Syngeneic transplantation has not been widely used since an identical genetic repertoire dose not guarantee the presence of a graft versus effect, although a GVL effect has been demonstrated in several studies [3,4]. In our case its rationale was the consolidation high-dose therapy with a disease-free stem cells rescue, associate with a low GVHD occurrence rate and with a possible GVL effect.

GVHD ha been observed rarely in the setting of a syngeneic transplant, and was initially described in animal and human beings after cyclosporine administration [3,5]. Skin, liver and digestive tract may be involved, causing clinical signs or symptoms that usually resolve without therapy, although sometimes immunosoppression has been needed and fatal case have also been described [4].

Cyclosporine inhibits lymphocyte function but causes a decreased thymic function and control of tolerance, resulting in persistence of auto-reactive clones [6]. Other studies described the presence of auto-aggressive T-cells not correlated with cyclosporine administration, suggesting that central and peripheral tolerance mechanism are probably less active also after syngeneic BMT [7]. Nelson et al. attempted to discover risk factors connected with its occurrence such as donor/recipient parity, CMV and viral status and age in relation to thymic function. Especially donor/recipient parity could be related to GVHD occurrence through microchemirism [5]. In our opinion it is important to underline that a graft versus-tumor effect probably exist afters syngeneic transplantation as already shown from clinical observation in high-grade lymphoma [4]. Some authors did not report any improvement in both overall survival and disease free survival in presence of GVHD [8].

In our patient a skin and stomach GVHD occurred during first 3 months after transplant and during the 3 years of follow up disease monitoring confirmed molecular CR even without imatinib therapy. Patient quality of live was good without nor chronic GVHD signs or symptoms nor severe infective complications. Regarding a risk factors hypothesized in some studies, donor and recipient were not young (age 50 years) suggesting a lower thymic function.

In the era of CML new treatment strategies, in presence of a twin a syngeneic transplantation could be considered due to its safer profile concerning infections and GVHD occurrence compared to allogeneic approach but in presence of a graft versus leukaemia effect.

Anyway more studies are needed in order to understand both the biology and clinical impact of GVHD and GVL in this type of transplant approach.

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M. Pelosini*
S. Galimberti
E. Benedetti
F. Caracciolo
R. Fazzi
M. Petrini
F. Papineschi
Hematology and Bone Marrow Transplantation Unit, Department of Oncology, Transplant and Advances in Medicine, University of Pisa, Italy

* Corresponding author. Tel.: +39 050993085; fax: +39 050992903.
E-mail address: mpelo78@hotmail.com (M. Pelosini)

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TPL-2 MEK kinase is not targeted by mutation in diffuse large B cell lymphoma and myeloid leukemia

To the Editor,

The ERK-1/2 mitogen-activated protein kinase (MAPK) signalling pathway plays a critical role in regulating cell division by transmitting proliferative signals from cell surface receptors to the nucleus. ERK-1/2 are phosphorylated and activated by the MAP 2-kinases, MEK-1 and 2, which in turn are phosphorylated and activated by a MAP 3-kinase, which for mitogenic receptors is a RAF family member. One of these, BRAF is constitutively activated by point mutations

Table 1
PCR primers used for *TPL-2* gene mutation screening

Primer name	Exon targeted	Sequence	Product size (bp)
3.1	Exon 3	Sense 5′ TGTATGTCAGTTTCCCATGGGTC 3′ Antisense 5′ GGGTTCATAAACTGCTGGCTC 3′	236
3.2	Exon 3	Sense 5′ CTTTATGCAAGTGAAGAGCCAGC 3′ Antisense 5′ CTGAGCACATAGCACCCAACT 3′	279
4	Exon 4	Sense 5′ GAAGTTCTCACCGTCTCACA 3′ Antisense 5′ CCAGAATGCCAAACACACAGT 3′	255
5.1	Exon 5	Sense 5′ GGGGTTATTTAGAATCTCG 3′ Antisense 5′ CCTTGAGAACATGCTTTGTC 3′	253
5.2	Exon 5	Sense 5′ CTCTTTATGGAAGCAGGCGAG 3′ Antisense 5′ TCTGGAGAAGGAAGACGGACG 3′	295
6	Exon 6	Sense 5′ TGTTTTCATTTGACTTATGG 3′ Antisense 5′ ATAGTAACCCTTTTCATGTG 3′	202
7	Exon 7	Sense 5′ AGCTGAATGTTTCCCACTTC 3′ Antisense 5′ CGGGATGCATAACTCAACGC 3′	241
8.1	Exon 8	Sense 5′ GCTTTTGCTGTTGAGGGTGT 3′ Antisense 5′ CAGGGCCTCATGTTTTAGTAG 3′	200
8.2	Exon 8	Sense 5′ GGAGAGAAACCCCAATCACC 3′ Antisense 5′ ACATCAGAGGTTTGATGTGG 3′	237
9	Exon 9	Sense 5′ CACTGCAGAAGGAACAGGTA 3′ Antisense 5′ TTTAGAGCAAACATGGCATC 3′	227

in a range of human cancers, resulting in uncontrolled ERK activation and cell proliferation [1].

Activation of ERK by receptors involved in innate immune responses utilizes an alternative MEK kinase, tumour progression locus 2 (Tpl-2), also known as COT or MAP3K8 [2]. *TPL-2* was originally identified as a transforming oncogene from a human thyroid carcinoma cell line and subsequently as an oncogene expressed in a Ewing sarcoma cell line [3,4]. *TPL-2* was also identified as the site of proviral insertion in Moloney murine leukaemia virus-induced T cell lymphomas [5]. Oncogenic activation of *TPL-2* always results in the production of protein that is truncated at its carboxy-terminus. The generation of transgenic mice expressing Tpl-2 under the control of the *Lck* promoter confirmed that C-terminal truncation confers oncogenic activity to Tpl-2 in T cells [6]. Together, these findings raise the possibility that Tpl-2 may be an oncogenic target in human cancer.

Consistent with this possibility, Tpl-2 is over-expressed in early stage breast cancer [7], large granular lymphocyte proliferative disorder [8], EBV-related nasopharyngeal carcinoma and Hodgkin lymphoma [9]. Furthermore, Clark et al. identified a novel mutation in the *TPL-2* gene from a primary lung cancer, which caused a carboxy-terminal truncation, and dramatically increased the transforming ability of Tpl-2 in vitro [10].

Somatic mutation analysis of the *TPL-2* gene has been reported only in lung cancer and it remained to be investigated whether *TPL-2* is activated by somatic mutation in other human cancers. In this study, we have investigated this possibility in 75 cases of diffuse large B-cell lymphoma (DLBCLs), 30 cases of acute myeloid leukaemia (AML) and 20 cases of chronic myeloid leukaemia (CML). Local ethical guidelines were followed for the use of archival pathological specimen for research and such use was approved by the local ethics committees of the authors' institution.

All DLBCL (formalin-fixed and paraffin-embedded tissues) and leukaemia samples (AML: 23 bone marrow and 7 peripheral blood; CML: 12 bone marrow and 8 peripheral blood) used for this study were diagnostic tissue biopsies, which contained a high proportion of tumour cells (>70% in DLBCL, 35–95% in AML samples and 86–100% in CML samples). DNA was extracted using standard methods and subjected to PCR of the exons 3–9 of the *TPL-2* gene. In total, 10 sets of PCR primers were designed and one of the primer pair was fluorescently labelled. Each primer pair targeted a short fragment of the *TPL-2* gene (Table 1), thus suitable for application to DNA samples extracted from formalin-fixed paraffin-embedded tissues as well as for mutation screening. Typically, PCR was performed with a single set of primers in a 25 μl reaction mixture using a touchdown protocol as previously described [11]. A heteroduplex formation step was included at the end of each PCR program consisting of heating the products to 95 °C and allowing it to cool to 40 °C over a period of 90 min [11]. The PCR products were then analysed on an ABI377 DNA sequencer using the protocols established by one (RAH) of the authors in a previous study [11]. Samples showing heteroduplex peaks, thus indicating presence of a mutation, were further investigated by sequencing. DNA samples with known *P53* gene mutation were analysed in parallel as positive controls.

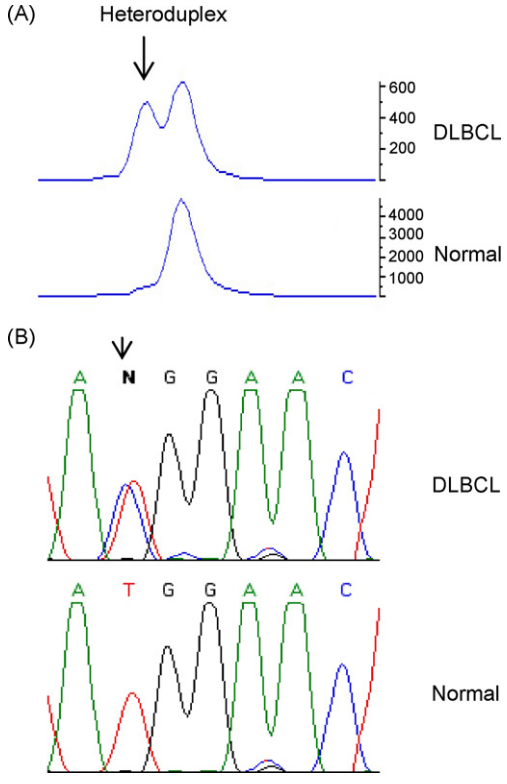

Fig. 1. Detection of *TPL-2* gene mutation by heteroduplex analysis, followed by sequencing. (A) Example of detection of heteroduplex peak in a case of DLBCL and (B) DNA sequencing shows T → C change in exon 3.2.

Of the lymphoma and leukemia cases studied, 19/75 (25%) cases of DLBCL, 11/30 (37%) cases of AML and 11/20 (55%) cases of CML showed presence of a heteroduplex peak with PCR product of exon 3.2 (Fig. 1). Sequencing of these PCR products all revealed a T → C change, a silent mutation, at the third base of codon 78 (nucleotide 930 of the cDNA sequence, NM_005204). A search of the Human Genome Database (http://www.ncbi.nlm.nih.gov/SNP) showed that this is a previously reported polymorphism (polymorphism ID is 1042058) with similar frequencies (34–43%) to that seen in our cases (33%). Our data therefore indicate that *TPL-2* is not targeted by mutation in diffuse large B cell lymphoma and myeloid leukemia.

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Sima Shirali
Rifat A. Hamoudi
Division of Molecular Histopathology, Department of Pathology, University of Cambridge, P.O. Box 231, Level 3, Lab Block, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, United Kingdom

Anthony Bench
Mike Scott
Department of Haematology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom

Steven C. Ley
Division of Immune Cell Biology, National Institute for Medical Research, London, United Kingdom

Ming-Qing Du *
Division of Molecular Histopathology, Department of Pathology, University of Cambridge, P.O. Box 231, Level 3,

Lab Block, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ, United Kingdom

* Corresponding author. Tel.: +44 1223 767 092; fax: +44 1223 586 670.

E-mail address: mqd20@cam.ac.uk (M.-Q. Du)

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