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Haematologica 2009 [Epub ahead of print]
doi:10.3324/haematol.2009.041528

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Clonal heterogeneity in the 5q− syndrome: p53 expressing progenitors prevail during lenalidomide treatment and expand at disease progression

Martin Jädersten,1,2 Leonie Saft,3 Andrea Pellagatti,1 Gudrun Göhring,6 James S. Wainscoat,4 Jacqueline Boulwood,4 Anna Porwit,3 Brigitte Schlegelberger,6 and Eva Hellström-Lindberg1,2

1Karolinska Institutet, Department of Medicine, Division of Hematology; 2Center of Experimental Hematology; 3Department of Pathology, Karolinska University Hospital Stockholm Sweden; 4The LRF Molecular Haematology Unit, NDCLS, John Radcliffe Hospital, Oxford, UK, and the Institute of Cell and Molecular Pathology, Hannover Medical School, Hannover, Germany

ABSTRACT

Clonal heterogeneity has not been described in patients with myelodysplastic syndrome (MDS) with isolated del(5q), for which lenalidomide has emerged as a highly potent treatment. However, transformation to acute myeloid leukemia (AML) is occasionally observed, particularly in patients without a cytogenetic response to lenalidomide. We performed molecular studies in a patient with classical 5q− syndrome with complete erythroid and partial cytogenetic response to lenalidomide, who evolved to high-risk MDS with complex karyotype. Immunohistochemistry of pre-treatment marrow biopsies revealed a small fraction of progenitors with over-expression of p53 and sequencing confirmed a TP53 mutation. TP53 mutated subclones have not previously been described in MDS with isolated del(5q) and indicate a previously unknown heterogeneity of this disease. The aberrant subclone remained stable during the treatment with lenalidomide and expanded at transformation, suggesting that this pre-existing cell population had molecular features rendering it insensitive to lenalidomide and prone to disease progression.

Key words: clonal heterogeneity, p53, lenalidomide treatment.


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Introduction

The immunomodulatory drug lenalidomide is a potent treatment for low-risk myelodysplastic syndrome (MDS) with a karyotype including the deletion of 5q31 (del[5q]), with 67% achieving transfusion independency, and 45% obtaining complete cytogenetic remission. However, 50% of responders relapse within 2 years, and the estimated risk of leukemic transformation at 10 years is 15% for cytogenetic responders and 67% for patients without a cytogenetic response.

Design and Methods

Patients and treatment

A patient with classical 5q− syndrome (MDS with isolated del[5q]) according to the WHO 2008 classification3 received lenalidomide within a clinical trial (Celgene MDS-004). The clinical and laboratory studies were approved by the Ethical Committee for Research at the Karolinska Institutet, Stockholm, Sweden. Informed consent was obtained from the patient.

Bone marrow assessment and immunohistochemistry

Histopathology and morphology was assessed on consecutive bone marrow (BM) samples. Immunohistochemistry on paraffin-embedded sections was performed using mouse monoclonal antibodies: p53 (clone DO-1; Santa Cruz Biotechnology, Santa Cruz, CA, USA); CD34 (DakoCytomation, Glostrup, Denmark). Ten normal BMs were stained and demonstrated p53 expression in less than 0.01% of cells.

Chromosome banding analysis

After 20-48 hours of culture, metaphases of BM were prepared and fluorescence R-banding was performed.

Acknowledgments: we express our warmest gratitude for excellent technical support to Lalla Forsblom (cell separation and culture) and Monika Jansson (FISH analysis), at the Karolinska Institutet, Department of Medicine, Division of Hematology, to M. Hagedorn, M. Schoop, J. Espenkötter, R. Rodde, W. Arndt, and M. Meyer (cytogenetics) at Hannover Medical School, and Cristina Fernández-Santamaría (TP53 sequencing), The LRF Molecular Haematology Unit, NDCLS, John Radcliffe Hospital, Oxford, UK. This work was supported by the Competence Network “Acute and chronic leukemias”. Finally, we thank Dr. Robert Knight, Celgene, for allowing us to use cytogenetic data obtained within the Celgene MDS-004 clinical trial. Funding: the work was financially supported by grants from the The International MDS Foundation (M.J.), Swedish Cancer Foundation (070678:E.H.-L.), Swedish Medical Research Council (K2005-71X-15273-01A:E.H.-L.), the Cancer Society in Stockholm (051143; E.H.-L), and the Leukaemia Research Fund of the U.K. (IB). Manuscript received on May 17, 2009. Revised version arrived on June 12, 2009. Manuscript accepted on June 16, 2009. Correspondence: Martin Jädersten, Karolinska Institutet, Department of Medicine, Center for Experimental Hematology, Karolinska University Hospital Huddinge, SE-141 86 Stockholm, Sweden. E-mail: martin.jadersten@ki.se
Karyotypes were described according to the International System for Human Cytogenetic Nomenclature.  

**Fluorescence in situ hybridization (FISH) and M-FISH**

Interphase FISH was performed using a probe for the locus 5q31. Depending on the cytogenetic aberrations detected, probes (Abbott, Wiesbaden, Germany) were applied for the MLL-locus (11q23), RB1-locus (13q14), TP53-locus (17p13), BCL2-locus (18q21), and AML1-locus (21q22). At least 200 nuclei were evaluated. M-FISH analysis was carried out as previously described.

**Cells and cultures**

BM sampling for *in vitro* culture was performed pre-lenalidomide and at time of disease progression. CD34+ progenitors were selected from BM mononuclear cells and cultured for 7 days ± lenalidomide 10 µM, as recently described.

**Gene expression profiling and gene sequencing**

RNA and DNA were extracted from cultured progenitors at day 7. Gene expression profiling analysis was performed as described elsewhere. Pathway analysis was performed using Ingenuity Pathway Analysis software (Ingenuity Systems, Redwood City, CA, USA).

DNA-sequences spanning exons 5-8 of TP53 were amplified by polymerase chain reaction (PCR), using published primer sequences. PCR products were sequenced using the BigDye Terminator v1.1 kit (Applied Biosystems).

**Results and Discussion**

**Clinical observations**

A 76-year old woman was diagnosed with 5q- syndrome in April 2004. She was transfusion-dependent up-front (2 units of red blood cells per month), and had a transient response to EPO. Lenalidomide treatment was started in February 2006, leading to a complete erythroid response, and a reduction in the del(5q) clone from 75% to 32% by FISH. G-CSF was given during the first months. The transfusion-need recurred following pneumonia in October 2007, however, neutrophil and platelet counts were still normal. In November 2007, 22 months after start of treatment, BM examination showed 9% blasts and cytogenetic analysis demonstrated a large clone with complex karyotype, including del(5q). By FISH, a loss the TP53 locus at 17p13 and a gain of the MLL- and the RB1-loci could be confirmed (Table 1, Figure 1). In December 2007 she was started on 5-azacytidine and became transfusion independent.

| Table 1. Summary of clinical data and laboratory investigations. |
|-----------------|----------------|-----------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **t\* Clinical status† WHO‡ Karyotype FISH 5q§ FISH additional PLT (x10\(^{9}\)) ANC (x10\(^{9}\)) Blasts CD34\* NPMc\* p53\+ |
| Primary diagnosis 5q| 46,XX,del(5)(q14q34)[24] | ND 13q14(RB1x2) 100% | 13q14(RB1x2) 96% | 17p13(P53x2) 97% | 482 1.8 2% 9% 5% 2% |
| RBC transfusions 5q| 46,XX,del(5)(q14q34)[24] | 85% | 11q23(MLLx2) 100% | 13q14(RB1x2) 100% | 17p13(P53x2) 99% | 450 2.3 4% 2% 5% 4% |
| Pre-LEN sampling‡ 5q| 46,XX,del(5)(q14q34)[21] | 76% | ND 369 1.9 | <5% ND ND ND |
| LEN, CER, on G-CSF 5q| 46,XX[5] | ND ND ND | 135 1.3 2% 2% 5% 1% |
| LEN, CER, on G-CSF 5q| 46,XX[18] | 25% | ND ND ND | 207 3.1 ND ND ND ND |
| LEN, CER, on G-CSF 5q| 46,XX[12] | 32% | ND 197 3.2 | ND ND ND ND |
| LEN, CER, on G-CSF 5q| 46,XX,del(5)(q14q34)[10] | ND 46,idem,+10,der(11)(t)(11;16) | 11q23(MLLx3) 48% | 13q14(RB1x3) 31% | 17p13(P53x1) 35% | 21q22(AML1x2) 99% |
| LEN, CER, on G-CSF 5q| 46,XX,del(5)(q14q34)[9] | 51% | ND ND ND | 200 1.4 9% 10% 5% 10% |
| LEN, CER, on G-CSF 5q| 46,XX[16] | 11g23(MLLx3) 48% | 13q14(RB1x3) 31% | 17p13(P53x1) 35% | 21q22(AML1x2) 99% |
| LEN, CER, on G-CSF 5q| 46,XX[12] | ND ND ND | 200 1.4 9% 10% 5% 10% |
| 5-AZA, CER RAEB-2 5-AZA| ND ND ND | 146 0.5 13 ND ND ND ND |
| 5-AZA 5-AZA| AML ND ND | 271 0.2 27 ND ND ND ND |

\*t, time in months from initiation of treatment with lenalidomide. RBC, red blood cell; LEN, lenalidomide; CER, complete erythroid response. (19) WHO category: 5q-, 5q-syndrome; RAEB, refractory anemia with excess blasts; AML, acute myeloid leukemia. §FISH 5q, proportion of cells with 5q-deletion as determined by FISH for 5q31(EGR1). ND, not done.
although there was no decrease in bone marrow blasts. After 8 cycles she progressed to full leukemia and succumbed to an infection.

**Cell culture and gene expression**

Pathway analysis based on the gene expression profiles of cultured cells (86-96% del[5q] by FISH) demonstrated altered apoptosis and integrin signaling at treatment failure compared pre-treatment, which may reflect a more aggressive disease and an altered interaction between MDS progenitors and stroma.

We also assessed the effect of lenalidomide on mRNA expression of SPARC and Activin-A, based on data from a previous study. Both genes were up-regulated in post-treatment samples (3.6-7.1 and 2.5-5.1 fold, respectively), similarly to our findings in lenalidomide naïve samples.

As the complex karyotype at progression included trisomy 13 we assessed the expression level for the FLT3 gene, located at 13q12. There was an up-regulation of FLT3 at the time of progression, however, from a low pre-treatment level.

**Mutated TP53 pre-treatment**

Before treatment with lenalidomide, 1-2% of the BM progenitors over-expressed p53, which may indicate a mutation in the TP53 tumor suppressor gene. TP53 plays a crucial role in genomic integrity and stability. Interestingly, this subclone expanded in parallel with the blasts count at disease progression (Table 1, Figure 1). TP53 was sequenced in pre- and post-treatment samples. Before treatment, an A>G mutation in exon 5 (Y163C) was identified at a similar levels as the wild-type gene. At time of progression the mutated gene dominated, consistent with the cytogenetics demonstrating a complex karyotype including del(17p13) resulting in loss of the one TP53 allele (Table 1).

TP53 mutations are exceedingly rare in the 5q- syndrome, however, they frequently occur in MDS with complex karyotypes including del(5q) or in therapy-related MDS, invariably implying a poor outcome. The frequency of small fractions of BM progenitors with abnormal p53 expression, which may fall below the detection threshold for a mutational analysis, is unknown in MDS with isolated del(5q).
Potential prognostic implications of p53 expression
subclones pre-treatment

The p53 mutated clone in our case was present before treatment with lenalidomide, remained stable despite a partial cytogenetic response, and expanded at progression. We argue that this clone consisted of cells with inherent genomic instability which increased their probability of acquiring molecular features making them less sensitive to lenalidomide. The p53 expressing clone expanded in parallel to the blast count at time of transformation, and the complex karyotype evolving included deletion of the TP53 locus at 17p13 in addition to trisomy 13, incorporating the FLT3 gene, and rearrangements leading to a gain of the MLL locus on chromosome 11. Consequently, inactivation of p53 and acquisition of additional genetic aberrations are likely to have contributed to progressive disease in this patient. Moreover, the presence of trisomy 13 at time of disease progression during lenalidomide treatment is in contrast to a recent report suggesting that AML patients with trisomy 13 may respond particularly well to lenalidomide. The cytogenetic characteristics at time of transformation have not been well studied. A preliminary report indicated that 7 of 22 (32%) MDS patients with <10% BM blasts and a karyotype including del(5q) receiving lenalidomide developed complex karyotypes at time of progression, at a median of 44 months from diagnosis. Of the 7 patients who progressed, 2 patients had a true low-risk 5q- syndrome, while 5 had a blast count 5-9% or del(5q) with one additional chromosomal abnormality. In addition, a recent report described a patient with classical low-risk 5q- syndrome that transformed to AML with a highly complex karyotype after 8 months of treatment with lenalidomide. It is conceivable that lenalidomide may suppress a favorable clone and allow expansion of a more malignant subclone, however, it is equally conceivable that the potent effect of lenalidomide may delay progression. This question is best addressed in a randomized trial. A first step could be to analyze the association of p53 over-expression and TP53 mutation with the probability of disease progression in an expanded cohort of MDS del(5q) patients. This will not answer the question whether lenalidomide affects the natural course of the disease, however, it may identify a subgroup of patients at higher risk of transformation who should be carefully monitored and in which other treatment modalities should be explored. The presence of easily detectable subclones with inactivated p53 and thereby a more malignant potential represents a novel concept in low-risk MDS and may have important prognostic implications.

Authorship and Disclosures

Designed research: MJ, LS, GG, APo, BS, EHL; managed the patient: MJ, EH-L; generated and analyzed data: MJ, LS, AP, GG, JSW, JB, APo, BS, EH-L; wrote the paper: MJ, LS, GG, APo, BS, EH-L.

The following authors participated in the Celgene MDS-004 trial: Eva Hellström-Lindberg (Swedish principal investigator), Martin Jädersten (co-investigator), and Brigitte Schlegelberger and Gudrun Göhring were part of the central reference laboratory for cytogenetics.


