

LETTER TO THE EDITOR

Investigation of malignant cells in ovarian tissue from a patient with central nervous system primitive neuroectodermal tumor relapse after ovarian tissue transplantation

Sir,

Safety issues surrounding reimplantation of ovarian tissue retrieved at the time of a cancer diagnosis have been a cause of concern for many years.¹⁻³ Here, we report a case of a central nervous system (CNS) primitive neuroectodermal tumor (PNET) relapse 6 years after ovarian tissue transplantation. In 2001, a 17-year-old patient was diagnosed with supratentorial PNET on the right orbitofrontal lobe of the cerebrum and underwent two operations for tumor removal. Prior to adjuvant chemotherapy, ovarian tissue biopsy and cryopreservation by slow-freezing were carried out, and no malignant cells were detected in the retrieved ovarian tissue. Follicle density (primordial follicles/mm³) was 55.7 ± 14.6 (SD). The patient then completed chemotherapy with six VIDE cycles (vincristine, ifosfamide, doxorubicin and etoposide). In 2002, radical resection of extraneural PNET metastasis to the right lung was required. Intensive chemotherapy, including busulfan-melphalan, and stem cell autotransplantation were performed. Radiotherapy was indicated

at the tumor site at a total dose up to 54 Gy. The patient developed secondary ovarian failure. In 2008, she underwent transplantation of frozen-thawed ovarian tissue by laparoscopy. A first estradiol peak was detected (49 pg/mL) concomitant with a drop in follicle stimulating hormone (13 mIU/mL) 3.5 months after surgery. Nine months after transplantation, she became pregnant by natural conception and gave birth vaginally to three healthy children in 2010, 2011 and 2012. In 2014, the patient relapsed. She also received adjuvant radio-chemotherapy but developed refractory epilepsy due to tumor compression of the cervical cord with leptomeningeal carcinomatosis. After 2 weeks of treatment, the patient died from medullary compression caused by disseminated PNET. Patient history is detailed in Table 1.

After obtaining patient consent and approval to use human ovarian tissue, from the Ethics Committee of the Cliniques Universitaires Saint-Luc and the Institutional Review Board of the Université Catholique de Louvain (registration number B403201213872) and the approval document for the research 3.06.2019, her remaining frozen ovarian tissue was thawed, analyzed and xenotransplanted to immunodeficient mice for 5 months. The presence of cancer cells in the thawed and xenografted tissue at histology, expression of neuron-specific enolase (NSE) by immunostaining, and levels of enolase 2 (ENO2) in the tissue were quantified by real time-drop digital polymerase chain reaction (RT-ddPCR). All sections were negative for malignant cell presence at histology and none showed NSE immunostaining, whereas this marker was extensively expressed in the patient's primary CNS tumor and recurrent tumor. At RT-ddPCR, the ENO2 gene was not able to determine the presence of PNET cell contamination in the patient's ovarian tissue. Indeed, positive signals for ENO2 were also obtained in normal ovarian cortex from 10 healthy women. After 5 months of xenografting, there was no sign of disease in the two immunodeficient mice used for this study. Four human ovarian fragments from the patient were degrafted. They show no evidence of malignant cells by histology or immunohistochemistry (Figure 1).

To conclude, CNS-PNETs are rare, highly aggressive neoplasms.⁴ The prognosis of supratentorial PNET has been historically poor and recurrence is observed in 37%-56% of patients and is considered the main cause of patient mortality.⁵ Thorough investigations were therefore performed to discard the probability of reseeding of malignant cells in the frozen-thawed ovarian tissue of patients with CNS-PNET. In

TABLE 1 Outline of patient history

Year	Patient history
2001	September: diagnosed with CNS-PNET, underwent two surgical procedures November: laparoscopy for retrieval of ovarian tissue Chemotherapy and radiotherapy, resulting in menopausal status
2002	Diagnosed with lung metastasis and treated by radio-chemoradiotherapy
2008	Laparoscopic surgery for ovarian tissue transplantation
2009	First pregnancy, natural conception, vaginal birth
2011	Second pregnancy, natural conception, vaginal birth
2012	Third pregnancy, natural conception, vaginal birth
2014	Recurrence of PNET on the right frontal lobe
2015	Surgery for tumor removal with adjuvant radio-chemotherapy
2016	Death due to medullary compression and recurrent infiltrating PNET

Abbreviations: CNS, central nervous system; PNET, primitive neuroectodermal tumor.

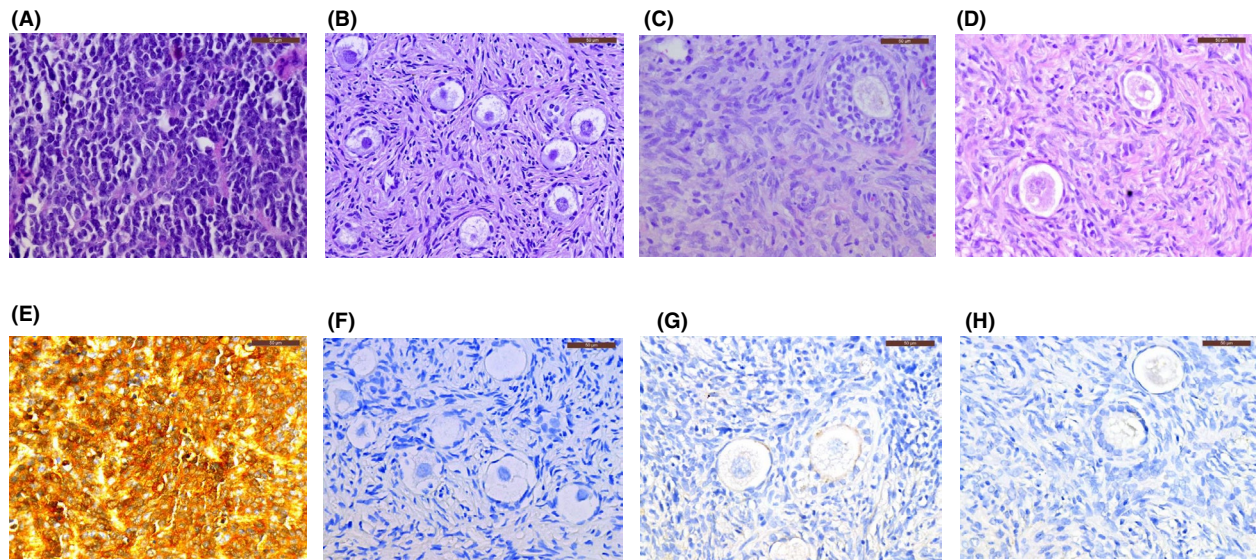


FIGURE 1 Representative photos of histological and immunohistochemical analysis. The patient's primary tumor (primitive neuroectodermal tumor [PNET]) (A) consisted of small round blue cells and rosette-like structures. Her frozen-thawed ovarian tissue (B) and ovarian tissue after long-term grafting (C, D) showed no cancer cell contamination. In immunohistochemistry for neuron-specific enolase (NSE), the patient's primary tumor (E) showed a strong positive expression, but staining in frozen-thawed ovarian tissue (F) and ovarian tissue after long-term grafting (G, H) were negative. Scale bars: 50 μ m

the present case, it is important to emphasize that the patient's transplanted ovarian tissue and remaining frozen tissue were confirmed to be without any detectable malignancy. There is therefore no evidence to suggest that ovarian tissue transplantation causes reseeding of the primary cancer. Nevertheless, we have to take into account the risk of mortality of some aggressive neoplasms when counseling women requiring ovarian tissue transplantation.

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