antibody (cat. no. ZA-0703; ZSGB-BIO), mouse anti-human CK (pan) antibody (cat. no. ZM-0069; ZSGB-BIO), rabbit anti-human Ki-67 antibody (cat. no. RMA-0731; MXB), mouse anti-human AFP antibody (cat. no. ZM-0009; ZSGB-BIO), rabbit anti-human Vim antibody (cat. no. ZA-0511; ZSGB-BIO), rabbit anti-human CD117 antibody (cat. no. ZA-0523; ZSGB-BIO), mouse anti-human OCT3/4 antibody (cat. no. MAB-0874, MXB), and mouse anti-human CD30 antibody (cat. no. MAB-0868; MXB). Subsequently, the slides were washed and incubated with a horseradish peroxidase-conjugated secondary antibody (cat. no. PV-6000; ZSGB-BIO) at 37˚C for 20 min. The immunostaining was carried out with 3,3'-diaminobenzidine chromogen (DAB), and counterstaining with hematoxylin for 5 min at 25˚C. Positive expression of cells was observed by two independent pathologists under a light microscope (BX43; Olympus Corporation).

Microscopic histopathology confirmed the diagnosis of pure testicular CC. It showed extensive parenchymal replacement by a biphasic pattern of syncytiotrophoblasts and cytotrophoblasts with haemorrhage and necrosis (Fig. 2A-D). Microscopically, haemorrhage and necrosis were observed in association with the tumour (Fig. 2A); and tumour cells were composed of syncytiotrophoblast and cytotrophoblast cells (Fig. 2B). Multinucleated syncytiotrophoblast cells were with sizeable and irregular nuclei (Fig. 2C), and demonstrated strong IHC staining of HCG (Fig. 2D).

This tumour was positive for HCG and pan-CK, and the percentage of Ki67-positive tumour cells (Ki67 index) was ~60% (data not shown). The other immune-markers, such as AFP, Vim, CD117, OCT3/4 and CD30 were negative (data not shown), which ultimately ruled out the possibility of mixed GCT. Final staging of the tumour was T2N0M1a S2 staged III-B according to the American Joint Committee of Cancer TNMS classification (14).

The patient then received chemotherapy with etoposide and cisplatin (EP). Considering the high-volume of lung metastases of the male patient, bleomycin was excluded due to its pulmonary toxicity. HCG levels decreased continuously after each cycle of chemotherapy (Fig. 3), and the patient no longer complained of haemoptysis. However, after two cycles of chemotherapy, a brain MRI revealed new malignant lesions in the (Fig. 4A) left frontal lobe, (Fig. 4D) left ventricle and (Fig. 4G) left occipital lobe. Meanwhile, chest CT showed that some lung lesions had decreased in size, while others had increased in size (Fig. 5A, B, E and F). Stereotactic body radiotherapy (SBRT) of the intracranial lesions was

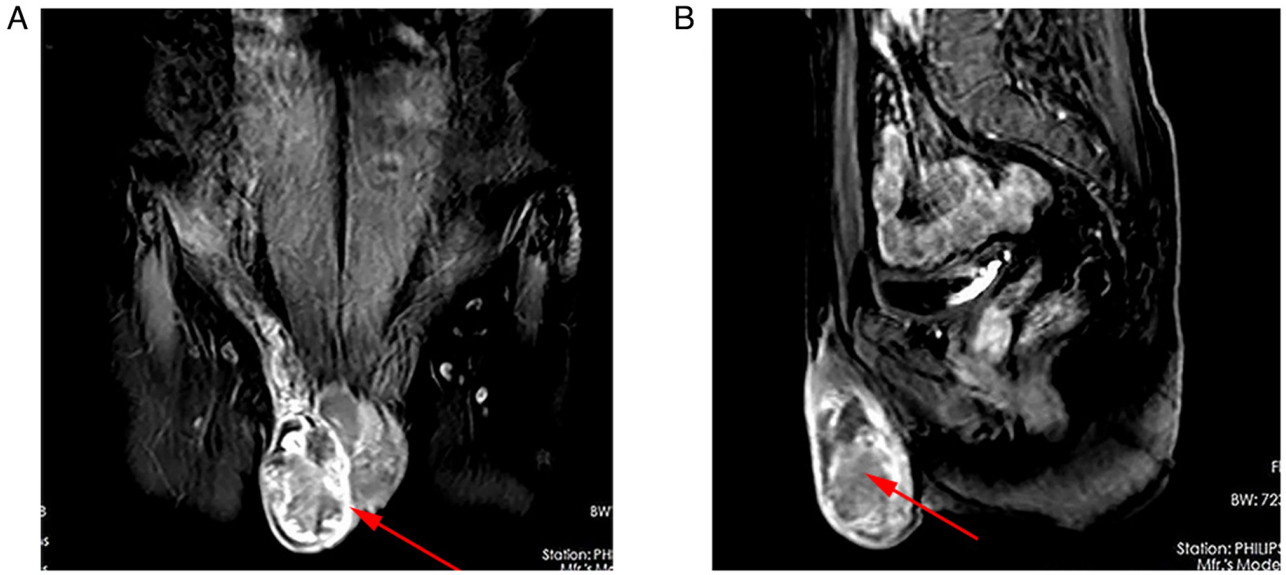

Figure 1. (A) Coronal and (B) sagittal cuts of the pelvis show a swelling mass of the right testis with malignant features (red arrows).

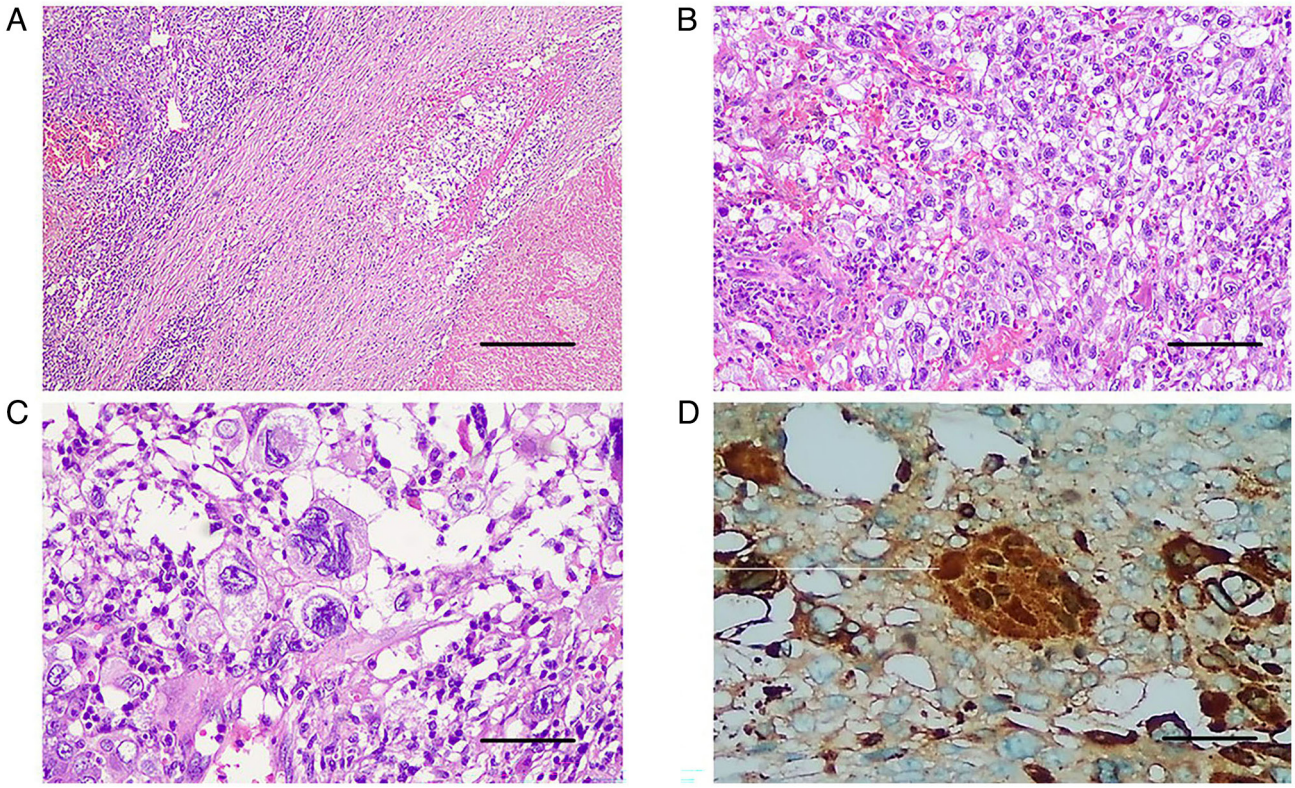

Figure 2. Histopathology images with typical microscopic appearance of choriocarcinoma. (A) H&E staining, revealing extensive areas of haemorrhage and necrosis associated with the tumour; magnification, x40 and scale bar, 200 μm. (B) A higher power image of the tumour revealed that tumour cells were composed of syncytiotrophoblast and cytotrophoblast cells (H&E stain; magnification, x100; scale bar, 100 μm). (C) Image of syncytiotrophoblast cells with large, multinucleated cells with sizeable, irregular nuclei (magnification, x200; scale bar 50 μm. (D) Immunohistochemical staining of human chorionic gonadotropin, revealed strong cytoplasmic staining of the syncitiotrophoblast tumour cells (magnification, x200; scale bar 50 μm). H&E, haematoxylin and eosin.

then administered (a dose of 40 Gy delivered in 10 fractions) with concurrent chemotherapy of the third cycle. As the patient suffered from severe bone marrow suppression during chemotherapy, the male patient refused further HDCT or ASCT after six cycles of EP therapy. Although the HCG levels decreased continuously and most of the metastases decreased in size, the treatment was suspended for 6 weeks. Although immunotherapy was recommended, the patient demanded to return to Tibet with oral medication for economic and physical reasons. Comprehensive assessment was then arranged. Laboratory results of the tumour markers showed that HCG was elevated to 572.9 U/l and LDH was 195 U/l. The brain

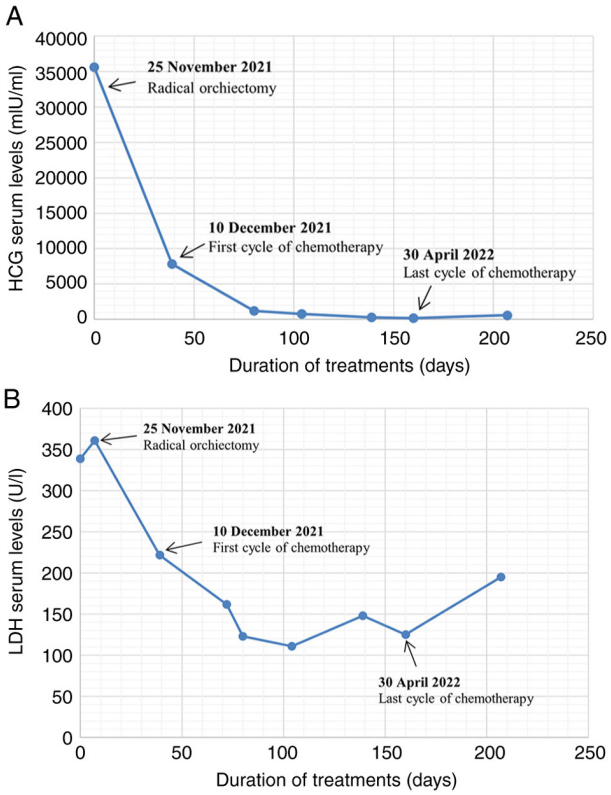

Figure 3. Variation in serum HCG/LDH levels at different time points. (A) HCG and (B) LDH levels in serum were continuously on the decrease during chemotherapy but were on the rise at the end of chemotherapy. HCG, human chorionic gonadotropin; LDH, lactate dehydrogenase.

MRI showed stable encephalic lesions with slight reduction in size (Fig. 4A-I). The chest CT revealed that most of the pulmonary lesions were decreasing in size, while others were slightly increasing in size (Fig. 5A-H). The patient returned to Tibet with oral etoposide. Notably, 4 weeks later the patient agreed to receive immunotherapy of sintilimab along with oral etoposide. However, without therapeutic evaluation the condition of the patient deteriorated 2 weeks later. The patient developed respiratory failure accompanied by massive haemoptysis and eventually succumbed 2 days later at home.

Discussion

Clinical characteristics. GCT is the dominant histology (95%) among all patients with TC, developed from a non-invasive tumour type known as germ cell neoplasia *in situ* (GCNIS) (14). The clinical classification of GCTs comprises two subgroups: Seminomas and NSGCTs (4). When presenting with a combination of both, mixed GCTs will be managed identically to NSGCTs (14). GCTs have a distinctive pathobiology whereby they primarily originate from gonocytes which fail to differentiate into spermatogonia. Seminomas are halted in the initial differentiation stage, exhibiting greater resemblance to gonocytes (15). By contrast, NSGCTs are highly heterogeneous and exhibit differing degrees of differentiation across various histological subtypes, including both embryonal carcinomas, the extraembryonic-derived CC and yolk sac tumours, and the most highly differentiated somatic-like teratomas (15). Generally, non-seminomas tend to be less common than seminomas, but frequently demonstrate greater aggression.

Among all subtypes of NSGCTs, testicular CC is the most aggressive neoplasm. It is composed of cytotrophoblast, intermediate trophoblast, and syncytiotrophoblast cells, and is capable of producing a high level of HCG hormone (2). Cytotrophoblasts are trophoblastic stem cells while the syncytiotrophoblast is a more differentiated cell. Typically, syncytiotrophoblast cells exhibit a plexiform pattern, surrounded by predominantly mononucleated cytotrophoblast cells situated near hemorrhagic foci. Nonetheless, particular samples may contain a syncytiotrophoblast component that is inconspicuous (16). While testicular CC is typically found alongside other histopathologic components in mixed GCTs (for example, seminoma, embryonal carcinoma, yolk sac tumour, and teratoma), pure CC accounts for <1% of testicular neoplasms (2).

Testicular GCTs tend to affect younger individuals and occur in sites along the migratory pathways of germ cells during embryo-fetal life (17). Testicular CC, as a rare type of testicular GCTs, occurs most commonly in young men between the ages of 20 and 39 (6). A PubMed literature search found that the oldest patient reported with testicular CC was 63 years old, and there was a markedly limited number of patients >50 years old (18). In the present study, to the best of our knowledge, the oldest patient with testicular CC ever reported in English publications, is presented. It is commonly difficult to distinguish this tumour in its early stages from other more frequent diseases in older individuals (18). Therefore, delaying diagnosis contributes to a reduction in the effectiveness of treatment in older individuals. Moreover, given that older patients experience more side effects from treatment than young adults, adequate antineoplastic treatment is often unattainable (19). Although it is unclear whether ageing plays a critical role in the disease process, it may be associated with a poorer prognosis.

Due to its rapid proliferation and vascular invasion, testicular CC tends to exhibit hematogenous spread to the lungs, liver, and brain at an early stage. Patients usually present with symptoms related to metastases rather than a swelling mass in the testis. Thus the clinical presentation of metastatic CC of the testis is so varied that each case may be a diagnostic challenge (12). Such patients usually present with advanced metastatic disease due to a delay in the diagnosis. Notably, ~30% of cases of testicular CC have metastatic disease at the time of diagnosis. The lungs (80%) are the most common site of metastasis, followed by the vagina (30%) and the liver (10%) (11). Moreover, a propensity for haemorrhage is well recognized in patients with gestational trophoblastic disease, including those with testicular CC. Haemorrhage was identified as the cause of death in 44% of the patients diagnosed with testicular CC during autopsy (20). Therefore, it is commonly observed that patients present with haemorrhage from metastatic sites. Otherwise, high-volume pulmonary metastases can cause acute impairments in breathing, resulting in fatal respiratory failure. In recent years, there have been a few reported cases of hyperthyroidism in patients with testicular CC. This could be attributed to the structural similarity of HCG and thyroid-stimulating hormone (TSH) (21).

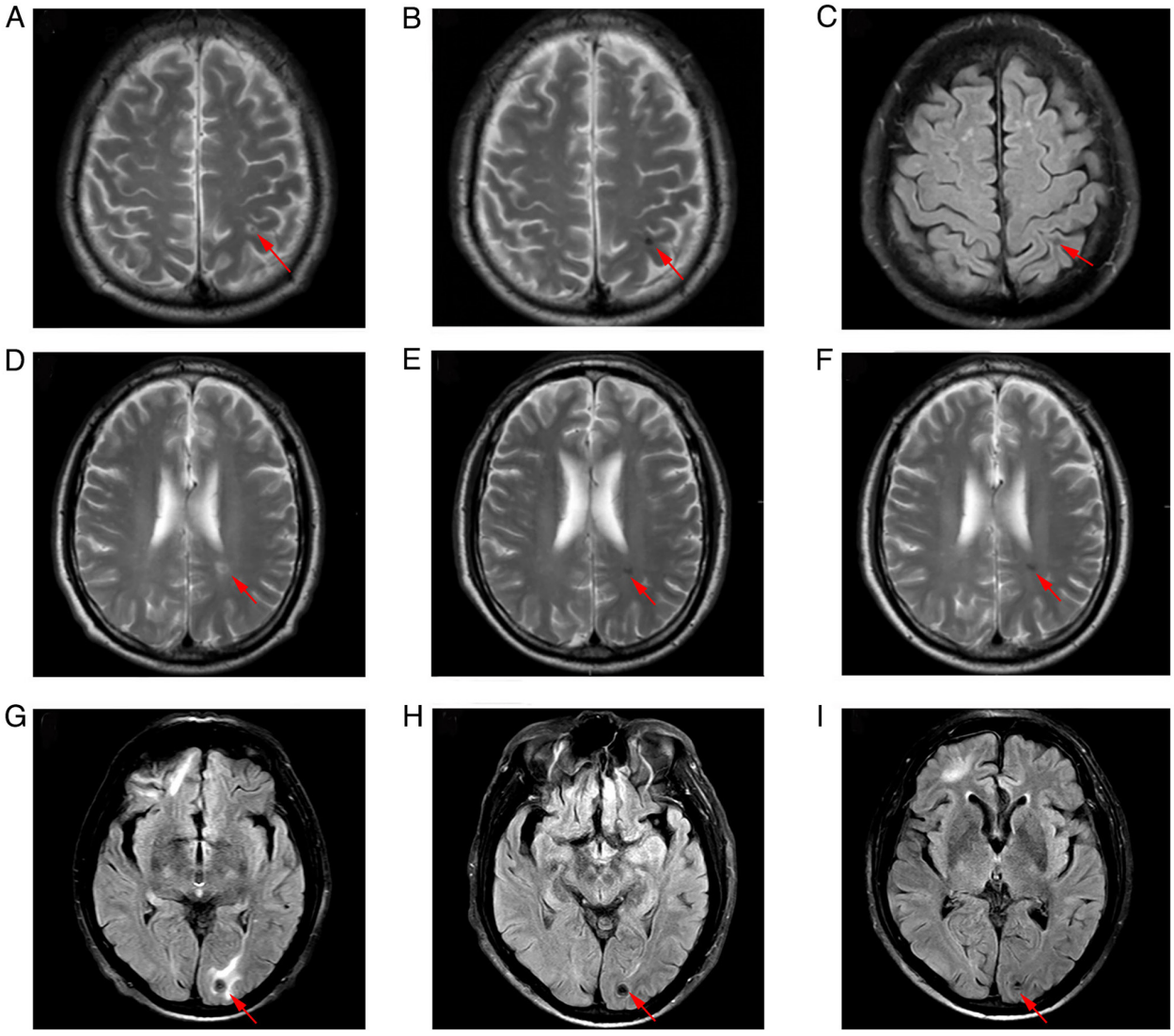

Figure 4. Magnetic resonance imaging of the brain. (A) The new lesions (red arrows) in the left frontal lobe, (D) left ventricle and (G) left occipital lobe after two cycles of etoposide and cisplatin chemotherapy. The lesions (red arrows) in the (B and C) left frontal lobe, (E and F) left ventricle and (H and I) left occipital lobe decreased in size after radiotherapy and four cycles of chemotherapy, and remained regressed after six cycles of chemotherapy.

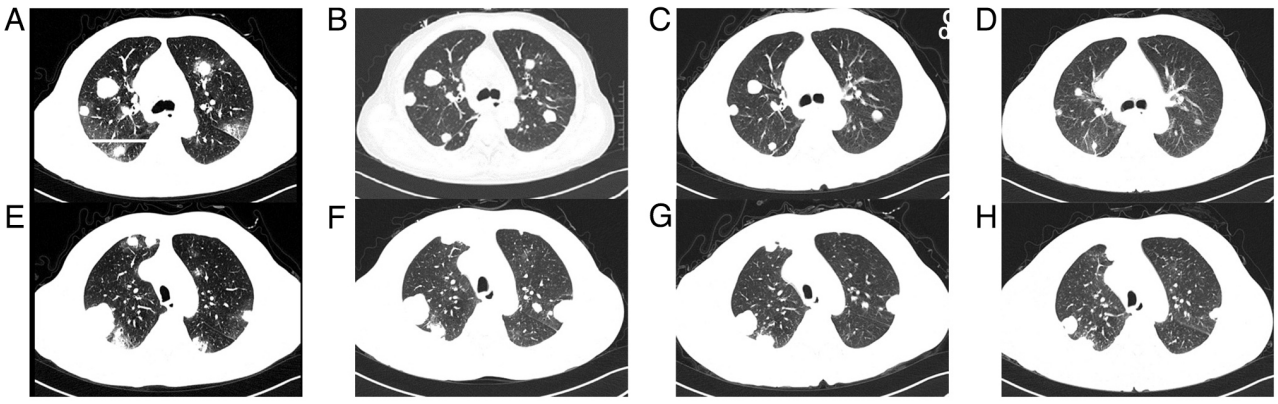

Figure 5. Tumour response to treatment. Computed tomography scans of the metastatic masses in the lungs (A and E) before treatment, (B and F) after two cycles, (C and G) after four cycles, (D and H) after six cycles of etoposide and cisplatin chemotherapy. Some pulmonary lesions decreased in size while others enlarged in size after two cycles of chemotherapy. In the last four cycles of chemotherapy, most of the pulmonary metastases continued to decrease in size.

Patients with advanced GCT could develop choriocarcinoma syndrome (CS), which is characterized by rapid hematologic spread of malignancy to multiple organs with intratumoural bleeding (22). It occurs more frequently in those

with a high-volume of CC elements and an HCG level over 50,000 IU/l. Typically, CS occurs shortly after the administration of chemotherapy and is associated with high morbidity and mortality (23).

Treatment

Standard treatment. Typically, radical orchiectomy should be performed when clinically confirmed, allowing for proper diagnosis and primary tumour control (14). Prompt initiation of chemotherapy is also a well-known determinant of prognosis of metastatic CC. Cisplatin-based regimens are recommended for fist-line chemotherapy, including EP, bleomycin EP, oretoposide, ifosfamide and cisplatin (VIP).

Patients with testicular CC should receive at least four cycles of chemotherapy with ongoing monitoring of HCG (12). Based on the risk of developing CS or acute respiratory failure during chemotherapy, new alternative approaches have been developed for patients with advanced diseases in recent years. The effective approach is a shortened or reduced course of induction chemotherapy prior to administering the full-dose chemotherapeutic regimen (22). Furthermore, salvage treatments should be considered for cases of refractory disease, in which HCG levels plateau after several cycles. For those patients who relapse or show resistance to first-line chemotherapy, combination salvage chemotherapy is typically recommended, such as paclitaxel, ifosfamide and cisplatin (TIP), gemcitabine and oxaliplatin (GemOx), gemcitabine, oxaliplatin and paclitaxel (GOP), or irinotecan combined with nedaplatin (19,24). Additionally, HDCT with ASCT should be taken into consideration for specific patients with resistant testicular CC (13,25).

Taza *et al* (26) found daily oral etoposide therapy produced encouraging efficacy results in patients with relapsed NSGCT who completed HDCT and peripheral-blood stem-cell transplant (PBSCT) and achieved complete serologic remission and hematologic recovery. Therefore, oral etoposide may be used as supplementary treatment for patients who have already achieved complete remission, but not as maintenance treatment for patients with large amounts of residual tumour.

In the present case report, the patient presented with massive pulmonary metastases and a high serum HCG level, which are known to be associated with a poor prognosis (12). In order to avoid respiratory failure and tumour lysis syndrome, EP therapy was administered without bleomycin or ifosfamide after radical orchiectomy. The serum HCG levels of the patient gradually decreased after each cycle of chemotherapy, but remained elevated to normal levels after six cycles of chemotherapy. The patient suffered from severe bone marrow suppression throughout the chemotherapy, which was probably due to the advanced age of the patient. Instead of HDCT with stem cell rescue, the patient opted for oral chemotherapy, which ultimately failed to control the disease.

Generally, testicular GCTs are malignant neoplasms with excellent curative potential, even in advanced stages, primarily attributed to the incorporation of cisplatin into treatment protocols (27). However, testicular CC is a unique type of GCT with a markedly higher likelihood that patients may develop cisplatin-refractory or progressive disease despite high-dose salvage chemotherapy treatment. Failure to achieve complete tumour remission following multiple salvage treatments is a definite indicator of poor prognosis for this specific patient group (12).

Radiotherapy. The patient succumbed to rapidly progressing pulmonary metastases, but the brain lesions remained stable after radiotherapy until the patient succumbed. Historically, NSGCTs have been considered relatively radioresistant compared with seminomas, and radiotherapy is consequently excluded from curative strategies (28). In order to control the radioresistant disease, higher doses of radiation are required which would probably lead to severe side effects. However, modern techniques including stereotactic radiotherapy (SRT), which allows very high dose delivery in small volumes, have rendered radiotherapy applicable in multimodal treatments for NSGCTs. Several clinical trials have investigated the potential of SRT in NSGCTs, and the results appear to be encouraging (28,29). The potential applications of SRT in NSGCTs would likely be in areas of platinum-refractory disease and consolidation therapy for residual masses after primary systemic therapy.

Targeted therapy. Treatment of patients with advanced testicular CC has been a challenge for numerous years. It is particularly difficult for patients with high serum levels of HCG and a high volume of metastases, even after four cycles of chemotherapy (19). For these patients, additional cycles with a salvage regimen are warranted. In the case of refractory or relapsed disease, advanced treatments such as HDCT with ASCT should be considered (13). However, for patients ineligible or resistant to such aggressive treatments, further investigation of novel therapeutic agents is essential (30). In recent years, multiple studies have investigated the role of targeted therapies in refractory GCTs (30-34). These new targeted agents may offer longer response durations with fewer side effects, rendering maintenance therapy with improved overall survival (OS) feasible for refractory GCTs.

Several receptor tyrosine kinases, including KIT, ERBB2, PDGFR and VEGFR, have been implicated in the activation of the MAPK and PI3K/AKT/mTOR pathways in TC (32). The PI3K/AKT/mTOR pathway has been shown to inhibit tumour growth in *in vitro* and *in vivo* TC models. But clinical therapeutic response to sunitinib, pazopanib, imatinib, cabozantinib and everolimus has been limited to case reports and a few small phase II clinical trials, with objective response rates (ORRs) ranging from 0-20% (33,34).The combination of targeted agents with cisplatin has been shown to increase therapeutic efficacy in several preclinical studies (35-37). Therefore, these targeted therapies may not be recommended as single-agent treatment. However, in all of these preclinical and clinical studies, cases of testicular CC were extremely rare and no significant antineoplastic activity was demonstrated in the rarest subtype of TC (32-37).

Angiogenesis, the formation of new blood vessels from vascular endothelium, is a key event in tumour development. The vascular endothelial growth factor (VEGF) is currently recognized as the major inducer of angiogenesis and vascular permeability. VEGF receptors are almost exclusively expressed on the surface of endothelial cells, but are overexpressed in GCTs (38). Several clinical trials have attempted to achieve satisfactory results with a combination of chemotherapy and bevacizumab in refractory GCTs. Jain *et al* (39) reported that combination therapy with oxaliplatin and bevacizumab in patients with refractory GCTs showed an ORR of 27.6%

(8 out of 29 patients) in a phase II clinical trial (39). However, the contribution of bevacizumab to the effectiveness of the treatment cannot be identified. Another trial treated 43 patients with GCTs with high doses of bevacizumab in combination with chemotherapy (gemcitabine, docetaxel and carboplatin). An ORR of 89% was achieved, but the high rate of side effects rendered this regimen infeasible in some patients (40). Further studies of anti-angiogenic agents in GCTs, including CC, may shed light on combination therapy and maintenance treatment.

A translational research study investigated the expression of the poly (ADP-ribose) polymerase (PARP) pathway in 124 patients with GCTs. The expression rate of PARP in tumour tissue reached high levels in varied subtypes, such as 100% in intratubular germ cell neoplasia unclassified (IGCNU), 52.6% in seminomas, 47% in embryonic carcinomas, 33.3% in yolk sac tumours, 26.7% in teratomas and 25% in CC (41). Patients with low PARP expression in tumour tissue had improved overall survival (OS) than patients with high PARP expression, but the difference was not statistically significant (41). De Giorgi *et al* (42) reported that olaparib as a single agent showed no objective response in heavily pretreated patients with GCT (n=18), with only 5 patients (27.8%) achieving stable disease. Another clinical trial evaluated veliparib in combination with gemcitabine and carboplatin (n=15), also revealing limited efficacy with 4 partial remissions (26.7%) and 5 cases of disease stabilization (33.3%) (43).

The CD30 cell surface protein is commonly expressed in Hodgkin's lymphoma cells, anaplastic large cell lymphoma (ALCL) cells and embryonal carcinomas. However it can also be found on pure seminomas and seminomatous components of mixed GCTs (44,45). Notably, patients with GTC and with CD30-expressing tumours had worse progression-free survival (PFS) and OS compared with patients with CD30-negative tumours. In addition, 56-60% of CD30-positive GCTs would convert to CD30-negative during cisplatin-based chemotherapy. The maintenance of CD30 expression is a marker of poorer prognosis (46,47). Brentuximab vedotin is an anti-CD30 antibody linked to the antimitotic agent monomethyl auristatin E, which is FDA-approved for the treatment of Hodgkin's lymphoma, ALCL and cutaneous T-cell lymphoma. A phase II clinical trial enrolled 9 patients with $CD30^+$ refractory GCT, of which 1 patient achieved a complete response and 1 patient a partial response (ORR, 22.2%) (48). Both patients who achieved therapeutic response had mixed GCTs with embryonal carcinoma, and one of them had a CC component. In another study, 2 out of 5 $CD30^+$ patients with relapsed or refractory GCTs achieved an objective response [Partial response (PR), 1; and complete response (CR), 1], and 2 patients had a CC component [PR, 1; and stable disease (SD), 1] (49).

Immunotherapy. Immunotherapies have reached important milestones with clinical impact in recent years in numerous cancer models, including TCs. There are two main studies investigating the role of PD-L1 in the treatment of GCTs (50,51). Both studies reported a higher expression of PD-L1 in tumour compared with normal testicular tissue, and patients with low PD-L1 expression had significantly improved PFS and OS compared with patients with high PD-L1.

High PD-L1 expression was detected in CC (52.6%), embryonal carcinomas (12.5%), teratomas (9.1%) and seminomas (2.2%) (51). Additionally, Lobo *et al* (52) found that the tumour cell intensity of cytotoxic T-lymphocyte-associated antigen 4 was significantly higher in yolk sac tumours, teratomas and CC, while PD-L1 tumour cell positivity was significantly more frequent in CC. This is probably because CC is the only subtype of GCTs that expresses PD-L1 in tumour cells, whereas other subtypes express varying levels of PD-L1 primarily on tumour-associated macrophages (53). Therefore, in contrast to other subtypes of TC, PD-L1 may have more therapeutic potential in CC. However, Kawahara *et al* (54) reported that in a phase II trial of nivolumab monotherapy in chemo-refractory germ cell tumours, tumour mutation burden (TMB) was a potential biomarker of therapeutic response instead of PD-L1 expression. As the inclusion of testicular CC in this study remained unclear, further investigation into prognostic factors predicting response to immunotherapy in testicular CC was warranted.

There were some case reports on the efficacy of PD-1/PD-L1 blockade therapy in male patients with primary CC. Chi and Schweizer (55) reported that 1 male patient with metastatic CC achieved a partial but durable response to nivolumab treatment. However, Loh and Fung (56) reported that one patient with testicular CC did not respond to pembrolizumab treatment and progressed rapidly. Moreover, Han *et al* (57) reported that 1 patient with primary neck CC whose PD-L1 expression was 40%, achieved complete remission after pembrolizumab treatment combined with cytotoxic chemotherapy.

Despite these case reports, there were several case series or small phase II studies that have evaluated the effectivity of immunotherapy in refractory and relapsed patients with GCTs. Zschäbitz *et al* (58) reported that 3 out of 7 refractory GCTs responded to anti-PD1 (nivolumad or pembrolizumab) treatment. However, Adra *et al* (59) reported that out of 12 patients with refractory NSGCTs, only 2 patients were PD-L1 positive (one of them was with predominant CC) and did not respond to pembrolizumab. In another phase II study, Tsimberidou *et al* reported that 12 patients with refractory GCTs (10 men and 2 women) were treated with pembrolizumab. No objective response was observed, but 3 patients remained radiographically stable for 10.9, 5.5 and 4.5 months, respectively (60). Moreover, another study showed that avelumab was not effective in patients with multiple relapsed/refractory non-seminomas (61). Kawahara *et al* (54) reported that out of 17 patients with chemo-refractory GCTs treated with nivolumab, 1 patient achieved PR with a median duration of 90.1 weeks and 3 patients achieved SD with a median duration of 11.7 (range, 5.9-68.4) weeks. These results suggest that only selected patients with GCTs may benefit from immune checkpoint inhibitors as a potentially effective treatment. The low mutational burden and low number of neoantigens in TC tumours may contribute to the lack of clinical efficacy (31). Meanwhile, several clinical trials of PD-L1/PD1 combinations are underway, including nivolumab in combination with ipilimumab, durvalumab in combination with tremelimumab, nivolumab in combination with cabozantinib with or without ipilimumab (62-65). In conclusion, it is crucial to investigate prognostic factors that predict response to immunotherapy. Furthermore, the combination of different immunotherapy checkpoint inhibitors with cytotoxic chemotherapy may lead to improved clinical outcomes.

Table I. Clinical trials of promising targeted therapies for GCTs.

Target	Therapy	Trial phase	Main findings	Design	Status (Refs.)	NCT identifier	Histological subtype: Including CC or not
VEGFR	Oxaliplatin + bevacizumab	Phase II	OR 27.6% (CR, 1; PR, 7; and SD, 1)	29 pts of relapsed/ refractory GCTs (72.4% platinum-sensitive)	Completed (36)		N/A
VEGFR	Bevacizumab + ifosfamide high doses + carboplatin + etoposide	Phase II	OR 89% (PR, 15; CR, 4; and SD, 2), but high rate of side effects	43 pts of relapsed/ refractory GCTs (14% platinum-sensitive)	Completed (37)		N/A
PARP	Olaparib	Phase II	CR/PR, 0; and SD, 5	18 pts of relapsed/ refractory metastatic GCTs	Completed (39)	NCT02533765	N/A
PARP + DNMT	Veliparib + gemcitabine + carboplatin	Phase II	OR 26.7% (CR, 0; PR, 4; and SD, 5)	15 pts of relapsed/ refractory GCTs (80% platinum-sensitive)	Completed (40)	NCT02860819	N/A
CD30	Brentuximab vedotin	Phase II	OR 22.2% (CR, 1; PR, 1; and SD, 1)	9 pts of relapsed/ refractory GCTs	Completed (45)	NCT01851200	1 PR: Mixed GCT including CC
CD30	Brentuximab vedotin	Case series	CR, 1; PR, 1; and SD, 1	5 pts of relapsed/ refractory GCTs (67% platinum-sensitive)	N/A (46)		1 PR and 1 SD: Mixed GCTs including CC

GCTs, germ cell tumours; VEGFR, vascular endothelial growth factor receptor; OR, objective response; CR, complete response; PR, partial response; SD, stable disease; pts, patients; PARP, poly (ADP-ribose) polymerase; CC, choriocarcinoma; DNMT, DNA methyltransferase; N/A, not applicable.

All the aforementioned clinical trials of promising targeted treatments are summarized in Table I. Although immunotherapy has shown therapeutic potential for testicular CC in some studies, the patient in the present case report did not achieve a therapeutic response, which may be due to the extremely advanced stage of the cancer. As immunotherapy has been shown to have a slow but long-lasting effect, this patient may have benefitted more from immunotherapy on the condition of earlier intervention. However, it should be noted that only a few studies included a limited number of patients with CC, due to its extremely low incidence. Therefore, further investigations are warranted to evaluate the clinical activity and therapeutic side effects of new targeted agents in testicular GCTs, especially in testicular CC.

Other targeted agents. c-KIT is overexpressed in GCTs, predominantly in seminomas, and nearly absent in CC and teratocarcinomas (66-68). The c-KIT inhibitor, imatinib, has been demonstrated to be of low clinical effectiveness in GCTs (69,70). Therefore, c-KIT inhibitors should not be considered as therapeutic agents for testicular CC.

Cyclin-dependent kinases 4 and 6 (CDK4/6) are associated with cyclin D phosphorylate, Rb, which leads to cell cycle progression. Teratomas have been shown to have significantly higher expression levels of Rb protein than undifferentiated GCTs (71). In addition, the CDK4/6 inhibitors have been demonstrated to be potentially beneficial for teratomas in several clinical trials (72,73). Further investigation of CDK4/6 inhibitors should be initiated for the treatment of teratomas, but not for testicular CC.

Claudin 6 (CLDN6) is a tight junction membrane protein, which was found to be overexpressed ubiquitously in all elements of GCTs (74). Preclinical data indicates that >90% (n=97/104) of TC tissue samples with various histological components were positive for CLDN6, including seminomas, embryonal carcinomas, yolk sac tumours, CC, and teratomas (72). The anti-CLDN6 monoclonal antibody ASP1650, also known as IMAB027, has been shown to induce cell death as a single agent in preclinical studies (75). However, in a phase II clinical trial, ASP1650 did not appear to have clinically meaningful single-agent activity in relapsed/refractory GCTs. A change in CLDN6 expression over time and/or the mechanism of action of ASP1650 may explain the lack of

clinical activity (76). Therefore, further studies should focus on investigating other means of targeting CLDN6 with agents that have a different mechanism of action.

In conclusion, testicular CC is a rare subtype of testicular neoplasms, exhibiting a very heterogeneous behaviour, and is mainly aggressive with poor prognosis. Preoperative diagnosis is infrequent, and its treatment relies mainly on radical surgical resection and chemotherapy. Radiotherapy is now limited to the occasional circumstance. In refractory or relapsed cases, aggressive treatments such as HDCT with ASCT should be considered.

Recently, new targeted therapies, including immunotherapy, have shown potential benefit for selected GCTs. These targeted treatments emphasized longer duration of response and fewer side effects of therapy, which could enable long-term survival in refractory disease. However, clinical trials of targeted therapy for GCTs are limited, particularly for testicular CC. Reasons include the low incidence rate, the fact that these patients are heavily pre-treated and exhibit a poor prognosis. The future of targeted therapy for GCTs lies in the appropriate selection of patients with a certain molecular profile and in identifying predictive response and targeted drug resistance factors.

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Availability of data and materials

All data generated or analysed during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

NL, LLL, CHW and CRM contributed to acquisition, analysis and interpretation of the patient data presented in this case report. NL and CRM substantially contributed to the conception and the design of the study. All authors have made critical revisions. NL and CRM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

A relative of the patient provided written informed consent for participation.

Patient consent for publication

A relative of the patient provided written informed consent for publication of the data in the present study.

Competing interests

The authors declare that they have no competing interests.

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