Single agent lenalidomide induces complete remission of acute myeloid leukemia in patients with isolated trisomy 13

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Running title: Lenalidomide induces CRc in trisomy 13 AML.
Abstract

Patients with acute myeloid leukemia (AML) frequently fail chemotherapy due to refractory disease, relapse, or toxicity. Among older AML patients (age >60 years), there are few long-term survivors. Lenalidomide is a candidate for study in AML based on its clinical activity in a related disorder, myelodysplastic syndrome (MDS) with the 5q- chromosomal abnormality. We report induction of sustained morphologic and cytogenetic complete remission in two older AML patients treated with high-dose, single agent lenalidomide; each patient had trisomy 13 as the sole cytogenetic abnormality. We show for the first time that lenalidomide has clinical activity in this poor-risk cytogenetic subset of AML. The clinical trials described in this paper have been registered with www.clinicaltrials.gov under identifier NCT00466895 and NCT00546897.
Introduction

Advances in the treatment of AML, including cytogenetic and molecular risk-stratification, have improved clinical outcomes for younger patients (age < 60 years)\(^1\)-\(^3\). In contrast, older AML patients given standard cytotoxic treatments have a dismal prognosis with a median survival of less than one year\(^4\),\(^5\). Poor outcomes in older patients, who constitute the bulk of AML cases, are related to the combination of unfavorable leukemia biology and co-morbidities that limit treatment options\(^5\),\(^6\). Thus, successful development of novel AML therapy requires not only improved understanding of leukemogenesis but also tolerability of the drug in older patients. Novel drug development strategies are assisted by identification of molecular markers or biologic features of disease associated with increased likelihood of clinical response to the treatment.

Lenalidomide is active against a number of hematologic malignancies and FDA approved for both relapsed multiple myeloma and low risk MDS with the 5q- cytogenetic abnormality. Cytogenetic responses observed in previous clinical trials of lenalidomide in MDS suggest that lenalidomide eliminated the abnormal MDS clone\(^7\),\(^9\). The lenalidomide dose in these trials was limited to 10 mg/day by drug-related myelosuppression; this dose results in substantially lower plasma concentrations compared with higher dosing strategies\(^10\),\(^11\). Since therapy-related myelosuppression is routine in AML treatment and may be necessary to obtain complete remissions, we explored the activity of higher doses of lenalidomide in AML in two independent clinical trials. We report here sustained morphologic and cytogenetic complete remissions
(CRc) achieved with high-dose lenalidomide in two older AML patients harboring trisomy 13 as the sole cytogenetic abnormality.
Case Reports

At both institutions involved, protocols were approved by scientific review committees and then at local Institutional Review Boards and informed consent was obtained in accordance with the Declaration of Helsinki.

Case 1.

A 71 year old male with no prior history of MDS presented with dyspnea and pancytopenia. Bone marrow (BM) biopsy showed 90% myeloblasts, undifferentiated (FAB M0) AML (Fig 1A); flow cytometry revealed that the blasts expressed CD34, CD33, CD13, and CD117. Metaphase cytogenetics showed trisomy 13 in 5/20 metaphase cells but no other abnormalities (Fig 1B). FISH studies excluded abnormalities of chromosomes 5, 7, 8, and 11q23. Patient characteristics are summarized in Table 1.

After providing informed consent, the patient was treated on a human research protection office (HRPO)-approved clinical trial evaluating lenalidomide in untreated AML for patients aged > 60 years at Washington University in St. Louis, MO (clinicaltrials.gov: NCT00546897). Study therapy consisted of high-dose lenalidomide at 50 mg/day x 14 days, followed by 30 days off therapy, and then a second course of 50 mg/day x 21 days. Peripheral blood AML blasts cleared after the initial 14 day course of lenalidomide. At day 30, the BM biopsy showed 25% cellularity with 72% blasts (and was FLT3-ITD positive). Following cycle 2 of high-dose lenalidomide, BM biopsy showed aplasia with <10% cellularity, though blasts were present, and he remained transfusion dependent. Cycle 2 was complicated by two episodes of
neutropenic fever requiring hospitalization, for which he received G-CSF. Thirty days after completing the 2 high-dose cycles, he began low-dose lenalidomide (10 mg daily in 4 week cycles), as per the clinical protocol. After 21 days of low-dose lenalidomide, his blood cell counts normalized without transfusion or growth factors. At the completion of his first low-dose cycle (124 days from initiation of lenalidomide) BM showed 60% cellularity with < 5% blasts. Cytogenetics showed no clonal abnormalities, and FLT3-ITD was negative. This CRc was confirmed on two subsequent BM biopsies at 6 and 16 weeks following the initial CRc. The patient continued on low dose lenalidomide for 10 months, but subsequently relapsed (duration of CRc was 9 months).

Notably, 0/13 non-trisomy 13 abnormality AML patients treated on the Washington University clinical trial achieved CR, although two PRs were observed. There was one additional patient with trisomy 13 on the study, though the patient also had additional cytogenetic abnormalities as 48,XY+der(1)t(1;1)(p11;q25),+13. This patient received only 6 days of lenalidomide before going off study due to infection.

Case 2.

A 68 year old male presented with marked pancytopenia and was found to have relapsed AML. Approximately 3.5 years prior, he was diagnosed with AML arising from MDS; at that time, he had normal karyotype. Due to medical comorbidities including ischemic cardiomyopathy, at the original presentation he received induction with fludarabine, cytarabine, and G-CSF (FLAG), and achieved CR1. He then received one cycle of cytarabine (1.5gm/m2 x 6 doses) consolidation, completing all therapy 3 years prior to the current relapse. At relapse, BM biopsy showed 40% myeloblasts, FAB M2 AML(see Table 1), and flow cytometry revealed myeloid blasts expressing CD13, HLA-
DR, CD33, and CD34, with subpopulations expressing CD15, CD11b and CD7. Metaphase cytogenetics demonstrated doubling of a clone with trisomy for chromosome 13.

After providing informed consent, he was treated on a HRPO-approved, phase I dose escalation study of lenalidomide for relapsed AML at The Ohio State University (clinicaltrials.gov: NCT00466895). Study therapy consisted of lenalidomide 35mg, days 1-21 of repeated 28 day cycles. At the completion of cycle 1, the CBC showed WBC 1,200/uL with ANC 10/uL, and the patient remained red blood cell and platelet transfusion-dependent. BM biopsy showed 16% blasts. Cytogenetic analysis showed persistent disease in 8/20 metaphase cells. Four days after starting cycle 2, lenalidomide was held for fever, hypoxemia, and pneumonia, which resolved after IV antibiotics and supportive measures. Four weeks later (lenalidomide still on hold) the patient recovered his blood counts without G-CSF support. BM biopsy showed CR; cytogenetic analysis demonstrated a normal male karyotype in 20/20 metaphase cells, and FISH was negative. The CRc was confirmed with a repeat BM biopsy 5 weeks later. After achieving CRc, the patient received two additional cycles of lenalidomide at 35 mg/day and was then dose reduced to 10 mg/day (days 1-21 of 28 day cycles) due to drug-related myelosuppression. He subsequently relapsed after a 9 month CRc.

In the OSU trial, 2/18 non-trisomy 13 AML patients have responded to lenalidomide. A 74 year old male with isolated extramedullary relapse (skin only, no blood or marrow involvement) achieved a CR on lenalidomide lasting 8 months. Originally, the patient had normal karyotype AML and achieved CR1 with cytarabine, daunorubicin, and etoposide; CR1 lasted 4 years. At first relapse (marrow and
extramedullary relapse), he was re-induced with daunorubicin and cytarabine; CR2 lasted 9 months. At second relapse, he was treated with lenalidomide as noted above. Another patient, a 61 year old male with AML and monosomy 7 who had relapsed after allogeneic transplantation, achieved CRc after 3 cycles of lenalidomide treatment and continues on therapy. He had been transplanted in CR2 with stem cells from an unrelated donor following a non-myeloablative conditioning regimen and relapsed 9 months after transplant.
Discussion

We report induction of sustained CRc in two older AML patients with trisomy 13 treated with single agent lenalidomide. In AML, cytogenetic abnormalities remain the most important disease-related prognostic factor\textsuperscript{12-15}. Occurring in about 3\% of AML cases, AML with trisomy 13 is rare, and it is associated with a very poor prognosis\textsuperscript{13,16}. Each of the two patients achieved morphologic and cytogenetic CR after a prolonged delay (78 and 124 days, respectively) from initiation of intermittent, high-dose lenalidomide. No other anti-leukemic agent was administered; the remissions were a direct result of lenalidomide therapy. To our knowledge, this is the first report of high-dose lenalidomide inducing morphologic and cytogenetic CRs in AML or any other myeloid disorder with trisomy 13 as the sole chromosomal abnormality.

The biologic mechanism of lenalidomide clinical activity in hematologic malignancies remains unclear. Clinical and cytogenetic responses to lenalidomide in MDS patients have been strongly associated with the 5q- chromosomal abnormality\textsuperscript{7-9}, providing a genetic starting point for studies to define lenalidomide’s mechanism of action in this disease. Indeed, potential genes of interest in 5q- MDS have been identified including SPARC\textsuperscript{17} and RPS14\textsuperscript{18}. Alternatively, extrinsic effects such as NK or T cell activation, anti-angiogenesis, or cytokine modulation may contribute to its clinical activity. The clinical responses observed here, in the rare subset of AML with trisomy 13, again provide a narrow genetic framework to investigate potential targets of the drug in AML. Interestingly, retrospective analyses of AML patients have identified a strong association of trisomy 13 with mutations of the AML1 (RUNX1) gene and with increased expression of the FLT3 tyrosine kinase (located on chromosome 13)\textsuperscript{19,20},
suggesting potential targets that may be relevant to the lenalidomide activity observed in this report. Further analysis of lenalidomide activity in additional trisomy 13 AML patients may ultimately lead to better understanding of myeloid leukemogenesis and aid in the development of new targeted therapeutic approaches for AML.

Acknowledgments

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Authorship

Contributions:
T.A.F. designed the Washington University study, interpreted data, and wrote the paper;
J.C.B. designed study, interpreted data, and contributed to the paper;
G.M. interpreted data, and contributed to the paper;
C.N.A. collected clinical data, and contributed to the paper;
C.K. provided research support to the Ohio State study;
J.E.P. reviewed pathology, analyzed data, and contributed to the paper;
R.V. designed study, interpreted data, and contributed to the paper;
W.B. designed Ohio State study, interpreted data, and wrote the paper;

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References


Figure Legends.

Figure 1. Bone marrow morphology and karyotype of Case 1 at AML diagnosis.

(A) Bone marrow aspirate blast morphology (left, Wright-Giemsa, 100X) and biopsy (right, Leder stain, 100X) demonstrating AML at diagnosis. The blast cells are medium-sized, have a high nuclear:cytoplasmic ratio, agranular Leder-negative cytoplasm, and visible nucleoli. (B) AML karyotype showing trisomy 13 (arrow) at time of AML diagnosis (47,XY,+13[5]/46,XY[15]).
Figure 1

A.

B.
Table 1. Summary of AML cases.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case 1</th>
<th>Case 2</th>
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<tr>
<td>Age</td>
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<td>Performance status (ECOG)</td>
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<td>2</td>
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<tr>
<td>WHO/FAB Dx</td>
<td>AML M0</td>
<td>AML M2</td>
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<tr>
<td>Prior AML therapy</td>
<td>No</td>
<td>Yes, FLAG chemotherapy at original presentation</td>
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<tr>
<td>Duration of prior remission</td>
<td>N/A</td>
<td>3.5 years</td>
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<tr>
<td>Lenalidomide Treatment</td>
<td>Cycle 1: 50 mg/day days 1-14</td>
<td>Cycle 1: 35mg/day days 1-21</td>
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<tr>
<td></td>
<td>No therapy x 30 days</td>
<td>Cycle 2: 35mg days 1-4, therapy halted due to pneumonia</td>
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<td></td>
<td>Cycle 2: 50 mg/day x 21 days</td>
<td>Cycle 3: 35mg days 1-21</td>
</tr>
<tr>
<td></td>
<td>No therapy x 30 days</td>
<td>Cycle 4: (start of cycle delayed 3 weeks until resolution of Grade 2* neutropenia and thromobocytopenia) 35mg days 1-21</td>
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<tr>
<td></td>
<td>Low-dose cycles: 10 mg/day days 1-28 x 5</td>
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<td></td>
<td>Low-dose therapy 10 mg/day, ongoing</td>
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<td>Pre-Lenalidomide Data</td>
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<tr>
<td>White blood cells</td>
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<td>Hemoglobin</td>
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<td>Platelets</td>
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<td>ANC</td>
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<td>PB Blasts</td>
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<td>BM Cellularity</td>
<td>30%</td>
<td>40%</td>
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<tr>
<td></td>
<td>Case 1</td>
<td>Case 2</td>
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<tr>
<td>BM Blast %</td>
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<td>40%</td>
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<td>Karyotype</td>
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<td>94&lt;4n&gt;,XXYY,+13,+13(cp15)/46,XY(3)/non-clonal(2)</td>
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<td><strong>Data at CR</strong></td>
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</tr>
<tr>
<td># days to CR</td>
<td>124 days (35 days of high-dose therapy, 28 days of low-dose therapy)</td>
<td>78 days (25 days of treatment)</td>
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<td>White blood cells</td>
<td>6,100/uL</td>
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<tr>
<td>BM Cellularity</td>
<td>60%</td>
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<td>&lt; 5 %</td>
<td>&lt;5%</td>
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<td><strong>Duration of CR</strong></td>
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<td>9 months</td>
</tr>
<tr>
<td><strong>CR confirmation</strong></td>
<td>Yes (6 weeks, 16 weeks)</td>
<td>Yes (5 weeks)</td>
</tr>
</tbody>
</table>

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