Neoantigen-specific T-cell response after donor lymphocyte infusion associates with favorable outcome in a patient with i(12p) germ cell tumor, acute leukemia and sarcoma of the same clonal origin

A 22-year-old man was admitted to our hospital for bleeding diathesis with anemia and severe thrombocytopenia. He reported dry cough, fever, weight loss, and asthenia. Rare blast cells were observed on the blood smear. A 8.7 cm anterior mediastinal mass was seen on computed tomography (CT) scan. The levels of α-fetoprotein and lactate dehydrogenase were 45.7 μg/L and 7,380 U/L, respectively and the β-human chorionic gonadotropin level was normal. Biopsy through mediastinotomy revealed a placental alkaline phosphatase (PLAP)-positive mixed germ cell tumor (GCT), in which atypical large cells, later determined to be CD61-positive, were focally identified (Figure 1A-C). In addition to the seminoma, there were mature and immature teratomatous components represented by disorganized respiratory epithelium and a poorly differentiated mesenchymatous tissue, respectively. The bone marrow was massively infiltrated by similar, CD34-negative, CD61- and CD43- positive cells, with multilobulated nucleus and eosinophilic cytoplasm (Figure 1D, E). Staining for CD45, PLAP as well as lymphocytic and epithelial markers was negative. Cytogenetic analysis of the bone marrow showed a complex karyotype including polyploidy, +der(3) and an isochromosome i(12)p. Given these results, synchronous acute megakaryocytic leukemia (AML) and primary mediastinal mixed GCT was diagnosed, an association carrying a dismal prognosis.[1] Inspired by a report of combined chemotherapy targeting both tumoral components in a pediatric patient,[2] we designed a regimen based on cytarabine, mitoxantrone, etoposide phosphate, and cisplatin. After the first cycle of chemotherapy, bone marrow was seen to be leukemia-free, with a normal karyotype. The patient received three cycles of this chemotherapy regimen, with an intrathecal chemotherapy (methotrexate, cytarabine, and methylprednisolone) during the second cycle. Two additional cycles of etoposide phosphate and cisplatin were given at reduced doses due to hematotoxicity and infectious complications.

After completion of chemotherapy, the leukemia was in complete remission, the mediastinal mass had decreased in metabolism (SUV max 5.1 *vs*. 7.2) and size (4.7x.4.7cm *vs*. 8.6x5.8cm), and the α-fetoprotein level was normal. The patient underwent hematopoietic stem cells transplantation (HSCT) from a mismatched unrelated donor (human leukocyte antigen [HLA] compatibility 8/10, with incompatibility to 1 HLA-A and 1 DQB1 allele), with a dose of $0.37x10^6$/kg $CD34^+$ donor cells harvested from bone marrow ($1.3x10^8$/kg nucleated cells). Conditioning consisted of anti-thymocyte globulins 10 mg/kg/d for 5 days, cyclophosphamide 60 mg/kg/d for 2 days, etoposide phosphate 34 mg/kg, methylprednisolone 1 g for 2 days, and total body irradiation (total dose 12 Gy). The patient required numerous blood transfusions and experienced hemorrhagic cystitis but returned home 2 months after transplantation. A medullary biopsy performed at 3 months showed no residual leukemia.

Six months after HSCT, in the context of recurrent chest pain, a 18F-fluoro-2-deoxy-D-glucose positron emssion tomography (PET)/CT showed a 60% increase in size of the residual mediastinal mass with abnormal metabolic activity. α-fetoprotein and β-human chorionic gonadotropin levels were normal. Histopathological examination of the surgically resected mass showed a fusiform sarcoma with angiomatous differentiation (Figure 1F). The cells were negative for both CD61 and PLAP. The post-operative course was complicated by mediastinitis, pleural effusion, and lung edema.

Due to the appearance of mixed chimerism 10 months after HSCT, we administered two donor lymphocyte infusions (DLI) ($5x10^5$ and $6x10^6$ $CD3^+$ cells/kg). Complete donor chimerism was obtained and persistent complete hematological remission was confirmed. Almost 10 years after HSCT, the patient has no evidence of neoplastic disease, and an excellent performance status without any limitation in daily activities.

In order to explore the possible relation between the three tumors, we performed tumor exome sequencing. Forty-eight somatic mutations were detected in the GCT, 17 in the leukemia and 331 in the sarcoma. Phylogenetic reconstruction revealed 13 shared genetic events, suggesting a common clonal origin (Figure 2A). One of these was a driver splice site mutation in *TP53* (c.920-1 G>A), reported as COSM6917 in the COSMIC v76 database. The variant allele frequency (VAF) of this mutation was >2 times higher than median VAF of all other somatic mutations in the tumors (Figure 2B), suggesting a probable loss of the other allele and the lack of wild-type *TP53* in tumor cells. Another splice site mutation, also on chromosome 17, involved *CDK12*. Other shared genetic alterations included five loss of heterozygosity (LOH) events, including *PTEN* LOH.

Given the relatively high number of mutations detected in the sarcoma, we sought to identify whether mutation-specific anti-tumor T-cell responses played a role in the extraordinary course of this patient. We used somatic mutations from the three tumors to predict HLA class I-restricted

neoepitopes. These neoantigens were prioritized based on predicted major histocompatibility complex (MHC) binding affinity (absolute and quantile rank), excess affinity compared with the non-mutated protein, and protein expression in cancer (Protein Atlas). After filtering with these criteria, we retained 84 neo-peptides for further analysis. As expected, the number of potential neo-peptides was much higher in sarcoma than in AML and GCT. Some of these neo-peptides were shared by two tumors, and ten were common to the GCT, AML, and sarcoma. These 84 peptides were synthetized and tested in pools in interferon (IFN)-γ ELISPOT assays with the patient's peripheral blood mononuclear cells (PBMC) after 1 week of *in vitro* amplification. PBMC were obtained after complete donor chimerism. Positive response in two pools containing the common interleukin (IL) $36G_{16\text{-}24}$ YPSMCKPIT peptide was confirmed using the corresponding peptide (Figure 3). This neoantigen was restricted by HLA-B*54:01, an HLA allele shared between the donor and the patient and was detected in the sarcoma but neither in the GCT nor AML. The neoepitope-specific T-cell response was detected again in the patient's PBMC 7 years after HSCT.

Association between GCT and hematologic malignancies is a rare condition, occurring in about 2-5% of patients with primary mediastinal non-seminomatous GCT.[1] The median time from diagnosis of GCT to hematologic malignancy is 5 months but both tumors are diagnosed simultaneously in 33% of cases, with median survival from GCT diagnosis ranging from 1 to 6 months.[1,3] Sarcomas in patients with GCT are also rare. They are observed mainly in the context of primary mediastinal GCT, bear a poor prognosis and are thought to derive from yolk sac tumors.[4] We describe here a case of simultaneous AML and mediastinal GCT, followed by a mediastinal sarcoma. Treatment with combined chemotherapy, allogeneic HSCT/DLI and surgery resulted in a durable complete remission, possibly maintained through immunosurveillance.

A recent study analyzing 15 patients with GCT and associated hematological malignancies demonstrated that these malignancies evolve independently from a common precursor and not from further differentiation of the GCT:[1] Indeed, germ cells and hematopoietic cells are both em-

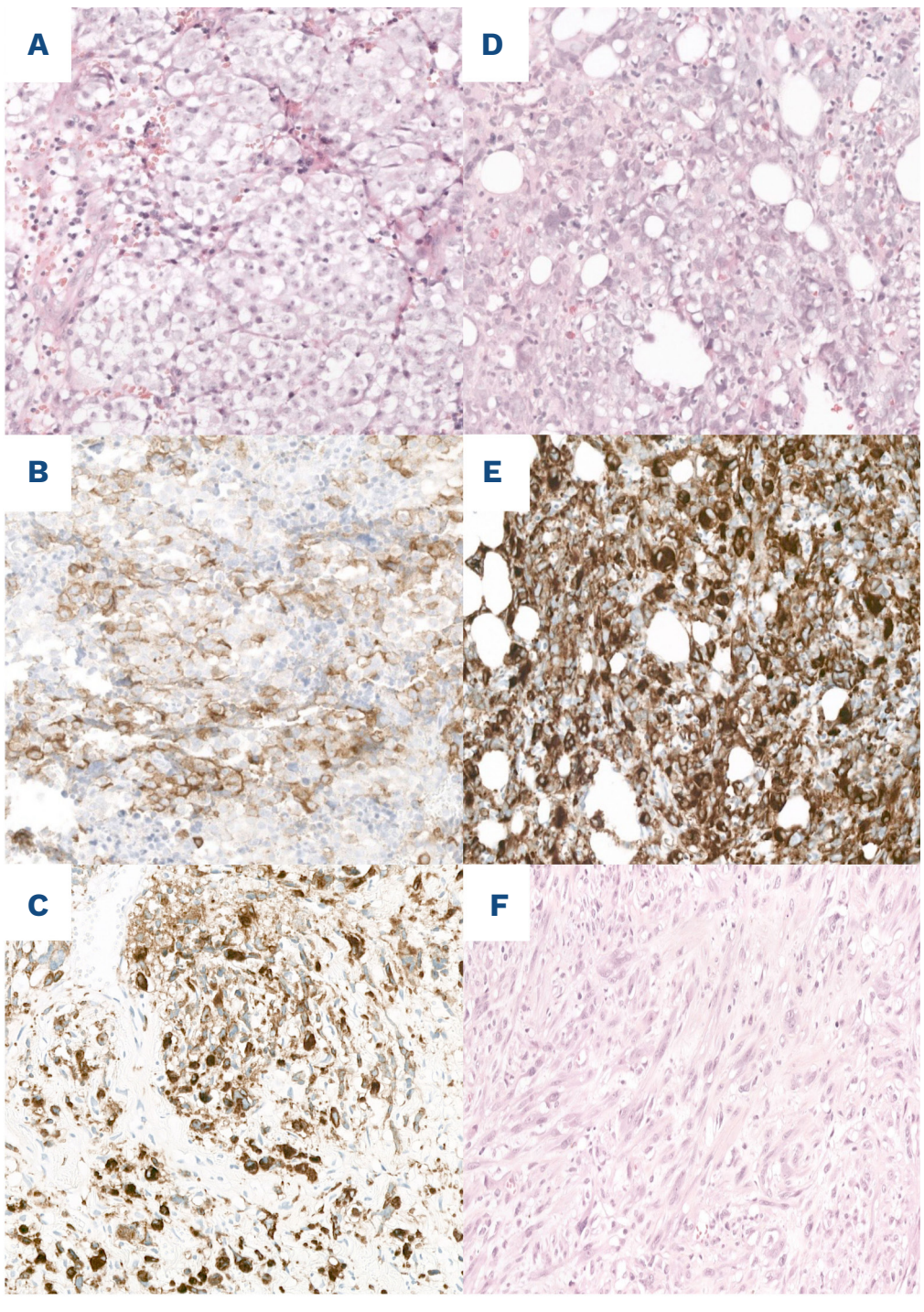

Figure 1. Histological analysis of the patient's tumors. (A-C) Seminomatous component of the mediastinal tumor: (A) hematoxilin and eosin (H&E) staining, (B) placental alkaline phosphatase (PLAP) staining, (C) CD61 staining showing the megakaryocytic differentiation. (D, E) Bone marrow biopsy at initial presentation: (D) H&E staining, (E) CD61 staining showing the megakaryocytic differentiation. (F) H&E staining of the sarcoma that developed in the mediastinum following bone marrow transplantation. The vascular differentiation can be seen by the presence of red blood cells in the cytoplasm of certain cells and was confirmed by ERG staining (*data not shown*). Magnification x400.

bryologically derived from the yolk sac and aorta-gonado-mesonephros. The timing of the hematologic malignancy diagnosis, nearly always during the year after GCT diagnosis and sometimes simultaneously, argues against secondary leukemia. Clues for a common clone are the presence of hematologic malignant cells in the yolk sac component of the GCT,[5] and the description of shared karyotype alterations, such as an isochromosome i(12p)[6] which was observed in the present case and typically occurs in GCT, but never in *de novo* hematological malignancies.[1] Our data also support a common origin since the three patient's tumors carried a rare *TP53* somatic variant and *PTEN* LOH, as previously described in similar situations.[7,8] Together, they may play a critical role in pathogenesis and sarcoma differentiation. If *TP53* inactivation is frequent in cancer, splice variants are far less common than missense variants.[9] No germline *TP53* alteration was found in this patient, although this variant has been reported in a hereditary syndrome. In addition, some of the 13 shared somatic variants detected, including *TP53* and *CDK12* splice site mutations, are considered putative driver mutations, which induce genomic instability and defective DNA repair, rendering tumor cells prone to further genetic alterations promoting tumor progression. Altogether, our molecular analysis confirmed the common origin of the three cancers and identified additional drivers that may contribute to the unusual switch to sarcoma phenotype. The extraordinary clinical outcome described here can potentially be explained by the combination of (i) an aggressive polychemotherapy regimen, (ii) the anti-tumor effect of the allogenic HSCT and subsequent DLI, and (iii) a neoantigen-specific T-cell response, for which the direct causality with clinical outcome could not be tested because of unavailability of viable tumor material. Debulking of AML/GCT was initially obtained with a chemotherapy regimen combining compounds targeting AML (cytarabine, mitoxantrone TD, etoposide) and GCT (etoposide and cisplatin), that may deserve further investigation in other patients with this rare entity. A beneficial role of allogenic HSCT and DLI, which

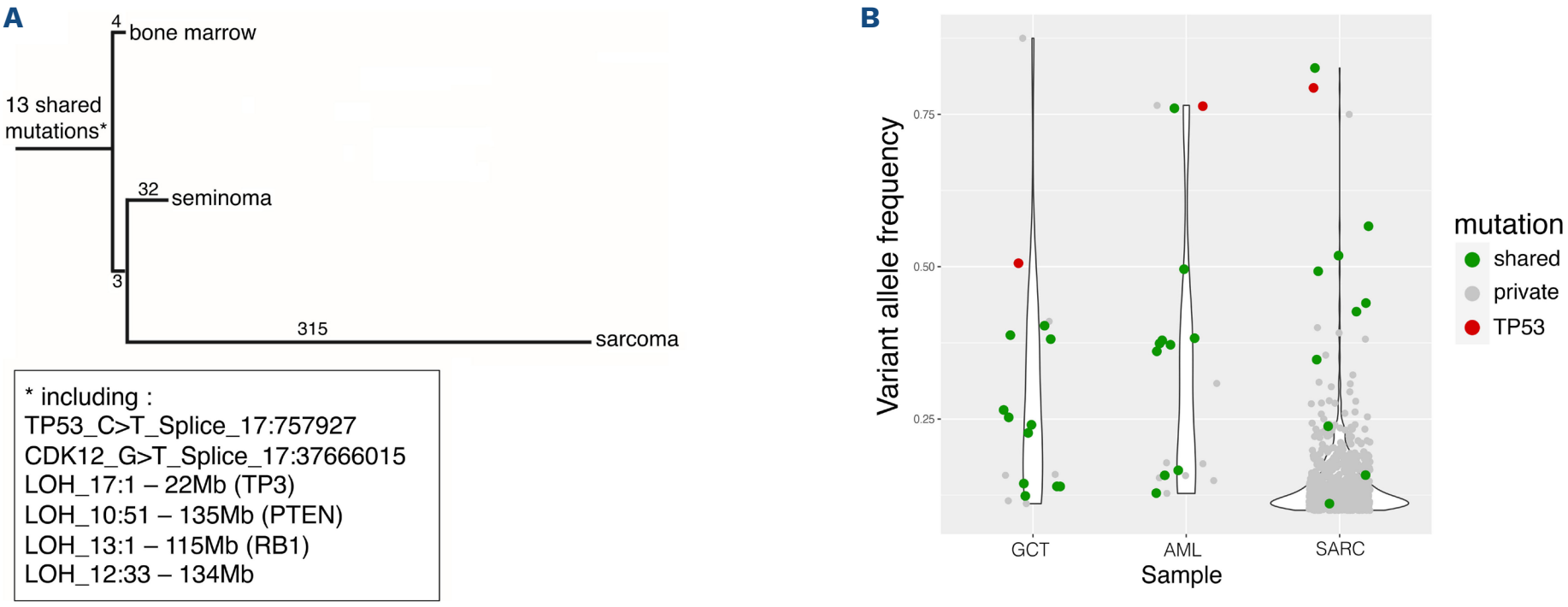

Figure 2. Molecular analysis of the patient's tumors. (A) Phylogenetic tree based on shared and private exomic mutations in different tumors from the patient. Putative driver events are marked in boxes. (B) Violin plots of variant allele frequency (VAF) of mutations in tumors. Red - *TP53* driver mutation. Green - common mutations. GCT: germ cell tumor; AML: acute megakaryocytic leukemia; SARC: sarcoma.

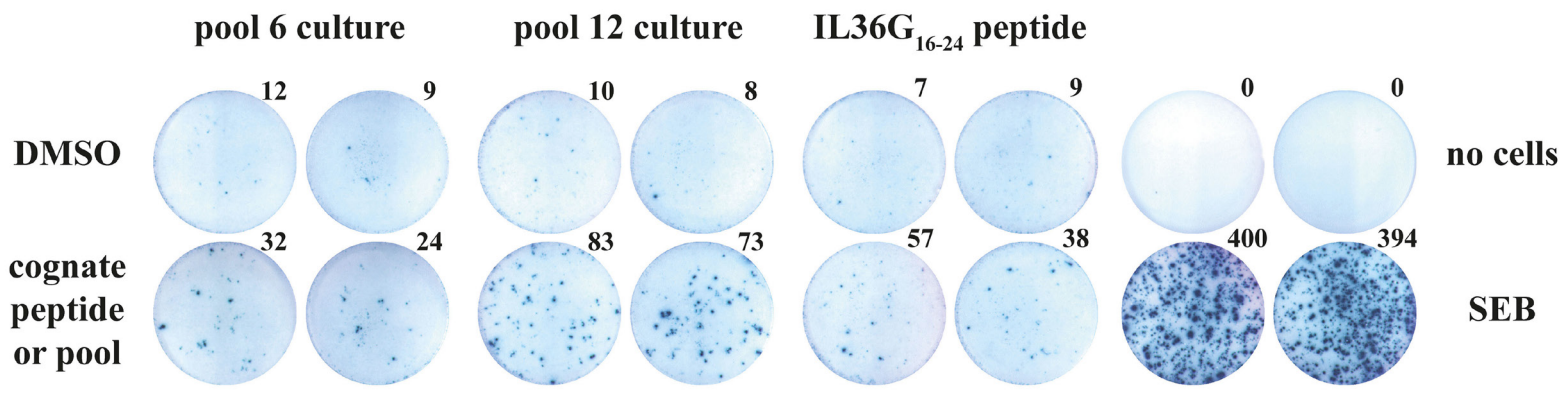

Figure 3. Interferon-γ ELISPOT assay showing recognition of peptide pools 6 and 12, and of the interleukin 36G$_{16-24}$ peptide common to the two pools. Displayed number above the spot is per 200,000 peripheral blood mononulcear cells. DMSO: dimethyl sulfoxide; IL: interleukin; SEB: Staphylococcal enterotoxin B.

mediated the recovery of a full complete chimerism, is highly probable and in accordance with the series reported by Taylor and colleagues[1] where the only patient with long-term survival had received HSCT. In addition to the anti-tumor effect mediated by the allogenic reaction, we were able to identify a neoantigen-specific T-cell response restricted by an HLA allele shared between the donor and the patient (HLA-B*54), detectable at two time points. The long-term persistence of this neoantigen-specific T-cell response suggests a potential role in the favorable outcome of this patient with an extremely poor prognosis and provides an additional clinical observation supporting the importance of genomic instability, mutational load and neoantigens in both anti-tumor immunity and response to immunotherapy.[10]

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Contributions

VG, VD and NT conceived and wrote the article. AJ and PT analyzed and interpreted the patient molecular data. TMK performed the histological examination. YC and PYD managed the patient. PYD conceived the treatment protocol. All authors read and approved the final manuscript.

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Written consent from the patient was obtained to publish this case report.

Data-sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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