A novel *ETV6::FGFR1* fusion gene in a myeloid/lymphoid neoplasm with *FGFR1* rearrangement sensitive to specific *FGFR1-2-3* inhibition

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Introduction

Myeloid/lymphoid neoplasms with *FGFR1* rearrangement (FGFR1r) are rare entities, characterized by the aberrant expression of the tyrosine kinase involving the fibroblast growth factor receptor 1 (*FGFR1*) in a pluripotent, both myeloid and lymphoid, progenitor cell[1]. Clinical and hematological features are determined by the partner gene involved where patients with the same fusion gene tend to present a similar disease phenotype. Prognosis is poor and patients have an aggressive course, being allogeneic hematopoietic stem cell transplantation the only curative treatment nowadays[2].Herein, we describe a myeloid/lymphoid neoplasm with *ETV6::FGFR1* rearrangement refractory to intensive salvage chemotherapy, which showed response to specific inhibition of the abnormally activated *FGFR1* tyrosine kinase with pemigatinib, a pan-*FGFR1-2-3* inhibitor. To our knowledge, this is the first report of a myeloid/lymphoid neoplasm with *FGFR1* rearrangement involving *ETV6* as partner.

Case report

A 55-year-old male presented with dyspnea and bilateral cervical lymph nodes. Laboratory tests showed anemia (hemoglobin 123 g/L) and lymphopenia ($0.5x10^9$/L). Lymph node biopsy showed a monomorphic T-lymphoblastic infiltration with a high Ki-67 proliferation index (80%). Full body imaging showed enlarged lymph nodes at both sides of the diaphragm with moderate FDG captation (SUVmax: 5). Bone marrow aspirate ruled out bone marrow involvement. Accordingly, he was diagnosed with a T-lymphoblastic lymphoma

and treated according to PETHEMA-LAL-19 chemotherapy protocol for acute lymphoblastic leukemia/lymphoma (vincristine, daunorubicin, PEG-asparaginase and prednisone in induction), achieving a metabolic complete response (CR), and followed the consolidation phase with alternate courses of high-dose methotrexate, PEG-asparaginase, vincristine and dexamethasone and high-dose cytarabine, PEG-asparaginase and dexamethasone. Nonetheless, after the third course he presented a clinical relapse with diffuse lymphadenopathy without peripheral blood involvement. A lymph node biopsy confirmed a relapse of the T-lymphoblastic lymphoma. Unexpectedly, bone marrow aspirate showed 18% myeloid blasts and mild eosinophilia (Figure 1). Eosinophilia was not found in peripheral blood and had not been previously observed. Next-Generation Sequencing (NGS) DNA analysis showed two mutations in *STAG2* (c.382C>T;p.Arg110Ter, VAF: 3.21 and c.2464C>T;p.Gln822Ter, VAF: 8.21%), and RNA NGS revealed a non-previously described *ETV6::FGFR1* fusion gene, later confirmed by specific break-apart FISH probe and Sanger sequencing (Figure 2). With these findings, he was diagnosed with a myeloid/lymphoid neoplasm with eosinophilia and *FGFR1* rearrangement, with a dissociated phenotypic presentation at relapse, both a T-lymphoblastic lymphoma phenotypic cell population at lymph node and acute myeloid leukemia phenotype in bone marrow.

He received salvage therapy with FLAG-IDA regimen (idarubicin, fludarabine, high-dose cytarabine and granulocyte-colony stimulating factor as priming strategy), achieving a morphologic CR, with positive measurable residual disease (Flow citometry: 1.8% of myeloid blasts) in the bone marrow

compartment. After a consolidation cycle, based on high-dose cytarabine and ponatinib, he presented with another morphological relapse in peripheral blood. This relapse showed exclusively a myeloid phenotype, in form of an acute myeloid leukemia (25% of blasts)

The patient was enrolled in the phase II FIGHT-203 clinical trial with pemigatinib (13.5 mg daily), a *FGFR1-2-3* selective inhibitor, obtaining a complete response with undetectable measurable residual disease. Response was maintained, even though he interrupted treatment with pemigatinib due to a grade IV toxic epidermal necrolysis related to treatment. Allogeneic hematopoietic stem cell transplantation (alloHCT) from his HLA-identical brother with a reduced-intensity conditioning (fludarabine and 8 Gy total body irradiation) was performed with PTCy and tacrolimus GVHD prophylaxis, maintaining the CR 13 months after the first obtained CR with pemigatinib.

Targeted NGS analysis

Targeted NGS analysis was performed on genomic DNA and total RNA using the Oncomine Myeloid Research Assay (ThermoFisher Scientific) (Waltham, MA). This panel studies the 'hotspot' region of up to 23 myeloid gene mutations in DNA, the coding region of 17 genes in DNA, and the rearrangement of 29 fusion genes in RNA. A fusion between exon 5 of *ETV6* and exon 10 of *FGFR1* was identified (chr12:12022903 - chr8:38275891)(Figure 2)

RT-PCR and Sanger sequencing

One microgram of total RNA was retrotranscribed to cDNA using random primers and the M-MLV reverse transcriptase (Invitrogen). RT-PCR was

performed on cDNA with the following primers: ETV6-E5 F 5'-ATCATGGTCTCTGTCTCCCC -3' and FGFR1-E10 R 5'-CGAACCAGAAGAACCCCAGA-3'. The amplified RT-PCR product (158 bp) was then purified and sequenced on an ABI3500XL DNA sequencer (ThermoFisher Scientific) using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific). Sequencing analysis confirmed that exon 5 of *ETV6* is fused with exon 10 of *FGFR1*.

G-Banding Karyotype and FISH

G-banding karyotype was performed showing trisomy of chromosomes 8, 12 and 19 (ISCN: 49,XY,+8,+12,+19[15]/46,XY[5]). ETV6 break-apart probe (Metasystems) confirmed the trisomy of chromosome 12 and no rearrangement of this gene was observed. The analysis with FGFR1 break-apart probe plus CEP8 probe confirmed the trisomy of chromosome 8 and shows the presence of 2 extra copies of the telomeric region of FGFR1. The metaphase analysis of these probes revealed that extra copies of FGFR1 were located at the distal portion of the short arm of two chromosomes 12 (Figure 2)

Discussion

Herein, we describe a novel *ETV6::FGFR1* fusion gene in a patient diagnosed with a myeloid/lymphoid neoplasm with *FGFR1* rearrangement, presenting as a T-ALL and relapsing with a mixed T-lymphoid and myeloid cell population. Specific targeted agent pemigatinib made a complete response possible, which allowed bridging to an alloHCT to maintain it.

Myeloid/lymphoid neoplasms with *FGFR1* rearrangement were defined by McDonald et al in 1995 as 8p11 myeloproliferative syndrome, due to the *FGFR1* gene location[3], and it is nowadays included in the renamed myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK)[4]. *FGFR1* is a promiscuous gene with at least 13 partners described so far[5]. A subfamily of receptor tyrosine kinases, the *FGFR* family plays an important role in normal development and oncogenesis. Abnormal dimerization and kinase activation of the FGFR protein by the partner gene constitutes the pathological mechanism in FGFR-rearranged neoplasia[6]. They present with a variable disease phenotype depending on the partner gene, evolving to acute leukemia shortly after the diagnosis.

Previously, *FGFR1* fusion genes have shown refractoriness to most commonly used tyrosine kinase inhibitors with the exception of ponatinib and futinatinib[7,8], both showing individually clinical response reported in one patient with FGFR1r lymphoid/myeloid leukemia and alloHCT has been considered the only therapeutic strategy able to provide long-standing responses performed in initial chronic phase or following intensive ALL/AML-type chemotherapy in patients in blastic phase[1,9]. Nonetheless, the combination of ponatinib with high-dose cytarabine was not effective in the patient herein reported, as shown by the overt relapse emerging after the first treatment course with this treatment regimen. Refractoriness to small TK inhibitors has changed since the development of the *FGFR1-2-3* inhibitor pemigatinib. Results of the phase II FIGHT-203 clinical trial[10] with patients treated with pemigatinib, administered 13.5 mg daily were presented in the 2021 ASH meeting, showing a composite

response rate of 77% among 31 patients diagnosed with a myeloid/lymphoid neoplasm, 85% of them previously treated. Grade >3 adverse events in at least a 10% of the patients were anemia, pain in extremity and stomatitis. In our case, the patient maintained the molecular response although he presented a grade 4 skin toxicity, which prompted to discontinuation of this experimental agent.

ETV6 gene, located on chromosome 12p13, relates to many abnormalities in leukemia, including more than 30 described fusion genes[11]. As a member of the E-26 transformation-specific family (ETS), *ETV6* functions as a transcription repressor that regulates hematopoiesis and embryonic development[12]. Interestingly, it is one of the few well-known tyrosine kinase activators in hematologic malignancies, since one of the functional domains of the *ETV6* gene, the helix-loop-helix domain (HLH), has a homodimerization motif which activates constantly the tyrosine kinase domain (TK)[13].

Two fusion genes between *ETV6* and other members of the *FGFR* family have already been described, all of them with rearrangements involving the exon 5 of *ETV6* and leading to fusion of 5' extreme of the gene with 3' part of the *FGFR* gene. Thus, the HLH domain of *ETV6* rearranged is retained in all resulting fusion gene in both cases, leading to constitutive oligomerization of the TK of the *FGFR* gene without specific ligand binding. Firstly, a fusion of exon 5 of *ETV6* and exon 10 of *FGFR3* was described in a peripheral T-cell lymphoma[14], and in 2020, Carll T. et al. reported a case with a fusion of upstream exon 5 of *ETV6* and downstream exon 5 of *FGFR2* in a patient with similarities to this

report in terms of clinical presentation and aggressive course, characterized by refractoriness to all treatment lines, including alloHCT[15].

This report briefly describes for the first time the *ETV6::FGFR1* fusion gene in a myeloid/lymphoid neoplasm who presented a mixed, dissociated relapse in two different compartments, showing refractoriness to diverse salvage chemotherapy lines. Genetic screening of fusion genes and targetable driver events at diagnosis and relapse is essential for a more precise risk assessment and to consider the use of novel targeted agents. Due to the promiscuity of the *FGFR* family, it is likely for new rearrangements to be described in the future. Specific *FGFR1-2-3* inhibitors may help to obtain a response, later to be consolidated with an alloHCT in patients with a high relapse risk such as those harboring FGFR1r.

Acknowledgements

The patient could receive treatment with pemigatinib because he was enrolled in phase II FIGHT-203 clinical trial.

Author Contributions

CJV collected data and wrote de manuscript; MG designed the study and contributed to manuscript writing; JE supervised the study and contributed to manuscript writing; MR DC and MRB supervised the study; MLG performed molecular analysis; EV performed cytogenetic analysis; FG, AMR, OB, AAL and

JCHB collected data. All authors revised and approved the final version of the manuscript.

Competing Interests

The authors declare no competing interests.

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Figure legends

Fig. 1: Morphology and immunophenotype of both T-lymphoid and myeloid parts of the disease

A-B H&E lymph node stain (A: 4x objective lens, B: 50x objective lens) with a homogeneous lymphoblastic proliferation and an effacement of the normal nodal architecture.

C-D Immunochemostain of the lymph node, and negative for CD34(C), positive por T markers such as CD3(D).

E-H Bone Marrow Smear (May-Grünwald-Giemsa, 4x objective lens(E), 100x objective lens(F-H)) with a 18% of big-sized, basophil myeloid blasts with the presence of nucleoli, megakaryocytic dysplasia in up to 25% of the series and eosinophilia.

I-L Immunophenotype of the myeloid phenotypic relapse showing a 15% of blasts with the following phenotype: CD34+, CD33 ++, CD64+ (40%), CD14-, HLA-DR+, CD7 +/-. Analyzed by flow cytometry following the Euroflow standardized antibody panels

Fig. 2: Genetic and molecular characteristics

A G-Banding Karyogram shows trisomy 8, 12 and 19 in 15 of 20 metaphases (ISCN: 49,XY,+8,+12,+19[15]/46,XY[5]).

B RNA-NGS analysis performed with the Oncomine myeloid panel showing the fusion of exon 5 of *ETV6*, located in chromosome 12 and the exon 10 of *FGFR1* gene, located in chromosome 8.

C-E Specific break-apart FISH probe hybridized to G-banding karyotype confirmed the translocation of the short arm of chromosome 8 (*FGFR1* gene region) to the distal portion of the short arm of chromosome 12 (der(12)). **F** Scheme of the *ETV6::FGFR1* fusion gene. NH2: NH2 domain, HLH: helix-loop-helix domain, ETS: ETS transcription protein, COOH: COOH domain, SP: signal peptide, TM: transmembrane domain, Ig1-3: Ig-like domains, TK1 and TK2: Tyrosine kinase domains, KI: kinase insert region

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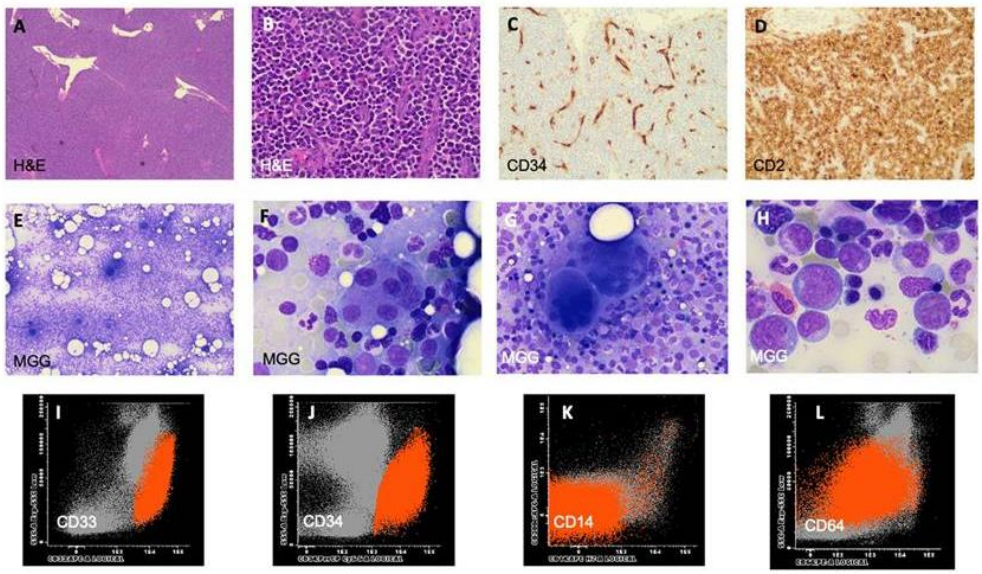
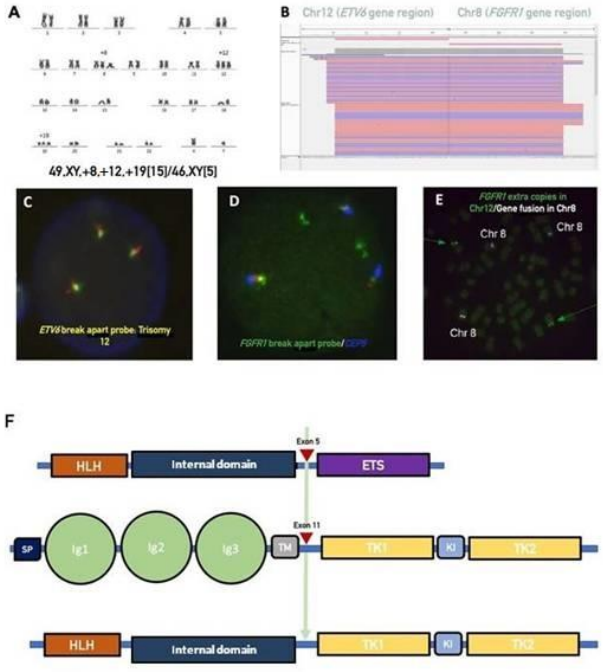


254
255 *Figure 2*



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