Efficacy of escalated imatinib combined with cytarabine in newly diagnosed patients with chronic myeloid leukemia

Wendy Deenik,[1] Jeroen J.W.M. Janssen,[2] Bronno van der Holt,[1] Gregor E.G. Verhoef,[3] Willem M. Smit,[4] Marie José Kersten,[5] Simon M.G.J. Daenen,[6] Leo F. Verdonck,[7] Augustin Ferrant,[8] Anton V.M.B. Schattenberg,[9] Pieter Sonneveld,[1] Marinus van Marwijk Kooy,[7] Shulamit Wittebol,[10] Roelof Willemze,[11] Pierre W. Wijermans,[12] H. Berna Beverloo,[1] Bob Löwenberg,[1] Peter J.M. Valk,[1] Gert J. Ossenkoppele,[2] and Jan J. Cornelissen[1]

[1]Erasmus University Medical Center, Rotterdam, The Netherlands; [2]VU Medical Center, Amsterdam, The Netherlands; [3]University Hospital Gasthuisberg, Leuven, Belgium; [4]Medical Spectrum Twente, Enschede, The Netherlands; [5]Academic Medical Center, Amsterdam, The Netherlands; [6]University Medical Center Groningen, Groningen, The Netherlands; [7]Isala Clinic, Sophia, Zwolle, The Netherlands; [8]University Hospital St-Luc, Brussels, Belgium; [9]University Medical Center Nijmegen, Nijmegen, The Netherlands; [10]Meander Medical Center, Amersfoort, The Netherlands; [11]Leiden University Medical Center, Leiden, The Netherlands, and [12]Haga Hospital, The Hague, The Netherlands

ABSTRACT

Background
In order to improve the molecular response rate and prevent resistance to treatment, combination therapy with different dosages of imatinib and cytarabine was studied in newly diagnosed patients with chronic myeloid leukemia in the HOVON-51 study.

Design and Methods
Having reported feasibility previously, we hereby report the efficacy of escalated imatinib (200 mg, 400 mg, 600 mg or 800 mg) in combination with two cycles of intravenous cytarabine (200 mg/m^2 or 1000 mg/m^2 days 1 to 7) in 162 patients with chronic myeloid leukemia.

Results
With a median follow-up of 55 months, the 5-year cumulative incidences of complete cytogenetic response, major molecular response, and complete molecular response were 89%, 71%, and 53%, respectively. A higher Sokal risk score was inversely associated with complete cytogenetic response (hazard ratio of 0.63; 95% confidence interval, 0.50-0.79, P<0.001). A higher dose of imatinib and a higher dose of cytarabine were associated with increased complete molecular response with hazard ratios of 1.60 (95% confidence interval, 0.96-2.68, P=0.07) and 1.66 (95% confidence interval, 1.02-2.72, P=0.04), respectively. Progression-free survival and overall survival rates at 5 years were 92% and 96%, respectively. Achieving a major molecular response at 1 year was associated with complete absence of progression and a probability of achieving a complete molecular response of 89%.

Conclusions
The addition of intravenous cytarabine to imatinib as upfront therapy for patients with chronic myeloid leukemia is associated with a high rate of complete molecular responses (*Clinicaltrials.Gov Identifier: NCT00028847*).

Key words: imatinib, cytarabine, escalated therapy, combination therapy, chronic myeloid leukemia.

Citation: Deenik W, Janssen JJWM, van der Holt B, Verhoef GEG, Smit WM, Kersten MJ, Daenen SMGJ, Verdonck LF, Ferrant A, Schattenberg AVMB, Sonneveld P, van Marwijk Kooy M, Wittebol S, Willemze R, Wijermans PW, Beverloo HB, Löwenberg B, Valk PJM, Ossenkoppele GJ, and Cornelissen JJ. Efficacy of escalated imatinib combined with cytarabine in newly diagnosed patients with chronic myeloid leukemia. Haematologica 2010;95:914-921. doi:10.3324/haematol.2009.016766

Acknowledgments: the authors would like to thank Isabel Chu and Chantal Goudswaard from the molecular diagnostic laboratory, Department of Hematology Erasmus MC, and our colleagues from the associated molecular diagnostic laboratories for all molecular analyses and for providing material for central analysis, and Silvia Verelst from the HOVON data-center for excellent central data management.

Funding: the Queen Wilhelmina Fund (KWF)-Kankerbestrijding provided support for the data management. Novartis Oncology, The Netherlands provided support for the standardization and centralization of RQ-PCR.

Manuscript received on September 6, 2009. Revised version arrived on November 22, 2009. Manuscript accepted on December 3, 2009.

Correspondence:
J.J. Cornelissen, PhD, MD
Erasmus University Medical Center Department of Hematology Groene Hilledijk 301 3075 EA Rotterdam The Netherlands
Telephone: (+31)10.704.1797
Fax: (+31)10.704.1004
E-mail: j.cornelissen@erasmusmc.nl

Introduction

The introduction of imatinib, a specific kinase inhibitor of the BCR-ABL protein, has dramatically changed prospects for patients with chronic myeloid leukemia (CML).[1] Most patients with newly diagnosed chronic phase CML nowadays achieve a complete cytogenetic response, which subsequently predicts for relatively long survival.[2] Moreover, patients achieving a major molecular response do even better, as not a single patient who attained such a response at 18 months had progressed at 5 years.[2,3] The recently presented 7-year follow-up data of the International Randomized Study of Interferon and STI571 (IRIS) confirmed durability of cytogenetic responses and a low rate of progression.[4] However, the estimated 5-year event-free survival was 83%, and an estimated another 16% of patients discontinued imatinib for various reasons within the first 5 years.[2] Comparable results were observed in a recent large single center study,[5] indicating that although the majority of patients enter a stable cytogenetic remission, more than one third of patients may still be in need of alternative therapy.

Patients needing second-line therapy include patients who do not tolerate imatinib and patients acquiring resistance. Primary or acquired resistance against imatinib is currently defined at hematologic, cytogenetic, and also molecular levels.[6] It may be caused by different mechanisms, including point mutations in the *BCR-ABL* kinase domain, overexpression of *BCR-ABL*, additional chromosomal abnormalities in the Philadelphia (Ph)-positive clone, and a relative insensitivity of quiescent leukemic stem cells to imatinib.[7-10] Prevention of resistance and improving the cytogenetic and molecular response rates may be achieved by different approaches, including dose escalation of imatinib, second-generation tyrosine kinase inhibitors, or combination therapy.[11-15] Several combinations have been explored *in vitro* and also in early clinical studies.[12,16-19] Among the combinations of imatinib and cytostatic drugs, the combination of cytarabine and imatinib was found to result in a synergistic effect, especially at higher concentrations of either drug.[18,19] Based on these findings, the HOVON cooperative study group set out to explore the clinical feasibility and efficacy of the imatinib plus cytarabine combination, applying a step-wise dose-increase of either drug. Recently, feasibility results of that combination were reported.[12] Here, the efficacy of the combination of imatinib and intravenous cytarabine is reported with emphasis on the rate and duration of molecular responses as well as their major determinants.

Design and Methods

The HOVON-51 was a multicenter study designed to investigate the feasibility and efficacy of escalated imatinib in combination with intravenous cytarabine in patients with early chronic phase CML. Inclusion criteria included: age between 18 and 65 years, presence of the Ph chromosome or *BCR-ABL* rearrangement, adequate organ function, registration within 6 months of diagnosis, and no previous treatment except for hydroxyurea. The ethics committees of all participating centers approved the study and informed consent was obtained from all patients in accordance with the Declaration of Helsinki. Patients were recruited from August 2001 to November 2005.

Study design and treatment

The design of the study has been described recently.[12] In brief, patients were assigned to one of seven predefined, successive dose levels. Dose levels were open for inclusion only when the preceding dose level had met the criteria of acceptable toxicity and safety. First, a prephase of imatinib (400 mg) monotherapy was given to all patients for 2 to 3 weeks. This was followed by combination therapy of two cycles of intravenous cytarabine (200 mg/m^2 or 1000 mg/m^2 days 1 to 7) with imatinib (200 mg, 400 mg, 600 mg or 800 mg once daily). Imatinib (400 mg, 600 mg or 800 mg) maintenance therapy was continued after the second cycle until disease progression, intolerance of treatment, or eligibility for allogeneic stem cell transplantation (SCT), whichever occurred first. Dose adjustments during imatinib maintenance therapy were made in the case of non-hematologic toxicity of Common Toxicity Criteria (CTC) grade 2 or higher as reported before,[12] and as described in detail at *www.hovon.nl*.

Definition of end-points

The definition of molecular response was adapted in order to be compatible with the international scale.[20,21] A laboratory-specific conversion factor to the international scale has been acquired via EUTOS for CML, which promotes quality controlled molecular monitoring using standardized real-time quantitative polymerase chain reaction (RQ-PCR) technologies and establishment of an international definition of major molecular response (*http://www.eutos.org/*). A complete molecular response was defined as no residual *BCR-ABL* transcripts by RQ-PCR (in duplicate), corresponding to a greater than 4.5 log-reduction of *BCR-ABL* copies. Only *BCR-ABL* values resulting from assaying with a level of sensitivity of at least 0.01% in duplicate were considered appropriate. If cytogenetic results were not available during follow-up, RQ-PCR measurement of *BCR-ABL* was used as a surrogate for complete cytogenetic response, with *BCR-ABL* values below 1% being considered as indicating a complete cytogenetic response.[13,22] Molecular response was centrally assessed at the Erasmus University Medical Center in Rotterdam using RQ-PCR on peripheral blood and/or bone marrow. Molecular analysis was done at baseline, after cycles 1 and 2, at 6 months, and at least every 3 to 6 months thereafter. All patients who failed to achieve a major molecular response at 1 year were evaluated for point mutations in the *ABL* kinase domain, and the investigation was repeated during follow-up as long as patients failed to achieve a major molecular response. Patients who lost their initial response or progressed during follow-up were also evaluated for mutations. *BCR-ABL* mutation analyses were performed as previously described.[23]

Cumulative incidences of response are expressed as the time from registration to complete hematologic response, major cytogenetic response, complete cytogenetic response, major molecular response, and complete molecular response. Loss of complete hematologic response was defined as a white blood cell count (WBC) greater than 20×10^9/L or progression to advanced phase CML; loss of major cytogenetic response as an increase of Ph-positive metaphases by at least 30% points to 35% or more Ph-positive metaphases; loss of complete cytogenetic response by the detection of one or more Ph-positive metaphases, loss of major molecular response as a 0.5 log increase of *BCR-ABL* to a *BCR-ABL* level greater than 0.1%; and loss of complete molecular response as renewed detection of *BCR-ABL* transcript levels. In the case of loss of hematologic, cytogenetic or molecular responses, confirmation by a subsequent evaluation at least 1 month later was required. Progression was defined as the development of accelerated phase or blast crisis CML, whichever came first. Failure of imatinib treatment was defined as progression (to advanced phase CML), loss of complete hematologic response, loss of major cytogenetic response, or an increasing WBC (defined as doubling of the WBC to greater than 20×10^9/L on two occasions at least 1 month apart in a patient who had never attained a complete hematologic response despite receiving maximally tolerated doses of therapy).

Progression-free survival was defined as the time from registration

until progression or death, whichever came first. Failure-free survival was defined as the time from registration until failure on imatinib treatment or death, whichever came first. Of note, primary hematologic resistance is not included in the definition of failure-free survival due to cytopenias associated with combination treatment, which precludes an early evaluation of hematologic response. Event-free survival was defined as the time from registration until failure on imatinib treatment, discontinuation of imatinib treatment, going off protocol treatment for any reason, or death, whichever occurred first. Overall survival was calculated as the time from registration until death of any cause. Patients still alive at the date of last contact were then censored.

Statistical methods

The cumulative incidences of complete hematologic response, major cytogenetic response, complete cytogenetic response, major molecular response and complete molecular response were calculated using competing risk analysis. Competing risks were disease progression, discontinuation of treatment before achieving response, or death without previous response. As an allogeneic SCT was allowed as off protocol treatment if no cytogenetic response was acquired within 12 months or according to the physician's preference, patients who underwent this treatment were censored at the date of the transplant. Progression-free, event-free, failure-free and overall survival rates were estimated by the Kaplan-Meier method, and 95% confidence intervals (CI) were determined. Patients who underwent allogeneic SCT were censored at the date of transplantation. Time to response and survival end-points were illustrated by Kaplan-Meier curves until 5 years.[24] In our trial, patients had been assigned to receive standard- or intermediate-dose cytarabine, as well as low/standard-dose (200 and 400 mg) or high-dose (600 mg and 800 mg) imatinib. Univariate and multivariate Cox regression analyses,[25] without and with interaction terms, were performed to evaluate the effect of higher dose levels and the impact of the Sokal risk score and Euro score on clinical outcome. Hazard ratios (HR) with 95% CI were determined. All reported *P* values are two-sided, and a significance level of α=0.05 was used.

Table 1. Baseline characteristics of all patients.

Characteristic	All patients (N=162)
Age at diagnosis, years	
median	47
range	19-65
Sex, n. (%)	
male	95 (59%)
female	67 (41%)
Sokal risk group, n. (%)	
low (< 0.8)	59 (36%)
intermediate (0.8-1.2)	50 (31%)
high (> 1.20)	43 (27%)
unknown	10 (6%)
Euro score, n. (%)	
low (≤ 780)	70 (43%)
intermediate (> 780-1480)	57 (35%)
high (> 1480)	23 (14%)
unknown	12 (7%)
Dose of imatinib, n. (%)	
low/standard-dose (200 mg and 400 mg)	49 (30%)
high-dose (600 mg and 800 mg)	113 (70%)
Dose of cytarabine, n. (%)	
standard-dose (200 mg/m^2)	107 (66%)
intermediate-dose (1000 mg/m^2)	55 (34%)

Percentages may not sum up to 100% due to rounding.

Results

The patients' characteristics are presented in Table 1. The median age at diagnosis was 47 years (range, 19-65 years); patients were fairly evenly distributed among the three Sokal risk categories. One hundred and sixty-two patients received a first cycle of combination therapy and 140 patients (86%) also received a second cycle of combination therapy. One hundred and fifty-seven patients (97%) started with imatinib maintenance therapy. The current analysis is based on data collected up to December 18, 2008, resulting in a median follow-up of 55 months (range, 10-84 months). Currently, 112 patients (69%) are still on protocol treatment, and 50 patients went off protocol treatment for various reasons including progression to accelerated phase or blast crisis in 7 patients, loss of hematologic or cytogenetic response in 7 patients, no complete hematologic response at 6 months in 2 patients, toxicity in 12 patients, proceeding to allogeneic SCT in 18 patients and other reasons in 4 patients. Second-line therapy included allogeneic SCT from either a related or a matched unrelated donor in 18 patients, nilotinib or dasatinib in 14 patients, chemotherapy in 7 patients, and other treatment modalities in 7 patients.

Hematologic, cytogenetic, and molecular responses

The patients' responses are presented in Table 2, and Figures 1A-C and 2A-B. One hundred and fifty-four patients achieved a complete hematologic response, 146 patients a major cytogenetic response, and 135 patients a complete cytogenetic response, based on cytogenetic evaluation in 130 patients and quantitative PCR in 5 patients. The median time to a complete cytogenetic response was

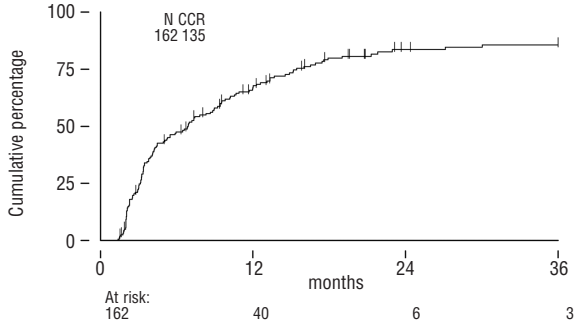
Table 2. Patients' responses (N=162).

Type of Response	N.
Complete hematologic response	
No	8
Progression to accelerated phase or blast crisis	2
Yes	154
Loss of complete hematologic response	9
Progression to accelerated phase or blast crisis	7
Complete cytogenetic response	
No	27
Yes	135
Loss of complete cytogenetic response	17
Loss of complete hematologic response	5
Progression to accelerated phase or blast crisis	4
Major molecular response	
No	55
Yes	107
Loss of major molecular response	6
Loss of complete cytogenetic response	1
Loss of complete hematologic response	1
Progression to accelerated phase or blast crisis	-
Complete molecular response	
No	84
Yes	78
Loss of complete molecular response	10
Loss of major molecular response	2
Loss of complete cytogenetic response	-
Loss of complete hematologic response	-
Progression to accelerated phase or blast crisis	-

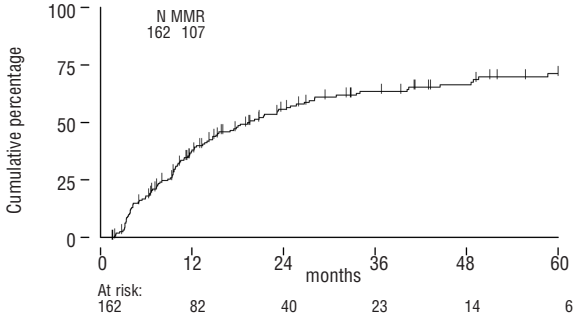
approximately 4.5 months. In total, 107 patients achieved a major molecular response, and 78 patients developed a complete molecular response on protocol treatment. In addition, we performed nested PCR in 51 of the 78 patients negative by real-time PCR, corresponding to a greater than 4.5-log reduction of *BCR-ABL* copies. All but nine of these patients were also negative by nested PCR. The median time to major molecular response was 11 months and the median time to complete molecular response was approximately 22 months. With a median follow-up of 55 months, 9 patients lost their complete hematologic response, 16 patients lost their previously established major cytogenetic response and 17 patients lost their complete cytogenetic response. Of all 107 patients with a major molecular response, 6 patients lost that response, and loss of complete molecular response was observed in 10 patients (Table 2). At 5 years, the cumulative incidences of a complete cytogenetic response, major molecular response, and complete molecular response were, respectively, 89%, 71%, and 53% (Figure 1A-C). Of note, 89% of the patients who achieved a major molecular response at 1 year subsequently developed a complete molecular response. Furthermore, none of the 107 patients with a major molecular response subsequently progressed to advanced phase CML, while 4 out of 135 patients with a complete cytogenetic response and 5 out of 27 patients who failed to achieve a complete cytogenetic response progressed to advanced phase CML. Among the 103 patients with a complete cytogenetic response at 1 year, 91 (88%) subsequently obtained a major molecular response and 71 patients (69%) ultimately developed a complete molecular response. In contrast, among 41 patients continuing protocol treatment, but who failed to achieve a complete cytogenetic response at 1 year, 27 patients (66%) subsequently developed a complete cytogenetic response at later time points (Figure 1A), 15 patients (37%) attained a major molecular response, and only 6 patients (15%) ultimately developed a complete molecular response.

There were significant differences in the rates of major

A Cumulative incidence of complete cytogenetic response (CCR)



B Cumulative incidence of major molecular response (MMR)



C Cumulative incidence of complete molecular response (CMR)

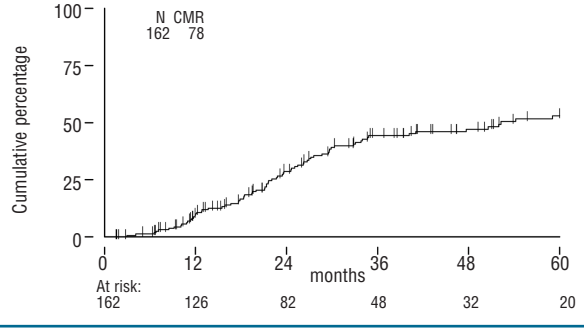
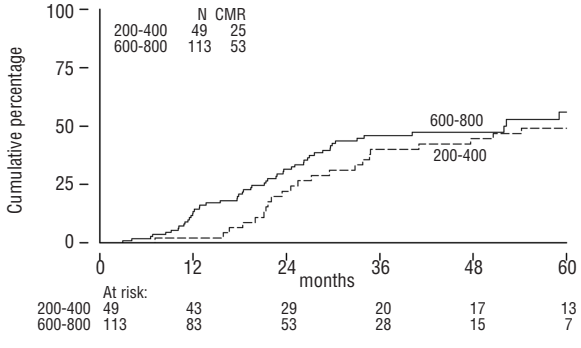


Figure 1. Cumulative incidences of (A) complete cytogenetic response, (B) major molecular response, and (C) complete molecular response.

A Cumulative incidence of complete molecular response (CMR) by dose of imatinib



B Cumulative incidence of complete molecular response by dose of cytarabine (CMR)

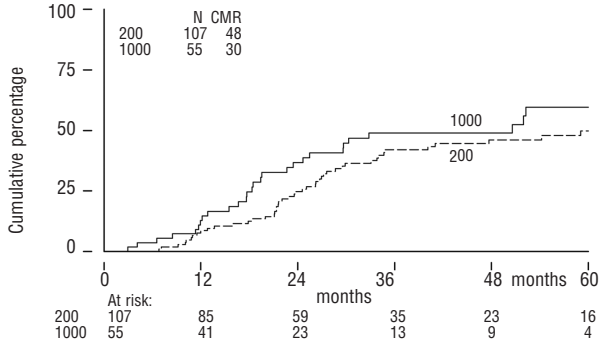


Figure 2. Cumulative incidences of (A) complete molecular response by dose of imatinib (HR = 1.60; 95% CI, 0.96-2.68, *P*=0.07) and (B) complete molecular response by dose of cytarabine (HR=1.66; 95% CI, 1.02-2.72, *P*=0.04)

and complete cytogenetic responses among patients according to Sokal risk and Euro scores in univariate analysis. A higher Sokal risk score remained adversely associated with major and complete cytogenetic responses (HR=0.63; 95% CI, 0.50-0.79, *P*<0.001) (Table 3) in multivariate analysis. At 1 year the cumulative incidences of a complete cytogenetic response was 76% in patients with a low Sokal score, 74% in patients with an intermediate Sokal score, and 40% in patients with a high Sokal score. However, at 5 years these differences in response rates were less pronounced, being 89%, 93%, and 81%, in low, intermediate, and high-risk patients, respectively. The latter higher response rate in high-risk patients at 5 years appeared primarily due to a slower developing response rate. A higher Sokal score was also inversely associated with major molecular response (HR = 0.74; 95% CI, 0.58-0.96, *P*=0.02) (Table 3), but not with complete molecular response. In contrast, the dose of imatinib and the dose of cytarabine were not associated with cytogenetic response, but a higher dose of imatinib appeared to be associated with a better major and complete molecular response rate (HR = 1.60; 95% CI, 0.96-2.68, *P*=0.07) (Table 3, Figure 2A). Independently, also the higher dose of cytarabine was associated with a better complete molecular response rate. Sixty percent of patients receiving the higher dose of cytarabine developed a complete molecular response at 5 years as compared to 50% of the patients receiving a standard-dose of cytarabine (HR = 1.66; 95% CI, 1.02-2.72, *P*=0.04) (Table 3, Figure 2B).

Progression-free, overall, failure-free, and event-free survival

After a median follow-up of 55 months, nine patients had developed advanced phase CML and three patients had died resulting in a 5-year progression-free survival rate of 92% (95% CI, 85%-95%) (Figure 3A). The estimated annual rate of progression was 5.0% in the first year, 0.7% in the second year, 0.8% in the third year, 2.2% in the fourth year, and 0% in the fifth year. Due to the limited number of events, prognostic factors for progression-free survival were not evaluated. In total, six patients died, resulting in an overall survival rate at 5 years of 96% (95% CI, 92%-98%). The causes of death of these six patients were blast crisis CML in three patients, excessive toxicity in two patients and an unrelated cause in one patient. Recipients of an allogeneic stem cell graft were censored at the time of transplantation for the latter analysis in concordance with earlier reports and to facilitate comparison.[2,5] Twenty-seven patients ultimately underwent allogeneic SCT as second- or third-line therapy, predominantly because of primary (8 patients) or secondary resistance (9 patients). Other reasons for performing allogeneic SCT included intolerance of imatinib (3 patients) and physicians' preference (7 patients). Twelve out of these 27 patients died due to either non-relapse mortality (n=11) or progressive disease (n=1). Survival without censoring the allogeneic SCT recipients at the time of transplantation was estimated to be 88% at 5 years.

Imatinib treatment failed in 20 patients, of whom 7 progressed to the accelerated phase or blast crisis as the first event of treatment failure, 2 patients had an increasing WBC count, 2 patients lost their complete hematologic response, and 9 patients lost a major cytogenetic response. Another three patients died without prior failure on imatinib treatment. The estimated 5-year failure-free survival rate was 86% (95% CI, 79-91%) (Figure 3A). Neither the dose of cytarabine or imatinib, nor Sokal risk and Euro scores were associated with failure-free survival. However, time-dependent analysis showed that early cytogenetic and molecular responses had a favorable impact on failure-

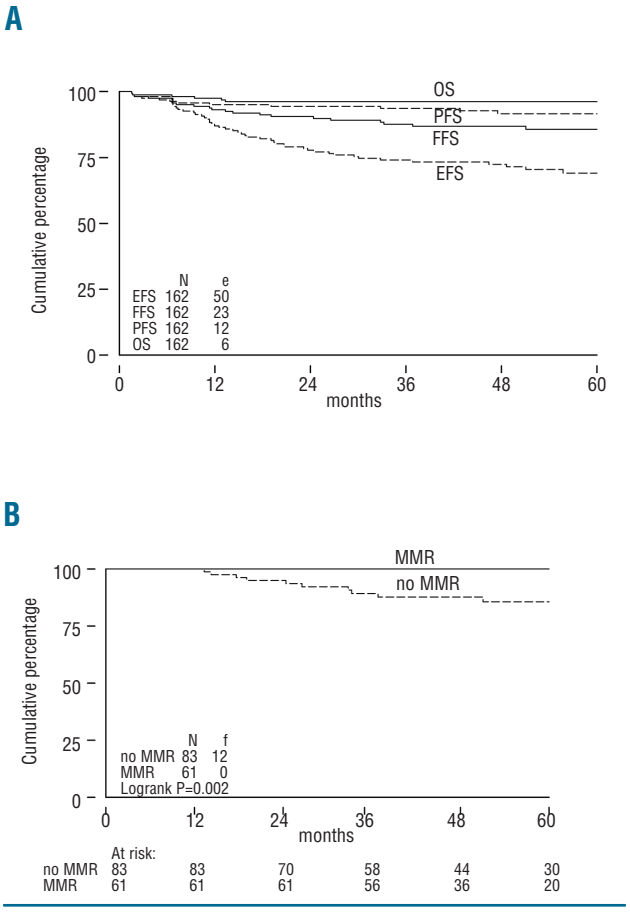


Figure 3. (A) Event-free survival (EFS), failure-free survival (FFS), progression-free survival (PFS), and overall survival (OS). (B) Landmark analysis of failure-free survival by major molecular response (MMR) at 1 year.

Table 3. Results of the multivariate analysis.

Parameter	Major cytogenetic response		Complete cytogenetic response		Major molecular response		Complete molecular response	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Dose of cytarabine	1.08 (0.75-1.57)	0.66	1.02 (0.69-1.51)	0.91	1.16 (0.75-1.78)	0.50	1.66 (1.02-2.72)	0.04
Dose of imatinib	1.07 (0.74-1.55)	0.73	1.38 (0.93-2.04)	0.11	1.67 (1.06-2.61)	0.03	1.60 (0.96-2.68)	0.07
Sokal risk score	0.56 (0.45-0.70)	< 0.001	0.63 (0.50-0.79)	<0.001	0.74 (0.58-0.96)	0.02	0.90 (0.67-1.22)	0.51

free survival. A landmark analysis of the 103 patients who had achieved a complete cytogenetic response at 1 year revealed a superior estimated 5-year failure-free survival of 97% as compared to 77% in 41 patients without a complete cytogenetic response at 1 year ($P<0.001$) (*data not shown*). Moreover, the 5-year failure-free survival rate of 61 patients who rapidly achieved a major molecular response by 12 months was higher than that of the 83 patients who had failed to attain a such a response by 1 year (100% *versus* 86%; $P=0.002$) (Figure 3B).

Event-free survival was also assessed. Overall, 50 events were noted, resulting in a 5-year event-free survival rate of 69% (95% CI, 61%-76%) (Figure 3A). Higher Sokal risk and Euro scores were associated with worse event-free survival in univariate analysis. A higher Sokal risk score remained adversely associated with event-free survival (HR = 1.55; 95% CI, 1.08-2.22, $P=0.02$), when adjusted for dose of cytarabine and imatinib.

Point mutations in the BCR-ABL *kinase domain*

Patients failing to achieve a major molecular response at 1 year, and at subsequent evaluation time points thereafter were evaluated for point mutations within the *BCR-ABL* kinase domain. In addition, patients with primary or secondary hematologic or cytogenetic resistance and all patients who, at any time, progressed to advanced phase CML were evaluated for mutations. In total, 153 samples were evaluated for point mutations in the kinase domain, showing a cumulative incidence of mutations of 10% at 5 years. In total, 14 different mutations were detected in 15 patients, including 2 patients with a T315I mutation. Nine of these 15 patients with a mutation subsequently lost their response, and 3 patients progressed to advanced phase CML.

Tolerance of protocol treatment

Adverse events and side effects during the phase of combination therapy have already been reported in detail.[12] During maintenance, the most frequent adverse events of CTC grade 2 or more included constitutional symptoms (34%) and gastrointestinal complaints (33%); toxicity of CTC grade 3 or 4 occurred infrequently. Both the incidence and severity of these side effects were essentially similar to the those of the side effects that can be observed in patients receiving monotherapy with imatinib as reported before.[2] Combination therapy and maintenance were well tolerated as illustrated by the fact that only 9% of patients discontinued treatment because of side effects (n=15), which represent all discontinuations including the toxic deaths, an event-free survival of 69%, and a total number of 112 patients still continuing protocol treatment.

Discussion

Imatinib treatment is associated with high rates of complete cytogenetic and major molecular responses in patients with first chronic phase CML, although complete molecular responses occur significantly less frequently and the majority of patients continue to harbor minimal residual disease, necessitating prolonged treatment with imatinib.[2,3,5] With the ultimate aim of improving the complete molecular response rate, the HOVON study group set out to explore combination therapy of escalated doses of imatinib and cytarabine. With a median follow-up of 55 months, the long-term efficacy of this combination therapy is presented here. The most important findings of our study include a relatively high complete molecular response rate, a low incidence of primary cytogenetic and molecular resistance, and a relatively high number of patients still continuing protocol treatment, while maintaining their remission.

The cumulative incidence of a complete molecular response was 53% at 5 years. A higher dose of imatinib monotherapy may already be associated with faster and better responses, although different results were observed in distinct risk-categories of patients.[11,26-29] In addition, an association has been observed between plasma trough levels and outcome.[30,31] A modest dose-dependent effect of imatinib was also apparent in our study (Table 2, Figure 3A). Furthermore, the earlier observed *in vitro* synergistic or additive effect of cytarabine[18,19] seems to have been mirrored here clinically. An additive effect of cytarabine is further supported by the significant dose-dependent effect of cytarabine observed in our study. Moreover, up to the latest follow-up, none of the patients receiving the higher dose of cytarabine has developed progressive disease. A high complete molecular response rate of approximately 50% was reported earlier by Branford *et al.*[32] These results cannot be compared directly with those from the present study, as we estimated cumulative incidences with competing risk-analysis. However, the median time to complete molecular response differed markedly, being 18 months in the present study and approximately 4 years in the Australian study.[32] Recently, Cortes *et al.* reported results obtained with 800 mg imatinib in newly diagnosed patients.[33] Approximately 50% of patients evaluable at 18 months after the start of treatment had obtained a complete molecular response, which comes close to what was observed in the present study, but these favorable results were obtained in a relatively good-risk group in that 70% of the patients had a low-risk Sokal score. The issue of an additive effect of cytarabine does, therefore, remain open, but may be settled by a prospective randomized trial that is currently underway. Two other cooperative groups explored the combination of cytarabine and imatinib. A French cooperative group demonstrated the feasibility of imatinib and low-dose cytarabine,[16] but their long-term results are not yet available. An Australian cooperative group developed a protocol including addition of cytarabine for patients failing to obtain a sufficient response 3 months after dose escalation of imatinib. However, only a minority of patients actually received the combination, which precludes any definite conclusion as regards the additive value of cytarabine in their study.[13]

By inducing a high complete molecular response rate, combination therapy may prevent primary resistance at the various levels, and it may also prevent secondary resistance in patients relapsing from an earlier established response. Primary cytogenetic resistance, defined as failure to achieve a complete cytogenetic response at 18 months, was observed in 36 patients (22%) in our study. Nineteen out of these 36 patients (53%) who failed to achieve a complete cytogenetic response by 18 months had a high-risk Sokal score. While a 22% failure rate may be somewhat lower than that which can be observed following imatinib only (approximately 30% in the Hammersmith study[5]), primary cytogenetic resistance is still of concern and combination therapy only partially

prevented cytogenetic resistance. It indicates that a subset of high-risk patients is still in need of a more efficient therapeutic approach. Furthermore, additional parameters apart from those incorporated in the Sokal and Euro scores may be needed to more accurately identify the patients at highest risk of primary cytogenetic resistance. New diagnostic techniques such as gene expression profiling and single nucleotide polymorphisms may possibly add to the well-established risk scores.[34,35] Secondary resistance percentages were rather low and progression-free survival estimated at 92% at 5 years. As outlined by de Lavallade *et al.*, another important outcome estimate is the 5-year probability of achieving and maintaining a major cytogenetic response, while continuing imatinib. It was 63% for patients with early chronic phase CML receiving a standard-dose of imatinib in the Hammersmith series of patients.[5] For comparison, 69% of the patients in the present study maintained at least an earlier established major cytogenetic response and were still on imatinib according to protocol. Apart from an encouraging efficacy of combination therapy, this high percentage of patients continuing protocol treatment also illustrates that combination therapy was rather well tolerated.

Our results, as well as those by several others, clearly suggest that patients with a more pronounced response, such as a major molecular response, benefit in terms of a lower risk of disease progression and prolonged progression-free survival.[2,3,36,37] Therefore, aiming for a major molecular response has been advocated as an important treatment goal by several investigators.[3,35] Is a further improvement up to the level of a complete molecular response of additional benefit? Recent preliminary reports have suggested that a subset of patients in continued complete molecular response may potentially be cured, as was suggested by absence of molecular relapse following cessation of imatinib maintenance initially described by Rousselot *et al.*[38] A more recent follow-up and inclusion of a total of 50 patients essentially showed the same picture with approximately 50% of patients maintaining PCR-negativity after cessation of imatinib.[39] A similar observation was made in Australia, with a relatively high failure-free survival rate, but longer follow-up may be needed to determine definitely to what extent patients may be cured.[40]

In conclusion, following earlier *in vitro* findings,[18,19] our clinical results may mirror the contributing effect of cytarabine to that of imatinib in patients with first chronic phase CML. The additive value of cytarabine in first chronic phase CML seems to be better eradication of residual disease, as reflected by a relatively high rate of complete molecular responses. While cytogenetic resistance may partially be prevented, a subset of high-risk patients still represents a category of patients for whom better therapeutic approaches are needed. The ultimate advantage of an increased complete molecular response rate should be assessed in future studies, including well-monitored trials evaluating the cessation of imatinib in patients with a long-lasting complete molecular response.

Authorship and Disclosures

WD, JJWMJ, BH, GEGV, BL, GJO, and JJC were responsible for the initial design of the present analysis, actual evaluation, and writing the paper; all authors were responsible for the design of the HOVON study, treatment of patients, critical review of the paper, suggestions for additional analysis, and finalizing the writing of the paper.

PS, GJO, and JJC have received consulting fees from Novartis Oncology. No other potential conflicts of interests relevant to this article were reported.

References

1. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med. 2001;344(14):1031-7.
2. Druker BJ, Guilhot F, O'Brien SG, Gathmann I, Kantarjian H, Gattermann N, et al. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. N Engl J Med. 2006;355(23):2408-17.
3. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, et al. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. N Engl J Med. 2003;349(15):1423-32.
4. O'Brien SG, Guilhot F, Goldman JM, Hochhaus A, Hughes TP, Radich JP, et al. International Randomized study of Interferon versus STI571 (IRS) 7-year follow-up: sustained survival, low rate of transformation and increased rate of major molecular response (MMR) in patients (pts) with newly diagnosed chronic myeloid leukemia in chronic phase (CMLCP) treated with imatinib (IM). Blood. 2008;112: Abstract 186.
5. De Lavallade H, Apperley JF, Khorashad JS, Milojkovic D, Reid AG, Bua M, et al. Imatinib for newly diagnosed patients with chronic myeloid leukemia: incidence of sustained responses in an intention-to-treat analysis. J Clin Oncol. 2008;26(20):3358-63.
6. Baccarani M, Saglio G, Goldman J, Hochhaus A, Simonsson B, Appelbaum F, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2006;108(6):1809-20.
7. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, et al. Clinical resistance to STI571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science. 2001;293(5531):876-80.
8. Marktel S, Marin D, Foot N, Szydlo R, Bua M, Karadimitris A, et al. Chronic myeloid leukemia in chronic phase responding to imatinib: the occurrence of additional cytogenetic abnormalities predicts disease progression. Haematologica. 2003;88(3):260-7.
9. Branford S, Rudzki Z, Walsh S, Parkinson I, Grigg A, Szer J, et al. Detection of BCR-ABL mutations in patients with CML treated with imatinib is virtually always accompanied by clinical resistance, and mutations in the ATP phosphate-binding loop (P-loop) are associated with a poor prognosis. Blood. 2003;102(1):276-83.
10. Graham SM, Jørgensen HG, Allan E, Pearson C, Alcorn MJ, Richmond L, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002;99(1):319-25.
11. Kantarjian H, Talpaz M, O'Brien S, Garcia-Manero G, Verstovsek S, Giles F, et al. High-dose imatinib mesylate therapy in newly diagnosed Philadelphia chromosome-positive chronic phase chronic myeloid leukemia. Blood. 2004;103(8):2873-8.
12. Deenik W, van der Holt B, Verhoef GEG, Smit WM, Kersten MJ, Kluin-Nelemans HC, et al. Dose-finding study of imatinib in combination with intravenous cytarabine: feasibility in newly diagnosed patients with chronic myeloid leukemia. Blood. 2008;111(5):2581-8.
13. Hughes TP, Branford S, White DL, Reynolds J, Koelmeyer R, Seymour JF, et al. Impact of early dose intensity on cytogenetic and molecular responses in chronic-phase CML patients receiving 600 mg/day of imatinib as initial therapy. Blood. 2008;112(10):3965-73.
14. Cortes J, O'Brien S, Jones D, Ferrajoli A, Konopleva M, Borthakur G, et al. Efficacy

of nilotinib (formerly AMN107) in patients (pts) with newly diagnosed, previously untreated Philadelphia chromosome (Ph)-positive chronic myelogenous leukemia in early chronic phase (CML-CP). Blood. 2008;112: Abstract 446.
15. O'Hare T, Eide CA, Deininger MW. New Bcr-Abl inhibitors in chronic myeloid leukemia: keeping resistance in check. Expert Opin Investig Drugs. 2008;17(6): 865-78.
16. Gardembas M, Rousselot P, Tulliez M, Vigier M, Buzyn A, Rigal-Huguet F, et al. Results of a prospective phase 2 study combining imatinib mesylate and cytarabine for the treatment of Philadelphia-positive patients with chronic myelogenous leukemia in chronic phase. Blood. 2003;102(13):4298-305.
17. Baccarani M, Martinelli G, Rosti G, Trabacchi E, Testoni N, Bassi S, et al. Imatinib and pegylated human recombinant interferon-α2b in early chronic-phase chronic myeloid leukemia. Blood. 2004; 104(13):4245-51.
18. Thiesing JT, Ohno-Jones S, Kolibaba KS, Druker BJ. Efficacy of STI571, an Abl tyrosine kinase inhibitor, in conjunction with other antileukemic agents against Bcr-Abl-positive cells. Blood. 2000;96(9):3195-9.
19. Topaly J, Zeller WJ, Fruehauf S. Synergistic activity of the new ABL-specific tyrosine kinase inhibitor STI571 and chemotherapeutic drugs on BCR-ABL-positive chronic myelogenous leukemia cells. Leukemia. 2001;15(3):342-7.
20. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, Kaeda J, Baccarani M, et al. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. Blood. 2006;108(1):28-37.
21. Branford S, Cross NCP, Hochhaus A, Radich J, Saglio G, Kaeda J, et al. Rationale for the recommendations for harmonizing current methodology for detecting BCR-ABL transcripts in patients with chronic myeloid leukemia. Leukemia. 2006;20(11): 1925-30.
22. Ross DM, Branford S, Moore S, Hughes TP. Limited clinical value of regular bone marrow cytogenetic analysis in imatinib-treated chronic phase CML patients monitored by RQ-PCR for BCR-ABL. Leukemia. 2006;20(4):664-70.
23. Branford S, Rudzki Z, Walsh S, Grigg A, Arthur C, Taylor K, et al. High frequency of point mutations clustered within the adenosine triphosphate-binding region of BCR/ABL in patients with chronic myeloid leukemia or Ph-positive acute lymphoblastic leukemia who develop imatinib (STI571) resistance. Blood. 2002;99(9): 3472-5.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc. 1958;53:457-81.
25. Cox DR. Regression models and life tables. J R Stat Soc. 1972;34(2):187-220.
26. Baccarani M, Rosti G, Castagnetti F, Haznedaroglu I, Porkka K, Abruzzese E, et al. A comparison of imatinib 400 mg and 800 mg daily in the front-line treatment of patients with high risk, Philadelphia-positive, chronic myeloid leukemia. a European LeukaemiaNet Study. Blood. 2009;113(19): 4497-504.
27. Kantarjian HM, Talpaz M, O'Brien S, Giles F, Garcia-Manero G, Faderl S, et al. Dose escalation of imatinib mesylate can overcome resistance to standard-dose therapy in patients with chronic myelogenous leukemia. Blood. 2003;101(2):473-5.
28. Cortes J, Giles F, O'Brien S, Thomas D, Garcia-Manero G, Rios MB, et al. Result of high-dose imatinib mesylate in patients with Philadelphia chromosome-positive chronic myeloid leukemia after failure of interferon-alpha. Blood. 2003;102(1):83-6.
29. Castagnetti F, Palandri F, Amabile M, Testoni N, Luatti S, Soverini S, et al. Results of high-dose imatinib mesylate in intermediate Sokal risk chronic myeloid leukemia patients in early chronic phase: a phase 2 trial of the GIMEMA CML Working Party. Blood. 2009;113(15):3428-34.
30. Larson RA, Druker BJ, Guilhot F, O'Brien SG, Riviere GJ, Krahnke T, et al. Imatinib pharmacokinetics and its correlation with response and safety in chronic-phase chronic myeloid leukemia: a subanalysis of the IRIS study. Blood. 2008;111(8):4022-8.
31. Picard S, Titier K, Etienne G, Teilhet E, Ducint D, Bernard M-A, et al. Trough imatinib plasma levels are associated with both cytogenetic and molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood. 2007;109(8):3496-9.
32. Branford S, Seymour JF, Grigg A, Arthur C, Rudzki Z, Lynch K, et al. BCR-ABL messenger RNA levels continue to decline in patients with chronic phase chronic myeloid leukemia treated with imatinib for more than 5 years and approximately half of all first-line treated patients have stable undetectable BCR-ABL using strict sensitivity criteria. Clin Cancer Res. 2007;13(23): 7080-5.
33. Cortes JE, Kantarjian HM, Goldberg SL, Powell BL, Giles FJ, Wetzler M, et al. High-dose imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: high rates of rapid cytogenetic and molecular responses. J Clin Oncol. 2009;27(28):4754-9.
34. Terragna C, Durante S, Astolfi A, Palandri F, Castagnetti F, Testoni N, et al. Gene expression profile (GEP) of chronic myeloid leukemia (CML) patients at diagnosis: two distinguished subgroups of CML patients identified, based on a molecular signature, irrespective of their Sokal risk score. Blood. 2008: 112: Abstract 3190.
35. Dulucq S, Bouchet S, Turcq B, Lippert E, Etienne G, Reiffers J, et al. Multidrug resistance gene (MDR1) polymorphisms are associated with major molecular responses to standard-dose imatinib in chronic myeloid leukemia. Blood. 2008;112(5): 2024-7.
36. Cortes J, Talpaz M, O'Brien S, Jones D, Luthra R, Shan J, et al. Molecular responses in patients with chronic myelogenous leukemia in chronic phase treated with imatinib mesylate. Clin Cancer Res. 2005;11(9):3425-32.
37. Iacobucci I, Saglio G, Rosti G, Testoni N, Pane F, Amabile M, et al. Achieving a major molecular response at the time of a complete cytogenetic response (CCgR) predicts a better duration of CCgR in imatinib-treated chronic myeloid leukemia patients. Clin Cancer Res. 2006;12(10):3037-42.
38. Rousselot P, Huguet F, Rea D, Legros L, Cayuela JM, Maarek O, et al. Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. Blood. 2007;109(1):58-60.
39. Mahon FX, Huguet F, Guilhot F, Legros L, Nicolini FE, Charbonnier A, et al. Is it possible to stop imatinib in patients with chronic myeloid leukemia? An update from a French pilot study and first results from the multicentre <<Stop Imatinib>> (STIM) study. Blood. 2008;112: Abstract 187.
40. Ross DDM, Grigg A, Schwarer A, Arthur C, Loftus K, Mills AK, et al. The majority of chronic myeloid leukemia patients who cease imatinib after achieving a sustained complete molecular response (CMR) remain in CMR, and any relapses occur early. Blood. 2008;112: Abstract 1102.