Association of the germline BRCA2 missense variation Glu2663Lys with high sensitivity to trabectedin-based treatment in soft tissue sarcoma

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ABSTRACT

We report an interesting clinical case of a patient carrying a specific BRCA2 germline variant affected by bone and hepatic metastases from a high grade uterine stromal sarcoma who obtained a complete metabolic response after only 3 cycles of trabectedin treatment (1.5 mg/m^2 given intravenously over 24 hours every 21 days). Molecular investigations linked this outstanding positive pharmacological response with the loss of heterozygosity (LOH) of the mutated BRCA2 gene. These data support the hypothesis that the response to trabectedin may be positively conditioned by the different DNA repair defects present in the neoplasm and that BRCAness tumor genotype is important in determining the efficacy of trabectedin-based chemotherapy.

Abbreviations: CT, computed tomography; DHPLC, denaturing high performance liquid chromatography; EAP, expanded access program; HR, homologous recombination; LOH, loss of heterozygosity; MLPA, multiplex ligation dependent probe amplification; MRI, magnetic resonance imaging; ORR, objective response rate; PET, positron emission tomography; STS, soft tissue sarcoma; ULMS, uterine leiomyosarcoma; VUS, variant of uncertain significance

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KEYWORDS

BRCA2 germline variant; DNA damage; mutations; soft tissue sarcoma; trabectedin; tumor genotype

Introduction

Soft tissue sarcomas (STS) are relatively uncommon tumors accounting for approximately 1% of all adult cancers.[1] Doxorubicin and ifosfamide remain the most active agents against these diseases inducing, when given as combination therapy, objective response rates (ORRs) in the range of approximately 20% to 40%.[2,3] Trabectedin, although approved for the treatment for advanced STS, was licensed on the basis of a randomized trial comparing 2 different treatment regimens in patients with predominantly leiomyosarcoma and liposarcoma.[4] Recently, these data were confirmed in a larger randomized phase III trial in which trabectedin administration resulted in superior disease control compared with conventional treatment with dacarbazine.[5]

Trabectedin binds to the N2 position of guanine in the minor groove of DNA, causing structural changes to the DNA that lead to double-strand DNA breaks as well as to the inhibition of transcription in a promoter and gene dependent fashion.[6] The homologous recombination (HR) pathway seems to be essential for the genesis of this drug's cytotoxicity and cells that lack this pathway are extremely sensitive to the drug.[7]

BRCA proteins have a crucial role in DNA repair being essential for the repair of double-strand breaks by HR[8] and cancers carrying mutation in the BRCA1 or BRCA2 genes that reduced protein activity as for ovaries and breast cancers may increase activity of the drugs that exert their cytotoxicity through the DNA double strand breaks.[9]

Data from a phase II study on metastatic breast cancer patients reported a strong association between trabectedin efficacy and the status of deleterious germline BRCA1 or BRCA2 mutation.[10] Within this specific population 17% of the patients reported a partial response whereas stable disease was observed in 22%. This favorable pharmacological outcome suggests that tumors with constitutional defect of BRCA1 or BRCA2 genes may be more sensitive to DNA damage induced by trabectedin treatment.

Previously investigations have pointed out that specific single nucleotide polymorphisms (SNPs) within the BRCA1 gene identified haplotypes associated with trabectedin sensitivity.[11] In this study, 59 patients with advanced STS were grouped into 2 categories based on the presence of the most frequent BRCA1's haplotype AAAG in tumor specimens. Forty-six patients had at least one AAAG allele and 13 patients had no AAAG allele. It was observed that patients who had at least one allele of the AAAG haplotype showed a statistically significantly longer progression-free survival (median: 5.6 vs 2.5 months; $p = 0.03$) and overall survival (median: 14.1 vs 5.4 months; $p = 0.0095$), compared with patients who had no AAAG

allele.[11] Moreover, in uterine leiomyosarcoma (ULMS), one of the histotypes more sensitive to trabectedin, it has been observed that the BRCA1 protein was absent in 29% of human ULMS.[12] Both studies support the crucial role of the function of the BRCA1 gene in modulating the response to trabectedin.

So far, no data have been reported with regard to the role played by germline mutations in combination with tumor BRCA1 or BRCA2 genetic status on trabectedin efficacy in STS.

Here we report a clinical case of a patient carrying a specific BRCA2 germline variant affected by bone and hepatic metastases from high grade uterine stromal sarcoma who obtained a metabolic complete response after the administration of trabectedin. This positive pharmacological response associated with the loss of the wild-type BRCA2 allele observed in tumor tissue, supports the hypothesis that the BRCAness tumor genotype has a reduced DNA repair activity that may improve the efficacy of a trabectedin-based chemotherapy.

Case report

In 2002 a 52 year-old patient underwent an external periareolar parenchymal excision of breast. The pathological report was compatible with a multifocal medullary carcinoma (pT3Nx). Therefore, radical mastectomy with axillary dissection was performed. The histopathology showed a microscopic focus of infiltrant ductal carcinoma (RpT1a pN0/18, G2). A histological specimen was examined for estro-progestinic and c-erbB-2 receptors expression. Estrogen receptors were positive, progesterone receptors were negative, whereas c-erbB-2 showed a moderate intensity. Based on the histopathological features of the tumor, treatment with tamoxifen for 5 years was begun.

In February 2006 hysterectomy plus bilateral salpingo-ovariectomy was performed because an abdominal CT (CT) scan revealed a pelvic mass in the right ovary. Pathological examination revealed an serous carcinoma poorly differentiated with vascular invasion in right ovary and a focal involvement of a serous carcinoma poorly differentiated also in the contralateral ovary. In addition, a synchronous stromal uterine sarcoma of high grade (pT1b,pNx,pMx, G3) was identified. Immunohistochemical analysis showed that the cells were positive for smooth actin, negative for S100, desmin, caldesmon and myogenin whereas EMA, CKMNF116 and D10 were poorly expressed.

In April 2006 an explorative laparotomy with radical omentectomy with systemic pelvic and lomboaortic lymphadenectomy with multiple biopsies of pelvic peritoneum was performed. The histopathology did not show any neoplastic tissue. However, complementary chemotherapy with carboplatin AUC5 (total dose 400 mg/die every 3 weeks) was administered for 4 cycles.

A strict follow up was maintained until December 2010 when a positron emission tomography (PET)/CT scan showed a hepatic lesion and a round-shaped area within the medullary canal without cortical involvement in the right femoral diaphysis. Liver biopsy revealed that the lesion was microscopically composed of spindle, plurinucleated and large sized cells with a sarcomatous morphology. Consistent with the diagnosis of uterine stromal sarcoma with muscular differentiation, the immunohistochemical analyses showed that the cells were positive for estrogen receptor, progesterone receptor, vimentin, and negative for S100, desmin, EMA, calponin, Pan Keratin, CD117/cKit, CD31, HMB45, DOG1 and NGFR. HHF35, 1A4 and CD10 were shown to be poorly expressed, whereas Ki67 was expressed in about 15%.

Neoadjuvant chemotherapy with epirubicin and ifosfamide was administered and a hepatic resection followed by intraoperative–radiation-treatment was performed. Histologically, the lesion was compatible with a secondary localization of stromal sarcoma. The cells were described as spindle pleomorphic and with necrosis areas. Immunohistochemical analyses showed that the cells exhibited a higher muscle differentiation consequently to a strong increase of EMA, desmin 1A4 and calponin expression.

A strict follow up was made until 2012 when both a magnetic resonance imaging (MRI) and PET/CT scan showed an important increase of the femoral diaphyseal lesion without apparent destructive phenomena of the cortex. Thus the patient underwent endoprosthetic replacement of the femoral diaphysis; the histopathologic examination was consistent with a cerebroid mass characterized by spindle cells with a sarcomatous morphology and a high mitotic index. Immunohistochemical analyses still supported the muscle differentiation of the lesion.

In March 2014 the patient was admitted to our hospital for mild pain of the right thigh. A PET/CT scan showed a bone and hepatic and abdominal lymphonodal relapse of the disease and a chemotherapy based on trabectedin was administered (1.5 mg/m^2 given intravenously over 24 hours every 21 days; total dose 2.3 mg). After only 3 administrations a complete metabolic response was achieved at metastasis sites (Fig. 1 a, b, c). To date, this complete response has been maintained for 20 cycles, and the patient remains on treatment.

Materials and methods

Family history of the patient revealed that her mother was affected by basal cell carcinoma at the age of 77 and a ductal breast cancer at the age of 40, and 2 maternal aunts suffered from ductal breast cancer diagnosed at age 55 and colon cancer at age 69, respectively. On the ground of pedigree's analysis genetic testing for BRCA1/BRCA2 mutations was offered and written consent was obtained from the patient.

Genomic DNA was purified from the patient's peripheral blood cells and from tumor tissue. Screening for mutations in the BRCA1 and BRCA2 genes was carried out by a combination of denaturing high performance liquid chromatography (DHPLC),[13] direct DNA Sanger-sequencing and multiplex ligation dependent probe amplification (MLPA) techniques (https://www.mlpa.com).

The microsatellite analysis of the tumor sample was performed considering from telomere to centromere the D13S171, D13S1701, D13S1698 markers flanking BRCA2 gene. The polymerase chain reaction primer sequences were obtained from the UniSTS NCBI database (http://www.ncbi.nlm.nih.gov/unists/). Polymerase chain reaction product size was evaluated by capillary electrophoresis on an ABI3130 Sequencer using GeneMapper 4.0 software (Applied Biosystems/Life technologies corp., Carlsbad, CA, USA).

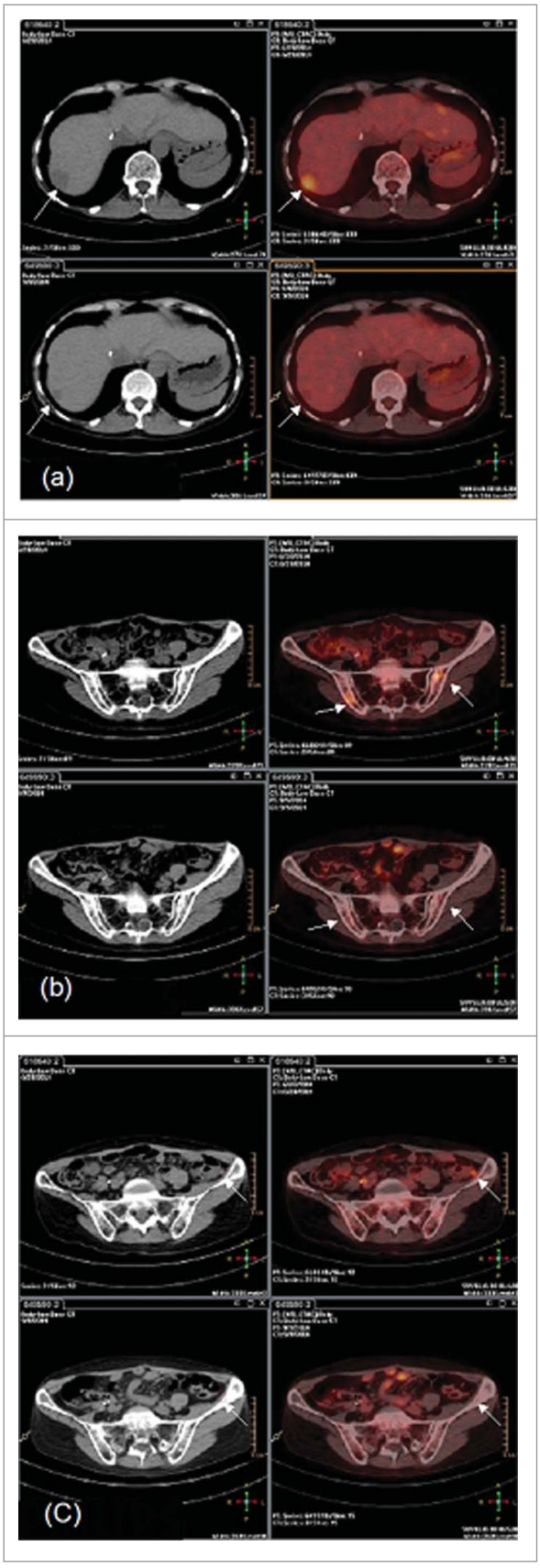

Figure 1. Positron emission tomography/computed tomography scan performed before the start of trabectedin treatment and 3 months later showing a complete metabolic response in all metastatic sites. (a) liver (b) pelvis (c) abdominal lymph node.

Results

A screening test for mutations in BRCA1 and BRCA2 genes performed on DNA isolated from peripheral blood cells of the patient showed a germline variant in BRCA2 gene of uncertain significance (VUS; c.7987G>A; p.Glu2663Lys) (Fig. 2 a, b, c). The same testing carried out on DNA isolated from liver metastasis sarcoma tissue confirmed the presence of this alteration and the microsatellite analysis showed the LOH of normal allele (Fig. 3 a, b, c).

Discussion

In this case study, in addition to breast cancer, the patient developed a cancer of the ovary and a synchronous poorly differentiated stromal uterine sarcoma which later caused liver and bone metastases.

Genetic testing for mutations in BRCA1 and BRCA2 genes performed on DNA isolated from peripheral blood cells identified a VUS in the BRCA2 gene, suggesting that the abnormality was constitutional. This alteration is predicted to result in the substitution of a glutamic acid for lysine at position 2663, but not to lead to a premature protein truncation.

No functional *in vitro* studies are available for this specific mutation however, it is possible to investigate its potential role by a computational approach that integrates informations

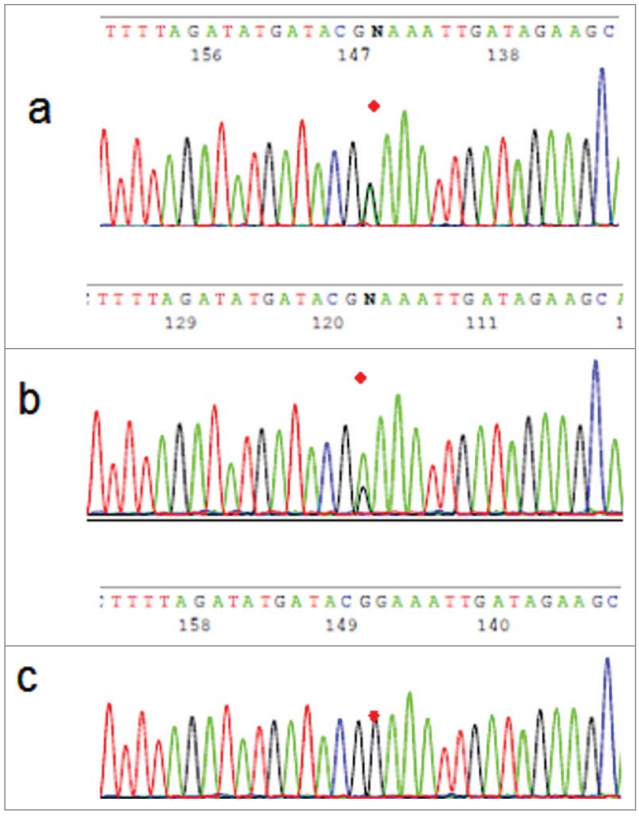

Figure 2. Direct DNA sequencing performed on DNA extracted from blood peripheral cells (a) and tumor tissue (b) as compared with the wild type control (c). The electropherograms identified a BRCA2 c.7987G>A germline mutation in the patient's blood (A, green peak) and the loss of the wild type allele in the tumor tissue (G, black peak), suggesting loss of heterozygosity of wild type and the retention of the mutated allele respectively.

Figure 3. The 3-microsatellite panel (D13S171, D13S1701, D13S1698) analysis of blood DNA (upper) and tumor tissue DNA (lower) confirmed loss of heterozygosity. Quantitative estimates of the peak areas indicate loss of about 50% of one allele.

about protein sequence, sequence conservation, and data from protein X-ray crystal structures to produce a probabilistic likelihood ratio predictive of whether a missense change impairs protein function.[14]

The mutations with ratio value in the range of 0.0 to 0.6 are considered have no significant effect on protein function and are classified as neutral, the functional effect of mutations that generate ratio in the range of 0.6 to 6.8 are uncertain while ratio value higher than 6.8 up to 190 are classified as deleterious.

The mutation Glu2663Lys observed in this clinical case, by using such computational predictive approach, gives a protein likelihood ratio of 20, that classified the mutation as "deleterious."

Moreover, the germline mutation involving the substitution of the negative charged glutamic acid with the positive charged lysine amino acid is located on the helical domain of the BRCA2 DNA binding domain and may influence the hydrogen bond network between the helical-OB1 interface protein regions which is important for retention of BRCA2 homology-directed repair activity.[15]

Interestingly, in liver metastasis tissue of the sarcoma we identify the same mutation and the microsatellite analysis in liver metastasis showed positive LOH of the wild-type allele.

The loss of the wild-type allele in the tumors from patients harboring deleterious germline mutations in any of the BRCA genes is the most frequent mechanism of complete gene inactivation.[16] In agreement with this mechanism of inactivation, we observed in our patient a selective retention of the mutated allele that further support a negative effect of the Glu2663Lys mutation on the integrity of the HR pathway.

Trabectedin-based chemotherapy has shown a high clinical benefit in STS, despite a low response rate, ranging from 7.4% to 9.9%.[4,5,17] Data from the expanded access program (EAP) of trabectedin showed that complete responses were observed only in 0.004% of treated patients.[18] Taken together, these data show that trabectedin-based treatment is effective in stabilizing STS giving a clinical benefit of about 50% despite the low rate of total responses.

The outstanding early complete metabolic response observed in this clinical case seems be associated with a germline potentially deleterious BRCA2 mutation and with the tumor loss of the wild-type BRCA2 allele due to a somatic aberration that likely leads to the deregulation of cellular HR function responsible for increased sensitivity to trabectedin.

In conclusion, this clinical report seems to indicate that the response to drugs that may directly create double-strand breaks, such as trabectedin, may be positively conditioned by specific tumor DNA repair defects. This finding supports the importance of the evaluation of the BRCA status before treatment because it may have a significant impact on the pharmacological response prediction of trabectedin treatments.

Ethical statement

Written informed consent was obtained from the patient for publication of this Case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal upon request.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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