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CORRESPONDENCE

Detection of *BCR/PDGRFα* Fusion Using Dual Colour Dual Fusion *BCR/ABL1* Probe: An Illustrative Report

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Dear Sir,

The diagnostic work up for a suspected patient of myeloproliferative neoplasm (MPN), at first, involves to investigate for the presence of t(9;22)/*BCR–ABL1* fusion either through molecular or cytogenetic techniques. Here, we report a case of *BCR–PDGFRα* fusion diagnosed on the basis of an atypical FISH signal pattern on *BCR–ABL1* dual colour dual fusion probe, and further confirmed by doing karyotyping and matched metaphase FISH studies.

Case Report

A 37 years aged male presented with abdominal discomfort and palpable spleen (4 cm below costal margin in left mid clavicular line). His baseline investigations showed Hb 10.0 g/dl, TLC 50.2 × 10^9/l, Platelet count 482 × 10^9/l with 82% neutrophils, 1% eosinophil and basophil each, shift to left and no blasts. Interphase FISH analysis on peripheral blood sample using *BCR/ABL1* dual colour dual fusion probe (Zytovision Bremerhaven, Germany) was negative for *BCR/ABL1* fusion in 100% of cells. However in 64% of cells additional spectrum green (*BCR*) signals were observed. The extra green signal was seen on a probable B-group chromosome on a clumped poor quality metaphase making the definite identification of the chromosome difficult. On the basis of clinical findings and FISH results, suspecting him as a case of myeloproliferative neoplasm with an atypical FISH pattern, the patient was started on Imatinib treatment empirically awaiting karyotyping report, which was carried out on the peripheral blood sample as the patient was unwilling to undergo for the bone marrow examination. The karyotyping revealed a balanced translocation between the long arms of chromosomes 4 and 22 in all the analysed metaphases [t(4;22)(q22;q11)]. The matched metaphase FISH analysis confirmed the rearrangement of *BCR* gene, the extra spectrum green signal being present on the long arm of chromosome 4 at 4q22 locus that houses the *PDGFRα* gene. FISH analysis using *PDGFRα/FIP1L1* tricheck probe (Zytovision Bremerhaven, Germany) confirmed the rearrangement of *PDGFRα* and the presence of *BCR–PDGFRα* fusion transcript (Fig. 1). Patient responded to Imatinib therapy with normal WBC counts within a month of starting therapy. The patient was followed closely on a monthly basis and was in haematological remission at the last follow up, ∼ 2 years after starting therapy (Fig. 1).

Discussion

Chromosomal rearrangements in MPNs other than Ph chromosome that result in aberrant tyrosine kinase activity and are amenable to therapy with a tyrosine kinase inhibitor (TKI) include rearrangements of the *PDGFRα* or *PDGFRβ* genes. These are a rare group of MPNs and share some overlapping features, the most common being

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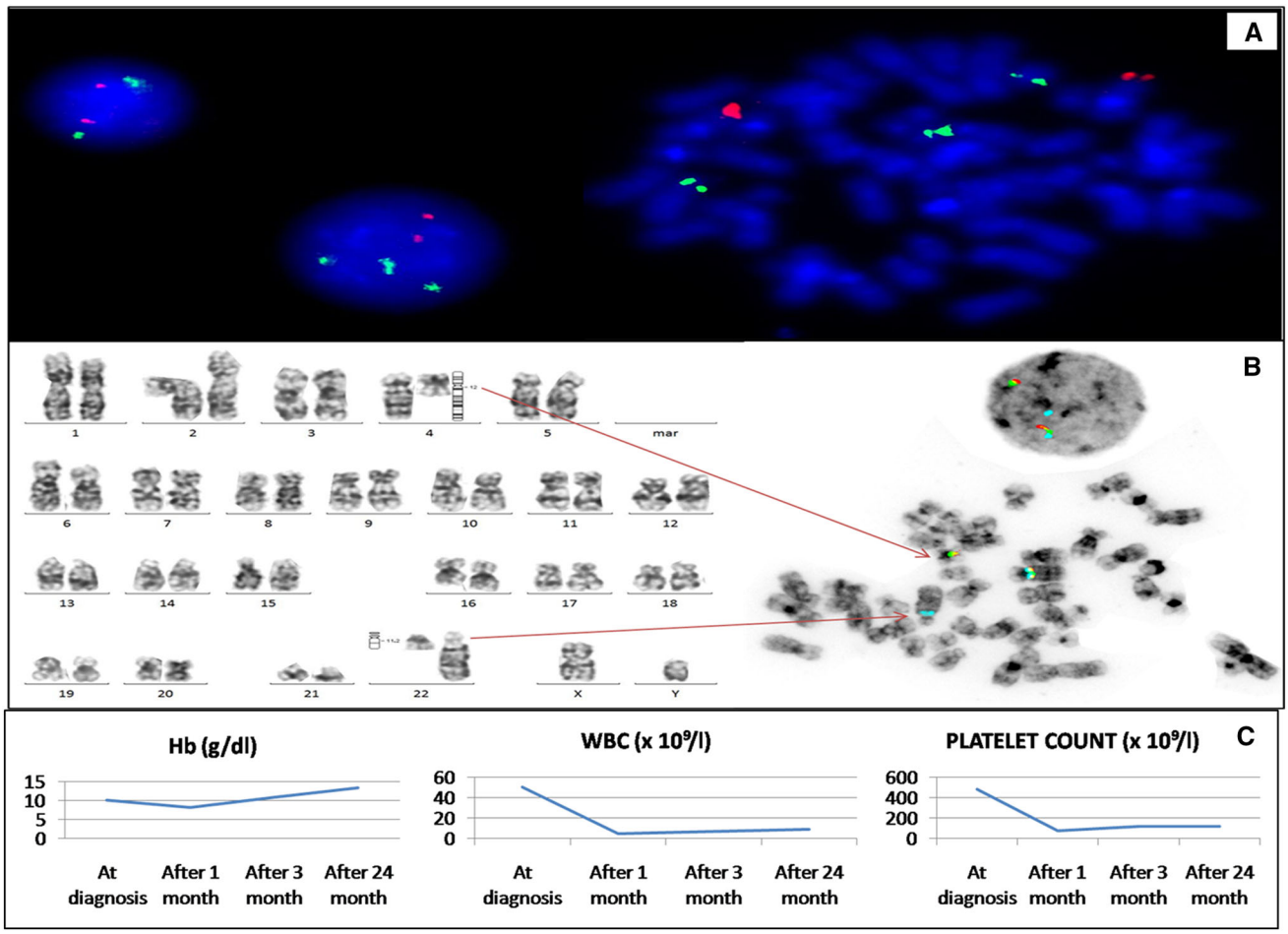

Fig. 1 **a** FISH analysis using *BCR/ABL1* dual colour dual fusion probe (*BCR*—Green, *ABL1*—Orange; Zytovision Bremerhaven, Germany) shows three spectrum green signals in an interphase nucleus and a clumped metaphase with an extra spectrum green signal on a probable group B chromosome, **b** karyotyping analysis from peripheral blood sample shows a balanced translocation between the long arms of chromosomes 4 & 22; Matched metaphase FISH analysis using *PDGFRα/FIP1L1* tricheck probe (5′ *FIP1L1*—Green, 5′ *PDGFRα*—Orange, 3′ *PDGFRα*—Aqua/Blue; Zytovision Bremerhaven, Germany) highlights the derivative chromosomes 4 & 22, **c** line diagram exhibiting patient's blood counts (colour figure online)

eosinophilia. The most common rearrangement involving the *PDGFRα* gene is the *FIP1L1/PDGFRα* fusion. Several fusion partners of *PDGFRα* in MPNs associated with eosinophilia, for e.g. *BCR*, *ETV6*, *KIF5B*, *CDK5RAP2*, *STRN*, *TNKS2*, and *FOXP1* have been documented in the form of case reports/-series [1–8]. Molecular techniques using specific primers directed towards the *FIP1L1* fail to detect these variant *PDGFRα* rearrangements.

Karyotyping identifies non cryptic translocations involving the *PDGFRα* gene and the same can be confirmed by using *PDGFRα* tricheck probe irrespective of the partner gene. In this case, we highlight that a systematic cytogenetic testing that includes karyotyping and FISH using the commonly available *BCR/ABL1* dual colour dual fusion probe can also diagnose some of these rare *PDGFRα* variants. As was reported previously, the extra *BCR* signal could be a result of *BCR* rearrangement and the partner was identified by studying metaphases using a the standard dual colour dual fusion *BCR/ABL1* probe [4, 5]. In our patient, a metaphase FISH study matched with GTG-banded karyotype, helped identify the variant fusion highlighting the importance of metaphase FISH in patients with atypical interphase FISH patterns.

The summary of reported cases of t(4;22)(q22;q11)/*BCR–PDGFRα* is described in Table 1. Contrary to the expectation peripheral blood eosinophilia was not seen in most of these patients. The presence of altered tyrosine kinase activity in these cases, which can be targeted with TKIs, had also been identified in subsequent literatures [4, 5]. Four of the seven cases for which the treatment follow up was available, were treated with Imatinib and showed durable responses.

This brief communication adds to the literature on this rare entity, highlighting the importance of conventional

Table 1 Overview of cases published in literature

S. no.	References	Patients	Diagnosis	Cytogenetics	Fusion transcript breakapoints	Followup
1.	Baxter et al. [3]	37/M	Atypical CML	t(4;22)(q12;q11) in metaohases from BM aspirate culture	*BCR* **exon 7** followed by 24 bp of the beginning of *BCR* **intron 7**, followed by *PDGFRA* sequence, **exon 12**	Progressed to accelerated phase with eosinophilia on Hydroxyurea
		Leukocytosis				Matched allotransplant
		Splenomegaly				Alive and healthy (85 months followup)
2.	Baxter et al. [3]	3/M	CML like MPD with extramedullary T-lymphoid blast crisis	t(4;22)(q12;q11) in all the metaphases from both BM and LN culture	*BCR* **exon 12** followed by a 12 bp insert followed by *PDGFRA* sequence, **exon 12**	Induction chemotherapy
		Leukocytosis		FISH using BAC bK143F12 (*BCR*) & BAC RP11-231C18 (*PDGFRA*)		Autologous PB stem cell transplant
		Lymphadenopathy				Myeloid hyperplasia with increase in abnormal metaphases with t(4;22)
		Hepatomegaly				Reinduction
		Splenomegaly				Allotransplant (MUD)
						Died on D + 50
3.	Trempat et al. [4]	47/M	Pre-B cell ALL	t(4;22)(q12;q11) in 95% of the cell	*BCR* **exon 1** in-frame with *PDGFRA* **exon 13**	Induction (LALA 2020 protocol)
		Leukocytosis		Post chemotherapy, remission marrow showed t(4;22)(q12;q11) in 87% of the cells		Intensification with high dose Cytosar & Mitoxantrone
		Lymphadenopathy		FISH using *BCR-ABL* probe: no fusion, extra *BCR* signal on chromosome 4 in matched metaphase		Blats decreased but Leucocytosis increased
		Hepatomegaly				Started on Glivec @400 mg/day
		Splenomegaly				Cytogenetic response within 6 weeks
						CNS relapse
						Intrathecal chemotherapy
4.	Safley et al. [5]	57/M	Atypical CML	t(4;22)(q12;q11) in 14 metaphases	*BCR* **intron 17** (position 143,925) and *PDGFRA* **exon 12** (position 1836)	Started on Imatinib @100 mg/day
		Past treated c/o DLBCL		FISH using *BCR-ABL* probe: no fusion, extra *BCR* signal (suggesting *BCR* rearrangement in 56.5% of the cells), partial karyotype showed chromosome 4 as partner		Hematologic response within 1 month
		Leukocytosis				A 7 months followup normal blood counts
		Lymphadenopathy				
5.	Erben et al. [6]	36	CEL	Not available	Used a generic quantitative RT-PCR to detect overexpression of the 3′-regions of *PDGFRA*	Not available

Table 1 continued

S. no.	References	Patients	Diagnosis	Cytogenetics	Fusion transcript breakapoints	Followup
6.	Erben et al. [6]	47	CEL	Not available	Same as above	Not available
7.	Wang et al. [7]	45/F	Mixed phenotypic acute leukemia (B/myeloid)	t(4;22)(q12;q11.2) in 12 of 13 metaphases	Reverse transcriptase–PCR showed ~ 590 bp product, consistent with the presence of a BCR/PDGFRα fusion gene	Treated with Imatinib, cytarabine, and idarubicin as induction and cytarabine and Imatinib as consolidation therapy
		Past treated case of breast cancer		FISH using *BCR-ABL* probe: no fusion, extra *BCR* signal (suggesting *BCR* rearrangement in 85% of the cells)—FIP1L1/CHIC2/PDGFRα deletion/fusion probe: signal separation in 96.5% of interphase nuclei		Complete cytogenetic, and molecular cytogenetic remission at days 14 and 28
		Leukocytosis				Remained disease free for 5 months since the initial diagnosis
						MUD transplant
8.	Yigit [8]	56/M	T lymphoblastic leukemia/lymphoma (T-ALL)	t(4;22)(q12;q11.2) in 19 of 20 metaphase	Not available	CALGB protocol
		Leukocytosis		Metaphase		Complete remission after 3 months (morphological and cytogenetic)
		Splenomegaly		FISH analysis showed that the BCR gene was translocated to chromosome 4, and the PDGFRA gene was translocated to chromosome 22		2 years maintenance therapy
		Lymphadenopathy				Remained in CR for 4 years since his last dose of maintenance therapy
9.	Present case	37/M	Myeloproliferative neoplasm	t(4;22)(q12;q11) in all the metaphases	Not done	Started on Imatinib
		Leukocytosis		FISH using *BCR-ABL* probe: no fusion, extra *BCR* signal, matched metaphase FISH analysis showed extra BCR on chromosome 4		Hematologic response within 1 month
						At 5 months followup normal blood counts

karyotyping and awareness of atypical signal patterns on FISH analysis in Ph negative myeloproliferative neoplasms.

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