The -7 chromosomal abnormalities with signs of myelodysplasia in chronic myeloid leukemia as a major red signal

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Chronic myeloid leukemia (CML) is a leukemic disorder the prognosis of which has been revolutionized by the use of several generations of tyrosine kinase inhibitors (TKI). During the course of treatment, patients can develop chromosomal abnormalities in addition to Philadelphia-positivity (Ph^+).[1] These Ph^+ clonal chromosomal abnormalities (CCA/Ph^+) could be considered markers of disease progression. A subgroup of patients has been clearly identified with fewer responses and worse outcome.[2] In addition, a few patients can also develop clonal chromosomal abnormalities in Ph-negative (Ph^-) cells (CCA/Ph^-). Based on small case series and anecdotal reports, the European Leukemia Net (ELN)[3] and the National Comprehensive Cancer Network Guidelines[4] did not consider that the presence of CCA/Ph^- negatively affects the prognosis provided there was no sign of bone marrow (BM) dysplasia, but chromosome -7/del(7q) abnormalities were identified as a red signal. Since 2001, a number of cases with chromosome 7 abnormalities have been reported, some of them harboring signs of myelodysplastic syndrome (MDS) or of acute myeloid leukemia (AML).[5-7] The precise risk of MDS or AML with 7/del(7q) abnormalities is unclear and most of the cases have been reported with a short follow up.

In this issue of *Haematologica*, Bidet *et al.*[8] report on the largest group of 26 CML patients presenting -7/del(7q) abnormalities treated front line with TKI with a median follow up of 6.47 years. These patients achieve lower cumulative incidence of deep molecular response, more frequently present BM signs of dysplasia, and are more frequently switched to second-generation TKI.

It is important to point out that CCA/Ph^- clones can only be identified when patients with CML achieve a cytogenetic response. Thus, if physicians want to capture these clones, they should propose that patient undergoes BM cytogenetic analysis during the course of the disease, and at least for the first two years. In addition, BM smears are also essential to detect signs of MDS/AML. Clonal additional abnormalities are usually identified as abnormalities present in ≥2 out of 20 metaphases or if the abnormalities are present in one metaphase in ≥2 assessments.

The first case with a deletion of 7q was reported by Gambacorti-Passeri,[9] and subsequently more details were provided by several groups on occasional individual cases. Soon after, Andersen *et al.*[10] reported on a patient who developed monosomy 7 after 12 months of imatinib therapy with BM hypoplastic signs and dysplastic features. Although CCA/Ph^- abnormalities is a rare observation, the risk of developing MDS or AML has not been precisely defined, and the proportion of patients with chromosome 7 abnormalities is not really known. Thus, it is important to perform studies on a large group of CML patients developing additional clonal abnormalities. By 2011, Groves *et al.*[5] had reviewed all cases published since 2002. The study cohort included 53 patients. The majority were in chronic phase but six had accelerated phase at the time of starting TKI. Previous treatment was interferon for 39 patients and only six patients were previously untreated. Of the 53 patients, -7 was the sole abnormality in 29 patients, -7 with +8 as an additional abnormal Ph^- clone in 14 patients, and del(7q) in ten patients. The chromosome 7abnormality was present in two or more metaphases in all but three patients. In the nine patients who developed AML, only one patient survived despite intensive chemotherapy or allogeneic stem cell transplantation. Resolution of MDS (refractory anemia with ring sideroblasts) was noted in one patient after discontinuation of TKI. Based on this survey, it was suggested that the risk of a second malignancy with -7 was significantly higher since none of the patients with del(7q) developed MDS or AML. As AML is an important event, the risk of developing AML was analyzed. Factors influencing the onset of AML appeared to be persistence (compared to transience) of a Ph-7clone, particularly -7sole, and a clone size of 50% or more at diagnosis; time to diagnosis of MDS/AML should also be considered. Transformation to MDS/AML was observed within six months of Ph -7 detection in 15 patients, with only one patient diagnosed at 11 months. Of the 12 patients with follow up of more than six months, none developed a second myeloid malignancy, suggesting that the risk of MDS/AML is higher within the first six months of Ph -7 detection than at later time points ($P<0.0001$). Karimata *et al.*[11] reported a single case of a 60-year Japanese man who was treated for seven years with various doses of imatinib. He had several episodes of cytopenia, and five years after the start of imatinib, a cytogenetic analysis revealed a complete cytogenetic response but monosomy 7 was detected in 16 out of 20 cells. BM smears showed a refractory anemia with multilineage dysplasia which progressed to AML and death. Two additional cases of monosomy 7 out of 155 CML chronic phase patients were subsequently reported.[6] The median time of the first appearance of monosomy 7 was six months. The clone of monosomy 7 persisted for eight years in the patient who achieved sustained major molecular response (MMR) and six months until BM transplantation in the second patient. More recently, the group of Cortes conducted a retrospective analysis of patients treated front line with first-, second-, and third-generation TKI.[7] Among the 598 evaluable patients, 108 (18%) had CCA/Ph^-. Of these, 4 patients with monosomy 7 had the worst survival.

In this issue of *Haematologica*, Bodet *et al.* provide useful additional information on patients who are diagnosed with -7/del(7q) abnormalities during the course of their

Table 1. Chronic myeloid leukemia cases with -7 del(7q) in Philadelphia-negative cells.

Author, year (ref)	N. of cases /type	N. with other CCA/Ph-	TKI	Previous TT	MDS	AML	Onset of CCA/Ph-	Outcome
Gambacorti-Passeri 2001[9]	1 Del(7)(q22q23)	1 +8	Imatinib	Yes IFN	No	No	6 months	NA
Groves 2011[5]	53 (including 6 AP) -7 (29) -7 + 8 (14) Del7q (10)	14 +8	Nilotinib Imatinib Dasatinib	Yes IFN	7	9	10 months 2.8-53)	AML alive 1/9 MDS alive 6 1 NA
Karimata 2011[11]	1 -7		Imatinib	Yes IFN	Yes	Yes	5 years	Died of AML
Wasilewska 2017[6]	2 -7	2 +8	Dasatinib Nilotinib	Imatinib	No	No	6 months	-8 years MMR -AlloBMT alive
Issa 2017[7]	4 -7	None	Imatinib Dasatinib Nilotinib Ponatinib	No	1	1	9 months	5 years OS 37% (95%CI: 1-80) -1 died of BC -1 died post allo BMT for AML -1 MDS in MR4.5 on bosutinib -1 MR 4.5 on imatinib
Bidet 2019[8]	26 -7	+8 (19.5%)	Imatinib Dasatinib Nilotinib Bosutinib Ponatinib	Yes (50%° of Pts) IFN	50%	No	2.08 years (0.8-12.65)	Worse EFS and PFS Same OS

N: number; CCA: clonal chromosomal abnormalities; Ph: Philadelphia; TKI: tyrosine kinase inhibitor; TT: therapy; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; IFN: interferon; NA: not available; AP: accelerated phase; MR: molecular response; MMR: major molecular response; alloBMT: allogeneic bone marrow transplantation; Pts: patients; BC: blast crisis; EFS: event-free survival; PFS: progression-free survival; OS: overall survival.

TKI therapy. Patients were selected from French centers of the French CML group. They screened 102 CML patients with CCA/Ph-, of these 26 had an abnormality of chromosome 7 [-7/del(7q)]. A control group of 11 MDS patients, four with 7 [-7/del(7q)], was obtained for comparison. All karyotypes were reviewed by the Groupe Francophone de Cytogenetique Hematologique (GFCH) members and then classified according to the International System for Human Cytogenetic Nomenclature (ISCN 2016), molecular monitoring being performed according to the ELN recommendations. Importantly, BM smears were assessed by morphological central review in 48 cases. Morphological dysplasia was considered significant when it was observed in 10% or more cells in any hematopoietic lineage with or without excess blasts (>5%). Underlying MDS was documented both by centralized morphological analysis of BM smears and by sequencing a targeted panel of 27 genes frequently altered in MDS and AML performed by a next-generation sequencing (NGS) assay. Chromosome 7 abnormalities were isolated in 13 out of the 26, and associated with +8 in 19.5% of cases. Patients who developed -7/del(7q) CCA/Ph- were significantly younger than other CCA/Ph- abnormalities (mean, 48 *vs.* 55 years old; P=0.035) and mostly benefit second-generation TKI as first line of treatment. Twelve out of the 26 patients who developed -7/del(7q) CCA/Ph- were in complete cytogenetic response at time of detection. Median follow up since CCA/Ph- detection was 5.35 (1-14) years for -7/del(7q) and 7.41 (0-15) years for others CCA/Ph- (P≥0.05). MDS signs were more frequent in -7/del(7q) CCA/Ph- patients as compared to other types of CCA/Ph-. Molecular response (MR) was less frequently observed in these patients: 33% (n=4 out of 12) of the -7/del(7q) patients with MDS signs achieved MMR or better *versus* 75% (9 out of 12) of these patients without MDS signs. Using NGS, MDS morphological features were significantly associated with the presence of mutation. Among the 12 patients with -7/del(7q), 4 presented MDS morphological signs and 3 were mutated. Cumulative incidence of MR4.5 was lower in patients with -7/del(7q). Although type of CCA/Ph- did not impact on overall survival, landmark analysis at three years after first TKI initiation revealed a strong negative impact of -7/del(7q) CCA/Ph- on event-free survival and progression-free survival.

The study of the French Group reports on the largest cohort with a long follow up (median 6.47 years) of CML patients with -7/del(7q) abnormalities.[8] These patients are younger, achieved lower cumulative incidence of MR4.5, and are more frequently associated with dysplastic features. One patient with -7 CCA/Ph- presented EZH2 mutations and then progressed to advanced CML phase; however, the role of this mutation can only be speculated as it was at low variant frequency.

Considering all the rare cases of -7/del(7q) CCA/Ph- abnormalities reported in the literature (Table 1), it

appears that such abnormalities are mostly observed during the first two years of TKI therapy. While there is a significant risk of a second myeloid malignancy in patients with -7 CCA/Ph$^-$, less than half of these patients will develop MDS/AML. The aggregate data provide evidence in support of the commonly held view that preemptive therapeutic strategies are not justified in all patients with detectable -7 CCA/Ph$^-$. Nevertheless, once a diagnosis of AML is confirmed in these patients, intensive treatment strategies, including allogeneic BM transplantation, are ineffective in most patients. One may speculate on the role of TKI in the mechanism of MDS development and the presence of -7/del(7q) CCA/Ph$^-$ abnormalities. The mutagenic effect of TKI on hematopoietic stem cells is not yet fully understood. However, it has been reported that a gastrointestinal stromal tumor patient developed MDS with monosomy 7 during imatinib treatment, suggesting that imatinib plays a direct role in causing MDS.[12]

The routine monitoring of CML patients is currently molecular assessment of the response. However, cytogenetic analysis is still relevant and should be performed with a BM smear certainly in cases of cytopenia during TKI therapy. Signs of dysplasia with -7 CCA/Ph$^-$ cells should be considered as a red signal and a switch to alternative treatment be discussed.

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Another piece of the puzzle added to understand t(4;11) leukemia better

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The story about t(4;11) leukemia, involving the *MLL/KMT2A* gene from chromosome 11q23.3 and the *AF4/AFF1* gene from chromosome 4q21, is still a mystery. The study by Agraz-Doblas *et al.*, published in this issue of *Haematologica*, adds some new and important information regarding the mysterious pathomechanism.[1] Agraz-Doblas *et al.* showed, for the first time, that the therapeutic outlook of patients with expression of both reciprocal MLL fusions, MLL-AF4 and AF4-MLL, is promising, but only 50% of the investigated patients seem to have this favorable condition; patients expressing only the *MLL-AF4* allele have an event-free survival of 10% and an overall survival of 30%. Moreover, only leukemic cells expressing both fusion alleles display the typical *HOXA* signature.

The fact that t(4;11) patients can be divided into two subgroups on the basis of *HOXA* transcription was first recognized by Trentin *et al.* in 2009,[2] and later confirmed by Stam *et al.* and Kang *et al.* in 2010 and 2012, respectively.[3,4] The missing *HOXA* transcription was correlated with overexpression of either IRX1 or IRX2[2,4] and a 3-fold higher relapse rate[3,4] Experimental overexpression of IRX1 revealed an interesting mechanism because it resulted in EGR1-3 expression.[5] EGR1 and EGR2 both control the $p21^{CIP1}$ gene and, thus, shut down the cell cycle and may even induce cellular quiescence, a known mechanism of resistance to treatment. CDK6 counteracts the actions of EGR proteins.[6] The second mechanism involves the IRX proteins, which are able to turn on HOXB4, a known stem cell marker of hematopoietic cells that activates factors such as TAL1, GATA factors, TGFB1, etc. Thus, expression of MLL-AF4 alone - with upregulated IRX proteins but without HOXA expression - may provoke treatment resistance or a stem cell-like mechanism which is