POPULATION-BASED REGISTRY AND RESPONSE MONITORING IN CHRONIC MYELOID LEUKEMIA

Inge G.P. Geelen

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POPULATION-BASED REGISTRY AND RESPONSE MONITORING IN CHRONIC MYELOID LEUKEMIA

Populatie-gebaseerde registratie en monitoring van behandelresultaten bij chronische myeloïde leukemie

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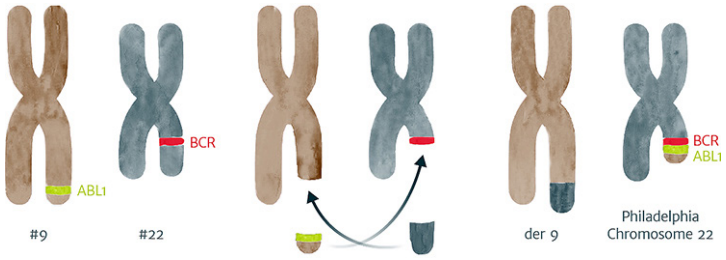
CHAPTER 1

General introduction

Chronic myeloid leukemia

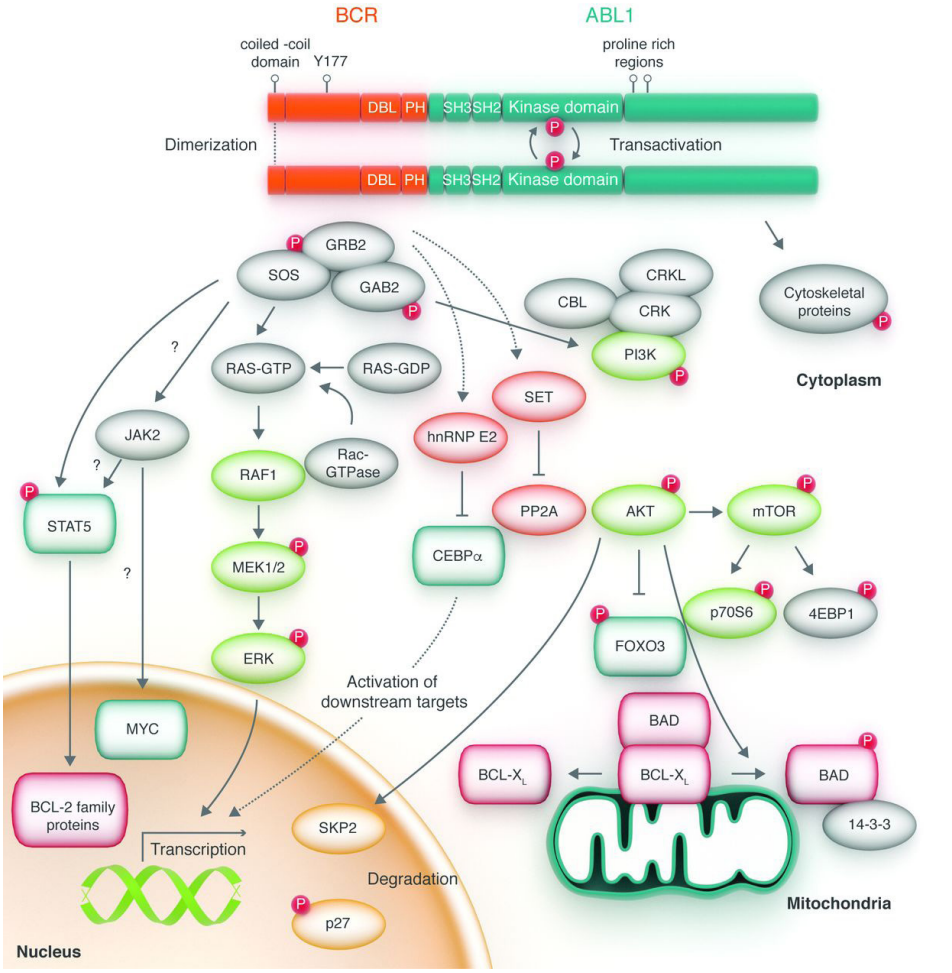
Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder defined by the presence of a *BCR-ABL1* fusion gene. The disease is characterized by an excessive accumulation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors (metamyelocytes, myelocytes, promyelocytes) in the bone marrow and peripheral blood. The discovery of the Philadelphia chromosome (Ph) in 1960 by Nowell and Hungerford was an important first step in unraveling the pathogenesis of CML.[1] A decade later it was recognized that this truncated chromosome 22 is the result of a reciprocal translocation between chromosome 9 and 22, t(9;22)(q34.1;q11.21).[2] The translocation leads to the fusion of the Abelson1 (*ABL1)* gene originally located on chromosome 9 with the Breakpoint Cluster Region (BCR) gene on chromosome 22 (figure 1). The *BCR-ABL1* fusion gene encodes a constitutively active tyrosine kinase which activates numerous signal transduction pathways leading to increased proliferation and differentiation of the CML progenitors and decreased apoptosis and adhesion to the bone marrow stroma (figure 2).[3,4]

Figure 1. Philadelphia translocation



The raw incidence of approximately 1 patient per 100 000/year[5] illustrates that CML is a rare disease, comprising 15% of all leukemias.[6] The 20-year prevalence of CML in the Netherlands was 1738 patients in 2016[7] and the Dutch CML population is growing with approximately 165 new CML patients per year.[8] A slight male predominance (53.7%) is observed and the median age at presentation is 55 years.[5] Fatigue, night sweats and weight loss are commonly reported presenting symptoms. Pain in the left upper quadrant, abdominal discomfort and early satiety are typically described by patients with an enlarged spleen at diagnosis (40-50%). In asymptomatic patients (20-50%) the incidental diagnosis is often revealed by an elevated white blood count in blood tests performed for unrelated reasons.[9,10]

Figure 2. Cytoplasmic BCR-ABL1 activates a myriad of signal pathways.



Note: Reproduced from 'Chronic myeloid leukemia: Reminiscences and Dreams', Mughal *et al.*, Haematologica, 2016.[4]

The CML diagnosis can be confirmed by either demonstrating the Philadelphia chromosome, *BCR-ABL1* fusion gene or BCR-ABL1 fusion transcript.[11,12] The presence of the Philadelphia chromosome can be determined by chromosome banding analysis of bone marrow metaphases, also called conventional cytogenetics. Fluorescence in situ hybridization (FISH) can detect the *BCR-ABL1* co-localization of the fluorescent probes for BCR and ABL, resulting in a single fusion signal. The *BCR-ABL1* mRNA transcript can be demonstrated with reverse transcriptase polymerase chain reaction (RT-PCR) using fusion transcript specific primers. An advantage of the latter two techniques is that the CML diagnosis can also be established in patients with a masked Philadelphia chromosome (± 5% of CML patients).

The natural course of CML is triphasic. More than 90% of CML patients present in chronic phase (CP),[5,13] an indolent phase in which the redundant amounts of myeloid cells still have the capability to undergo normal differentiation. In untreated patients, within three to five years the disease progresses to a rapidly fatal myeloid or lymphoid blast crisis (BC), a phase resembling acute leukemia. The transitional accelerated phase (AP) is characterized by an escalating quantity of immature blasts in bone marrow and circulation.[14-16] The three disease phases are defined by the European Leukemia Net (ELN) based on the percentage of blasts in blood or marrow (CP<15%, AP 15-29%, BC≥30%), basophils in peripheral blood (AP≥20%), persistent thrombocytopenia (AP), clonal chromosomal abnormalities (AP) and extramedullary blast proliferation (BC).[17]

Response monitoring

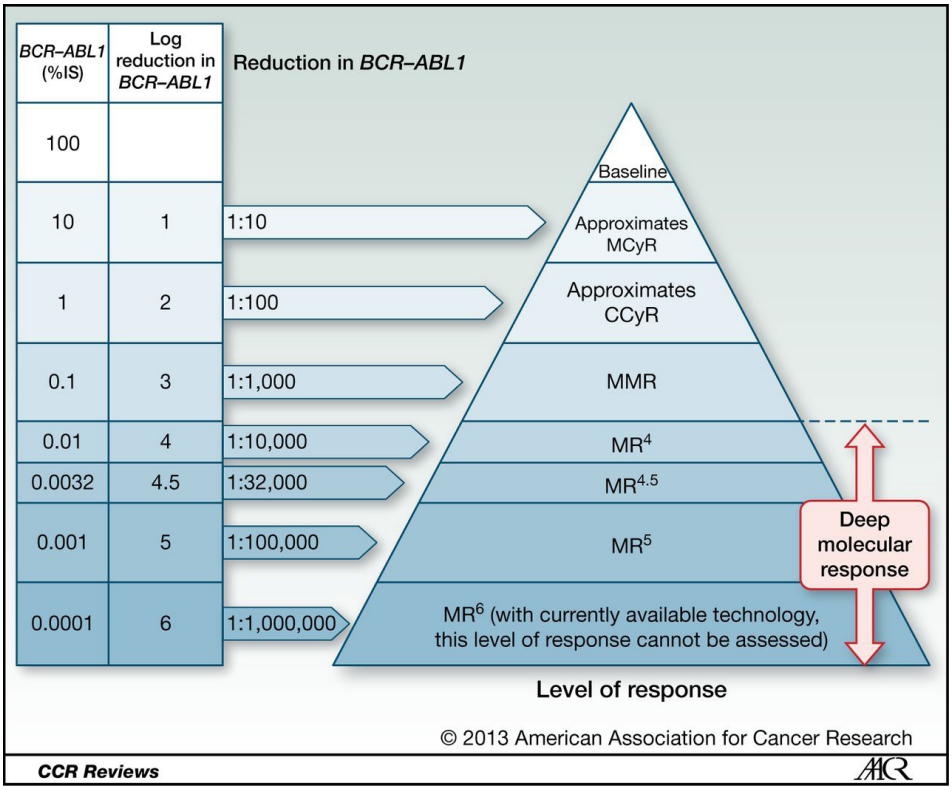
Treatment response can be divided into three categories: hematologic, cytogenetic and molecular response. Hematologic response is assessed by the white blood cell count (WBC), differential, platelet count and spleen size. A complete hematologic response (CHR) is defined as a WBC < 10 x 10^9/L, basophils <5%, no myelocytes, promyelocytes, myeloblasts in the differential, platelet count < 450 x 10^9/L and a nonpalpable spleen.[17,18] The beforementioned cytogenetic and molecular techniques used to confirm the diagnosis are also being used for the monitoring of cytogenetic and molecular response to treatment.

CML guidelines recommend that for response monitoring chromosome banding analysis should be performed in at least 20 bone marrow metaphases.[17] The percentage of Philadelphia chromosome positive (Ph+) metaphases is a measure for cytogenetic response (CyR) and can be categorized as none (noCyR, > 95% Ph+ metaphases), minimal (minCyR, 66-95% Ph+ metaphases), minor (mCyR, 36-65% Ph+ metaphases), partial (pCyR, 1-35% Ph+ metaphases) or complete (CCyR, 0% Ph+ metaphases). FISH can also be used for response monitoring, by evaluating the presence of the *BCR-ABL1* fusion gene in at least 200 cells derived from either bone marrow or peripheral blood. It has a higher sensitivity then chromosome banding analysis, but is much less sensitive than molecular assessments, such as real-time quantitative PCR (RT-qPCR) which is a quantitative variation on the abovementioned RT-PCR method.

RT-qPCR can be performed on either bone marrow or peripheral blood and it uses fluorescent DNA probes to quantify the number of complementary DNA (cDNA) copies that develop during the PCR reaction, relative to an internal reference gene, most commonly *ABL1*, *GUSB* or *BCR*. Since there is a high level of interlaboratory variability for this technique, a standardized international scale (IS) has been introduced.[19] This is a standardized outcome measure which assigns a value of 100% to the median BCR-ABL1 mRNA transcript level of baseline samples from 30 CML patients included in the IRIS trial as external reference.[20] Molecular laboratories have to acquire a laboratory-specific conversion factor by sample exchange or use kits and reagents that have been calibrated, in order to report results on the international scale. CCyR is equivalent to a BCR-ABL1 level of 1% on this scale. A major molecular response (MMR) is defined as 0.1%^{IS}. Deep molecular responses such as MR$^{4.0}$, MR$^{4.5}$ and MR$^{5.0}$ represent a residual disease of 0.01%^{IS}, 0.0032%^{IS} and 0.001%^{IS} respectively (figure 3). Since detectability of the BCR-ABL1 transcript at these deep levels of molecular response is also dependent of the sensitivity of the technique used, strict definitions of deep molecular responses have been formulated:[21]

- $MR^{4.0}$ (≥4-log reduction from IRIS baseline): either detectable disease ≤0.01% $BCR\text{-}ABL^{IS}$ or undetectable disease in cDNA with 10.000-31.999 *ABL1* transcripts or 24.000-76.999 *GUSB* transcripts.
- $MR^{4.5}$ (≥4.5-log reduction from IRIS baseline): either detectable disease ≤0.0032% $BCR\text{-}ABL^{IS}$ or undetectable disease in cDNA with 32.000-99.999 *ABL1* transcripts or 77.000-239.999 *GUSB* transcripts.
- $MR^{5.0}$ (≥5-log reduction from IRIS baseline): either detectable disease ≤0.001% $BCR\text{-}ABL^{IS}$ or undetectable disease in cDNA with ≥100.000 *ABL1* transcripts or ≥240.000 *GUSB* transcripts.

Figure 3. Levels of molecular response in CML.



Note: Reproduced from 'Deep Molecular Response in Chronic Myeloid Leukemia: The New Goal of Therapy?', Mahon *et al.*, Clinical Cancer Research, 2014.[23]

More sensitive PCR technique such as digital PCR with sensitivity levels between $MR^{5.0}$-$MR^{7.0}$ are currently being evaluated for their applicability in clinical practice.

Recommendations on monitoring frequency and milestones at specific time points are clearly outlined in international guidelines for the management of CML.[17,22] In short, monitoring should be performed three-monthly until MMR is reached, and 4-6 monthly thereafter. Response milestones are defined as optimal, warning and failure responses. Optimal responses require no change in treatment, warning responses demand more frequent monitoring and failure obligates additional evaluation of the cause of this failure, such as *BCR-ABL1* kinase domain mutations (explained below), patient compliance, and a switch to an alternative treatment.

Treatment

Splenic irradiation and conventional chemotherapy

In the late 1800s and early 1900s CML-patients were treated with arsenicals and splenic irradiation for symptomatic relief.[24,25] The oral alkylating agent busulfan introduced in 1959 was the first agent able to lower leukocyte counts. Busulfan acts on the primitive stem cells and it is accompanied with an inconvenient toxicity profile including gonadal failure, hypoadrenalism, skin pigmentation, pulmonary and marrow fibrosis and in occasional patients busulfan unpredictably causes irreversible marrow hypoplasia.[24] Ten years later, treatment with the better tolerated hydroxycarbamide (hydroxyurea) demonstrated a modest survival benefit (median survival 4.7 years with hydroxycarbamide vs. 3.8 years with busulfan, p=0.008).[26] Hydroxycarbamide is a ribonucleotide reductase inhibitor and acts on relatively late myeloid progenitors compared to busulfan, but does not eradicate the Philadelphia positive clone.[24,25]

Hematopoietic stem cell transplantation

In 1979 a first successful cure from CML was established in 4 patients with CML in CP by intensive (myeloablative) chemoradiotherapy followed by a transplantation of healthy bone marrow donor cells from their genetically identical (syngeneic) twins.[27] This treatment strategy was adopted by physicians and applied in a selected patient population with Human Leukocyte Antigen (HLA)-identical siblings available as donors, resulting in a 3-year survival of 63%, 36% and 12% for patients transplanted in CP, AP and BC respectively.[28] In the 1990s hematopoietic allogeneic stem cell transplantation (HSCT) became the treatment of choice for all relatively young patients (<55 years) without comorbidities, presenting with CP-CML, resulting in 5-year overall survival and leukemia-free survival probabilities of 60-80% and 55-77%, respectively, with a 10-20% relapse rate.[24,29] Results of transplants with HLA-matched unrelated donors were inferior, due to the increased rates of graft failure, graft-versus-host disease and subsequent transplant-related mortality.[30] A strong correlation was found between graft-versus-host disease and leukemia-free survival in CML patients who received a HSCT.[31-33] T-cell depletion of the bone marrow transplant resulted in reduced mortality rates but increased the relapse rate of CML significantly, implying an critical role for the T-lymphocytes in the success of allogeneic stem cell transplantation in CML, the so-called graft-versus-leukemia effect hypothesis.[34,35] This hypothesis was further underlined by the observation that donor lymphocyte infusions (DLI) induced complete remissions in CML patients who relapsed after HSCT.[36-38] The introduction of DLI enable the application of reduced intensity conditioning transplants, making HSCT available for patients of advanced age and with associated co-morbidities.[39-41] Overall the outcomes of HSCT for CML have improved over time: EBMT registry data showed an overall survival of 53%, 59% and 61% between 1980-1990, 1991-1999 and

2000-2003 respectively with accompanying transplant-related mortalities of 41%, 34% and 30%.[29] Nevertheless, HSCT continues to be associated with substantial transplant-related morbidity and mortality, even in selected patients.[42]

Interferon-alpha

In the early 1980s interferon-alpha (IFN-α) was introduced for the management of CML in chronic phase. Interferons are cellular glycoproteins with antiproliferative, antiviral and immunoregulatory properties.[43] Researchers from the M.D. Anderson Cancer Center were the first to administer IFN-α to 51 patients in CP-CML and were able to observe that this therapeutic agent was capable of bringing 71% of patients in CHR.[44] The same research group demonstrated that IFN-α was the first nonmyelotoxic drug to show a marked reduction in Philadelphia positivity in some patients (19% CCyR).[45] A meta-analysis of seven randomized trials showed an improved 5-year survival compared to treatment with conventional chemotherapy (57% vs. 42%).[46] The addition of cytarabine to IFN-α further improved the probability of achieving a major cytogenetic response at 12 and 24 months, but an observed improvement of overall survival was not confirmed.[47,48] Allogeneic stem cell transplantation became the treatment of choice for patients below 55 years with a HLA-matched donor available. For the 70% of CML patients not eligible for allografting, the combination of IFN-α plus cytarabine was the best available treatment option at that time, but it was accompanied with considerable side effects.

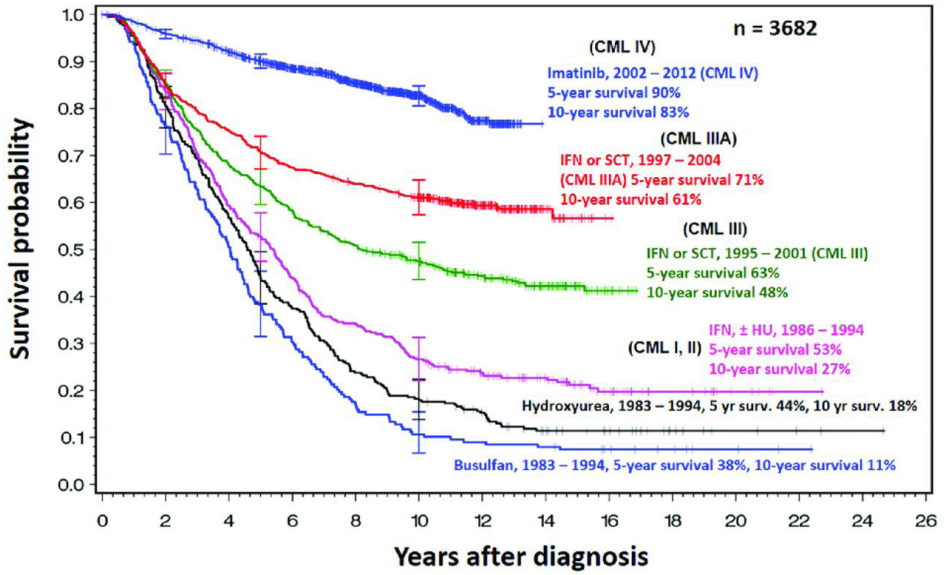
Imatinib

A revolutionary change in the treatment landscape of chronic myeloid leukemia occurred in 1998 with the initiation of a phase I trial designed to determine the safety and efficacy of imatinib (STI571), an orally administered tyrosine kinase inhibitor (TKI) targeting the ABL1-tyrosine kinase. In 98% of the patients who received at least 300 mg per day, a CHR was achieved within 4 weeks, cytogenetic responses occurred in 54% of the patients and side effects were mild to moderate.[49] Imatinib blocks the ATP binding site of the BCR-ABL1 protein, thereby impairing BCR-ABL1-mediated transfer of phosphate to its substrates, resulting in blockage of downstream signaling pathways that are responsible for the pathophysiology of the disease. Furthermore, imatinib causes off-target inhibition of Platelet Derived Growth Factor (PDGF) receptor and c-Kit (receptor for stem-cell factor).[50] Reports of three phase 2 trials included more than 1000 patients and confirmed the efficacy and safety of imatinib in CP, AP and BC-CML.[51-53]

In 2000, a large phase 3 trial, named the International Randomized study for Interferon and STI571 (IRIS) trial, enrolled 1106 patients randomized to receive either IFN-α plus cytarabine (combination-therapy group) or imatinib. The results of this trial were spectacular: 97% and 76% of patients in the imatinib group achieved CHR and CCyR at 18

months respectively, compared to 69% and 15% in the combination-therapy group. At 12 months only 4% of imatinib-treated patients demonstrated disease progression as compared to 20% in the combination-therapy group.[20] This resulted in an increase of the 5-year overall survival of imatinib-treated CML patients to 89-97% (figure 4).[54-57] In a total of 13 trials evaluating 400mg and 800mg imatinib, CCyR and MMR rates after 12 months varied between 49%-88% and 18%-47% respectively.[56-68] Two randomized controlled trials did not observe differences in cytogenetic and molecular response rates when comparing imatinib 400 mg with 800 mg[64,69], whereas two other clinical trials did show significantly higher CCyR and MMR rates, most likely due to a flexible dosing schedule that allowed more patients on the high-dose arms to remain on-study.[63,70] This did however not result in a better progression-free survival or overall survival.

Figure 4. Survival with CML as observed in five consecutive randomized treatment optimization studies of the German CML Study Group 1983-2014.



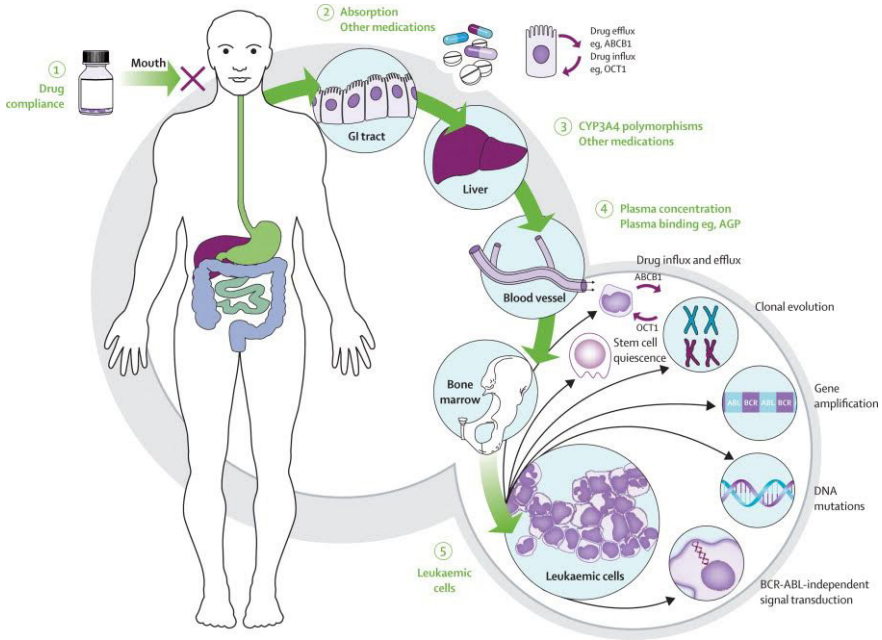
Note: Reproduced from 'CML – Where do we stand in 2015', by Hehlmann, Annals of Hematology, 2015.[55]

Deep molecular responses have been assessed in multiple clinical trials, but are difficult to compare, given the lack of standardization of PCR sensitivity at the time these trials were performed, differences in assay techniques, study design and follow-up duration: $MR^{4.0}$ and $MR^{4.5}$ after 5 years of imatinib treatment were achieved in 42%-68%, and 31%-58% of patients respectively.[71-73] Ten year results of the CML IV study showed an $MR^{4.0}$ and $MR^{4.5}$ rate of 83% and 70% respectively, whereas the 10-year $MR^{4.5}$ rate in the IRIS trial was 63%.

In both trials the numbers of patients at risk and/or still evaluable for molecular response were very low after 10 years.[73,74]

With a median follow-up duration of 10.9 years, the IRIS trial has proven that long-term administration of imatinib was not associated with unacceptable cumulative or late toxic effects.[74] The adverse events of imatinib were generally mild (grade 1 or 2), most frequently edema, muscle cramps, diarrhea, nausea, musculoskeletal pain, rash, abdominal pain, fatigue, joint pain and headache. More severe (grade 3 or 4) adverse events occurred in 9% of patients: neutropenia, thrombocytopenia, anemia, elevated liver enzymes and other drug-related adverse events.[20,54] These toxicities are reflected in a negative impact of long-term imatinib treatment on health-related quality-of-life, especially in younger patients (18-39 years) and women.[75]

Figure 5. Mechanisms of TKI resistance.



Note: Reproduced from 'Part I: mechanisms of resistance to imatinib in chronic myeloid leukaemia', by Apperley *et al.*, Lancet Oncology, 2007.[77]

Unfortunately not all patients responded to imatinib treatment due to primary resistance (failure to achieve the response milestones), secondary resistance (loss of a previously achieved response milestone) or intolerance. Secondary resistance is more likely to be

caused by BCR-ABL1 dependent mechanisms and primary resistance tends to be triggered by BCR-ABL1 independent mechanisms. Point mutations in the *BCR-ABL1* kinase domain (KD) are the most common cause of BCR-ABL1 dependent TKI resistance (figure 5).[76] They can be detected by sanger sequencing and lead to a less effective binding of TKIs in the kinase domain resulting in a decline of the inhibitory effect of the TKI. More than 50 different imatinib-resistance KD mutations have been described.[77] A study of 297 patients with resistance to imatinib reported KD mutations in 27% of CP-patients, 52% of AP-patients, 75% of myeloid BC and 83% of lymphoid BC.[78] Other mechanisms of resistance are low intracellular drug availability due to low activity of Organic-cation transporter-1 (OCT-1), a cellular influx pump for imatinib,[79] and overexpression of members of the ATP-binding cassette (ABC) transporter family, including ABCB1 and ABCG2, which behave as drug exporters.[80,81] Moreover, multiple alternative survival signaling pathways have been implicated in *BCR-ABL1* independent resistance, such as STAT3, PI3K/AKT, RAF/MEK/ERK, EZH2, XPO1 and RAN.[76]

Dasatinib

Dasatinib is a dual Src-Abl kinase inhibitor with a 325-fold higher *BCR-ABL1* inhibitory activity against *in vitro* wild-type *BCR-ABL1* than imatinib.[82] In 2006, this second generation TKI dasatinib became available for second-line therapy. The DASISION trail randomizing 519 newly diagnosed CP-CML patients to upfront treatment with dasatinib 100 mg daily or imatinib 400 mg daily, demonstrated higher CCyR and MMR rates at 12 months (77% vs. 66% and 46% vs. 28%).[62] This led to the approval of dasatinib as first line treatment in CP-CML patients in 2010. Five-year results showed that progression to AP/BC was lower in the dasatinib arm (4.6% vs 7.3%), but this difference did not result in a significantly better 5-year progression-free survival and overall survival.[72] A deep molecular response of $MR^{4.5}$ was achieved by 42% of patients after 5 years. Results from the Dasision trial were confirmed by a randomized controlled trial which enrolled 253 patients.[66]

In a second line setting outcomes were generally better in patients with prior imatinib-intolerance than imatinib-resistance. In the Phase II START-C trial, 39% of patients with imatinib-resistant CP-CML achieved major cytogenetic response and 28% achieved CCyR after 8 months of dasatinib treatment, while 80% and 64% of the imatinib-intolerant patients attained these endpoints. In the subgroup of imatinib-resistant patients 8% progressed or died within 8 months, compared to 0,5% in the imatinib-intolerant subgroup.[83] In the phase 3 CA180-034 study, 51/124 (43%) patients resistant to imatinib treated with dasatinib 100 mg once daily achieved MMR, compared to 22/43 (55%) patients with imatinib-intolerance. Progression-free survival and overall survival were 39% vs. 51% and 63% vs. 70% respectively.[84]

Pulmonary toxicities, such as pleural effusions and pulmonary hypertension are most closely linked to therapy with dasatinib and are rare with other TKIs. Only 15% of the adverse events reported with dasatinib were grade 3 or 4 in the DASISION trial. Withdrawal from the trial due to adverse events occurred in 16% of the patients. Drug-related pleural effusion was more common with dasatinib (28%) than with imatinib (0.8%) and was managed with dose interruption and/or dose reduction, diuretics, corticosteroids or therapeutic thoracocentesis. Pulmonary hypertension and arterial ischemic events were both reported in 5% of the patients.[72] Cytopenias were common but could be managed effectively with dose reductions or temporary interruptions. Headache, gastrointestinal disorders, fatigue and dyspnea were the most common nonhematologic events.[83]

Nilotinib

Nilotinib was developed by rational drug design based on the crystal structure of an Abl-imatinib complex and led to substantially increased binding affinity and selectivity for the Abl kinase compared with imatinib. This second generation TKI inhibits unmutated *BCR-ABL1* with a 20-fold higher potency than imatinib in *in vitro* experiments.[82] Nilotinib was approved for second-line treatment of CP-CML in 2007 and registered for newly diagnosed CP-CML patients in 2010 based on results from the ENESTnd trial. In this trial 846 newly diagnosed CP-CML patients were randomized to receive nilotinib 300 mg twice daily, nilotinib 400 mg twice daily or imatinib 400 mg once daily. The rates of CCyR and MMR at 12 months were significantly higher for nilotinib 300 mg and 400 mg than for imatinib (80% vs. 78% vs. 65% and 44% vs. 43% vs. 22%).[61] Similar results were observed in the ENESTchina comparing nilotinib 300 mg twice daily with imatinib 400 mg once daily.[85] Progression within 5 years occurred in 0.7%, 1% and 4,2% of patients on nilotinib 300 mg, nilotinib 400 mg and imatinib 400 mg. This did result in a better progression-free survival and overall survival in patients treated with nilotinib 400 mg, compared to imatinib 400 mg, but a difference with nilotinib 300 mg was not observed.[71] A deep molecular response of $MR^{4.0}$ and $MR^{4.5}$ was observed in 66% vs. 63% vs. 42% and 54% vs. 52% vs. 31% of patients after 5 years of treatment.

In patients with imatinib-resistance or -intolerance, nilotinib as a second line treatment was able to induce major cytogenetic responses and CCyR rates of 59% and 44% after 24 months. As expected, like in second line studies with dasatinib, the outcomes were better in patients with imatinib intolerance than with imatinib resistance: 66% major cytogenetic response and 51% CCyR after 24 months in the imatinib-intolerant subgroup and 56% and 41% after 24 months in the imatinib-resistant group.[86] Long-term results of this phase II trial demonstrated a 4-year progression-free survival of 57% and an overall survival rate of 78%. Of the 102 patients with progression-free survival events, only 11 (3%) progressed to AP or BC.[87]

Long-term follow-up from the ENESTnd trial has demonstrated an association between nilotinib and cardiovascular toxicity: hypercholesteremia and hyperglycemia were observed in 27% and 50% of nilotinib patients. Cardiovascular events (ischemic heart disease, ischemic cerebrovascular events and/or peripheral arterial disease) were reported in 7.5%, 13% and 2.1% of patients treated with nilotinib 300 mg, nilotinib 400 mg and imatinib. Also elevated liver enzymes and increased lipase levels are most commonly seen with nilotinib.[61,88]

Bosutinib

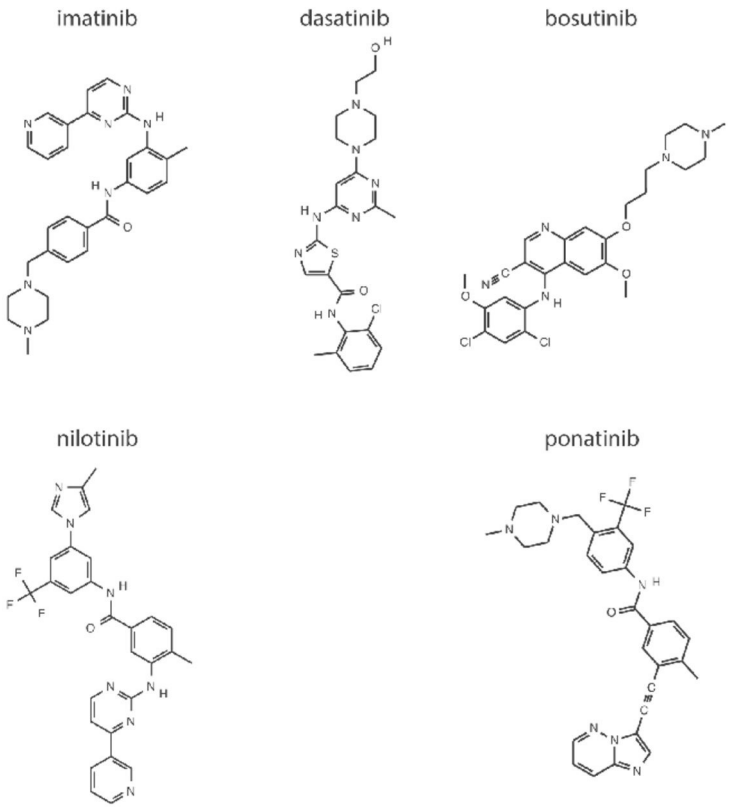
Bosutinib is a dual inhibitor of the Src and Abl tyrosine kinases, with minimal inhibitory activity against c-Kit or PDGF receptor. This second generation TKI was approved for second-line treatment of CP-CML in 2012, following a phase I/II trial in imatinib-resistant or intolerant patients. This trial enrolled 288 patients and demonstrated that among patients without a CCyR at baseline, the major cytogenetic response rate was 57% for imatinib-resistant and imatinib-intolerant patients treated with bosutinib 500 mg. An MMR was achieved in 35% of the patients. Notably the proportion of patients achieving an MMR was similar for imatinib-resistant (34%) and imatinib-intolerant (35%) patients, but MMR occurred faster in patients intolerant to imatinib (median time to MMR 12.2 vs. 35.9 weeks). Two-year progression-free survival and overall survival were 81% and 91% respectively.[89,90]

The phase III Bosutinib Efficacy and Safety in Newly Diagnosed Chronic Myeloid Leukemia (BELA) trial (n=502) failed to demonstrate a superior rate of CCyR after 12 months in patients treated upfront with bosutinib 500mg compared with imatinib 400 mg (70% vs. 68%, primary endpoint), but a superior rate of MMR was observed (41% vs 27%).[65] Two-year follow-up results comparing the two frontline treatments did also not show convincing differences.[91] Based on the results of the BELA trial, bosutinib was not approved for upfront therapy. A second phase III trial, named the BFORE (Bosutinib Trial in First-Line Chronic Myeloid Leukemia Treatment) study (n=590), used a lower starting dose of bosutinib (400 mg) and MMR at 12 months as primary and point to compare with upfront imatinib 400 mg. The MMR rate at 12 months was significantly higher among patients receiving bosutinib versus imatinib (47% vs 37%).[92] An approval of bosutinib for frontline treatment based on the BFORE results is expected soon.

The difference in selectivity of bosutinib was expected to result in fewer side effects than, because many toxicities associated with other TKIs can be tracked to the inhibition of PDGF-receptor and/or c-KIT. Gastrointestinal side effects however, occurred to have a significant impact on therapy. The most common adverse events of bosutinib are diarrhea (70%), nausea (35%), thrombocytopenia (35%) and elevated liver enzymes (22%-

30%). Diarrhea may necessitate dose interruption or dose reduction, in combination with anti-diarrheals.[91,92]

Figure 6. Chemical structures of imatinib, dasatinib, nilotinib, bosutinib and ponatinib.



Note: Reproduced from 'Chronic myeloid leukemia: Reminiscences and Dreams', Mughal *et al.*, Haematologica, 2016.[4]

Ponatinib

Third generation TKI ponatinib is a pan-BCR-ABL inhibitor, specifically designed to bind in the presence of a T315I mutation.[93] This is the only known *BCR-ABL1* KD mutations in which none of the four first and second generation TKIs were capable to induce a treatment response. The Phase II PACE (Ponatinib Ph-positive acute lymphoblastic leukemia [ALL] and CML evaluation) trial included 203 patients with CP-CML and resistance to or unacceptable side effects of dasatinib or nilotinib and 64 patients with CP-CML and the T315I mutation. A major cytogenetic response was observed in 56% of the patients after 12 months: 51% resistant or intolerant to dasatinib or nilotinib and 70% with T315I mutations. A CCyR was achieved by 46% of patients and MMR in 34%.[94] It led to the

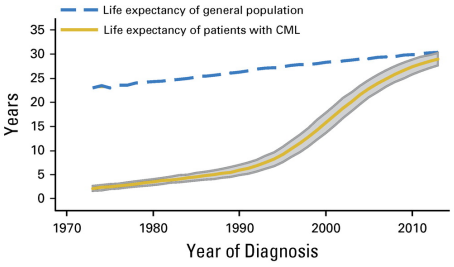
approval of ponatinib for refractory CML in 2012. The frontline Evaluation of Ponatinib versus Imatinib in Chronic Myeloid Leukemia (EPIC) study comparing ponatinib 45 mg with imatinib 400 mg in newly diagnosed CP-CML patients was terminated preliminary following concerns about vascular adverse events observed in patients given ponatinib in other trials. The proportion of patients achieving a MMR at any time was significantly higher for patients given ponatinib (41% vs 18%), but the interpretation of this results is restricted due to the preliminary termination. Eleven of 154 (7%) patients given ponatinib and three of 152 (2%) patients given imatinib had arterial occlusive events. Rash, headache, gastrointestinal symptoms, increased lipase, hypertension and increased liver enzymes were other commonly reported adverse events.[95] Results from ongoing clinical trials, including a dose-ranging study with ponatinib, are expected to provide further information regarding the benefit-risk balance in ponatinib-treated patients. Disease state, mutational status, line of treatment, reason of change of therapy and specific comorbidities play an important role in the decision to prescribe ponatinib.

The need for allogeneic stem cell transplantation in the CML treatment has reduced with the introduction of tyrosine kinase inhibitors, but it is still an important salvage strategy for the small number of patients who present in advanced disease, show progression during TKI treatment, fail on several lines of TKI treatment or are intolerant for multiple TKIs.

Baseline risk prediction

Risk scores use baseline characteristics of CML patients in chronic phase, such as age, spleen size and blast count, to predict outcome. Until recently, risk stratification of CML patients was used based on scores developed in the pre-imatinib era (Sokal and Hasford risk score)[96,97] with overall survival as the end point. After the introduction of imatinib, the EUTOS score was established to predict the chance of achieving CCyR at 18 months, as a proxy for survival.[98] The life expectancy of CML patients is currently approaching the life expectancy of the general population (figure 7).[99] Since the major causes of death of CML patients are no longer CML related, the required outcome parameter for baseline risk prediction has shifted from overall survival towards disease specific mortality. Therefore, recently the EUTOS long-term survival (ELTS) score was introduced to predict the risk of dying of CML in patients treated with first line imatinib.[100]

Figure 7. Life expectancy of the general population and of male patients, 55 years old, with chronic myeloid leukemia in Sweden.



Note: adopted from 'Life Expectancy of Patients With Chronic Myeloid Leukemia Approaches the Life Expectancy of the General Population', Bower *et al.*, Journal of Clinical Oncology, 2016.[99]

Treatment free remission

Unfortunately, TKIs do not have the ability to eradicate all leukemic stem cells and therefore they cannot provide a definite cure. In theory the inability of TKIs to eradicate all leukemic stem cells would mean that all CML patients would stay dependent on TKI treatment for the rest of their life. Although the majority of patients only experience mild discomfort of TKI treatment, this lifelong TKI treatment does affect their quality of life.[75] Moreover, TKI treatment is very costly, therefore long-lasting TKI treatment for an expanding group of CML patients with a near to normal life-expectancy would mean an ever-growing financial burden for health-care systems worldwide.[99]

Already in the pre-imatinib era it was observed that some patients treated with IFN-α reaching undetectable molecular residual disease could stop IFN treatment and stay in molecular remission, or a so-called treatment-free remission (TFR).[101] The Stop Imatinib (STIM) study was the first large TKI discontinuation study and it reported a 6-month TFR rate of 43% in patients with at least 2 years undetectable molecular residual disease[102] which has shown to be durable with a 5-year TFR rate of 38%.[103] Since then several imatinib discontinuation trials have been performed, reporting TFR-rates of 47-61%, depending on the patient characteristics required for stopping and the relapse definition.[104-108] TKI cessation trials with second generation TKIs showed TFR rates of 49-69% after 6 months (dasatinib)[109,110], 52% (nilotinib)[111] and 63% (dasatinib or nilotinib)[112] after 12 months. All of these studies observed successful recovery of molecular response after reintroduction of TKI treatment in case of relapse. A history of resistance or suboptimal response[109,112], digital PCR negativity[106,107] and treatment duration[102,107,108] have been identified as predictors of successful TFR.

Outline of the Thesis

This thesis focuses on the quality of treatment, monitoring and outcome prediction in the Dutch population-based CML registry, (PHAROS, Population-based HAematological Registry for Observational Studies) and explores a treatment strategy to deepen molecular responses.

Genetic variants and abnormalities
In addition to the Philadelphia chromosome, *BCR-ABL1* fusion gene and BCR-ABL1 fusion transcript, cytogenetic and molecular tests, used for the diagnosis and monitoring of treatment responses in CML, can uncover genetic variations and additional abnormalities. These variants and abnormalities are the result of genetic instability[113] and some, but not all, have consequences for disease management and outcome. Currently, a comprehensive overview of genetic variants, additional abnormalities and their clinical relevance in CML is lacking. In **chapter 2**, we provide a practical guide on cytogenetic and molecular variants and abnormalities in CML treatment, based on an extensive review of the most recent literature. This information is complemented by knowledge on the underlying pathophysiology, current and future diagnostic possibilities in CML.

Real world treatment patterns and responses
Multiple randomized controlled trials (RCTs) have provided solid evidence for the efficacy and safety of TKIs as treatment for CML, but analyses from observational studies, gathered in patients who did not participate in clinical trials ('the real world') are scarce. Clinical trials use tight inclusion criteria, strict rules for monitoring and treatment algorithms and may therefore not fully reflect results in the general treatment population.[114-116] Moreover, RCTs mainly focus on the outcome of the core study treatment, while a proportion of patients will not be able to continue their initial study treatment and is subsequently switched to an alternative treatment outside the trial.[72,88] To study treatment choices and patient outcome across different treatment lines, 'real-world' data contain important information for clinical practice. We provide a detailed overview of all aspects of CML care including responses to first and subsequent treatment lines with a specific focus on the impact of first line treatment with imatinib compared to second generation TKIs (2GTKIs) dasatinib and nilotinib (**chapter 3**). Also, we sought to evaluate what proportion of patients become eligible to attempt to stop their TKI treatment.

Baseline risk prediction
Since the major causes of death in patients with CML are no longer CML-related, it has become important to use baseline risk prediction models that predict disease specific mortality rather than overall survival.[117] The recently introduced ELTS-score was devel-

oped in chronic phase CML 'in-study' patients treated upfront with imatinib between 2002 and 2006.[100] In the meantime, the introduction of the second generation TKIs such as dasatinib (2006) and nilotinib (2007) have further improved patient outcomes, as more rescue options became available in case of TKI failure. We evaluated the predictive value of the ELTS-score for molecular response, death due to CML and overall survival in a recent, independent, population-based cohort of CML patients treated with upfront imatinib or 2GTKIs (**chapter 4**). In addition, we compared its performance with the previously developed risk scores (Sokal, Hasford and EUTOS).

Quality of response monitoring

Adequate treatment cannot be provided without frequent response monitoring. Timely recognition of TKI failure should trigger the physician to assess the *BCR-ABL1* KD mutation status and switch to an alternative TKI treatment, in order to prevent progression of the disease. US-based evaluations have revealed that response monitoring is often not performed according to international guidelines.[118-120] Knowledge on the 'real-world' practice of TKI response monitoring and *BCR-ABL1* KD mutation testing of CML patients in Europe is lacking. In **chapter 5**, we assessed the quality of response monitoring in the first year of TKI treatment in an unselected population-based Dutch patient cohort. Furthermore, we evaluated the effects of inadequate response monitoring on survival and looked for predictors of adequate response monitoring.

Assessment of the need for routine cytogenetic response monitoring

Chronic myeloid leukemia guidelines continue to recommend performing routine cytogenetic response assessments, even when adequate molecular diagnostics are available.[17,121] Compared to molecular response monitoring, cytogenetic monitoring has a lower sensitivity, is more expensive and requires invasive bone marrow sampling. However, it is the only technique that can detect prognostically unfavorable Additional Cytogenetic Abnormalities (ACAs) that can arise in the Ph+ clone.[122-125] Previous studies have demonstrated the strong correlation between cytogenetic and molecular response results.[126-128] By assessing the disease course of patients with simultaneously performed cytogenetic and molecular response assessments at 3, 6 or 12 months after first line TKI treatment initiation and patients who developed ACAs, we evaluated the addition value of routine cytogenetic response assessments in a population-based cohort (**chapter 6**).

Second line treatment strategy for deepening molecular responses

Treatment-free remission has proven to be feasible in approximately half of the patients with CML in sustained deep molecular remission on TKI therapy. Unfortunately, only 37-46% of patients in chronic phase at diagnoses achieve these durable deep responses after 8 years of imatinib monotherapy.[129,130] Therefore, novel treatment strategies that

increase the proportions of patients that will attain a deep molecular response are of great interest. We performed a phase II, single arm, multicentre trial (NordDutchCML009) to assess if CML patients who did not achieve an $MR^{4.0}$ or better after long-term imatinib therapy, could attain $MR^{4.0}$ after a switch to nilotinib combined with pegylated interferon-α2b (PegIFN). **Chapter 7** presents the efficacy, safety and tolerability of this treatment strategy.

Finally, the main findings of this thesis are discussed and implications for clinical practice and future perspectives outlined in **chapter 8**.

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CHAPTER 2

Cytogenetic and molecular abnormalities in chronic myeloid leukemia: pathophysiology and implications for clinical practice

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Abstract

Genomic instability is an important pathophysiological mechanism underlying chronic myeloid leukemia (CML). It results in formation of the Philadelphia chromosome (Ph, t(9;22)(q34;q11)/*BCR-ABL1*) with various breakpoints and variant translocations, but also additional chromosomal abnormalities and mutations in and outside the *BCR-ABL1* kinase domain. Cytogenetic and molecular assessment techniques are used for the diagnosis and monitoring of CML, but can also uncover these additional chromosomal and genetic variants and abnormalities. Understanding the diversity of chromosomal and molecular manifestations in CML is of importance for the clinician. In this review we discuss the underlying pathophysiology, different methods for disease monitoring and the clinical relevance of genetic variations and additional abnormalities.

Introduction

More than half a century ago in Philadelphia, Peter Nowel and David Hungerford discovered the relationship between a small and aberrant chromosome 22 and chronic myeloid leukemia.[1] In 1972, Janet Rowley recognized that this so-called Philadelphia chromosome (Ph) was the result of a balanced translocation between the long arms of chromosome 9 and 22, t(9;22)(q34.1;q11.21), resulting in a short derivative chromosome 22 (der(22) / Ph), and an abnormal long derivative chromosome 9 (der(9)).[2] At the breakpoint of the aberrant chromosome 22 a fusion of the Abelson (*ABL1*) oncogene (normally located on 9q34) with the Breakpoint Cluster Region (*BCR*) gene (normally located on 22q11) arises. This *BCR-ABL1* fusion gene is translated into a constitutively active tyrosine kinase with proliferative and anti-apoptotic features, which causes further genomic instability through increased production of oxygen radicals and abnormal DNA repair.[3] Blocking the function of the BCR-ABL1 tyrosine kinase with specific kinase inhibitors, such as imatinib, was one of the biggest successes achieved in the last decades in the treatment of hematologic malignancies.[4]

The Philadelphia chromosome, *BCR-ABL1* fusion gene and *BCR-ABL1* mRNA transcript are disease specific tumor markers, which can be used for both diagnosis and monitoring of the disease by cytogenetic and molecular assessment techniques.[5] Besides quantifying the amount of residual leukemic cells, these tests can also uncover additional chromosomal and genetic variants and abnormalities, which may result from the aforementioned genomic instability. In this review we discuss the underlying pathophysiology, different methods for disease monitoring and the clinical relevance of genetic variations and additional abnormalities.

Cytogenetic assessment in CML

Chromosome banding analysis

The study of chromosomes and their abnormalities is known as cytogenetics. During the mitotic phase of the cell cycle (metaphase) chromosomes can be visualized under the microscope using a specific staining method called Giemsa banding (G-banding), which creates unique banding patterns on the chromosomes.[6] Chromosome banding analysis (CBA), also called karyotyping, is a conventional cytogenetic technique that can detect genetic defects that involve large regions of chromosomes resulting from translocations, inversions, aneuploidies or large deletions.

The diagnosis of CML can be confirmed by CBA when the presence of the Ph chromosome is demonstrated in bone marrow metaphases. CBA can also be used for the monitoring of response to treatment. CML guidelines recommend that for response monitoring CBA should be performed in at least 20 bone marrow metaphases.[7] The percentage of Philadelphia chromosome positive (Ph+) metaphases is a measure for cytogenetic response (CyR) and can be categorized as none (noCyR, > 95% Ph+ metaphases), minimal (minCyR, 66-95% Ph+ metaphases), minor (mCyR, 36-65% Ph+ metaphases), partial (pCyR, 1-35% Ph+ metaphases) or complete (CCyR, 0% Ph+ metaphases). Guidelines have defined the response levels which should be considered as optimal, warning or failure at different times after the start of TKI therapy.[7] Patients achieving a CCyR have a favorable prognosis[8,9] and therefore, the achievement of CCyR was accepted an important treatment goal and served as surrogate clinical endpoint for clinical studies.

Additional chromosomal abnormalities

Additional chromosomal abnormalities (ACAs) can develop in the Ph^+ clone, which means that other chromosomal abnormalities are present in addition to the Philadelphia translocation. This could be deletions, inversions, isochromosomes, monosomies, trisomies or translocations in which the Ph chromosome is not included. The frequency of ACAs varies between 10-20% in chronic phase CML to 60-80% of CML patients in blast crisis.[10] The classification of ACAs in 'major route' and 'minor route' is based on the frequency of appearance in studies from the 70s and 80s.[7,10-13] ACAs were considered as 'major route' if detected in at least 10% of patients with ACAs and minor route was defined as present in less than 10% of patients with ACAs (table 1).[10] Also, a distinction is made between balanced and unbalanced ACA karyotypes. The majority of ACAs in CML are genomically unbalanced: trisomies, monosomies and deletions,[10] in which the deviant quantity of genetic code leads to disturbance of protein synthesis and cell behavior. In genomically balanced karyotypes (translocations, inversions) the total amount of genetic code is unchanged, in general with less consequence for protein synthesis, with the rare exception of fusion genes influencing cell function.[14]

Table 1. Major and minor ACAs

Major route ACAs [a]	Minor route ACAs[b]
Trisomy 8	Loss of Y-chromosome
(+8)	(-Y)
Second Philadelphia chromosome	Trisomy 21
(+Ph)	(+21)
Isochromosome 17q	Trisomy17
(i(17q))	(+17)
Trisomy 19	Loss of chromosome 7 or deletion 7q
(+19)	(-7/del(7q))

[a] with a frequency of ≥10% of all patients with ACAs[10]

[b] with a frequency of <10 but ≥5% of all patients with ACAs[10]

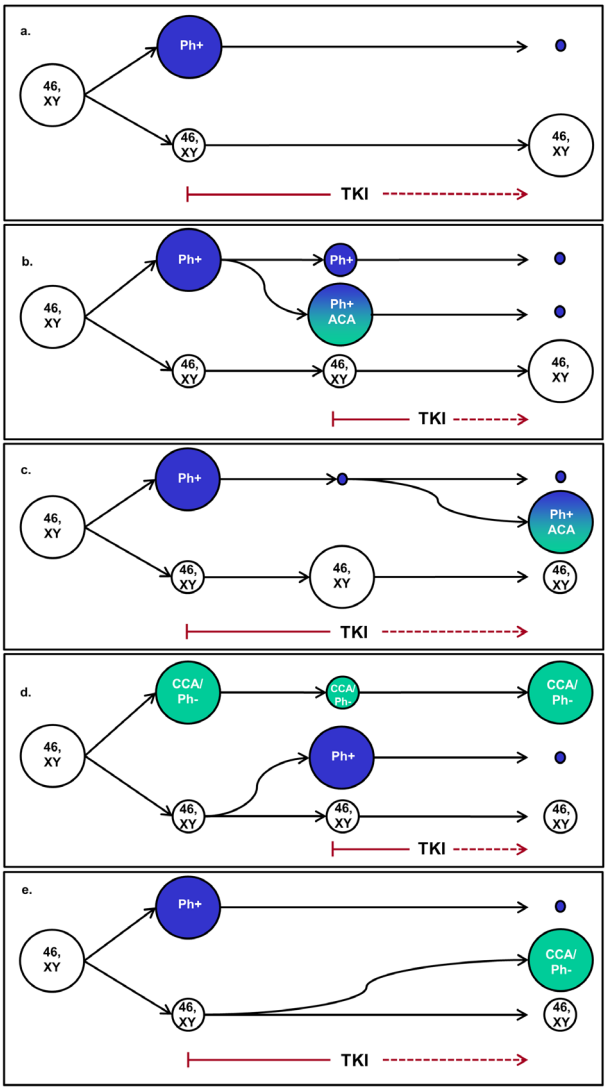
ACAs at diagnosis

In CML patients presenting in AP/BC, the presence of one or more ACAs at diagnosis is relatively common (figure 1a-b). Moreover, the presence of ACAs in patients with CML in chronic phase at diagnosis is unfavorable for the prognosis.[12,13,15] This particularly concerns 'major route' ACAs,[12] as well as complex karyotypes (≥3 ACAs) and 3q26.2 abnormalities.[16] Therefore the presence of these particular abnormalities at diagnosis is now classified as AP according to the in 2016 revised WHO classification of myeloid neoplasmata[16] and should to be treated as such with high dose imatinib or dasatinib, considering an allogeneic stem cell transplantation in eligible patients in case of suboptimal response.[7] Patients with 'minor route' ACAs have a similar response on treatment and survival as patients with no ACAs at diagnosis.[12] Furthermore, no difference in outcome between unbalanced and balanced 'minor route' ACAs has been observed.[14] Data on the prognostic implications of the loss of the Y-chromosome at diagnosis are contradictory.[12,14,17]

De novo ACAs in the Ph$^+$ clone during treatment

The development of ACAs during treatment in the Ph$^+$ clone (CCA/Ph$^+$), also known as clonal evolution (figure 1c), is a sign of progression to AP and it is associated with a negative effect on survival.[18,19] Even patients with acquired ACAs but no other features of progression in the blood count or bone marrow are considered to be in AP.[7,16] This specific group of patients with clonal evolution but morphological preservation of remission, however, has a good prognosis when this occurred during imatinib treatment and when they are switched to a 2GTKI.[20] The ELN recommendations consider major route CCA/Ph$^+$ acquired during treatment as progression to AP, whereas the current WHO classification still recommends considering each type of clonal evolution as progression to accelerated phase[16], but perhaps more stratification is needed. There is evidence that the type and number of ACA could be of prognostic significance. A negative effect on survival was for example observed in the presence of i(17)(q12), -7/del7q and 3q26.2/MECOM (MDS1 and EVI1 Complex Locus) rearrangements or the presence of more than one ACA, while this was not true for simple trisomy 8, -Y or an additional aberrant chromosome 22.[21]

Figure 1. Scenarios of the development and selection of Ph$^+$ clones, Ph$^-$ clones and additional cytogenetic abnormalities.



The dominance and suppression of the clones is represented by the variance in size of the clones.

a. The development and successful treatment of a Ph$^+$ clone in the absence of ACAs, CE and CCA/Ph$^-$
b. ACA at diagnosis, developed as a result of a second hit due to genomic instability
c. The rise of an ACA during treatment (clonal evolution)
d. The presence of a pre-existing Ph$^-$ clone at diagnosis which reveals during TKI treatment due to suppression of the dominant Ph$^+$ clone
e. The rise of a CCA/Ph$^-$ under the influence of TKI inhibition and/or other factors that cause genomic instability

Abbreviations: 46,XY, normal hematopoiesis in male; Ph$^+$, Philadelphia positive clone; ACA, additional chromosomal abnormality; CE, clonal evolution; CCA/Ph$^-$, clonal chromosomal abnormality in Philadelphia chromosome negative clone

Clonal cytogenetic abnormalities (CCAs) in the Ph- clone

Routine cytogenetic response assessments during TKI treatment revealed clonal chromosomal abnormalities in the Philadelphia-chromosome-*negative* (Ph-) cells (CCA/Ph-) in 3-15% of the CML patients; +8, -7 and -Y were observed the most frequently.[22-25] The occurrence of CCA/Ph- could be explained by the preexistent presence of a Ph- clone that becomes detectable under TKI treatment due to suppression of a dominant Ph+ clone (figure 1d). Considering the remarkably high frequency of emerging Ph- clones in patients with CML under TKI therapy, it is speculated that an underlying genomic instability predisposes for multiple abnormalities including the formation of the Ph chromosome.[22] There are also signs that TKI inhibition has a negative effect on DNA repair mechanisms, which could increase the chance of *de novo* CCA/Ph- formation (figure 1e).[22,23]

Although CCA/Ph- are associated with a modest risk of the development of myelodysplastic syndrome and acute myeloid leukemia in the Ph- clone,[22,24] an effect of CCA/Ph- on survival and progression free survival was not found.[24] The development of a secondary hematological malignancy is the most common in patients with -7 or del(7q).[24] In a majority of the cases these CCA/Ph- are however transient. Therefore, clinicians should be aware of the increased risk of second hematological malignancies, but the appearance of a CCA/Ph- does not have direct clinical consequences and should certainly not be perceived as clonal evolution of the Ph+ CML clone.

Variant translocations

In approximately 5% of the newly diagnosed CML patients a *BCR-ABL1* fusion gene arises from a variant on the standard translocation between 9q34 and 22q11, e.g. masked translocations or involvement of one or more chromosomes in addition to chromosome 9 and 22.[12,26] These variant translocations used to be categorized as simple translocations (t(v;22) or t(v;9)), complex translocations (t(v;9;22)) and Philadelphia chromosome negative (Ph-) CML (46,XX or 46,XY).

Two distinct mechanisms for the development of variant translocations have been described. In the one-step mechanism multiple breakpoints are present simultaneously, enabling a three-, four- or five way translocation. The two-step mechanism involves two or more subsequent translocations, resulting in the formation of a t(9;22) translocation in addition to others.[10,27] Although the participation of all 23 chromosomes in variant translocations has been described, there is a clear pattern of breakpoints that are preferably involved. It frequently concerns GC-rich, gene-rich parts of the genome (light bands), which are probably more sensitive to breakage.[10,27,28] Moreover, variant translocations in which *ABL1* is involved are associated with a significantly higher incidence of deletions of *ABL1* on der(9) (40-60%) compared with the incidence of deletions in classical t(9;22) translocations (10-15%).[28,29]

In the pre-imatinib era, a variant *BCR-ABL1* translocation was associated with a worse prognosis, presumably because of the increased incidence of deletions on der(9).[30] In the context of TKI treatment, the presence of a variant *BCR-ABL1* translocation or der(9) deletion does not influence the prognosis of patients anymore.[12,26,31] There may be an exception in the subgroup of complex variant translocations (at least 4 breakpoints), encompassing a fifth of all variant translocations, as recent work showed an inferior overall survival and higher rates of transformation to blast crisis in a contemporary patient cohort.[32]

FISH

An alternative technique for the detection of cytogenetic abnormalities is fluorescence in situ hybridization (FISH). It uses fluorescent DNA probes and detects and localizes the presence or absence of specific DNA sequences on chromosomes in either metaphase and/or interphase cells derived from bone marrow or peripheral blood. The chromosomal position of the *BCR* and *ABL1* genes can be detected with these fluorescent probes. Co-localization of the probes results in a single fusion signal, indicating the genetic juxtapositioning of *BCR* and *ABL*. This method of cytogenetic analysis is especially helpful when CML is suspected and the marrow cells are not assessable with CBA or do not reveal a Philadelphia chromosome despite high clinical suspicion. FISH can also be used for response monitoring, by evaluating the presence of the *BCR-ABL1* fusion gene in at least 200 cells derived from either bone marrow or peripheral blood. It is more sensitive that CBA, but not capable of recognizing ACAs and much less sensitive than molecular assessments, which will be discussed in the second part of this review.[33]

Philadelphia chromosome negative CML

With the development of the FISH technique it became clear that simple translocations and Ph$^-$ CML were frequently the result of complex translocations with masked involvement of chromosome 9 and/or 22.[27] In case of a masked Ph chromosome, an insertion of a small part of the *BCR* gene in the *ABL1* gene on chromosome 9 often occurred, or an insertion of the *ABL1* gene in the *BCR* gene on chromosome 22. For this reason, the presence of the *BCR-ABL1* gene fusion product is a requisite for the diagnosis of CML, independent of the presence of a Philadelphia chromosome detectable by karyotyping.[34] Atypical CML should be considered in case of a morphological suspected CML without *BCR-ABL1* involvement based on not only negative karyotyping but also a negative FISH and/or *BCR-ABL1* PCR.

Since cytogenetic monitoring via karyotyping will not be informative in Ph$^-$ CML patients, the presence of a masked translocation will impact response monitoring. This can be performed only using FISH analysis or PCR for *BCR-ABL1*. Although the studied case series are small, there are no indications that patients with a masked Ph chromosome respond differently to TKI therapy.[35-37]

Molecular assessment in CML

Reverse transcription quantitative polymerase chain reaction

A third way to confirm the CML diagnosis is by demonstrating the BCR-ABL1 mRNA transcript with reverse transcriptase polymerase chain reaction (RT-PCR). Fusion transcript specific primers are used to convert the RNA into complementary DNA (cDNA) using reverse transcriptase. The cDNA is then amplified using 'regular' PCR and the presence of the BCR-ABL1 mRNA transcript is evaluated with gel electrophoresis. This technique is qualitative in determining the presence and size of the BCR-ABL1 mRNA transcript, but is not quantitative and is therefore not suitable for disease monitoring.

Real-time quantitative PCR (RT-qPCR) uses fluorescent DNA probes to quantify the number of cDNA copies that develop during the PCR reaction. The number of PCR cycles necessary to detect a signal above the threshold is called the cycle threshold (CT) and is proportional to the amount of target present at the beginning of the reaction. A standard curve of CT values can be established by using standards with a range of known concentrations of copies of the gene of interest. This standard curve can be used to extrapolate the absolute quantity in an unknown sample. The same sample volume and amount of cDNA as used for the BCR-ABL1 gene amplification, should always be tested against an internal control gene (e.g. *BCR, ABL1, GUSB*), to correct for the quality and quantity of the sample. RT-qPCR can be performed both on bone marrow and peripheral blood with very similar and thus comparable results.[38] Peripheral blood is used most frequently in common routine practice.

There is substantial interlaboratory variability in the performance and results of RT-qPCR, caused by the use of different sample types, primers, assays etc.[39] To make interlaboratory results comparable, a standardized international scale (IS) was introduced. This is a standardized outcome measure which assigned a value of 100% to the median BCR-ABL1/BCR expression level of baseline samples from 30 CML patients included in the IRIS trial[4] and a 3-log reduction from this standardized baseline was defined as a major molecular response (MMR, 0.1%^{IS}).[40] Reference standards can be used to calculate an IS conversion factor for each molecular assay in each molecular diagnostic laboratory. Alternatively, commercial kits have become available that have standardized their assay to IS and incorporate an conversion factor in their analysis software.[41]

One of the advantages of RT-qPCR is the much lower levels of residual disease than can be detected and quantified, compared to CBA and FISH. CCyR is roughly equivalent to a BCR-ABL1 level of 1%^{IS}.[42] A major molecular response (MMR) is defined as 0.1%^{IS}, whereas MR$^{4.0}$ and MR$^{4.5}$ represent a residual disease of 0.01%^{IS} and 0.0032%^{IS} respectively. Long

term treatment with tyrosine kinase inhibitors leads to the achievement of deep molecular responses in the majority of patients.[43] Since approximately half of the patients in stable deep molecular response can stop their TKI treatment and remain in 'treatment free remission',[44] measurement of these deep molecular responses is becoming increasingly important.

Breakpoint variations

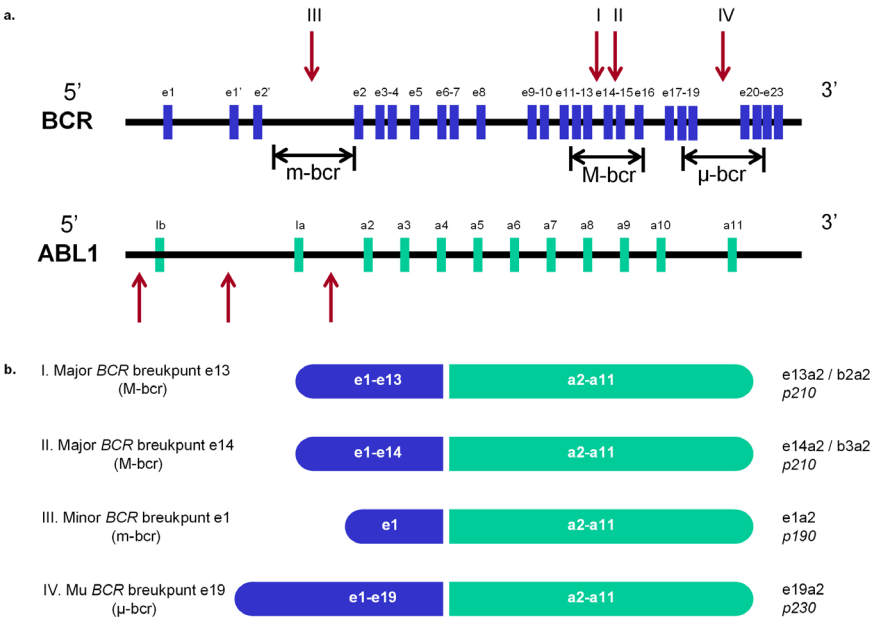
As a result of the diversity in the location of the chromosomal breakpoints that are involved in the Philadelphia translocation, the molecular weight of the BCR-ABL1 fusion protein is variable. The *ABL1*-gene on chromosome 9q34 consists of 11 exons, but the breakpoint on chromosome 9 is usually established upstream of exon 2 in the region where the alternative exons 1a and 1b are also located (figure 2a). The molecular weight of the final BCR-ABL1 fusion-protein is however determined by the location of the *BCR*-breakpoint on chromosome 22q11.[45] The majority (>95%) of CML patients and a third of patients with a BCR-ABL1^{+} B-cell acute lymphoblastic leukemia (Ph^{+} B-ALL) have a so called 'major' breakpoint (M-bcr) in the intron between *BCR* exon 13 (e13, formally called b2) and exon 14 (e14, formally called b3) or between exon 14 and 15, resulting in the e13a2 and e14a2 fusion gene respectively, coding for a BCR-ABL1 protein with a molecular weight of 210 kDa (p210$^{BCR\text{-}ABL1}$) (figure 2a-b). In one out of six patients both transcripts (e13a2 and e14a2) are expressed. The so-called 'minor' breakpoint (m-bcr) is involved in 1-2% of CML patients, but in two thirds of the patients with a Ph^{+} B-ALL and comprises a fusion between *BCR* exon1 and *ABL1* exon 2 (e1a2) with a p190$^{BCR\text{-}ABL1}$ protein (190 kDa) as the end product (figure 2a-b).[45,46] Alternative *BCR-ABL1* breakpoints such as e19a2 (resulting in a 230 kDa or p230$^{BCR\text{-}ABL1}$ fusion protein), e13a3 and e14a3 are rare.[45]

The molecular techniques for the determination of the *BCR-ABL1* transcript levels during therapy are often specifically adjusted for the measurement of these patient-specific breakpoints. Therefore the breakpoint has to be established for every patient at diagnosis using PCR with breakpoint-specific primers, gel electrophoresis and/or next generation sequencing. Commercial kits can only be used for quantification of the p210 *BCR-ABL1* transcript.[41] With the aforementioned conversion factor the *BCR-ABL1* levels between the different laboratories becomes comparable on the international scale (IS). This is however only standardized for the e13a2 and e14a2 transcripts. For the e1a2 breakpoint and other rare transcript types, quantification on the international scale IS is not possible, but it is possible to reliably quantify the e1a2 *BCR-ABL1* levels in relation to a laboratory-specific standard of the patient at diagnosis.

The *BCR-ABL1* breakpoint type is of prognostic value for the response to therapy. Although the difference is limited, several studies have found CML patients with the *BCR-ABL1*

e13a2 transcript type to achieve lower rates of optimal molecular response and increased chance of progression on imatinib and second generation TKI treatment, compared to patients with the *BCR-ABL1* e14a2 type.[47,48] A difference in long-term survival was not observed.[49] Recently, it has been noted by two expert groups in CML molecular diagnostics, that some of the observed differences may in fact represent a technical and not biological variation: commonly used protocols for *BCR-ABL1* quantification give slightly but consistently higher values on the international scale for the e14a2 transcript than the e13a2 transcript for equal sample concentrations.[50,51]

Figure 2. Schematic overview of breakpoint variations.



a. Variant breakpoints in the major-breakpoint region (I,II), minor-breakpoint region (III) and µ-breakpoint region (IV) of the *BCR*-gene and three variant *ABL1* breakpoints in the exon Ib-Ia region (after posttranscriptional splicing of exon 1, this will generally result in a fusion of the *BCR* breakpoint with exon a2).[45]

b. Variant *BCR-ABL1* mRNA transcripts, fusion genes and protein products. [45]

The rare *BCR-ABL1* e1a2 transcript type has shown to be associated with a significant worse response to TKI treatment in both *in vitro*[3] and *in vivo*[46,52] evaluations; 29-56% of the CP-CML patients progress to acceleration phase (AP) and blast crisis (BC) within 48 months. CML patients with a *BCR-ABL1* e1a2 transcript type should therefore be considered as high risk patients, in which more frequent monitoring is indicated and an

allogeneic stem cell transplantation should be considered in an early stage. There is no evidence available on whether patients with e1a2 transcript type would benefit from treatment with first line 2GTKIs over first line imatinib, but considering the bad prognosis linked to this transcript type we think that an initial treatment strategy with a more potent 2GTKI is justifiable.

Digital PCR

As discussed above, the accurate measurement of deep molecular responses is becoming increasingly important, since treatment free remission is becoming a novel treatment goal, but RT-qPCR has limitations in its accuracy at low levels of disease ($MR^{4.0}$-$MR^{5.0}$). Therefore more sensitive and accurate assays such as digital PCR (dPCR) might be interesting alternatives for measuring deep molecular responses to select patients eligible for attempting to stop their TKI and achieve treatment-free remission. Digital PCR dilutes the DNA into hundreds/thousands/millions of reaction chambers/ droplets, depending on the platform used and a PCR reaction is carried out in each partition individually. The presence of PCR product of interest is analyzed per partition using fluorescent probes.[53] A sample is partitioned prior to PCR amplification such that each reaction chamber contains 0 or ≥1 copies of target DNA or mRNA. Poisson statistics can be used to correct the results to account for partitions that contain more than one molecule, and an absolute count of the target sequence can be estimated. [54,55] The sample partitioning enables detection of extremely rare copy numbers that would normally be undetectable by conventional RT-qPCR. In two study patients with undetectable *BCR-ABL1* levels using conventional RT-PCR (n=13 and n=18), 94-100% were *BCR-ABL1*-positive using dPCR.[56,57] In both the Korean Imatinib Discontinuation (KID) study and Imatinib Suspension and Validation (ISAV) study, patients who were dPCR-*BCR-ABL1*-negative had a higher chance of remaining in treatment free remission after TKI discontinuation than patients with detectable *BCR-ABL1* by dPCR.[58,59] Also, the increased accuracy in quantifying BCR-ABL copy numbers may be useful in determining if a patient is achieving molecular treatment goals which are set at absolute numbers, e.g. ≤10% *BCR-ABL1* at three months TKI treatment, particularly if the concentration in the sample is close to the cut-off value. The additional value of dPCR in clinical CML practice is subject of current research.

Sanger sequencing and next generation sequencing

Sanger sequencing is a method used to read the base pairs of a specific piece of DNA and detect if there are any mutations present. It uses heat to denaturate the DNA and primers to select the genetic area of interest. Complementary deoxynucleosidetriphosepates (dATP, dGTP, dCTP and dTTP) and chain-terminating dideoxynucleotides (ddATP, ddGTP, ddCTP and ddTTP) are used to complement the single stranded DNA until the DNA polymerase meets a ddNTP. This results in DNA fragments of different lengths. Gel elec-

trophoresis is used to sequence the DNA fragments and it results in the complementary sequence of the DNA sample. In CML this technique can be used to detect mutations in the *BCR-ABL1* kinase domain (KD-mutations), but also to detect mutations outside the kinase domain.

Next generation or high-throughput sequencing platforms are capable of analyzing millions of sequencing reactions at the same time. In CML it can be used for ultra-deep sequencing, using the high throughput to focus on sequencing the KD-domain. It can identify even a few mutated amplicons and therefore has a much higher sensitivity than Sanger Sequencing to identify low level mutations.[60]

Kinase domain mutations

Mutations in the *BCR-ABL1* kinase domain (KD) are a common cause of TKI resistance. They lead to a less effective binding of TKIs in the kinase domain resulting in a decline of the inhibitory effect of the TKI. More than hundred different KD-mutations, coding for more than 50 different amino acid substitutes, have already been identified.[61] They can be classified into four groups, based on the location of the mutation in the structure of *ABL1*: I. mutations in the phosphate binding loop (P-loop), (residue 248-255). II. Mutations in the 'drug contact site' (T315/F317) which prevent the binding of the TKI with the catalytic domain of the oncogene protein, III. Mutations in the catalytic domain (residue 350-363) and IV. mutations in the activation loop (A-loop) (residue 381-402).[61]

With the fairly insensitive routine technique of Sanger sequencing, which is currently used for routine KD-mutation analysis, hardly any KD-mutation is detected at diagnosis.[62,63] In a substantial part of the patients with primary resistance for imatinib, in which an optimal response is never achieved, a KD-mutation is detectable at the moment of TKI failure (21-48%).[63] Patients with secondary resistance, in which an initially achieved optimal response is lost, are identified with KD-mutations in 10-68% of cases.[63] The most frequent mutations observed in imatinib-treated patients are: T315I, G250E, M244V, M351T and E255K/V. CML patients treated with nilotinib or dasatinib in second line develop new *BCR-ABL1* KD-mutations in 14-33% of cases. The mutation pattern is different from that observed during imatinib therapy; in the course of dasatinib treatment T315I, F317L and V299L are the most frequent described; during nilotinib therapy E255K/V, T315I, F359C/V, G250E and Y253H are the most common KD mutations.[63]

The ability to identify KD-mutations is dependent of the sensitivity of the technique used for the mutation analysis. Mutation detection with Sanger sequencing has a relatively low sensitivity. Mutations with a low frequency, present in ≤20% of the Ph^+ cells, will often not be detected with this technique.[62] The Sanger sequencing technique is nevertheless suf-

ficient in common clinical practice, since the clinical importance of low level mutations is not yet clear. The additional clinical value of techniques with higher sensitivity, such as mass spectrometry and next-generation sequencing (NGS) are currently evaluated. In more than half of the patients who fail on second line TKI treatment, NGS is able to detect a low-level mutation in the sample at diagnosis, while this mutation was not identified with Sanger sequencing.[64]

It is important that all patients with treatment failure undergo mutational analysis. IC50 values can be helpful in determining which TKI will be effective in the presence of a particular KD-mutation. According to the 'provisional' response-to-TKI criteria of the 2016 WHO revision, the occurrence of 2 or more KD-mutations during TKI therapy should be considered as progression to AP.[16]

Additional mutations

Genetic instability caused by the *BCR-ABL1* expression can also provoke genetic mutations outside the *BCR-ABL1* fusion gene. These mutations are frequently observed in CML patients with advanced disease stages and typically involve tumor suppressor- and oncogenes which are also prevalent in other (hematologic) malignancies. In patients with a myeloid blast crisis, mutations in *TP53*, *RB1* and *RUNX1* are often observed, while *CDKN2A* and *IKZF1* mutations are frequently demonstrated in patients with a lymphoid blast crisis.[15,65] Also *EVI1/MECOM* overexpression and mutations in *GATA2*, *ASXL1* and *TET2* are previously described in advanced stage CML.[65]

Abovementioned mutations could be potential novel targets for the treatment of CML in advanced phase and TKI-resistant CML. Moreover, just like KD-mutations they can be considered as surrogate markers for genetic instability. For now, it is however unclear whether the detection of these mutations should have clinical consequences.

Conclusions

Several cytogenetic and molecular variations and abnormalities can influence the clinical course, monitoring and response to treatment of individual patients. It is therefore important for the physician to have knowledge of these variations, abnormalities and their consequences. Understanding the pitfalls of the underlying laboratory techniques helps prevent misinterpretations of results. Molecular monitoring is becoming the preferred monitoring method in CML, due to superior accuracy, less need for invasive bone marrow sampling and the ability to measure deep molecular responses. For the recognition of additional cytogenetic abnormalities and variant translocations, cytogenetic assessments are still relevant. Additional genetic abnormalities may be signs of genomic instability that can be associated with progression to accelerated and blast phase CML. The assessment of mutations in case of TKI resistance is essential for good clinical practice to rationally select a next-line TKI. New techniques, such as digital PCR and next generation sequencing, are entering clinical practice and show promise to further improve molecular diagnostic guidance in CML treatment.

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CHAPTER 3

Treatment outcome in a population-based 'real-world' cohort of chronic myeloid leukemia patients

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Abstract

'Real-world' evaluations of the efficacy and safety of tyrosine kinase inhibitors in patients with chronic myeloid leukemia are scarce. A nationwide population-based chronic myeloid leukemia registry was analyzed to evaluate (deep) response rates to first and subsequent treatment lines and eligibility for a treatment cessation attempt in adults diagnosed between January 2008 and April 2013 in the Netherlands. The registries covered 457 patients; 434 in chronic (95%) and 15 patients (3%) in advanced disease phase. 75% of the patients in chronic phase were treated with imatinib and 25% with a second generation tyrosine kinase inhibitor. At 3 years 44% of patients had discontinued their first line treatment, mainly due to intolerance (21%) or treatment failure (19%). At 18 months 73% of patients had achieved a complete cytogenetic response and 63% a major molecular response. Deep molecular responses ($MR^{4.0}$ and $MR^{4.5}$) were achieved in 69% and 56% of patients at 48 months. All response milestones were achieved faster in patients treated upfront with a second generation tyrosine kinase inhibitor, but ultimately also patients initially treated with imatinib reached similar levels of responses. The 6-year cumulative incidence of stop attempt eligibility according to EURO-SKI criteria was 31%. Our findings show that in a 'real-world' setting the long-term outcome of patients treated with tyrosine kinase inhibitors is excellent and the conditions for an attempt to stop tyrosine kinase inhibitor therapy are met by a third of the patients.

Introduction

Multiple randomized controlled trials (RCTs) have provided solid evidence for the efficacy and safety of tyrosine kinase inhibitors (TKI) as treatment of chronic myeloid leukemia (CML), but analyses from observational studies, gathered in patients who did not participate in clinical trials ('the real world') are scarce. Clinical trials use tight inclusion criteria, strict rules for monitoring and treatment algorithms and may therefore not fully reflect results in the general treatment population.[1-3] Moreover, RCTs mainly focus on the outcome of the core study treatment, while a proportion of patients will not be able to continue their initial study treatment and is subsequently switched to an alternative treatment outside the trial.[4,5]

To study treatment choices and patient outcome across different treatment lines, 'real-world' data contain important information for clinical practice. Incidence and survival were the main focus of the currently published reports of nationwide population-based registries. The large European population-based EUTOS registry was the first to provide insight into real-world first and second line treatment patterns in relation to cytogenetic and molecular response.[6] However, this report did not cover deep molecular responses and the proportion of patients meeting the criteria to attempt to stop TKI treatment. Discontinuing TKI therapy is a novel opportunity in CML for patients with a durable deep molecular remission, of whom approximately half may successfully stop their TKI treatment while retaining a "Treatment Free Remission" (TFR).[7,8]

In the current article, we report findings from a nationwide population-based CML registry in the Netherlands capturing data from newly diagnosed CML patients in the majority of hospitals in our country. Detailed information was gathered on patient and treatment characteristics, both at baseline and during follow-up. Importantly, all TKIs are available in the Netherlands and the Dutch health care system includes mandatory health care insurance which covers all CML care making it accessible to all patients. The aim of the current study was to provide a detailed overview of all aspects of CML care including responses to first and subsequent treatment lines with a specific focus on the impact of first line treatment with imatinib compared to second generation TKIs (2GTKIs) dasatinib and nilotinib. Also, we sought to evaluate what proportion of patients become eligible to attempt to stop their TKI treatment.

Methods

Data sources

Data from two complementary Dutch population-based registries on CML patients (PHAROS CML and Hemobase) were combined to cover the nationwide population of adult (≥18 years) CML patients diagnosed between January 2008 and April 2013 in all twelve Dutch provinces. PHAROS CML is an extension of the Dutch Cancer Registry and consists of real-world data collected by trained data managers from medical records of patients newly diagnosed with CML between January 2008 and April 2013, covering the Dutch population in 11 out of 12 provinces.[9] Approval for this comprehensive data collection was obtained by the individual hospital boards. The PHAROS CML registry is a joint initiative of the Dutch-Belgian Hemato-Oncology Group (HOVON), the institute of Medical Technology Assessment at the Erasmus University Rotterdam and the Netherlands Comprehensive Cancer Organisation. HemoBase is a multidisciplinary Web-based electronic patient record in the North-eastern part of the Netherlands covering the one province that was not included in the PHAROS CML registry, which is the province Friesland. The data in Hemobase were registered by physicians and laboratory employees[10] and extracted from HemoBase to be combined with the PHAROS CML registry by the first author (IG) who verified each record to ensure comparability. Together, data on all new CML patients in 75 of approximately 90 hospitals in the Netherlands were available. Additional molecular data were retrieved from all 15 Dutch molecular laboratories performing *BCR-ABL1* diagnostic testing. Data on vital status and causes of death were obtained from the Netherlands Cancer Registry with a follow-up until the 1st of February 2016. The Medical Ethics Committee of the Erasmus Medical Center in Rotterdam authorized this study and the exemption from informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Definitions and end points

Disease phase according to the ELN criteria[11], Sokal risk score[12], EUTOS long term survival (ELTS) score[13] and Charlson Comorbidity index[14] were calculated as described in the original publications. Complete cytogenetic response (CCyR) was defined as the absence of Philadelphia chromosome-positive metaphases examined by chromosome banding. *BCR-ABL1* levels of ≤ 0.1%, ≤ 0.01% and 0.0032% on the international scale or ≥3 log, ≥4 log and ≥4.5 log reduction of *BCR-ABL1* mRNA transcripts from baseline (in molecular labs not able to report on the international scale at the time) were defined as the molecular response end points major molecular response (MMR), $MR^{4.0}$ and $MR^{4.5}$ respectively. Undetectable *BCR-ABL1* levels were classified as major molecular response when control gene numbers were not available to determine the sensitivity of the test.

In case of a switch in TKI therapy, the clinical chart was reviewed for the underlying reason for the treating physician to change therapy ('treatment failure' or "TKI intolerance'). As a proxy for effective and tolerable first line treatment "effective first line treatment" was reached when patients continued their first line TKI for at least one year after achieving MMR. For the determination for TKI stop eligibility, the inclusion criteria for the EURO-SKI trial (ClinicalTrials.gov Identifier: NCT01596114) were applied. In short, this required TKI treatment for a minimum of three years, $MR^{4.0}$ for at least one year and no history of TKI switch for a less than optimal treatment response. Disease progression was defined according to ELN criteria.[11] Death due to CML was defined as death preceded by disease progression.

Data analysis

Descriptive statistics were used to compare baseline characteristics between treatment groups. Patients treated upfront with nilotinib and dasatinib were clustered in the 2GTKI group for comparison with imatinib treated patients. Overall survival was analyzed using the Kaplan Meier method with log rank test. All other time-dependent analyses were performed using the cumulative incidence competing risks method. Death and progression were always considered a competing risk. Additional competing risks per specific analysis are shown in supplementary table 1. A p-value of less than 0.05 was considered significant. All analyses were performed using SPSS version 24 and R-software[15] version 3.2.4.

Results

Population description

The registries covered 457 patients, newly diagnosed with CML between January 2008 and April 2013 in the Netherlands. The 75 out of 90 Dutch hospitals participating in the registry were variable in size and type (academic vs. non-academic) and therefore an accurate representation of the performance of CML treatment in the Netherlands. At diagnosis, 434 patients were in chronic phase (CP, 95%), 8 patients (2%) in accelerated phase (AP) and 7 patients (1%) in blast crisis (BC). Of 8 patients (2%) disease phase was unknown. For all 457 patients, follow-up information on survival status and death due to CML was available until February 1st 2016. More than 1 year disease specific (treatment and/or response) follow up was available for 413 patients. Disease specific follow up was not available for 5 patients (1%) and of 39 patients (9%) the disease specific follow-up was less than 1 year (26 died, 13 lost to follow up).

First line treatment

382 patients (97%) of patients with detailed treatment information available were treated with first line TKI therapy (imatinib (IM) n=295, 75%; nilotinib (NIL) n=65, 17%; dasatinib (DAS) n=22, 6%; table 1), of which 43% had received initial hydroxyurea prior to or simultaneous with the start of first line TKI treatment. Hydroxyurea was the only reported treatment in 6 patients (2%) and 2 elderly patients with major comorbidities did not receive any treatment at all (0.5%). One patient was pregnant at diagnosis and was therefore treated with first-line interferon. One patient was in chronic phase at diagnosis, but was initially treated with leukapheresis and daunorubicine because of a hyperviscosity syndrome at diagnosis in association with hyperleukocytosis (leukocytes 525 $*10^E9$/l). This patient died one day after diagnosis. Leukapheresis was performed in two other patients prior to imatinib treatment with WBC counts of respectively 421 and 606 $*10^E9$/l).

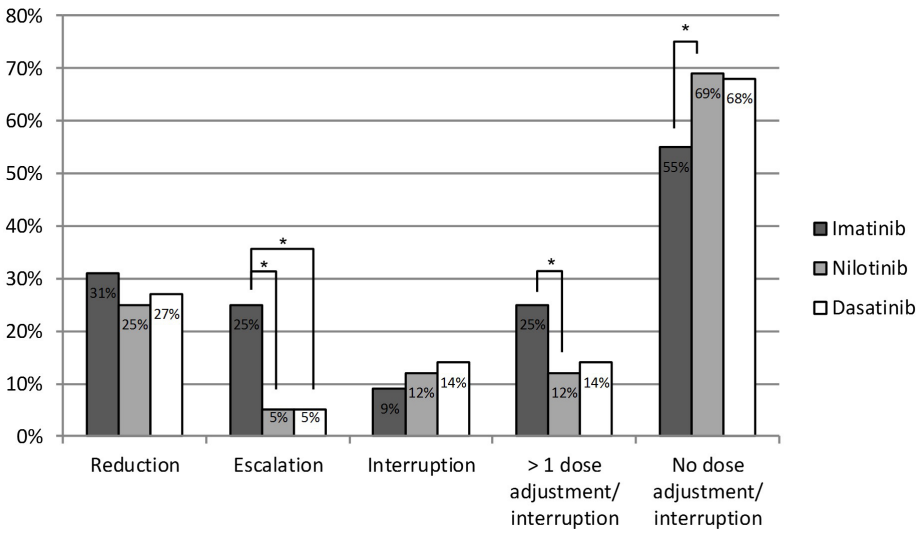
The majority of patients received the first line TKI in standard doses: imatinib 400mg QD (82%), nilotinib 300mg BID (89%) and dasatinib 100mg QD (95%). First line dose adjustments were most frequently observed during imatinib treatment, especially dose escalations and sequential dose adjustments and/or interruptions (figure 1).

Response

Cytogenetic response data were available for 246 out of 434 CP-CML patients (57%) and molecular response data for 326 out of 434 CP-CML patients (75%). Patients with no cytogenetic and/or no molecular response data available were significantly older, had a higher comorbidity index, were less frequently included in first line clinical trials and more frequently treated in non-academic hospitals. The median time to first CCyR was

10 months; the cumulative incidence of CCyR after 12 months was 55% (95% CI: 49-62%) and 73% (95% CI: 67-79%) after 18 months (figure 2). The median time to first MMR was 13 months. The cumulative incidence of MMR at 12 months was 47% (95% CI: 42-52%) and 63% (95% CI: 57-68%) at 18 months (figure 2). Deeper molecular responses were achieved after a median treatment duration of 30.5 months ($MR^{4.0}$) and 43 months ($MR^{4.5}$) with a cumulative incidence rate of 41% (95% CI: 35-46%) and 69% (95% CI: 63-74%) for $MR^{4.0}$ after 24 months and 48 months, respectively, and a cumulative incidence rate of 30% (95% CI: 25-35%) and 56% (95% CI: 50-62%) for $MR^{4.5}$ after 24 months and 48 months, respectively (figure 2).

Figure 1. First line TKI dose adjustments.



* p<0.05

Both cytogenetic and molecular response milestones were achieved faster in patients treated upfront with a 2GTKI (figure 3, supplementary figures S1-S3). On initial treatment cumulative incidence rates of the achievement of all response milestones was significantly lower in patients treated with first line imatinib (figure 3A, supplementary figures S1A, S2A and S3A), but eventually, after switching TKI treatment the same response rates were achieved (figure 3B, supplementary figures S1B, S2B and S3B).

TKI switch/discontinuation and time to effective treatment

Within the first 3 years after diagnosis, 44% (95%CI, 38-50%) of the 382 patients on first line TKI treatment discontinued their first line treatment; 21% (95%CI, 17-26%) due to

TKI intolerance, 19% (95%CI, 15-24%) due to treatment failure and 3% (95%CI, 1-5%) due to other or unknown reasons (figure 4A). The most frequently observed other reasons for first line discontinuation were trial inclusion within 4 months after start treatment (n=5), stop trial inclusion (n=3) and pregnancy (n=3).

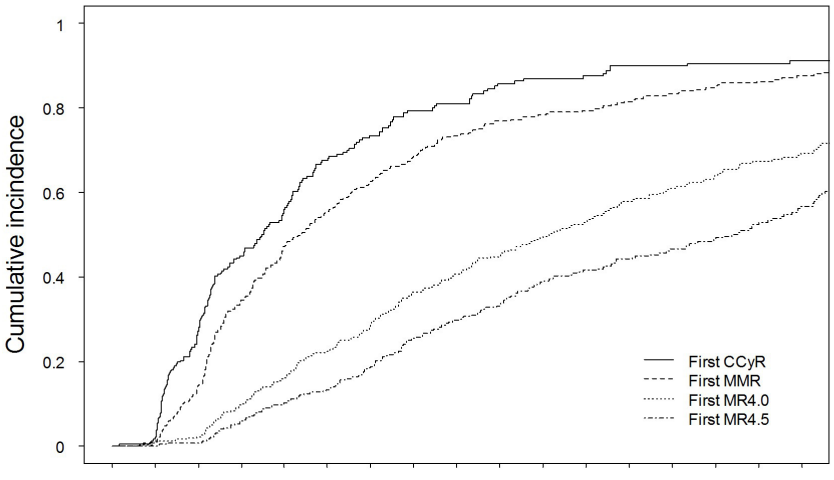
Table 1. Patient characteristics at diagnosis for the sub-analysis of 382 TKI-treated CP-CML patients

First line treatment	*Imatinib, n=295*	*2GTKI, n=87**
Male gender, n (%)	168 (57)	51 (59)
Age, years Median (IQR)	58 (42-69)	57 (45-68)
Year of diagnosis, n (%)		
2008	87 (30)	3 (3)
2009	77 (26)	0 (0)
2010	66 (22)	14 (16)
2011	40(14)	32 (37)
2012 – April 2013	25 (9)	38 (44)
Age-adjusted Charlson Comorbidity index, n (%)		
0	98 (33)	27 (31)
1-2	87 (30)	33 (38)
3-4	65 (22)	18 (21)
≥5	45 (15)	9 (10)
Sokal risk group, n (%)		
Low	66 (26)	14 (18)
Intermediate	112 (44)	36 (47)
High	77 (30)	26 (34)
Unknown	40	11
ELTS risk group, n (%)		
Low	124 (49)	33 (43)
Intermediate	88 (35)	30 (40)
High	43 (17)	13 (17)
Unknown	40	11
Treating hospital, n (%)		
Non-academic	226 (77)	54 (62)
Academic	69 (23)	33 (38) #
Unknown	0	0
Inclusion in 1st line clinical trial, n (%)		
No	247 (86)	66 (76)
Yes	40 (14)	21 (24) #
Unknown	8	0

*Nilotinib, n=65; Dasatinib, n=22; # p<0.05 compared to imatinib-group

Abbreviations: 2GTKI, second generation tyrosine kinase inhibitor; IQR, interquartile range; ELTS, EUTOS long term survival

Figure 2. Achievement of cytogenetic and molecular response milestones.



No. at risk									
CCyR	246	169	96	52	27	14	7	6	5
MMR	326	279	165	110	66	46	34	22	13
MR4.0	326	317	262	213	162	125	92	61	44
MR4.5	326	322	281	245	194	154	126	95	68

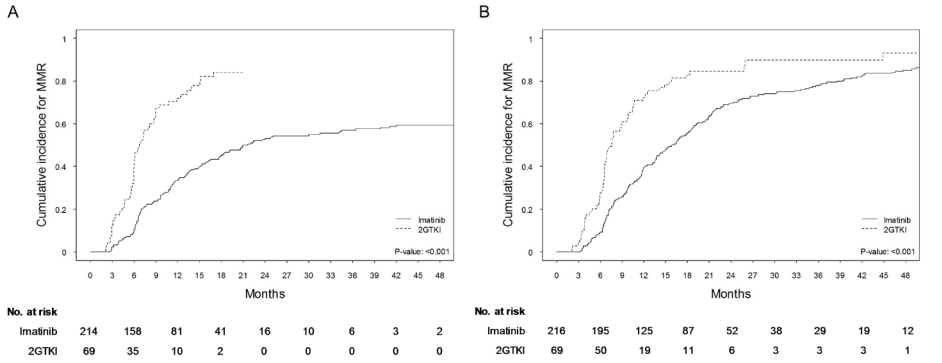
Cumulative incidence of first and unconfirmed achievement of CCyR, MMR, MR4.0 and MR4.5

Abbreviations: CCyR, complete cytogenetic response; MMR, major molecular response; MR4.0, 0.01% on international scale; MR4.5, 0.0032% on international scale.

The 3 year cumulative incidence of TKI discontinuation was not different between first line imatinib and 2GTKI treated patients (46% vs 38%, p=0.104, figure 4B). TKI discontinuation due to TKI failure was significantly more common in patients treated with first line imatinib (21% vs 13%, p=0.046, figure 4C). TKI discontinuation due to TKI intolerance was not different based on first line TKI type (21% vs 24%, p=0.854, figure 4D). In total, up to five subsequent treatment switches were observed (supplementary figure S4). An overview of the reported types of intolerance on all treatment lines and the actions that followed as a result of the intolerance can be found in the supplemental results section and supplementary table 2.

Median time to sustained effective and tolerable first line treatment was 33 months and the cumulative incidence was 56% (95% CI, 49-64%) after 4 years of treatment: 51% (95% CI, 43-59%) with first line imatinib treatment and 77% (95% CI, 57-97%) with first line 2GTKI treatment (p<0.001).

Figure 3. MMR achievement on frontline imatinib and 2GTKIs.



A. Cumulative incidence of MMR on initial treatment

B. Cumulative incidence of overall MMR rate (including switch)

Abbreviations: MMR, major molecular response, 2GTKIs, second generation tyrosine kinase inhibitors.

Progression during TKI therapy

The median observation period for progression of TKI treated CP-CML patients was 27 months (range 0-82 months). A total of 17 patients progressed within 3 years; a cumulative incidence of 3% after 1 year and 6% after 3 years. Progression was observed in 16 patients treated with first line imatinib whereas in only one patient treated upfront with a 2GTKI; cumulative incidence rates for progression after 3 years were 7% vs. 1% (p=0.193) in patients treated with frontline imatinib and frontline 2GTKI, respectively.

Survival

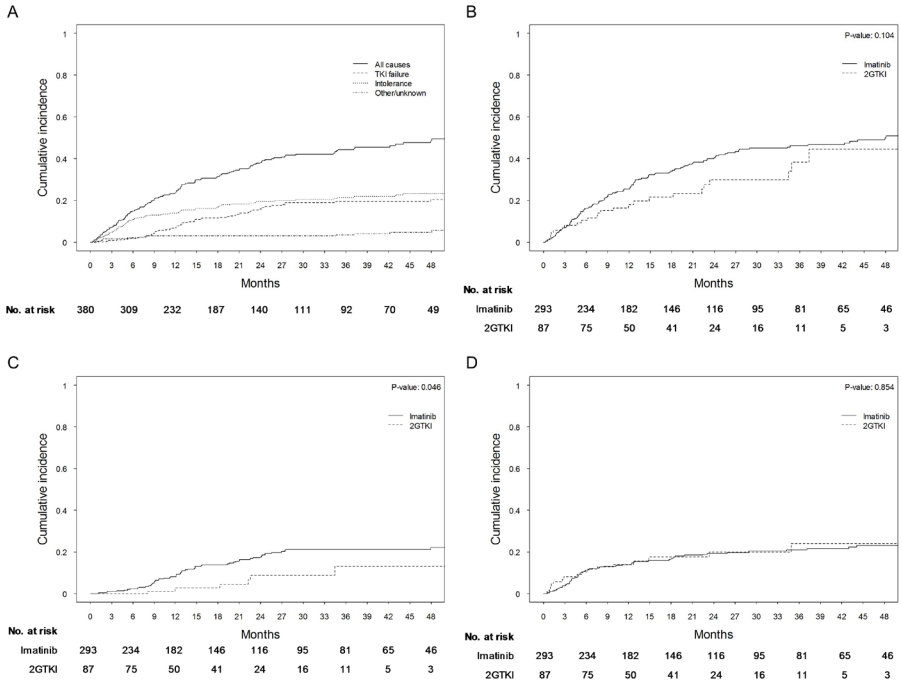
The median observation time for survival of patients in CP at diagnosis was 5 years and 7 months (range 34-97 months). Eighty-two patients died during follow-up (19%). Overall survival after 1, 2 and 4 years was 96% (95% CI 94-98%), 92% (95% CI 90-95%) and 85% (95% CI 81-88%), respectively. No significant differences in overall survival were observed when overall survival was stratified by type of first line TKI treatment (p=0.244). The cumulative incidence of death due to CML was 1% after 1 year, 2% after 2 years and 3% after 4 years. Again, death due to CML was not significantly different between patients treated with first line imatinib and 2GTKIs (p=0.208).

TKI stop eligibility

Eligibility for a TKI stop attempt according to the EURO-SKI criteria was evaluated for all 382 patients who started first line TKI treatment. A total of 43 patients (11%) met the eligibility criteria. During follow up 131 patients (34%) experienced a competing risk making them ineligible for a (EURO-SKI) stop attempt: first line TKI failure (43%), less than 3 years

on first and second line TKI (29%), death (20%) and progression (8%). Censoring occurred for 208 patients (55%): lost to disease specific follow up within 3 years (80%), not (yet) achieved 3x $MR^{4.0}$ in 1 year (19%), emigration (1%) and unknown reason for TKI switch (0.5%). The cumulative incidences of stop attempt eligibility after 4 and 6 years were 24% (95% CI, 17-30%) and 31% (95% CI, 23-38) respectively (figure 5A). The cumulative incidence of TKI stop eligibility after first line 2GTKI treatment was numerically higher than after imatinib treatment, but this did not reach statistical significance (p=0.147) (figure 5B).

Figure 4. First line TKI discontinuation.



A. Cumulative incidence of all causes of TKI discontinuation

B. Cumulative incidence of TKI discontinuation, a comparison of imatinib with 2GTKIs

C. Cumulative incidence of TKI discontinuation due to TKI failure

D. Cumulative incidence of TKI discontinuation due to TKI intolerance

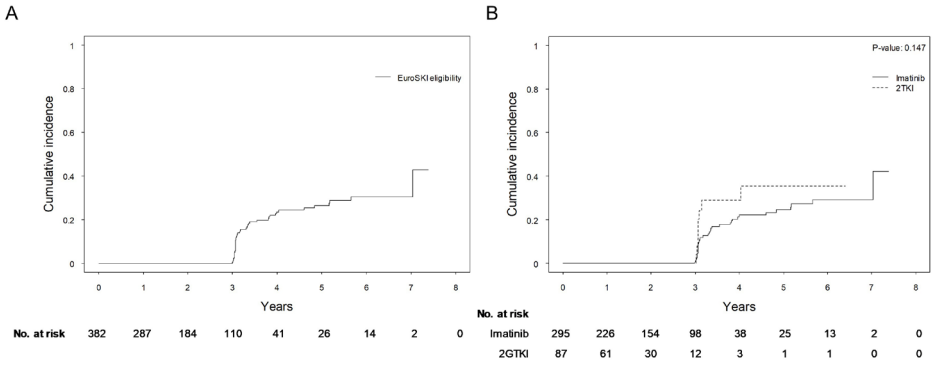
Abbreviations: 2GTKI, second generation tyrosine kinase inhibitor

Treatment and outcome of patients presenting in advanced phase CML

The 15 patients who presented in advanced disease phase at diagnosis (8 AP and 7 BC) were mainly treated upfront with a TKI (6 AP and 4 BC). One BC-CML patient was started on chemo + TKI treatment and of two patients, treatment was unknown. Allogeneic stem

cell transplantation was performed in 2 AP-CML and 3 BC-CML patients. Median survival after diagnosis was 1.4 years in CML-AP patients and not reached in CML-BC patients. 5-year overall survival was 38% and 57% respectively.

Figure 5. TKI stop attempt eligibility according to EURO-SKI criteria.



A. Cumulative incidence of overall eligibility of first line TKI treated CP-CML patients

B. Comparison of cumulative incidences of upfront treated imatinib and 2GTKI patients

Abbreviations: 2GTKI, second generation tyrosine kinase inhibitor

Discussion

Our nationwide population-based study confirms the excellent results of TKI treatment in CML patients observed in RCTs outside clinical trials and adds insights in the patterns of TKI switching and patient outcome. Moreover, this study presents the first real world data on the proportion of patients becoming eligible for an attempt to achieve TFR.

The baseline characteristics of the Dutch CML population are comparable to other population-based CML registries.[6,16,17] In contrast, patients included in the ENESTnd and DASISION RCTs[18,19] were 10 years younger than in our real world cohort. Since nilotinib and dasatinib were both registered for first line treatment in December 2010, the majority of patients treated with 2GTKI was diagnosed in the second half of the inclusion period and therefore experienced a shorter follow-up period. Because of the relatively low number of individual patients treated upfront with nilotinib and dasatinib, it was decided to combine these two groups of patients for comparisons with the imatinib treated patients.

Our real world observations show that TKI therapy is effective and tolerable in a majority of patients, but requiring frequent dose adjustments and temporary treatment interruptions (45% of patients treated upfront with imatinib and 31-32% of 2GTKI treated patients). Only half (54%) of all imatinib-treated patients with CP-CML were estimated to remain on first line TKI 3 years after treatment initiation in our observational cohort. This TKI persistence rate is notably lower than the rates observed in the ENESTnd and DASISION RCTs in which 62% and 69% of patients were still on their first line imatinib treatment after three years[20,21]. For nilotinib and dasatinib these rates were 71-74% and 71% respectively,[20,21] while we observed a 3 year persistence rate of only 62% in patients treated with 2GTKIs. These differences were mainly due to the higher TKI discontinuation rates due to TKI intolerance observed in our real-world setting. It can be hypothesized that the threshold for TKI switch due to intolerance for both physicians and patients is lower when treated outside a clinical trial. In concordance with our study, Castagnetti *et al.* reported a TKI persistence rate of imatinib treated patients of 59% after a median of 48 months follow-up in a real world setting in Italy, also because of frequent TKI switches due to intolerance.[22]

The observations of earlier and higher achievement of the important response milestones CCyR and MMR in patients treated with first line 2GTKI in our real-world cohort, confirm the reproducibility of the superior efficacy results of first line 2GTKIs found by the two large RCTs.[4,5] Of note, the 3 year MMR achievement rates of our real world data on first line 2GTKIs (84%) are even higher compared to the rates reported by the RCTs (69-73%).

Differences in methodology and study design of our observational study and the RCTs may have attributed to the variation in findings. A limitation to population-based cohort studies is that patient monitoring is not as strict as during the closely supervised clinical follow-up in trials. Also in our registry, patients with more favorable baseline characteristics more often had cytogenetic and molecular response analyses performed. This may in part explain the relatively high remission rates observed by us.

Real world data not only give insight in response on first line treatments, but also provide information on the overall response on subsequent treatment lines, in contrary to data from the currently published clinical trials. The data from our nationwide registry demonstrate for example that 91% of all CP-CML patients treated with a TKI eventually achieved CCyR and 88% MMR after 4 years, whereas the results from RCTs on core treatment were significantly lower at this time point. Therefore these observational data of overall response reflect relevant patient outcomes much better than the clinical trial results do. Our overall response rates are comparable to the overall response rates observed by other population-based registries.[16,22,23] The observation that both patients treated upfront with imatinib or a 2GTKI eventually achieved comparable CCyR and MMR rates, whether or not preceded by one or more treatment switches, has also been recognized before.[23] An analysis of long term molecular response on first line treatment even showed that patients treated with imatinib 400 mg QD only can reach MMR rates nearly similar to those treated with imatinib 800 mg QD, nilotinib and dasatinib after 5 years.[24]

To our knowledge, this is the first observational study, comparing deep molecular responses achieved on imatinib with deep molecular responses achieved on 2GTKIs. Of note, comparative analyses were hampered in our study by a relatively low number of patients receiving 2GTKI and shorter follow-up period. Despite this, we were still able to show that $MR^{4.0}$ and $MR^{4.5}$ were achieved significantly faster in the real-world when treatment was initiated with a 2GTKI compared to initial treatment with imatinib, independent of subsequent treatment lines. In the RCTs both higher[24] and lower[4,5] cumulative response rates were observed than in our real-life cohort. These deep responses are especially interesting in the light of eligibility for a TKI stop attempt. Together with the duration of TKI treatment and switching history, a durable deep molecular remission is the main selection criterion currently used in TFR trials. In a previous analysis of patients in first line clinical trials, after 8 years of imatinib treatment a cumulative incidence of stable (≥ 24 months) $MR^{4.5}$ of 36.5% was found on an intent-to-treat basis, suggesting that this proportion may be TFR eligible.[25] In our population-based study, the eligibility criteria of the largest TFR trial to date, EUROSKI, were used to evaluate this endpoint and showed a cumulative incidence of 31% after six years. Although a higher eligibility rate was observed by us in patients treated upfront with 2GTKIs, this did not reach statistical

significance with the relatively low number of patients that had started on a 2GTKI and had a follow-up beyond three years.

In conclusion, this population-based analysis showed overall favorable treatment responses compared to the RCTs, which could be attributed to dose adjustments and subsequent treatment lines in the real-world setting. Also, it shows that long-term outcome of patients initially treated with imatinib is excellent when switched to 2GTKI when needed. The cumulative incidence of patients eligible to attempt to stop their TKI to achieve TFR was 31% after six years when the EUROSKI criteria were applied.

Acknowledgements

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Supplementary results

TKI intolerance

Considering all treatment lines for analysis, side effects were reported in 177/305 patients treated with imatinib (58%), 58/139 patients treated with nilotinib (42%) and 61/116 patients treated with dasatinib (53%) in any line. Cytopenia (in particular thrombocytopenia), fatigue and infections were reported frequently (>5%) during all three forms of treatment (supplemental table S2). Gastrointestinal complaints (in particular diarrhea) were far more common in patients treated with imatinib and dasatinib, whereas rash was more often observed during imatinib and nilotinib treatment. Imatinib showed high rates of fluid retention (both superficial and periorbital) and musculoskeletal symptoms, while pleural effusion was observed frequently during dasatinib treatment. Lastly, cardiovascular events were observed at a high rate (5.8%) during nilotinib treatment. Actions following the different adverse events are shown in supplemental table S2.

Supplementary tables

SI table S1. Events, competing risks and censors per cumulative incidence competing risk analysis.

Analysis	Event
Time to CCyR (Overall)	First CCyR
Time to CCyR (On initial treatment)	First CCyR on first line treatment
Time to MMR (Overall)	First MMR
Time to MMR (On initial treatment)	First MMR on first line treatment
Time to $MR^{4.0}$ (Overall)	First $MR4^{.0}$
Time to $MR^{4.0}$ (On initial treatment)	First $MR^{4.0}$ on first line treatment
Time to $MR^{4.5}$ (Overall)	First $MR^{4.5}$
Time to $MR^{4.5}$ (On initial treatment)	First $MR^{4.5}$ on first line treatment
All cause TKI discontinuation	First line TKI discontinuation
TKI discontinuation due to failure	First line TKI discontinuation due to reported failure
TKI discontinuation due to intolerance	First line TKI discontinuation due to reported intolerance
Effective and tolerable first line treatment	First line TKI continuation at least one year after achieving MMR
CML-related death	Death proceeded by CML progression
TKI stop eligibility	Eligibility for a TKI stop attempt according to the EURO-SKI criteria

Abbr: CCyR, complete cytogenetic response; MMR, major molecular response; $MR^{4.0}$, BCR-ABL1 0.01% on international scale; $MR^{4.5}$, BCR-ABL1 0.0032% on international scale; TKI, tyrosine kinase inhibitor; HSCT, hematopoietic stem cell transplantation

	Competing risk	Censor
	- Death - Progression	- End of follow up without event or competing risk
	- Death - Progression - First line TKI discontinuation without CCyR achievement	- End of follow up without event or competing risk
	- Death - Progression	- End of follow up without event or competing risk
	- Death - Progression - First line TKI discontinuation without MMR achievement	- End of follow up without event or competing risk
	- Death - Progression	- End of follow up without event or competing risk
	- Death - Progression - First line TKI discontinuation without MR4.0 achievement	- End of follow up without event or competing risk
	- Death - Progression	- End of follow up without event or competing risk
	- Death - Progression - First line TKI discontinuation without MR4.5 achievement	- End of follow up without event or competing risk
	- Death	- End of follow up without event or competing risk
	- Death - TKI discontinuation due to all other reasons	- End of follow up without event or competing risk
	- Death - TKI discontinuation due to all other reasons	- End of follow up without event or competing risk
	- Death - Progression - HSCT	- End of follow up without event or competing risk
	- Death due to all other causes	- End of follow up without event or competing risk - HSCT
	- Death - Progression - First line TKI failure - Less than 3 years on first and second line TKI	- End of follow up without event or competing risk

SI table S2. Adverse events per TKI and actions taken on the adverse events

	Imatinib		**Nilotinib**		**Dasatinib**	
	n=305		n=139		n=116	
	n	%	n	%	n	%
Hematologic adverse events	**67**	**22,0**	**20**	**14,4**	**23**	**19,8**
Thrombocytopenia*	30	9,8	14	10,1	12	10,3
Leukopenia*	15	4,9	0	0,0	2	1,7
Anemia*	13	4,3	4	2,9	2	1,7
Pancytopenia*	9	3,0	2	1,4	7	6,0
Biochemical adverse events	**9**	**3,0**	**7**	**5,0**	**6**	**5,2**
Elevated serum creatinine	6	2,0	0	0,0	2	1,7
Elevated serum ALAT	2	0,7	2	1,4	1	0,9
Elevated serum bilirubine	0	0,0	2	1,4	0	0,0
Elevated serum GGT	0	0,0	0	0,0	2	1,7
Elevated serum amylase	0	0,0	0	0,0	1	0,9
Elevated serum ASAT	1	0,3	1	0,7	0	0,0
Elevated serum lipase	0	0,0	1	0,7	0	0,0
Electrolyte disorders	0	0,0	1	0,7	0	0,0
Hypoalbuminemia	0	0,0	0	0,0	0	0,0
Nonhematologic						
Gastrointestinal complains	**63**	**20,7**	**5**	**3,6**	**20**	**17,2**
Diarrhea	28	9,2	2	1,4	11	9,5
Nausea	15	4,9	1	0,7	5	4,3
Vomiting	13	4,3	1	0,7	3	2,6
Abdominal discomfort	6	2,0	1	0,7	1	0,9
Pancreatitis	1	0,3	0	0,0	0	0,0
Pulmonary	**8**	**2,6**	**8**	**5,8**	**21**	**18,1**
Pleural effusion	2	0,7	3	2,2	16	13,8
Dyspnea	5	1,6	4	2,9	4	3,4
Pleuritis	1	0,3	0	0,0	1	0,9
Pulmonary hypertension	0	0,0	1	0,7	0	0,0
Fluid retention	**38**	**12,5**	**7**	**5,0**	**4**	**3,4**
Superficial edema	18	5,9	5	3,6	3	2,6
Periorbital edema (+eyelid edema)	16	5,2	2	1,4	1	0,9
Macular edema	4	1,3	0	0,0	0	0,0
Musculoskeletal	**30**	**9,8**	**5**	**3,6**	**5**	**4.3**
Myalgia	11	3,6	2	1,4	3	2,6
Muscle cramps	10	3,3	1	0,7	1	0,9
Musculoskeletal pain	8	2,6	2	1,4	1	0,9
Gout	1	0,3	0	0,0	0	0,0

	No action		Interruption		Reduction		Switch		Stop		Unknown	
	n	%	n	%	n	%	n	%	n	%	n	%
	19	33	9	16	18	32	7	12	1	2	3	5
	9	50	3	17	5	28	1	6	0	0	0	0
	7	37	1	5	7	37	2	11	1	5	1	5
	2	11	2	11	6	33	6	33	2	11	0	0
	6	75	0	0	1	13	1	13	0	0	0	0
	1	20	2	40	2	40	0	0	0	0	0	0
	0	0	1	50	0	00	1	50	0	0	0	0
	1	50	0	0	0	0	0	0	0	0	1	50
	1	100	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	1	50	0	0	0	0	1	50
	1	100	0	0	0	0	0	0	0	0	0	0
	1	100	0	0	0	0	0	0	0	0	0	0
	0	0	0	0	0	0	0	0	0	0	0	0
	6	14	6	14	12	28	18	42	0	0	1	2
	2	10	0	0	10	48	9	43	0	0	0	0
	4	22	1	6	4	22	8	44	0	0	1	6
	1	13	0	0	1	13	6	75	0	0	0	0
	1	100	0	0	0	0	0	0	0	0	0	0
	0	0	3	14	2	10	14	67	2	10	0	0
	3	21	0	0	4	29	5	36	2	14	0	0
	0	0	0	0	0	0	1	50	1	50	0	0
	1	100	0	0	0	0	0	0	0	0	0	0
	3	12	0	0	12	46	10	39	1	4	0	0
	2	10	1	5	4	19	12	57	1	5	1	5
	1	25	0	0	0	0	3	75	0	0	0	0
	1	6	1	6	10	63	3	19	1	6	0	0
	4	29	1	7	6	43	3	21	0	0	0	0
	1	9	0	0	4	36	4	36	2	18	0	0
	1	100	0	0	0	0	0	0	0	0	0	0

SI table S2. Continued

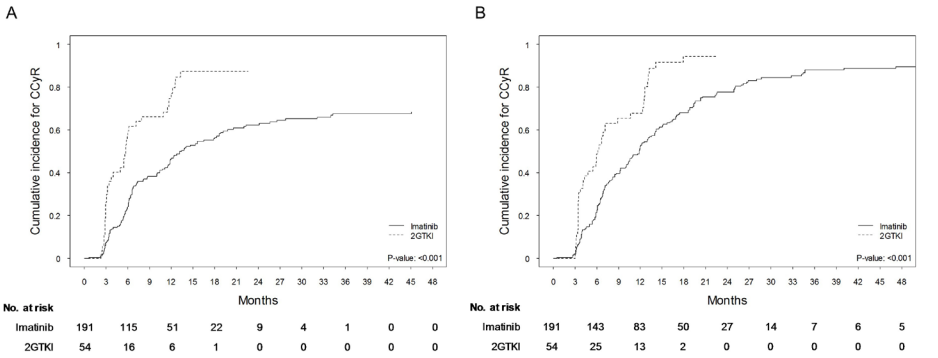
	Imatinib n=305		**Nilotinib** n=139		**Dasatinib** n=116	
	n	%	n	%	n	%
Cardiovascular events	**4**	**1,3**	**8**	**5,8**	**1**	**0,9**
Ischemic heart disease	1	0,3	3	2,2	1	0,9
Cerebro vascular event	0	0,0	2	1,4	0	0,0
Peripheral aterial occlusive disease	2	0,7	2	1,4	0	0,0
Venous tromboembolic event	1	0,3	1	0,7	0	0,0
Non-ischemic cardiac events	**9**	**3,0**	**5**	**3,6**	**1**	**0,9**
Cardiac arrhytmia	4	1,3	2	1,4	1	0,9
Cardiac valvular problem	1	0,3	0	0,0	0	0,0
Congestive heart failure	4	1,3	2	1,4	0	0,0
Symptomatic QT prolongation	0	0,0	1	0,7	0	0,0
Neurological	**5**	**1,6**	**6**	**4,3**	**3**	**2,6**
Headache	2	0,7	3	2,2	3	2,6
Dizziness	2	0,7	1	0,7	0	0,0
Polyneuropathy	1	0,3	2	1,4	0	0,0
Other						
Rash	34	11,1	10	7,2	4	3,4
Infection	33	10,8	7	5,0	7	6,0
Fatigue	25	8,2	8	5,8	6	5,2
Bleeding	5	1,6	0	0,0	3	2,6
Anorexia	4	1,3	0	0,0	3	2,6
Second malignancies	7	2,3	2	1,4	1	0,9
Pruritus	4	1,3	3	2,2	1	0,9
Surgical intervention	1	0,3	1	0,7	2	1,7
Alopecia	0	0,0	2	1,4	0	0,0
Mucositis	4	1,3	0	0,0	0	0,0
Hypertension	0	0,0	1	0,7	1	0,9
Raynaud phenomonom	0	0,0	0	0,0	1	0,9
Depression	0	0,0	0	0,0	1	0,9
Gynaecomasty	2	0,7	0	0,0	0	0,0
Alleric reaction	2	0,7	0	0,0	0	0,0
Diabetes mellitus	0	0,0	1	0,7	0	0,0
Insomnia	1	0,3	0	0,0	0	0,0

*Cytopenia ≥ CTC grade 3 and/or requiring adjustment of treatment

No action		Interruption		Reduction		Switch		Stop		Unknown	
n	%	n	%	n	%	n	%	n	%	n	%
3	60	0	0	0	0	2	40	0	0	0	0
2	100	0	0	0	0	0	0	0	0	0	0
1	25	0	0	1	25	2	50	0	0	0	0
1	50	0	0	0	0	1	50	0	0	0	0
7	100	0	0	0	0	0	0	0	0	0	0
1	100	0	0	0	0	0	0	0	0	0	0
6	86	0	0	1	14	0	0	0	0	0	0
0	0	0	0	1	100	0	0	0	0	0	0
0	0	0	0	3	38	5	63	0	0	0	0
0	0	1	33	1	33	1	33	0	0	0	0
1	33	2	67	0	0	0	0	0	0	0	0
6	12	2	4	11	22	30	61	0	0	0	0
40	82	4	8	5	10	0	0	0	0	0	0
8	20	2	5	14	35	16	40	0	0	0	0
5	63	0	0	0	0	1	13	0	0	2	25
0	0	2	29	2	29	2	29	1	14	0	0
10	100	0	0	0	0	0	0	0	0	0	0
0	0	1	13	2	25	4	50	1	13	0	0
3	75	0	0	0	0	0	0	0	0	1	25
0	0	0	0	2	100	0	0	0	0	0	0
0	0	1	25	1	25	2	50	0	0	0	0
1	50	0	0	0	0	1	50	0	0	0	0
0	0	0	0	0	0	1	100	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	100
0	0	0	0	0	0	2	100	0	0	0	0
1	50	0	0	0	0	1	50	0	0	0	0
1	100	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	100	0	0	0	0

Supplementary figures

SI figure S1. CCyR achievement on frontline imatinib and 2GTKIs.

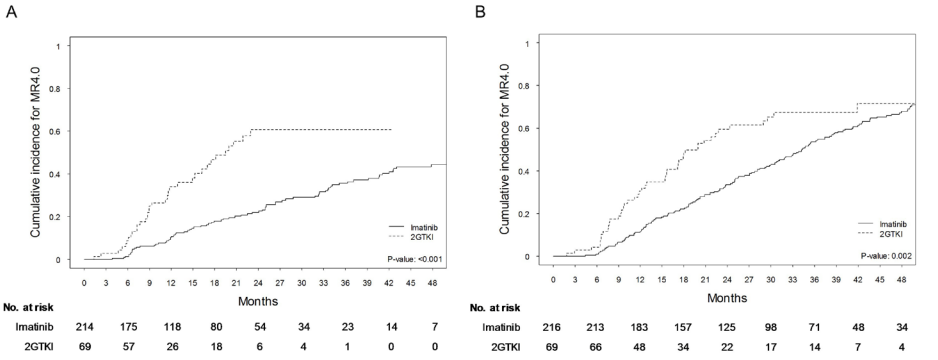


A. Cumulative incidence of CCyR on initial treatment

B. Cumulative incidence of overall CCyR rate (including switch)

Abbreviations: CCyR, complete cytogenetic response, 2GTKIs, second generation tyrosine kinase inhibitors.

SI figure S2. MR$^{4.0}$ achievement on frontline imatinib and 2GTKIs.

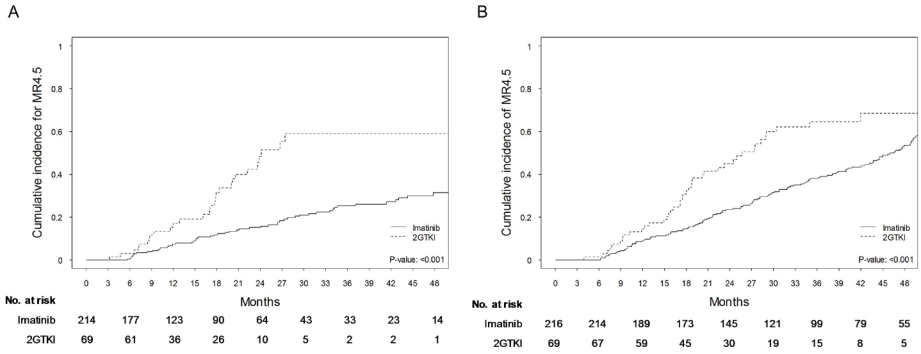


A. Cumulative incidence of MR$^{4.0}$ on initial treatment

B. Cumulative incidence of overall MR$^{4.0}$ rate (including switch)

Abbreviations: MR$^{4.0}$, BCR-ABL1 0.01% on international scale, 2GTKIs, second generation tyrosine kinase inhibitors.

SI figure S3. MR$^{4.5}$ achievement on frontline imatinib and 2GTKIs.

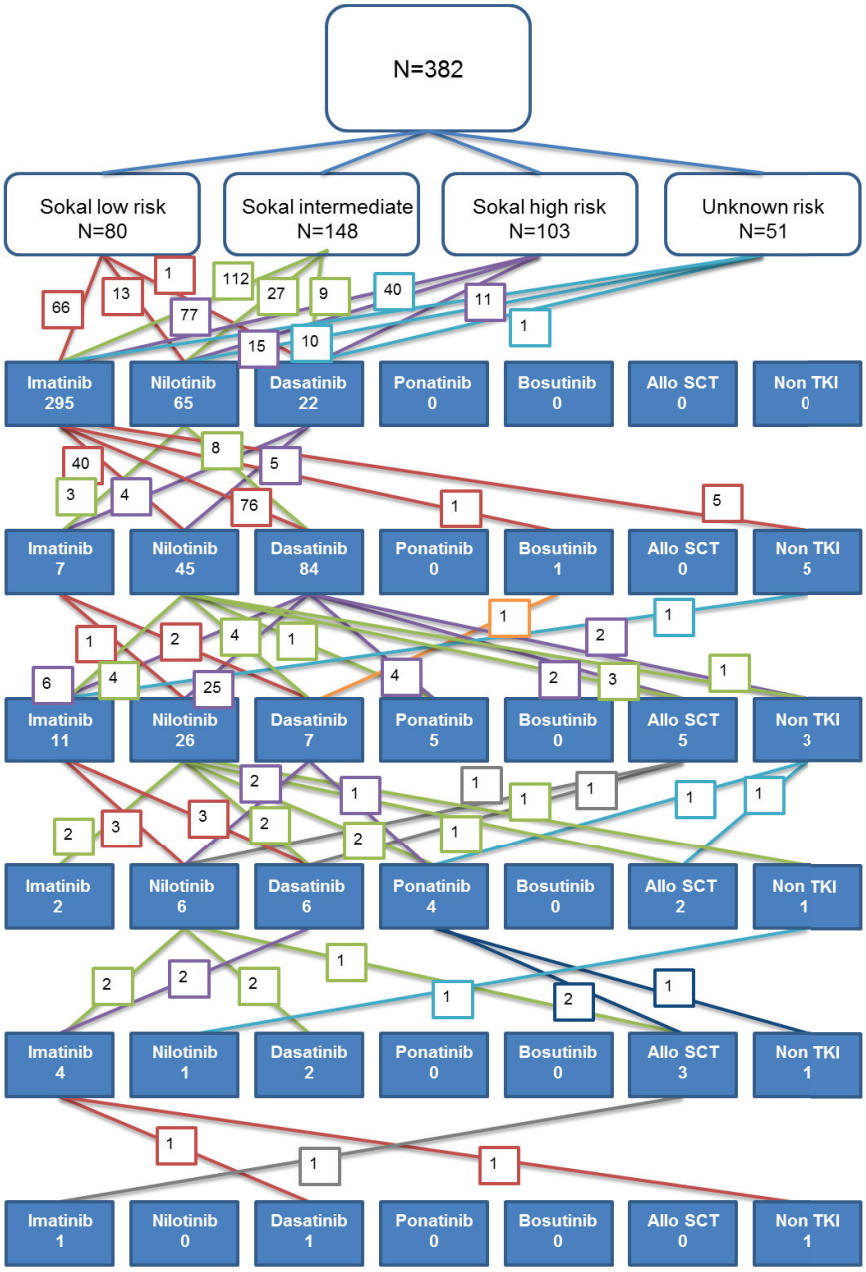


A. Cumulative incidence of MR$^{4.5}$ on initial treatment

B. Cumulative incidence of overall MR$^{4.5}$ rate (including switch)

Abbreviations: MR$^{4.5}$, BCR-ABL1 0.032% on international scale, 2GTKIs, second generation tyrosine kinase inhibitors.

SI figure S4. Treatment lines and patterns.



Treatment patterns for 382 CML patients in chronic phase at diagnosis treated with a first line TKI

Abbreviations: Allo SCT, allogeneic stem cell transplantation

CHAPTER 4

Validation of the EUTOS long-term survival score in a recent independent cohort of 'real world' CML patients

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Abstract

Risk prediction scores for patients with chronic myeloid leukemia (CML) should predict disease specific mortality rather than overall survival as most deaths are no longer CML-related. We evaluated the recently introduced EUTOS long-term survival (ELTS) score in an independent "real-world" population-based cohort of Swedish and Dutch chronic phase CML patients. Together, this included 953 patients newly diagnosed between 2007 and 2013 and treated upfront with imatinib (n=709) or second generation tyrosine kinase inhibitors (2GTKI) nilotinib or dasatinib (n=244). The ELTS score stratified for three risk groups with significantly different time to achieve MMR in both imatinib and 2GTKI treated patients. The 8-year probability of death due to CML in patients treated upfront with imatinib was 8%, 5% and 1% in the ELTS high, intermediate and low risk group respectively (high vs. low, p=0.001). For patients treated upfront with 2GTKI this was 6%, 0% and 0% respectively (high vs. low, p=0.010; high vs intermediate, p=0.030). The ELTS performed better than earlier risk scores (Sokal, Hasford and EUTOS) on both molecular and survival endpoints. Because of its superior predictive value on clinically relevant outcome parameters, our data support the preferred use of the ELTS-score for baseline risk stratification in CML.

Introduction

Prognostic risk scores, using patient characteristics such as age, spleen size and blast count, are used to predict survival at diagnosis in patients with chronic myeloid leukemia (CML) presenting in chronic phase. Until recently, risk stratification of CML patients was based on scores from the pre-imatinib era developed to predict overall survival probability. In 1984, the Sokal score was the first score developed to identify three CML patient subgroups based on overall survival when treated with busulfan or splenectomy in combination with intensive chemotherapy. The Sokal score included age, spleen size, blast percentage and platelet count.[1] The Hasford score, also known as the EURO score, was introduced in 1998 for CML-patients receiving interferon-α treatment. Eosinophil and basophil counts were added to the new risk score formula, and like the Sokal score, three risk groups with distinct survival probabilities could be distinguished.[2]

The spectacular improvement in life-expectancy of CML patients following the introduction of the tyrosine kinase inhibitor (TKI) imatinib[3,4], emphasized the need for a new prognostic risk score specifically designed for imatinib-treated patients. The EUTOS-score, including only two variables (basophils and spleen size), was introduced in 2011 for predicting the achievement of complete cytogenetic response (CCyR) after 18 months of imatinib-treatment.[5] Although CCyR has proven to be a strong predictor of overall survival[6,7], the EUTOS-score did not consistently perform well at predicting progression free survival and overall survival.[5,8-14] This may explain why many clinicians continue to use the Sokal or Hasford scores also for imatinib-treated patients. Interestingly, studies showed that these scores indeed retained some predictive power, even though they were not developed for TKI-treated CML patients.[15-18]

As a result of effective treatment with TKIs and reduced leukemia-related deaths, the need for baseline risk prediction has shifted from overall survival towards disease specific mortality.[4,19] In 2016 the EUTOS long term survival (ELTS) score was published, which was developed in chronic phase CML patients treated upfront with imatinib stratifying for three risk groups based on the probability of 'death due to CML'.[20] For this, data from patients diagnosed between 2002 and 2006 were used.

The introduction of the second generation TKIs (2GTKI) such as dasatinib (2006) and nilotinib (2007) have further improved patient outcomes; more salvage options became available for patients who develop first line imatinib failure (21%) or intolerance (19%)[21] and even less progressions occurred in patients treated upfront with nilotinib or dasatinib.[22,23] In the current study we further evaluated the ability of the ELTS score to distinguish three risk groups with different probabilities of 'death due to CML' in a population-based

cohort of CML patients treated upfront between 2007 and 2013 with imatinb or a 2GTKI. In addition, the predictive value for major molecular response (MMR) and overall survival were tested and the performance of the ELTS score was compared to Sokal, Hasford and EUTOS scores for all three endpoints.

Methods

Patients

Data of adult (≥ 17 years) CML patients newly diagnosed between 2007 and 2013 were obtained from the Swedish and Dutch population-based registries. Details on data sources have been published previously.[13,21] Patients were eligible when they were in chronic phase according to European LeukemiaNet criteria at diagnosis and were treated with first line imatinib, nilotinib or dasatinib.

Definitions and end points

Chronic phase, accelerated phase (AP) and blast crisis (BC) were defined according to European LeukemiaNet (ELN criteria).[24] Progression was characterized by the development of accelerated phase or blast crisis under treatment. The Sokal, Hasford, EUTOS and ELTS risk scores were calculated as stated in the original papers.[1,2,5,20] Recorded progression of CML prior to death was considered 'death due to CML', in line with the original ELTS paper,[20] and validated by reviewing the medical records of deceased patients. All four risk scores were evaluated on their predictive value for time to first MMR, 'death due to CML', and overall survival. The time to event analysis were calculated from the start of first line TKI treatment.

Molecular response assessments

BCR-ABL1 levels of ≤ 0.1% on the international scale or ≥3 log reduction of *BCR-ABL1* mRNA transcripts from baseline (in molecular labs not able to report on the international scale at the time) were defined as the molecular response end point MMR. Undetectable *BCR-ABL1* levels were classified as MMR when control gene numbers were not available to determine the sensitivity of the test. If molecular data were unavailable, patients were not included in the analyses regarding time to first MMR achievement. As the data were derived from population-based registries, the frequency of molecular monitoring was variable, as previously reported for the Dutch population-based registry.[25]

Statistical analysis

The study cohort was subdivided in a imatinib group and 2GTKI group based on their first line of treatment. Descriptive statistics (Mann-Whitney U and Chi-square tests) were used to compare the imatinib and 2GTKI upfront treatment groups. 'Death due to CML' and time to first MMR achievement were evaluated using a cumulative incidence competing risk analysis with censoring at the time a hematopoietic stem cell transplantation (HSCT) was performed in first chronic phase. For 'death due to CML' death due to other causes was considered a competing risk, whereas progression and death (any cause) were competing risks in the time to first MMR analyses. Differences in cumulative

incidence were tested using the Gray's test. Overall survival was estimated using the Kaplan Meier method and idfferences between groups were tested using a log rank test. All endpoints were evaluated for the groups of patients treated upfront with imatinib and upfront with second generation TKIs. A p-value of 0.05 was considered statistically significant. This study was performed in accordance with the declaration of Helsinki and approved by the Medical Ethics Committees of Uppsala University and the Erasmus Medical Center in Rotterdam. All analysis were performed using SPSS version 22 and R-software[26] version 3.2.4.

Results

Population description

The combined registries covered 1158 patients (694 Swedish and 464 Dutch) newly diagnosed between 2007 and 2013; 1062 patients (92%) were in chronic phase (CP) at diagnosis and 953 CP-CML patients were documented to have received imatinib (74%), dasatinib (7%) or nilotinib (19%) as first line treatment. An overview of patient characteristics is shown in table 1. In 7% of patients not all components of the four risk scores were available for calculation. The majority of patients (47%) were categorized as low risk according to the ELTS score, 36% as intermediate risk and 17% as high risk. A quarter of the patients were treated upfront with a second generation TKI. This patient subset was significantly younger, spleen size was slightly but significantly larger, presented more often in the EUTOS high risk category and more frequently participated in a first line clinical trial.

Data on the achievement of first major molecular response (MMR) were available for 830 (87%) patients; 607 (86%) patients on first line imatinib and 223 (91%) patients on first line second generation TKIs. The 12- and 24-month first MMR rates were 45% [95% CI: 42-49%] and 70% [95% CI: 67-74%] respectively with a median follow-up duration of 1.0 years (0-6.7); 1.2 years for the imatinib subgroup and 0.7 years for the 2GTKI subgroup. As expected the cumulative incidence of first MMR was significantly higher in patients treated upfront with a 2GTKI than patients treated upfront with imatinib (12 months: 37% vs. 68% and 24 months: 66% vs. 81%, p<0.001).

Allogeneic HSCT in first chronic phase was performed in 23 patients (2.4%). Progression to AP/BC was reported in 44 (5%) patients; 13 (30%) patients underwent a SCT and in 26 patients (59%) the progression was fatal. In total 165 patients died (140/709 in upfront imatinib and 25/244 in upfront 2GTKI group), of which 26 patients (16%) due to CML progression (23/709 in upfront imatinib and 3/244 in upfront 2GTKI group). Overall survival data were available for all patients, but 'death due to CML' was unknown for five deceased patients and therefore these five patients were excluded from the 'death due to CML' analysis. The 8-year cumulative incidence of 'death due to CML' was 3% [95%CI: 2-5%]: 4% vs. 1% (p=0.130) in patients treated upfront with imatinib and 2GTKI respectively. The 8-year OS was 76% [95%CI: 72-80%]: 74% vs. 84% (p=0.019) in patients treated upfront with imatinib and 2GTKI respectively. The median survival follow-up duration was 5.3 (0-9.9) years; 5.7 years for the imatinib subgroup and 4.6 years for the 2GTKI subgroup.

Risk score prediction of molecular response

In patients treated upfront with imatinib, the ELTS score was capable of identifying three risk groups with significantly different rates of first MMR attainment: a 2-year probability of 44%, 64% and 76% in the high, intermediate and low ELTS risk groups (figure 1a). The

Sokal and Hasford score were able to differentiate a high risk group with a significantly lower cumulative incidence of first MMR achievement from the other two risk groups (supplementary figure S1). The EUTOS score had no predictive capacities regarding time to first MMR. In patients treated upfront with 2GTKI the ELTS score distinguished a higher rate of MMR achievement in the low risk than in the intermediate and high risk groups (figure 1b). For 2G-TKI, the Sokal score predicted a higher first MMR probability in the low in comparison with the high risk group (88% vs. 75%, p=0.034, (supplementary figure S2), but otherwise the Sokal, Hasford and EUTOS scores did not differentiate in the 2GTKI subgroup analysis.

Risk score prediction of death due to CML

The 8-year probability of death due to CML in patients treated upfront with imatinib was 8% in the ELTS high risk group, 5% in the ELTS intermediate risk group and 1% in the ELTS low risk group (figure 1c). A significant difference in cumulative incidence was only found when comparing the high with the low risk group (p=0.001). Sokal, Hasford and EUTOS risk scores all did not predict significant differences in 'death due to CML' rates in patients treated with first line imatinib (supplementary figure S3). The two patients who died due to CML after upfront 2GTKI treatment were both classified as high risk (figure 1d), resulting in a significantly higher risk of death due to CML in the ELTS high risk group compared to the ELTS intermediate (6% vs. 0%, p=0.01) and low risk groups (6% vs. 0%, p=0.03), but even though this reached statistical significance, the small number of events limits the reliability of the ELTS validation in the 2GTKI group. All other risk scores only classified one out of two patients who died due to CML as high risk (supplementary figure S4).

Risk score prediction of overall survival

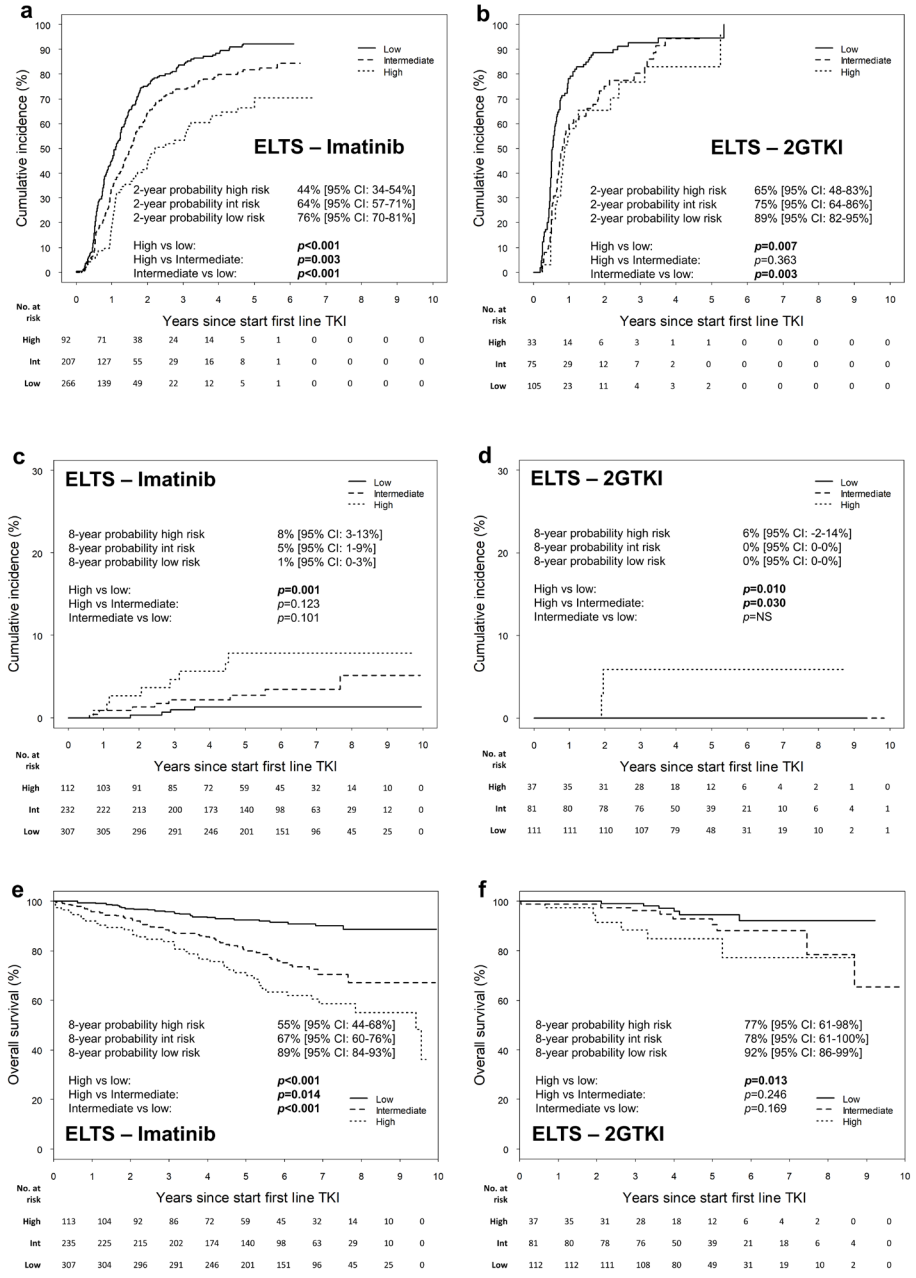
In the upfront imatinib subgroup the ELTS score clearly distinguished three risk groups in terms of overall survival, with a 8-year probability of 55%, 67% and 89% for patients with high, intermediate and low ELTS risk (figure 1e). Although the Sokal risk score separated a low risk group with a significantly lower risk of dying of all causes, it was unable to discriminate overall survival rates between the intermediate and high risk group (supplementary figure S5). The Hasford and EUTOS risk scores did not differentiate between overall survival rates. In patients treated upfront with 2GTKI, the ELTS score separated a high from a low risk group in terms of overall survival (8-year probability of 92% vs. 77%, p=0.013) with an intermediate risk group with an intermediate overall survival, although this did not reach statistical significance (figure 1f). Sokal, Hasford and EUTOS failed to predict overall survival probabilities in patients treated upfront with 2GTKI (supplementary figure S6).

Table 1. Patient characteristics

	Total (n=953) *Median (range) or n (%)*	*Number of missing cases*	**Imatinib (n=709)** *Median (range) or n (%)*	**2GTKI (n=244)** *Median (range) or n (%)*	**Imatinib vs 2GTKI** *P-value*
Age, years	59 (17-90)	0	60 (17-90)	55 (17-90)	0.003*
Sex (proportion male)	547 (57%)	0	401 (57%)	146 (60%)	NS†
Spleen size, cm below costal margin	0 (0-27)	41	0 (0-25)	0 (0-27)	0.007*
Hemoglobin, g/dl	11,8 (5.2-17.1)	18	11,8 (5,2-17,1)	11,9 (6-16,9)	NS*
White blood cell count, $x10^9$/l	104 (4-716)	2	103 (4,2-716)	105 (9-603)	NS*
Eosinophils, % in peripheral blood	2 (0-22)	17	2 (0-22)	2 (0-14)	NS*
Basophils, % in peripheral blood	3,5 (0-19)	18	3,3 (0-19)	3,8 (0-19)	NS*
Blasts, % in peripheral blood	1 (0-14)	23	1 (0-14)	1 (0-10)	NS*
Platelet count, $x10^9$/l	416 (35-5035)	7	417 (35-5035)	404 (87-4864)	NS*
Sokal score		68			NS†
Low risk	227 (26%)		165 (25)	62 (27)	
Intermediate risk	376 (42%)		282 (43)	94 (41)	
High risk	282 (32%)		208 (32)	74 (32)	
Hasford score		71			NS†
Low risk	308 (35%)		226 (34%)	82 (36%)	
Intermediate risk	450 (51%)		340 (52%)	110 (49%)	
High risk	124 (14%)		89 (14%)	35 (15%)	
EUTOS score		58			0.003†
Low risk	772 (86%)		586 (88%)	186 (81%)	
High risk	123 (14%)		78 (12%)	45 (19%)	
ELTS score		68			NS†
Low risk	419 (47)		307 (47%)	112 (49%)	
Intermediate risk	316 (36)		235 (36%)	81 (35%)	
High risk	150 (17)		113 (17%)	37 (16%)	
First line trial inclusion		14			<0.001†
Yes	158 (17%)		75 (11%)	83 (34%)	
No	781 (83%)		622 (89%)	159 (66%)	

* Mann-Whitney U, † Chi-Square test

Figure 1. The ELTS stratification in a low, intermediate and high risk group for imatinib and 2GTKI treated patients.



For achievement of MMR (a,b), CML-related death (c,d) and overall survival (e,f).

Abbr: MMR, major molecular response; 2GTKI, second generation TKI.

Discussion

In this independent, population-based and recent cohort of CML patients, we were able to confirm that the ELTS risk score was the only risk score reliably predicting 'death due to CML' in CML patients treated with frontline imatinib. In addition, we found that the ELTS score can identify a subgroup of patients at increased risk for dying of CML in patients receiving upfront 2GTKI treatment. Consistent with these findings, we found the ELTS risk score to also correlate with achievement of MMR.

The ELTS score segregated our imatinib cohort in three groups with distinct risk profiles for CML-related death, similar to its first publication with in-study (n=2205) and out-study (n=1120) cohorts[20] and a recent smaller single center Italian study (n=417).[27] Our validation in a population-based cohort with e.g. older patients (average 60 years vs. 51 and 49 years in the original publication[20]) shows its applicability in general CML practice. In addition, the period of diagnosis of our cohort was more recent (2007-2013 vs. 2002-2006[20]) in which more rescue options were available in case of TKI failure. Moreover, its applicability in children and adolescents treated upfront with imatinib has recently been confirmed.[28]

We observed that also in patients treated with frontline 2GTKI, the ELTS risk score was able to identify a high risk group with a significantly higher risk of disease specific mortality. However, since the subgroup of 2GTKI treated patients in our cohort was relatively small, had a limited duration of follow-up and particularly since the incidence of CML-related death was very low in patients treated upfront with 2GTKI in comparison to imatinib (8-year cumulative incidence of 4% vs.1%), the ELTS score for patients treated upfront with 2GTKI requires further validation.

Our study is the first to report on the predictive value of the ELTS score in imatinib treated patients on the achievement of MMR, a major surrogate endpoint for survival and clinical goal of therapy. We observed an excellent prediction of time to first MMR and overall rate of MMR by the ELTS risk score in patients treated with frontline imatinib. In this, the ELTS score outperformed the Sokal, Hasford and EUTOS scores. Also in patients treated with upfront 2GTKI, the ELTS risk score was able to identify a low risk group with a significantly higher cumulative incidence of MMR. This is in line with one other study (presented in abstract form).[29] Further studies should investigate whether ELTS is also able to predict early molecular response (e.g. *BCR-ABL1* <10% at 3 months) in patients receiving imatinib upfront.

In conclusion, our study adds to the body of evidence supporting the ELTS as an excellent risk stratification tool for contemporary CML patients treated with imatinib. We and others[27,29] show that the ELTS may also be used for 2GTKI treated patients. The ELTS outperforms historical Sokal, Hasford and EUTOS scores, which are non-predictive for CML-related death. The ELTS may be used in clinical practice to select patient subpopulations at high risk for CML-related death that may benefit most from 2GTKI therapy.

An ELTS risk score calculator is available in the 'Hematology app' (downloadable for free through Apple App Store and Google Play store for EU-based physicians) and on the following website: https://www.leukemia-net.org/content/leukemias/cml/elts_score

Acknowledgements

The authors would like to thank Peter Huijgens, currently chairman of the Netherlands Comprehensive Cancer Organisation (IKNL) of initiating the Pharos registry. We thank Tom Wiggers, Wencke de Jager, Sanne Nijssen and Jolie Cheung for entering patient data in the Pharos database. We thank Marianne van der Mark from the IKNL for retrieving survival data from the Netherlands Cancer Registry. We thank all the hospitals and molecular labs in the Netherlands who participated in the Pharos and Hemobase registries. The authors thank all Swedish hematologists who have carefully reported CML patients to the CML Registry, in particular the regional coordinators of the Swedish CML registry working group (Berit Markevärn, Ulla Olsson-Strömberg, Leif Stenke, Arta Dreimani, Hans Wadenvik and Johan Richter). The authors also appreciate the work of data managers at the respective RCCs.

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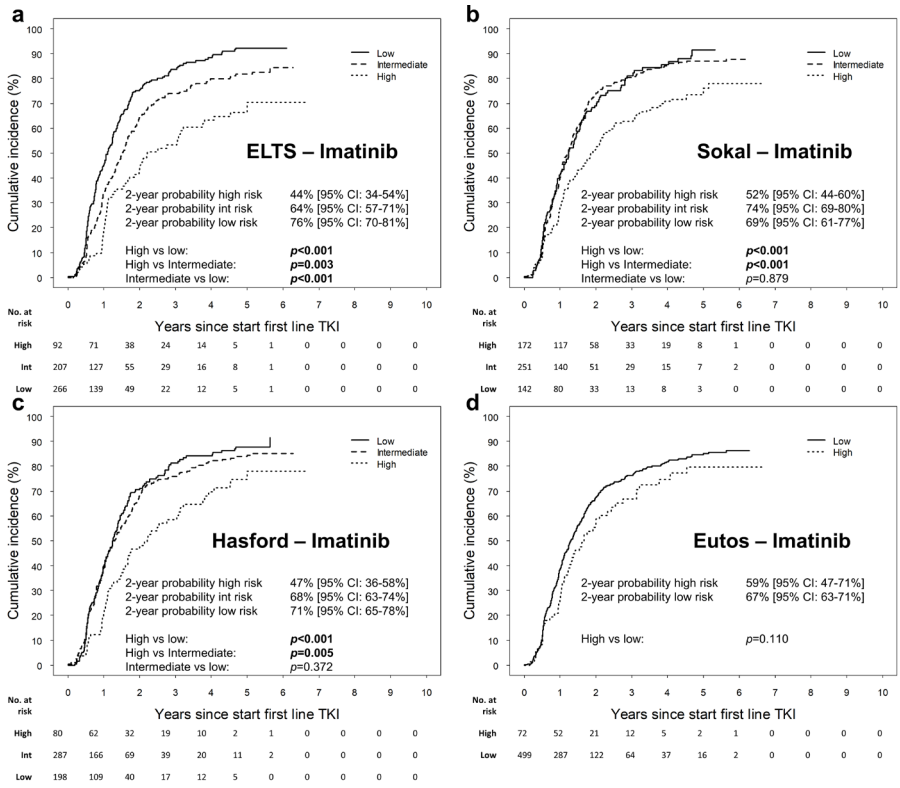
Supplementary figures

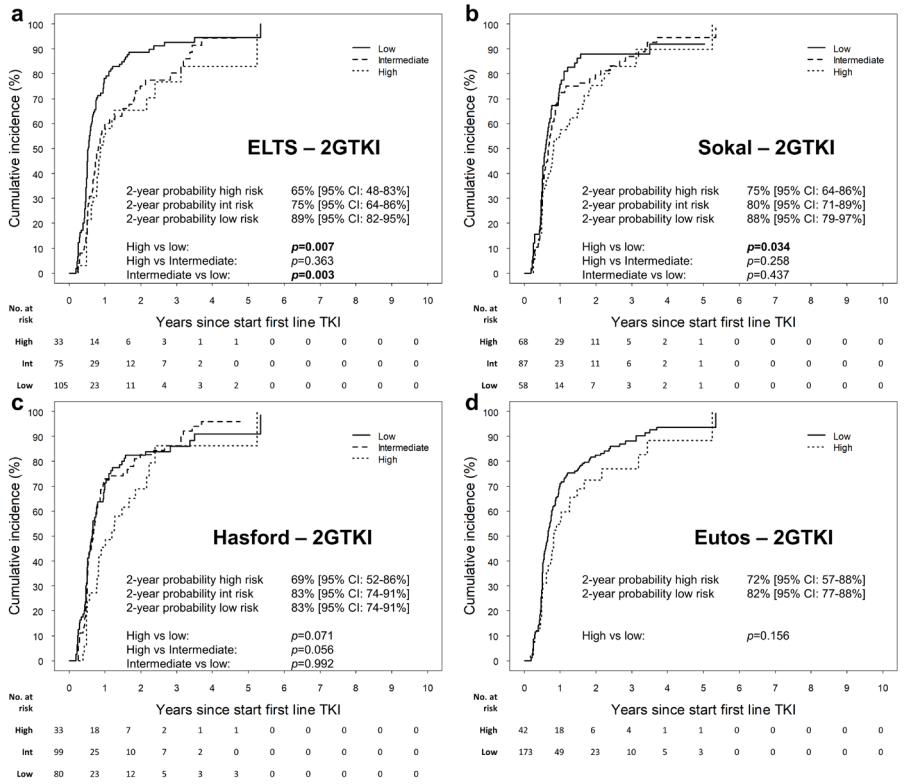
Figure S1. Cumulative incidence of first MMR in patients treated with frontline imatinib.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second generation TKI; ELTS, Eutos long-term survival

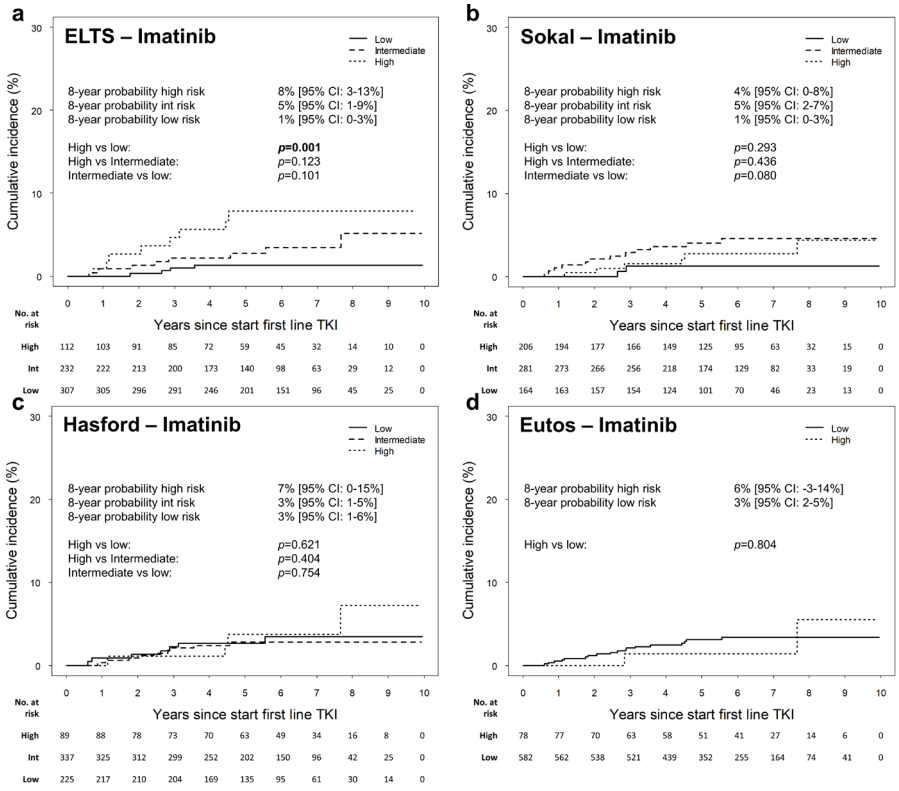
Figure S2. Cumulative incidence of first MMR in patients treated with frontline 2GTKI.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second generation TKI; ELTS, Eutos long-term survival

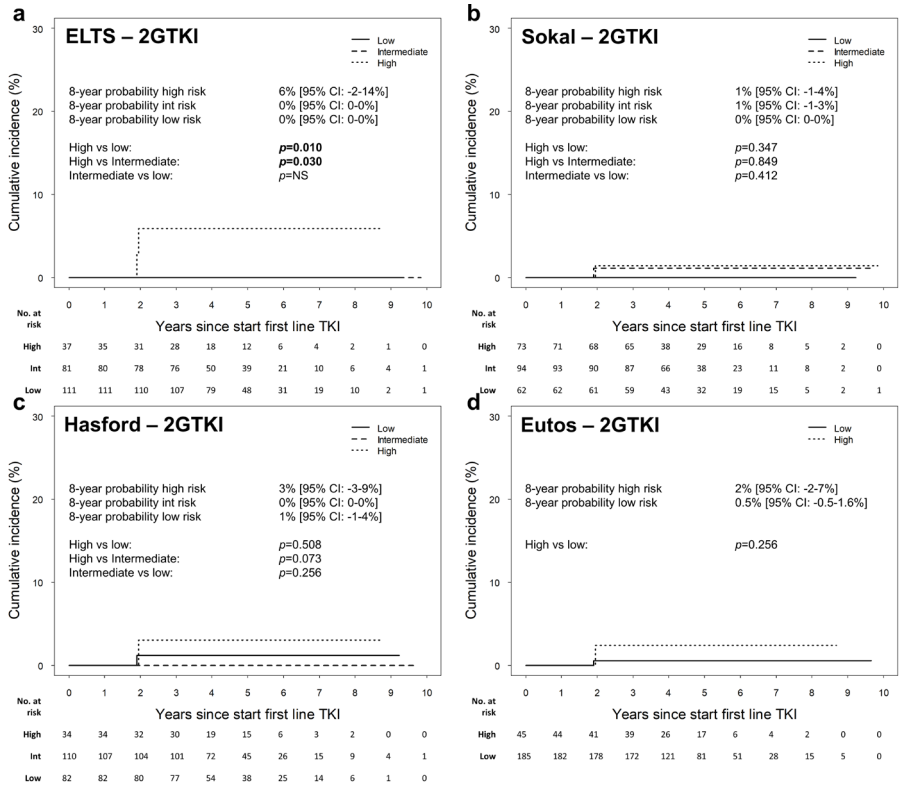
Figure S3. Cumulative incidence 'Death due to CML' in patients treated with front line imatinib.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second generation TKI; ELTS, Eutos long-term survival

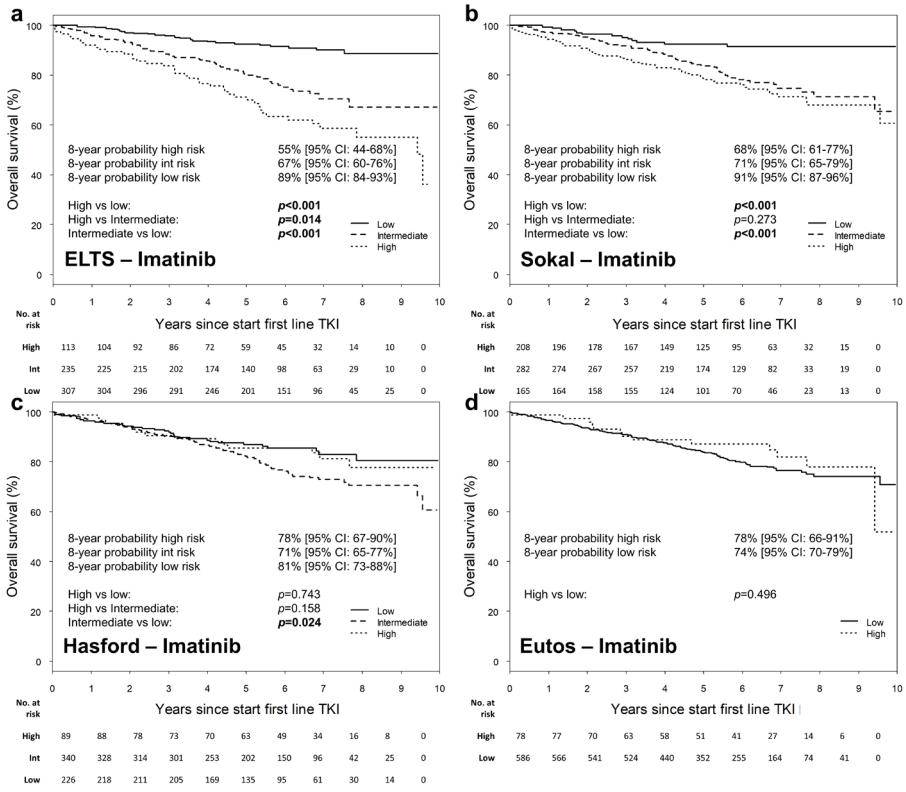
Figure S4. Cumulative incidence 'Death due to CML' in patients treated with front line 2GTKI.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second generation TKI; ELTS, Eutos long-term survival

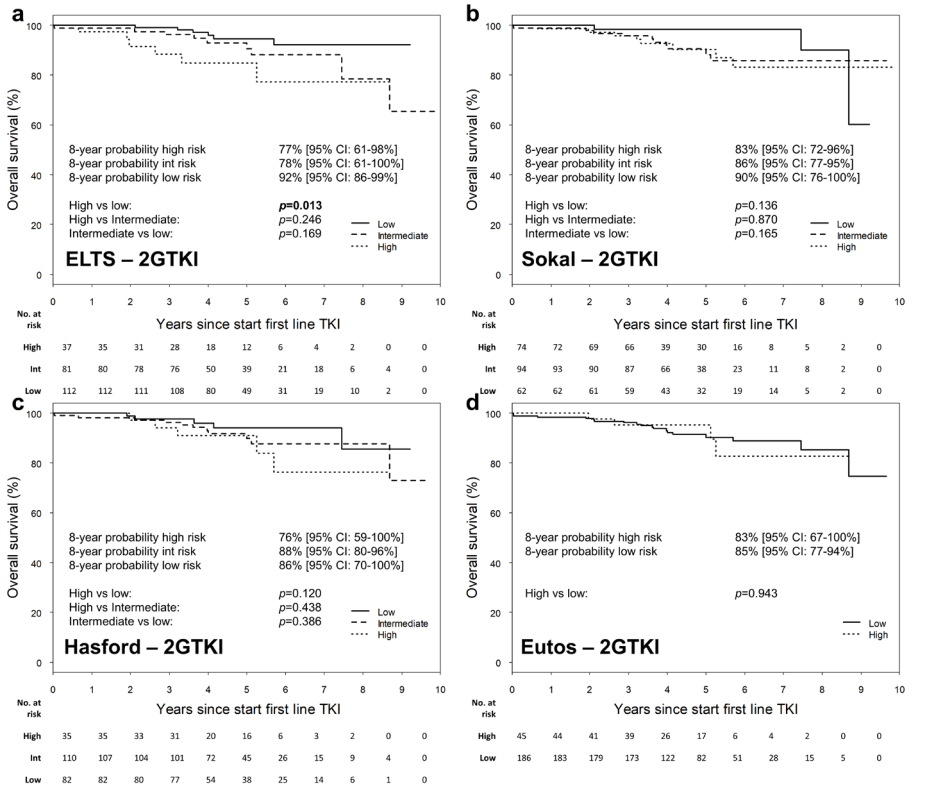
Figure S5. Overall survival in patients treated with front line imatinib.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second generation TKI; ELTS, Eutos long-term survival

Figure S6. Overall survival in patients treated with front line 2GTKI.



Stratified by: a. ELTS-risk score, b. Sokal-risk score, c. Hasford risk score, d. EUTOS risk score

Abbr: 2GTKI, second

CHAPTER 5

Impact of hospital experience on the quality of tyrosine kinase inhibitor response monitoring and consequence for chronic myeloid leukemia patient survival

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Haematologica. 2017 Dec;102(12):e486-e489

Introduction

The importance of adequate response monitoring during the treatment of chronic myeloid leukemia (CML) with tyrosine kinase inhibitors (TKIs) and testing for *BCR-ABL1* kinase domain (KD) mutations in case of TKI failure is generally acknowledged and clearly outlined in guidelines and recommendations on CML management.[1-6] Recent studies from the US have indicated that TKI response monitoring may be suboptimal in clinical practice.[7-9] This was found to be of major clinical importance as patients undergoing the recommended molecular assessments 3-4 times annually experienced a reduced risk of progression and mortality[7], had improved TKI adherence[8] and generated lower health care costs[9] compared to patients monitored less frequently. Since no population-based European data have been published on the quality of response monitoring in CML so far, we conducted an evaluation of response monitoring in an unselected population-based CML patient cohort in the Netherlands in the first year after diagnosis. In our study, we observed suboptimal monitoring of response to TKI treatment in a quarter of the patients. Inadequate monitoring was associated with a reduced overall survival and hospital CML treatment experience was the strongest predictor for proper monitoring. Also, we found that KD domain mutation testing was performed in only 34% of patients switching TKI therapy due to TKI failure.

Methods

Data were obtained from two complementary population-based registries for newly diagnosed CML patients in the Netherlands[10,11], together covering 75 out of 90 Dutch hospitals including 7 of the 8 academic hospitals. In the Netherlands, a total of 15 specialized laboratories perform cytogenetic, molecular and mutational analyses. The frequency of cytogenetic and molecular response assessments in the first year was calculated as the total number of tests performed between 15 and 407 days from the start of TKI treatment (allowing for a 6 weeks margin from the one year landmark). Assessments performed within 30 days between were counted as one. Based on NCCN and ELN guidelines,[1-6] we defined the minimum standard of care for response monitoring as at least 3 molecular and/or cytogenetic assessments performed in the first year after treatment initiation. The performance of mutational analysis was assessed during the entire follow-up period during first line treatment. Based on the number of CML treatment initiations over the 5 year inclusion period between 2008 and 2013, hospitals were categorized into three groups as a proxy for hospital CML treatment experience: less experienced (≤ 5 patients); medium experienced (6-10 patients) and most experienced (> 10 patients). Data on survival and causes of death were obtained from the Netherlands Cancer Registry with a follow-up until the 1st of February 2016. The Medical Ethics Committee of the Erasmus Medical Center in Rotterdam approved this study and the exemption from informed consent. The study was conducted in accordance with the Declaration of Helsinki. Details on statistical analysis are included in the supplement.

Results

The current analysis included a total of 382 patients aged 18 years or older that were in chronic phase at diagnosis and were treated with a TKI as first line treatment with at least one year of follow-up data available. Most patients were treated with first line imatinib (77%), in non-academic centers (73%) and were not included in a clinical trial (83%) (table 1). Slightly more than half (57%) of the patients were treated in one of the 18 most experienced hospitals; the remainder (26% and 17% respectively) in medium or less experienced hospitals. In the most experienced hospitals, the median age at diagnosis was significantly lower and patients experienced less comorbidities at baseline. Patients treated in the most experienced hospitals were more likely to have participated in clinical trials. Although the academic centers were mainly categorized as most experienced hospitals for CML treatment, the majority of CML patients across all hospital experience subgroups were treated in non-academic centers.

Monitoring relied predominantly on molecular testing. In 74% of patients three or more molecular assessments were performed (figure 1A). A minority of the patients (18%) underwent three or more cytogenetic tests, whereas almost one third of the patients did not receive any cytogenetic follow up in the first year of treatment. Together, 84% of patients met the minimum standard of care and had at least 3 molecular and/or cytogenetic assessments performed in the first year. The monitoring was therefore suboptimal in 26% of cases with 4% of patients not receiving any cytogenetic or molecular response assessment in the initial year after diagnosis at all.

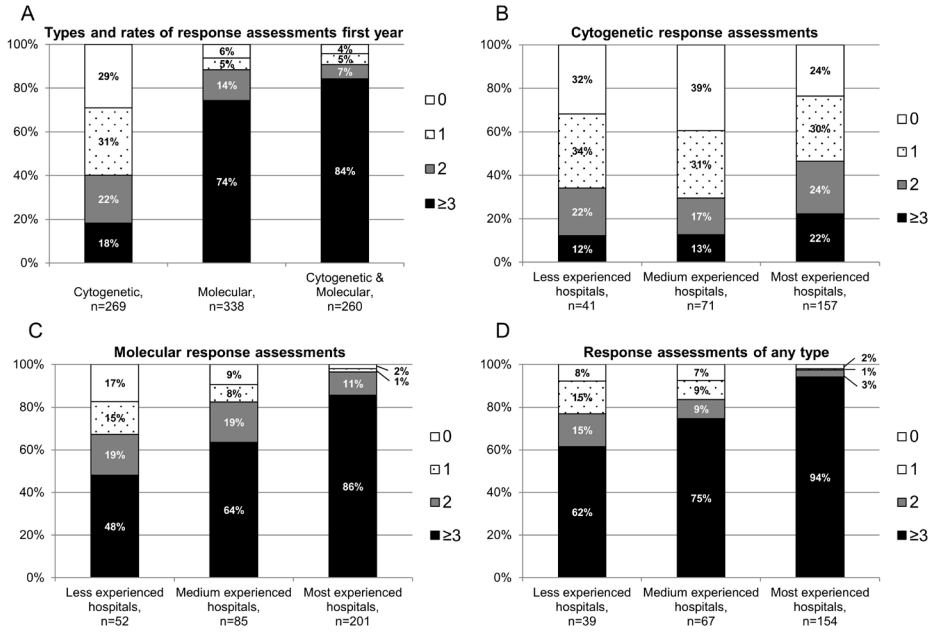
The median survival time of living patients was 5.6 years (2.8 to 8.0 years). During follow up 72 patients died (19%); 18 (5%) due to CML. Univariable analysis demonstrated that the performance of a minimum of one cytogenetic response assessment, three molecular response tests or three response tests of any type in the first year of treatment were all associated with a better overall survival (supplementary figures S1-S3). In a multivariable Cox proportional hazards model, a minimum of three molecular response tests in the first year was the only response monitoring method with a marginally significant positive association on overall survival (HR 0.52, 95%CI [0.27-1.00]) (supplementary tables S1-S3). The age-adjusted comorbidity index was negatively associated with overall survival in all three models.

Table 1. Baseline characteristics

	Total (n=382)	Less experienced hospitals (n=66)	Medium experienced hospitals (n=97)	Most experienced hospitals (n=219)	P-value §
Male gender, n (%)	219 (57)	38 (58)	56 (58)	125 (57)	0.993†
Age, years					**<0.001**‡
Median (IQR)	58 (43-69)	65 (55-75)	57 (46-69)	54 (39-68)	
Year of diagnosis, n (%)					0.329†
2008	90 (24)	10 (15)	31 (32)	49 (22)	
2009	77 (20)	16 (24)	15 (15)	46 (21)	
2010	80 (21)	14 (21)	17 (18)	49 (22)	
2011	72 (19)	16 (24)	16 (16)	40 (18)	
2012 – April 2013	63 (17)	10 (15)	18 (19)	35 (16)	
Charlson Comorbidity index*					**0.001**†
Age-adjusted, n (%)					
0	125 (33)	7 (11)	27 (28)	91 (42)	
1-2	120 (31)	27 (41)	34 (35)	59 (27)	
3-4	83 (22)	18 (27)	22 (23)	43 (20)	
≥5	54 (14)	14 (21)	14 (14)	26 (12)	
Sokal risk group, n (%)					0.443†
Low	80 (24)	10 (17)	24 (30)	46 (24)	
Intermediate	148 (45)	31 (53)	32 (40)	85 (44)	
High	103 (31)	17 (29)	25 (31)	61 (32)	
Unknown	51	8	16	27	
First line treatment					0.848†
Imatinib	295 (77)	51 (77)	78 (81)	166 (76)	
Nilotinib	65 (17)	11 (17)	13 (13)	41 (19)	
Dasatinib	22 (6)	4 (6)	6 (6)	12 (5)	
Treating hospital, n (%)					**<0.001**†
Non-academic	280 (73)	66 (100)	92 (95)	122 (56)	
Academic	102 (27)	0 (0)	5 (5)	97 (44)	
Inclusion in 1st line clinical trial, n (%)					**<0.001**†
No	309 (83)	59 (92)	89 (93)	161 (75)	
Yes	65 (17)	5 (8)	7 (7)	53 (25)	
Unknown	8	2	1	5	

Abbr: IQR, interquartile range; *2 points for CML not included, † Chi-square test, ‡ Kruskal Wallis test, § Unknown groups excluded from analysis

Figure 1. Monitoring frequencies during the first year of TKI treatment.

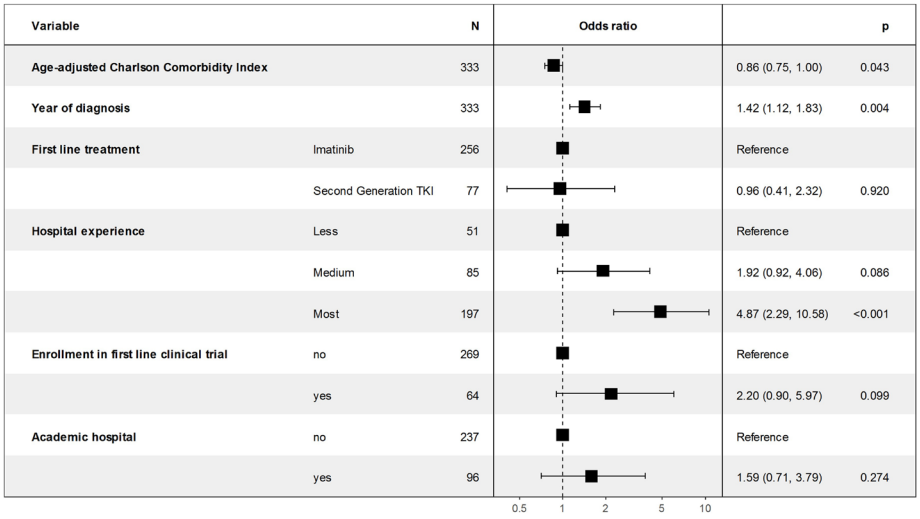


(A) per assessment type and (B-D) per hospital experience category

A binary logistic regression model (based on n=333) demonstrated that treatment in a hospital categorized as most experienced was the strongest independent predictor for the performance of a minimum of three molecular response assessments in the first year of treatment (OR 4.87, 95%CI [2.29-10.58]) (figure 2). A higher age-adjusted Charlson comorbidity index was negatively associated with the performance of three or more molecular tests in the first year (OR 0.86, 95%CI [0.75-1.00]). The odds of adequate molecular testing for a patient in our cohort increased per year of diagnosis and thus moment of entry of the patient in the cohort (OR 1.42, 95%CI [1.12-1.83]), indicating that the practice of molecular monitoring during the first year of TKI treatment improved over time. Response monitoring rates per hospital experience category are presented in figure 1 B-D.

After initiation of first-line TKI treatment, 97 patients met the criteria for TKI failure.[1,2] Mutational analysis was performed in only 24 of these patients (25%). Some patients (n=36) continued their TKI despite meeting treatment failure criteria. Of the 61 patients who switched to second line TKI due to failure, 21 patients underwent mutational assessments (34%) to potentially direct the choice of second line TKI. No association between hospital experience and KD domain mutation assessment was found.

Figure 2. The performance of 3 or more molecular assessments in the first year.



Forest plot with odds ratios for the performance of 3 or more molecular assessments in the first year of TKI treatment, based on a 1-year landmark binary logistic regression model of 333 patients. Hosmer and Lemeshow goodness of fit test: $X^2 = 5.18$, df = 8, p-value = 0.74

Abbr: TKI, tyrosine kinase inhibitor

Discussion

This is the first European population-based evaluation of the quality of TKI response monitoring in CML. In comparison with response monitoring evaluations performed in the US we found relatively high rates of adequate molecular response monitoring in the first year of TKI treatment (74%). A physician-administered chart-review of 402 CML-CP patients on first line imatinib therapy in the US showed the rate of 3 or 4 molecular tests per year to be 46%.[7] A claim-based analysis performed in the US showed a much lower rate of 3-4 molecular tests performed in the first year of TKI treatment (27%).[8,9] Goldberg *et al*. determined that patient resource barriers were an important factor negatively influencing physician adherence to CML monitoring guidelines in the US.[12] This factor might also explain the superior results found in the Dutch patient cohort studied by us, since healthcare insurance is mandatory in the Netherlands and it covers all laboratory assessment expenses without additional costs for the patient. We are confident that the large observational SIMPLICITY study will provide us with more information regarding factors that influence monitoring frequencies in the US and Europe.[13]

Although life-expectancy of patients with CML in general is approaching the life expectancy of the general population[14], the current study suggests that a potential survival benefit of 9% over a period of 4 years can be gained by optimization of molecular response monitoring in the first year of TKI treatment. Promoting adequate TKI response monitoring has been a priority of the HOVON (Dutch-Belgian Cooperative Trial Group for Hematology-Oncology) leukemia working group. The improvement in monitoring practice observed during our study observation period may indeed reflect a growing awareness. It has to be taken into account that potential (unmeasurable) confounders might have attributed to the observed association between molecular monitoring in the first year and overall survival. Whether centralization of CML care indeed improves patient outcome remains speculative from our observational study and should be prospectively monitored if applied. Of note, we did not find an association between hospital experience and overall survival, although this may relate to the fact that more than half of patients in less and medium experienced hospitals are monitored adequately. Lauseker *et al*. also did not observe an effect of hospital experience on overall survival, but an association between hospital type and overall survival was demonstrated.[15]

In conclusion, our study has further underlined the importance of close monitoring for response to TKI treatment in CML patients with a survival advantage for optimally monitored patients. Although we show relatively high rates of optimal monitoring in Dutch clinical practice, there is substantial room for improvement, particularly in hospitals with low CML patient numbers receiving treatment. In contrast, the use of KD mutation test-

ing was poor across the patient cohort, independent of the hospital experience. Physicians and patients should continue to work to improve the quality of CML care to optimize the benefits of available TKIs.

Acknowledgements

Peter Huijgens, currently chairman of the Netherlands Comprehensive Cancer Organisation (IKNL), initiated the PHAROS registry. Tom Wiggers, Wencke de Jager, Sanne Nijssen and Jolie Cheung entered patient data in the PHAROS database. Marianne van der Mark from the IKNL retrieved survival data from the Netherlands Cancer Registry. We thank all the hospitals and molecular labs in the Netherlands who participated in the PHAROS and Hemobase registries for their efforts.

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Supplementary methods

Statistical analysis

Data are presented as a median with interquartile range (IQR). For baseline comparisons between the three subgroups of hospitals based on experience, chi-square tests and Kruskal-Wallis analysis were used. Overall survival was analyzed using the Kaplan-Meier method, log rank test and a Cox-proportional hazards model. Proportional hazards assumptions were tested. Binary logistic regression modeling was applied to determine predictors of adequate response and mutational assessments performance. The goodness-of-fit of the models was evaluated with the Hosmer-Lemeshow test. A p-value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS version 24 and R-software[1] version 3.2.4.

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Supplementary Tables

SI table S1. Cox proportional hazards model including cytogenetic assessments in first year

		No. of patients	HR (95% CI)	P-value
Age-adjusted Charlson Comorbidity Index		265	1.46 (1.27-1.69)	<0.001
Hospital experience *		265	1.02 (0.91-1.15)	0.75
Enrollment in first line clinical trial	no	204	1.00	
	yes	61	0.78 (0.23-2.65)	0.70
Cytogenetic assessments first year	0	78	1.00	
	≥1	187	0.58 (0.30-1.10)	0.09

Hazard ratios on overall survival, derived from the 1-year landmark multivariable cox proportional hazards model including the variable 'no or at least 1 cytogenetic response assessments underwent in the first year of TKI treatment'

* Absolute number of treatment initiations per year.

Abbreviations: TKI, tyrosine kinase inhibitor; HR, hazard ratio

SI table S2. Cox proportional hazards model including molecular assessments in first year

		No. of patients	HR (95% CI)	P-value
Age-adjusted Charlson Comorbidity Index		333	1.39 (1.20-1.59)	<0.001
Hospital experience *		333	1.06 (0.94-1.19)	0.36
Enrollment in first line clinical trial	no	269	1.00	
	yes	64	0.60 (0.18-1.98)	0.41
Molecular assessments first year	<3	85	1.00	
	≥3	248	0.52 (0.27-1.00)	0.05

Hazard ratios on overall survival, derived from the 1-year landmark multivariable cox proportional hazards model including the variable 'less than 3 or at least 3 molecular response assessments underwent in the first year of TKI treatment'

* Absolute number of treatment initiations per year.

Abbreviations: TKI, tyrosine kinase inhibitor

SI table S3. Cox proportional hazards model including any type of assessments in first year

		No. of patients	HR (95% CI)	P-value
Age-adjusted Charlson Comorbidity Index		256	1.45 (1.24-1.70)	<0.001
Hospital experience *		256	1.05 (0.92-1.21)	0.45
Enrollment in first line clinical trial	no	195	1.00	
	yes	61	0.79 (0.22-2.83)	0.72
Assessments of any type first year	<3	40	1.00	
	≥3	216	0.50 (0.23-1.08)	0.08

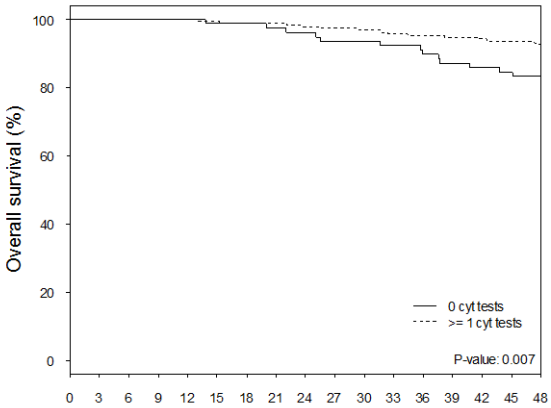
Hazard ratios on overall survival, derived from the 1-year landmark multivariable cox proportional hazards model including the variable 'less than 3 or at least 3 response assessments of any type underwent in the first year of TKI treatment'

* Absolute number of treatment initiations per year.

Abbreviations: TKI, tyrosine kinase inhibitor; HR, hazard ratio

Supplementary figures

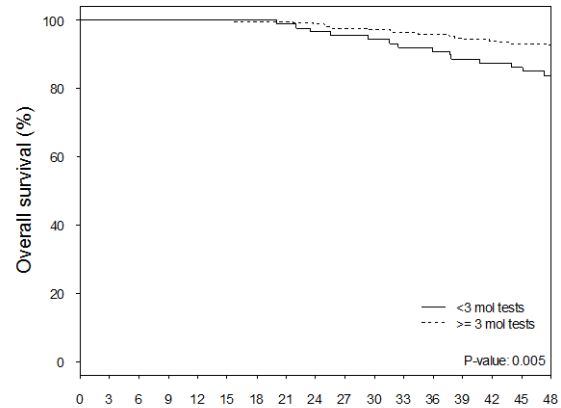
SI Figure S1. Kaplan-Meier survival plot of the 1-year landmark analysis of overall survival of patients who underwent no or at least one cytogenetic response assessment in the first year of TKI treatment.



No. at risk									
0 cyt	78	78	78	77	75	73	70	67	63
≥1 cyt	191	190	190	188	186	183	180	176	170

The log rank test was used to compare groups.
Abbreviations: cyt tests, cytogenetic response assessments; TKI, tyrosine kinase inhibitor

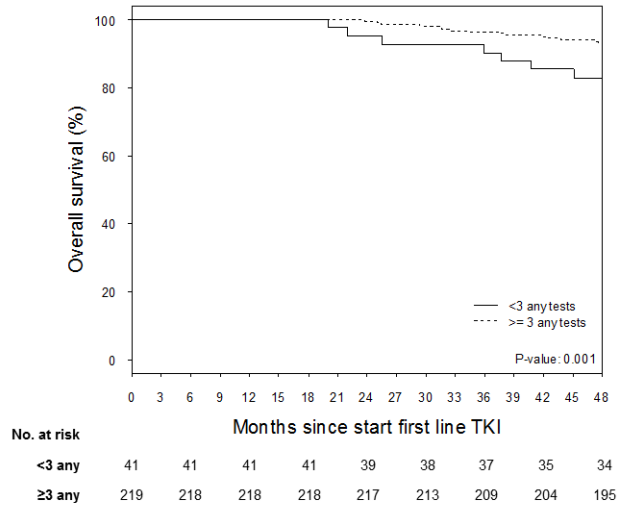
SI Figure S2. Kaplan-Meier survival plot of the 1-year landmark analysis of overall survival of patients who underwent less than 3 or at least 3 molecular response assessments in the first year of TKI treatment.



No. at risk									
<3 mol	87	87	87	87	84	82	79	72	63
≥3 mol	251	251	251	248	246	241	236	225	193

The log rank test was used to compare groups.
Abbreviations: mol tests, molecular response assessments; TKI, tyrosine kinase inhibitor

SI Figure S3. Kaplan-Meier survival plot of the 1-year landmark analysis of overall survival of patients who underwent less than 3 or at least 3 response assessments of any type in the first year of TKI treatment.



No. at risk									
<3 any	41	41	41	41	39	38	37	35	34
≥3 any	219	218	218	218	217	213	209	204	195

The log rank test was used to compare groups.

Abbreviations: any tests, response assessments of any type; TKI, tyrosine kinase inhibitor

CHAPTER 6

Omitting cytogenetic assessment from routine treatment response monitoring in CML is safe

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Abstract

Background: The monitoring of response in chronic myeloid leukemia (CML) is of great importance to identify patients failing their treatment in order to adjust TKI choice and thereby prevent progression to advanced stage disease. Cytogenetic monitoring has a lower sensitivity, is expensive, and requires invasive bone marrow sampling. Nevertheless, chronic myeloid leukemia guidelines continue to recommend performing routine cytogenetic response assessments, even when adequate molecular diagnostics are available.

Methods: In a population-based registry of newly diagnosed CML patients in the Netherlands all simultaneous cytogenetic and molecular assessments performed at 3, 6 and 12 months were identified and response of these matched assessments was classified according to European Leukemia Net (ELN) recommendations, The impact of discrepant cytogenetic and molecular response classifications and course of patients with additional chromosomal abnormalities were evaluated.

Results: The overall agreement of 200 matched assessments was 78%. In case of discordant responses, response at 24 months was consistently better predicted by the molecular outcome. Cytogenetic response assessments provided relevant additional clinical information only in some cases of molecular "warning". The development of additional cytogenetic abnormalities was always accompanied with molecular failure.

Conclusion: We conclude that it is safe to omit routine cytogenetics for response assessment during treatment and to only use molecular monitoring in order to prevent ambiguous classifications, reduce costs and reduce the need for invasive bone marrow sampling. Cytogenetic re-assessment should still be performed when molecular response is suboptimal.

Introduction

The importance of frequent monitoring of treatment response in patients with chronic myeloid leukemia (CML) is well established.[1-3] Timely adjustment of Tyrosine Kinase Inhibitor (TKI) therapy in patients with a failing response is essential to prevent progression from chronic to advanced stage disease as this carries a poor prognosis. Various guidelines, including the widely used European Leukemia Net (ELN) recommendations[4], have defined response targets at several time points by hematological remission status, cytogenetic response from metaphase karyotyping and molecular response by *BCR-ABL1* reverse transcription quantitative PCR (RT-qPCR). Compared to RT-qPCR, cytogenetics have low sensitivity due to the low number of analyzed metaphases, the need of a bone marrow aspiration makes it a painful procedure for the patient and karyotyping is costly. It is however the only technique to detect the prognostically unfavorable additional cytogenetic abnormalities (ACAs).[5-8]

Current treatment guidelines emphasize the central role of molecular monitoring when available on the international scale, but continue to recommend routinely performing cytogenetic response monitoring in parallel. The 2013 ELN recommendations do state that cytogenetics can be omitted if adequate molecular monitoring can be performed, but also that whenever possible, both cytogenetic and molecular tests are recommended until a complete cytogenetic response (CCyR) and major molecular response (MMR) are achieved.[4] Also in the 2017 update of the European Society of Medical Oncology (ESMO) guidelines, it is still recommended to assess the bone marrow karyotype at 3 and 6 months and every 6 months thereafter until CCyR has been achieved.[9] As may be expected, simultaneous assessment of cytogenetic and molecular response may result in discrepant response classifications between cytogenetic and molecular assessments.[10] We wondered if the routine monitoring of cytogenetic response provides critical information in clinical practice and if it would be safe to omit routine cytogenetic testing. We therefore used data from a population-based registry to evaluate how many simultaneous cytogenetic and molecular response assessments resulted in essential prognostic information, which could not have been recognized with routine hematological and molecular response monitoring alone.

Methods

Data were obtained from two complementary population-based registries (PHAROS CML and HemoBase) for newly diagnosed CML patients in the Netherlands in the period 2008-2013. Details on data sources and definitions have been previously published.[11] We included all patients aged ≥ 18 years presenting with CML in chronic phase who received first line TKI treatment and analyzed matched data for simultaneous cytogenetic and molecular response assessment (not more than two weeks apart) at 3, 6 and 12 months after treatment initiation (with a margin of 6 weeks). The cytogenetic assessment had to include at least twenty metaphase reviews. The Medical Ethics Committee of the Erasmus Medical Center in Rotterdam approved this study and the exemption from the requirement to obtain informed consent. The study was conducted in accordance with the Declaration of Helsinki.

Treatment responses were classified as 'optimal', 'warning' or 'failure' according to ELN recommendations.[4] All matched assessments were analyzed for the achievement of major molecular response (MMR, ≤0.1%IS) at 24 months. For all the patients with discordant responses, we analyzed the course of the disease in the first two years after treatment initiation focusing on cytogenetic and molecular responses at 3, 6 and 12 months, achievement of complete cytogenetic response (CCyR, 0% Philadelphia positive metaphases) and/or MMR at 18 and 24 months, TKI changes due to failure (including dose increases and TKI switch), progression to advanced stage disease and CML-related death. Also, we assessed the course of the molecular response in all patients with ACAs. Trisomy 8 (+8), trisomy Ph (+Ph), isochromosome 17q(i(17q)), trisomy 19 (+19) and isoderivative chromosome 22 (ider(22)(q10)) were defined as major route ACAs.[4]

Results

The two registries comprised 401 newly diagnosed CML-patients meeting the inclusion criteria. In this cohort, 200 simultaneous cytogenetic and molecular response evaluations complied with our definition of matched assessments; 70, 72 and 58 simultaneous assessments at 3, 6 and 12 months respectively. Table 1 provides an overview of the response classifications for the matched cytogenetic vs. molecular assessments performed in the first year. The overall agreement rate between the concomitant cytogenetic and molecular assessments was 78% (156/200) with a Kappa measure of agreement of 0.51 (95% CI [0.40-0.63], p<.001). Of the 44 discordant classifications in our cohort, 36 (18% of the total) involved a 'warning', but 8 (4% of total) scored an 'optimal' vs. 'failure' with the two different techniques.

Table 1. Matched assessments allocated into response categories

		Molecular response			
		Optimal	Warning	Failure	Total
Cytogenetic response	Optimal	126 (89%)	13 (37%)	4 (17%)	143
	Warning	12 (8%)	13 (37%)	2 (9%)	27
	Failure	4 (3%)	9 (26%)	17 (74%)	30
	Total	142 (100%)	35 (100%)	23 (100%)	200

A comparison of all matched assessments with molecular follow up available at 24 months (n=164) showed that the MMR achievement rate at 24 months was consistently associated with the molecular response categorizations during the first year of treatment, irrespective of the cytogenetic response category (Fig 1). In patients with an optimal molecular response, the 24 month MMR rate was high across cytogenetic response categories (80-100%), intermediate in patients with a molecular 'warning' (40-75%) and low in patients with molecular failure (0-31%).

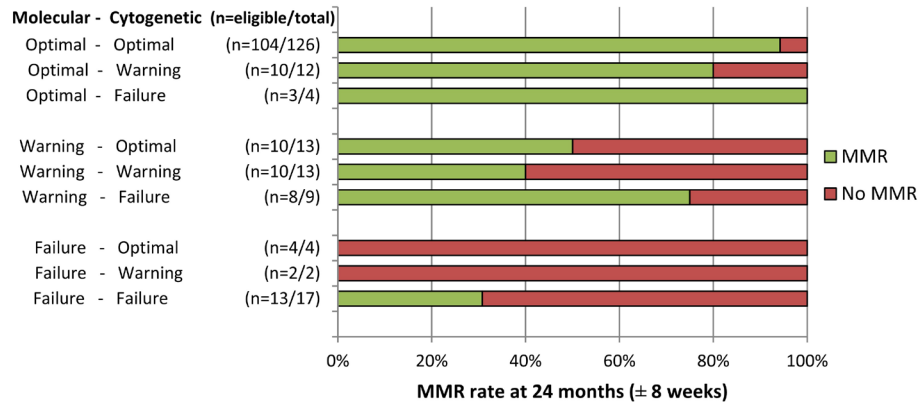
Of the assessments classified as molecular optimal (n=142), only 16 (11%) were accompanied by discordant cytogenetic responses; 12 (8%) cytogenetic warning responses and 4 (3%) cytogenetic failures. Supplementary table S1 shows the disease course of these patients. In 3 out of 4 patients the course of the molecular response after the discordant cytogenetic failures was excellent without any change in treatment. One patients with simultaneous molecular optimal and cytogenetic failure at 12 months, developed a loss of MMR at 14 months, which triggered the physician to switch TKI treatment. Simultaneous molecular optimal and cytogenetic warning results were only recognized at 3 and 6 months after the start of TKI treatment. They did not lead to TKI changes, but the majority of these patients (10/12) did develop subsequent molecular warning or failure responses within the first 24 months.

Only one third (37%) of the assessments in the molecular warning category had a corresponding cytogenetic warning test result. Simultaneous cytogenetic optimal results were seen at all three time points and were followed by the achievement of MMR in 8/12 (67%) patients in the absence of any treatment changes within the first 24 months of TKI treatment (supplementary table S2.). Likewise, concurrent molecular warning and cytogenetic failure results were observed at all three time points. This combination of outcomes demonstrated a more dramatic course of successive molecular responses and eventually resulted in an allogeneic stem cell transplantation due to TKI failure in two out of three patients with this specific discordance occurring at 3 months. Six out of nine patients (67%) achieved MMR in later assessments, which was preceded by a TKI change in three patients. After assessments with molecular warning and cytogenetic failure results, the rate of subsequent failures was higher than when the molecular warning was accompanied by cytogenetic optimal results (56% vs 38%, p=0.528).

A quarter (26%) of the molecular failures did not correspond with the cytogenetic results. Four patients had a simultaneous cytogenetic optimal test result, all observed at 12 months after TKI initiation (supplementary table S3). None of these patients achieved MMR in the subsequent assessments within the first 24 months. Whereas one out of two patients with concurrent cytogenetic warning responses both after 6 months of treatment, did manage to achieve MMR without a change in TKI treatment.

ACAs emerged on therapy in 2% (7/401) of the patients of which four patients developed major route chromosomal abnormalities. In six cases, a treatment failure signal based on molecular response arose preceding (n=2), simultaneously (n=2) or within six months after (n=2) the presentation of the ACA. The latter in the absence of hematological progression to advanced stage disease. For one patient, no molecular data were available in the time period surrounding the discovery of the ACA.

Figure 1. MMR rates at 24 months.



MMR rates for all matched assessments categories with molecular responses available at 24 months (± 8 weeks).

Abbr: MMR, major molecular response

Discussion

The reluctance to perform simultaneous cytogenetic and molecular response assessments is reflected in our population-based registry by the low number of matched assessments at 3, 6 and 12 months after treatment initiation (200 in 401 patients). The high overall agreement rate between the concomitant cytogenetic and molecular assessments is in line with previous findings, showing a strong correlation between molecular and cytogenetic assessments.[12-14]

There is one earlier study investigating discordant classifications according to the ELN criteria, which focused on patients with an 'optimal' versus 'warning' response.[10] This Italian study found a similar prevalence of discordant results as we did in our cohort (12.6 and 10.6% at three months; 17.4 and 17.5% at six months respectively). Moreover, this study found significantly lower MMR rates at 12 months in patients with discordant warning results at 3 and 6 months, mainly caused by molecular warnings. This is in line with our observations of a better predictive value of molecular response assessment. Additionally we observed that no TKI switch took place in any of the patients directly after an optimal molecular but cytogenetic warning or failure result illustrating that the treating clinicians decided to ignore these discrepant cytogenetic signals.

The occurrence of discrepant cytogenetic failure and warning responses can partly be explained by the stochastic effect involved in chromosome banding due to analyzing small sample sizes.[13] This phenomenon could be enhanced by selection bias of cells with potent mitotic capacity arising when harvesting metaphases from bone marrow aspirates.[13,15] The 1-log difference in the level of leukemic load considered as an optimal response for both assessments from 12 months onwards[4] can account for the manifestation of discordant molecular failures at 12 months.

Our data show that routine cytogenetic response monitoring did not provide relevant additional information in case of 142 molecular optimal responses, which would not have emerged on molecular monitoring alone. In patients with molecular failures, the course of the patients was not influenced by the results of the cytogenetic response either. In case of molecular warning responses, additional cytogenetic information could be used for the decision to change the treatment strategy as our data suggest that the cytogenetic response in these cases could help to predict future molecular failures, although no statistical significance could be demonstrated due to low numbers and effects of TKI changes in the cytogenetic failure group. In the patients who developed ACAs, not achieving molecular milestones or the loss of molecular remission provided an adequate signal to perform further diagnostics such as bone marrow cytogenetics. Previous work evaluating

828 matched assessments also showed that the additional value of routine cytogenetic value was limited, since only one patient with clonal evolution was identified without a prior or simultaneous significant increase in *BCR-ABL1* levels. This patient proceeded directly to allogeneic stem cell transplantation and therefore subsequent molecular response rates were not available for evaluation.[13]

A limitation to population-based registry studies is that there is no externally monitored standardized patient follow-up and outcome assessment. This is reflected in our study by the limited number of simultaneously performed cytogenetic and molecular response assessments. However, the strength of population-based registries is that the data reflect 'real-world' clinical practice without selection of included patients. The registry data therefore provide a unique opportunity to assess a research question as posed in our current study that focuses on daily practice.

Taken together, we can deduct that routine cytogenetic response assessments were only of additional value in case of molecular warning responses, which comprised 35 out of 200 routinely matched assessments. Discrepant results involving a 'failure vs. 'optimal' classification occur at a low rate, but in these cases, the outcome is most reliably predicted by the molecular response assessment. Moreover, the development of ACAs was always accompanied with molecular failure, which could have triggered performing cytogenetic assessment by indication. Omitting routine cytogenetic monitoring would prevent ambiguous classifications complicating the interpretation of response assessment, reduce costs and the need for invasive bone marrow sampling. In addition, it will simplify the assessment of response, which may facilitate adequate monitoring by clinicians. If regular molecular monitoring is available, cytogenetic assessment should be limited to sampling at diagnosis and in case of loss of hematological response, molecular warning or failure response.

Acknowledgments

Peter Huijgens, currently chairman of the Netherlands Comprehensive Cancer Organisation (IKNL), initiated the PHAROS registry. Tom Wiggers, Wencke de Jager, Sanne Nijssen and Jolie Cheung entered patient data in the PHAROS database. Marianne van der Mark from the IKNL retrieved survival data from the Netherlands Cancer Registry. We thank all the hospitals and molecular labs in the Netherlands who participated in the PHAROS and Hemobase registries for their efforts.

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Supplementary tables

Supplementary table S1. Course of patients with molecular optimal and discordant cytogenetic responses

	No.	Time discordant response	Cytogenetic response				
			3mo	6mo	12mo	CCyR 18mo	CCyR 24mo
Cytogenetic failure-molecular optimal	1	3	100% (20/20)	0% (0/20)	0% (0/20)	Yes	Yes
	2	12	NA	NA	5% (1/20)	NA	NA
	3[A]	12	33% (3/9)	0% (0/22)	4% (1/26)	NA	NA
	4[B]	12	NA	5% (1/20)	5% (1/20)	NA	NA
Cytogenetic warning-molecular optimal	5[C]	3mo	43% (9/21)	18% (4/22)	0% (0/23)	NA	NA
	6[D]	3mo	45% (9/20)	60% (12/20)	0% (0/20)	Yes	Yes
	7	3mo	60% (18/30)	13% (4/30)	6% (2/31)	yes	NA
	8	3mo	60% (12/20)	NA	NA	NA	NA
	9	3mo	45% (9/20)	NA	NA	NA	NA
	10	6mo	8% (2/24)	5% (1/22)	NA	NA	yes
	11[C]	6mo	43% (9/21)	18% (4/22)	0% (0/23)	NA	NA
	12	6mo	NA	3% (1/30)	NA	NA	yes
	13[E]	6mo	0% (0/30)	3% (1/30)	3% (1/30)	Yes	NA
	14[B]	6mo	NA	5% (1/20)	5% (1/20)	NA	NA
	15[F]	6mo	5% (1/20)	5% (1/21)	0% (0/24)	NA	NA
	16[G]	6mo	NA	3% (1/30)	3% (1/30)	Yes	NA

Green is an optimal response according to ELN recommendations, orange represents a warning response and a red cell illustrates a treatment failure.
No. are the numbers of discordant response, some patients can have different discordant results at different time points as indicated with the corresponding letters.

Molecular response					TKI change due to failure**	Progression**	CML-related death**	Transplantation**
3mo	6mo	12mo	MMR 18mo	MMR 24mo				
3%	0,45%	0,03%	Yes	Yes	No	No	No	No
NA	0,2%	0,06%	Yes	Yes	No	No	No	No
23,1%	1,6%	0,09%	yes	Yes*	No	No	No	No
4,0%	0,4%	0,05%	no	yes	Switch, 419	No	no	No
8,4%	0,7%	0,09%	no	Yes	Switch, 1030	No	no	no
0,5%	7,8%	0,09%	Yes	Yes	No	no	No	No
4,1%	2,9%	1,1%	yes	yes	Unknown	No	no	no
1,1%	0,6%	0,5%	no	no	Switch, 556	No	no	no
2,3%	1,1%	NA	NA	NA	Switch, 146	no	no	no
0,8%	0,09%	0,05%	NA	NA	No	no	no	no
8,4%	0,7%	0,09%	no	yes	Switch, 1030	No	no	no
4,6%	1,0%	0,03%	yes	yes	No	No	no	no
1,1%	0,8%	0,2%	Yes	Yes	Switch, 387	no	No	No
4,0%	0,4%	0,05%	no	yes	Switch, 419	No	no	no
3,1%	0,4%	0,2%	yes	no	No	AP/CE, 188	No	No
28,0%	0,6%	0,9%	Yes	Yes	Switch, 370	AP/CE, 362	No	No

*MMR maintained at 21 and 29 months, no molecular assessment performed at 24 months (+/- 8 weeks)

**The figures in this column represent the numbers of days from start TKI until event

Abbr: MMR, major molecular response; CCyR, complete cytogenetic response; AP/CE, accelerated phase with clonal evolution; NA, not available

Supplementary table S2. Course of patients with molecular warning and discordant cytogenetic responses

	No.	Time discordant response	Cytogenetic response				
			3mo	6mo	12mo	CCyR 18mo	CCyR 24mo
Molecular warning- cytogenetic optimal	1	3mo	29% (6/21)	0% (0/21)	NA	NA	NA
	2	3mo	33% (10/30)	0% (0/30)	NA	yes	NA
	3	6mo	NA	0% (0/21)	NA	NA	NA
	4[A]	6mo	33% (3/9)	0% (0/22)	4% (1/26)	NA	NA
	5[H]	6mo	NA	0% (0/30)	0% (0/30)	yes	yes
	6	6mo	10% (3/30)	0% (0/30)	NA	NA	NA
	7	12mo	NA	NA	0% (0/30)	NA	yes
	8	12mo	NA	NA	0% (0/30)	NA	NA
	9	12mo	NA	NA	0% (0/20)	yes	NA
	10[H]	12mo	NA	0% (0/30)	0% (0/30)	yes	yes
	11	12mo	75% (15/20)	0% (0/20)	0% (0/20)	NA	NA
	12[I]	12mo	NA (/0)	60% (12/20)	0% (0/20)	yes	yes
	12[F]	12mo	5% (1/20)	5% (1/21)	0% (0/24)	NA	NA
Molecular warning - cytogenetic failure	14[J]	3mo	100% (20/20)	20% (4/20)	5% (1/20)	Yes	NA
	15	3mo	100% (20/20)	35% (7/20)	NA	NA	NA
	16	3mo	100% (20/20)	90% (9/10)	30% (6/20)	NA	NA
	17	6mo	NA	40% (12/30)	NA	Yes	NA
	18[D]	6mo	45% (9/20)	60% (12/20)	0% (0/20)	Yes	Yes
	19[J]	6mo	NA (/0)	60% (12/20)	0% (0/20)	Yes	Yes
	20[G]	12mo	NA	3% (1/30)	3% (1/30)	Yes	NA
	21	12mo	NA	NA	9% (2/22)	No	NA
	22[E]	12mo	0% (0/30)	3% (1/30)	3% (1/30)	Yes	NA

Green is an optimal response according to ELN recommendations, orange represents a warning response and a red cell illustrates a treatment failure.

No. are the numbers of discordant response, some patients can have different discordant results at different time points as indicated with the corresponding letters.

Molecular response					TKI change due to failure**	Progression**	CML-related death**	Transplantation**
3mo	6mo	12mo	MMR 18mo	MMR 24mo				
16,8%	0,04%	NA	NA	NA	No	No	No	No
13,0%	0,8%	NA	yes	yes	No	No	No	No
NA	6,5%	NA	NA	NA	No	No	No	No
23,1%	1,6%	0,09%	yes	Yes*	No	No	No	No
NA	1,6%	0,2%	no	no	Dose ↑, 787	No	No	No
NA	3,5%	1,5%	no	no	Switch, 393	No	No	No
0,8%	0,44%	0,3%	yes	yes	No	No	No	No
NA	NA	0,8%	NA	yes	Dose ↑, 867	No	No	No
40,6%	4,9%	0,2%	yes	yes	No	No	No	No
NA	1,6%	0,2%	no	no	Dose ↑, 787	No	No	No
15,0%	NA	0,5%	no	no	No	No	No	No
1,2%	2,3%	0,1%	yes	yes	Switch, 1051	No	No	No
3,1%	0,4%	0,2%	yes	no	No	AP/CE, 188	No	No
89,5%	32,6%	5,1%	No	No	Switch, 639	no	Yes	Yes, 1213
13,3%	2,6%	0,8%	No	No	No	no	No	No
42,2%	30,2%	NA	NA	NA	Switch, 114	no	Yes	Yes, 428
11,0%	7,8%	1,5%	yes	Yes	Dose ↑, 332	no	No	No
0,5%	7,8%	0,09%	Yes	Yes	No	no	No	No
1,2%	2,3%	0,1%	Yes	Yes	Switch, 1052	no	No	No
28,0%	0,6%	0,9%	Yes	Yes	Switch, 370	AP/CE, 362	No	No
42,6%	1,5%	0,1%	No	Yes	No	No	No	No
1,1%	0,8%	0,2%	Yes	Yes	Switch, 387	no	No	No

*MMR maintained at 21 and 29 months, no molecular assessment performed at 24 months (+/- 8 weeks)
**The figures in this column represent the numbers of days from start TKI until event
Abbr: MMR, major molecular response; CCyR, complete cytogenetic response; AP/CE, accelerated phase with clonal evolution; NA, not available

Supplementary table S3. Course of patients with molecular failure and discordant cytogenetic responses

	No.	Time discordant response	Cytogenetic response				
			3mo	6mo	12mo	CCyR 18mo	CCyR 24mo
Cytogenetic optimal-molecular failure	1	12mo	NA	22% (2/9)	0% (0/20)	NA	NA
	2	12mo	NA	NA	0% (0/30)	NA	NA
	3	12mo	33% (10/30)	40% (4/10)	0% (0/30)	NA	NA
	4	12mo	NA	70% (14/20)	0% (0/30)	Yes	NA
Cytogenetic warning-molecular failure	5	6mo	45% (9/20)	25% (5/20)	NA	NA	NA
	6[j]	6mo	100% (20/20)	20% (4/20)	5% (1/20)	Yes	NA

Green is an optimal response according to ELN recommendations, orange represents a warning response and a red cell illustrates a treatment failure.
No. are the numbers of discordant response, some patients can have different discordant results at different time points as indicated with the corresponding letters.

Molecular response					TKI change due to failure**	Progression**	CML-related death**	Transplantation**
3mo	6mo	12mo	MMR 18mo	MMR 24mo				
NA	NA	1%	No	No	No	No	No	No
20%	6%	6%	No	No	No	No	No	No
10%	5%	1%	No	No	Switch, 276	No	No	No
81%	45%	4%	No	No	Switch, 209	No	No	No
18,0%	14,0%	0,9%	no	no	no	no	no	no
89,5%	32,6%	5,1%	No	No	Switch, 639	no	yes	Yes, 1213

*MMR maintained at 21 and 29 months, no molecular assessment performed at 24 months (+/- 8 weeks)
**The figures in this column represent the numbers of days from start TKI until event
Abbr: MMR, major molecular response; CCyR, complete cytogenetic response; AP/CE, accelerated phase with clonal evolution; NA, not available

CHAPTER 7

Improving molecular response by switching imatinib to nilotinib combined with pegylated interferon-α2b in chronic phase CML

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Abstract

Confirmed deep molecular response rates ($MR^{4.5}$, *BCR-ABL1*^{IS} <0.0032%) in chronic phase chronic myeloid leukemia (CML) are currently achieved by 37-46% of CML patients after 8 years of imatinib treatment. For the majority of patients treated with imatinib, a stopping attempt is therefore not feasible. In order to improve molecular response depth enabling a treatment cessation trial, we evaluated whether CML patients, who did not achieve at least a $MR^{4.0}$ (*BCR-ABL1*^{IS}<0.01%) after long-term imatinib therapy, could attain $MR^{4.0}$ after a switch to nilotinib combined with pegylated interferon-α2b (PegIFN). In this NordDutchCML009 study, the primary endpoint of confirmed $MR^{4.0}$ was reached by 44% (95% CI: 23-67%) of the patients (n=16), with 81% (95% CI: 57-93%) of patients achieving an unconfirmed $MR^{4.0}$. The scheduled combination was only completed by 56% of the patients, with discontinuations mainly due to mood disturbances after the introduction of PegIFN. In view of the low number of enrolled patients, variable molecular response levels at inclusion and most pronounced decline in molecular response observed prior to the addition of PegIFN and the uncontrolled design of the study, these results should be interpreted with caution. Moreover, we concluded that this treatment strategy is not feasible in this particular setting due to the high rate of PegIFN discontinuation, primarily due to a substantial incidence of mood disturbances. This trial was registered at www.clinicaltrials.gov as #NCT01866553.

Introduction

The introduction of tyrosine kinase inhibitors (TKIs) like imatinib has led to an impressive improvement in survival with life expectancy of the majority of chronic myeloid leukemia (CML) patients now approaching that of the general population.[1,2] After 8 years of imatinib treatment, confirmed deep molecular response rates ($MR^{4.5}$, *BCR-ABL1*^{IS} <0.0032%) were seen in 37-46% of patients.[3,4] In 40-50% of these patients, TKI treatment could successfully be stopped.[5-7] This is remarkable, as in vitro data show that TKIs are unable to eradicate all leukemic cells, probably since CML stem cells do not depend on *BCR-ABL1* activity for their survival.[8,9] Unfortunately, 50-60% of stopping patients relapse after discontinuation, mostly within 6-7 months of discontinuation.[10] Up to now, patients not attaining deep molecular remissions like $MR^{4.0}$ were not enrolled in stopping studies as they were expected to relapse in a very high percentage of cases. Thus, a large number of patients are destined to continue their TKI treatment life-long, with side effects potentially reducing the quality of life for patients and high medical costs. In an effort to improve the molecular response and potentially increase the number of patients eligible for a stopping attempt, we performed the NordDutch CML 009 study. In this phase 2, single arm, multicenter study, patients with a response level above $MR^{4.0}$ (*BCR-ABL1*^{IS} >0.01%IS) after at least two years of imatinib treatment were enrolled. We evaluated whether a switch to nilotinib and subsequent addition of pegylated interferon-α2b (PegIFN) could increase the number of patients with $MR^{4.0}$. The current paper presents the efficacy, safety and tolerability of this treatment strategy in a phase II study performed in 16 patients.

Methods

Study design

Chronic phase (CP)-CML patients (aged ≥ 18 years) with a *BCR-ABL1* level above $MR^{4.0}$ after more than two years of imatinib treatment could be enrolled. Persistent disease had to be demonstrated by two PCR positive tests ($BCR\text{-}ABL1^{IS}$ >0.01%) performed within 9 months before inclusion and with at least 10 weeks between positive tests. A detailed overview of in- and exclusion criteria is reported in the *Supplementary materials*. Novartis provided nilotinib tablets and MSD provided pegylated interferon-α2b (PegIFN) as study drugs. This study was conducted in accordance with the Declaration of Helsinki and all patients provided written informed consent. All local medical ethical committees approved the protocol. The NordDutchCML009 trial was registered at www.clinicaltrials.gov as NCT01866553.

Treatment

Patients were switched from imatinib to nilotinib 300 mg BID at the start of the study. In addition to the nilotinib treatment, subcutaneous injections of PegIFN were introduced at 25μg/week after three months and if well tolerated, this dose was increased to 40μg/weeks in month 6 and continued until month 12. The combination treatment phase was followed by 12 months of nilotinib monotherapy (300 mg BID) until end of study at 24 months. PegIFN dose reductions because of adverse events (AEs) down to 15 μg/2 weeks were allowed. For nilotinib, dose reductions down to 300 mg once daily were permitted.

Safety assessments

Blood chemistry and hematological laboratory assessments were performed at least every month in the first six months of the study and 3 monthly thereafter. ECG evaluation of the QTc time was performed at 1 and 4 months after nilotinib initiation. Adverse events (AEs) were assessed according to the common Toxicity Criteria for Adverse Events (CTCAE) version 3.0 and categorized as biochemical, hematological and non-hematological AEs. Grade 1 AEs were not reported. AEs occurring in the period between inclusion and Month 3 were attributed to nilotinib and the later occurring side effects to pegIFN or the combination. Dose reductions were made to the drug most likely to have caused the event. If unclear, pegIFN was stopped before nilotinib. Dose adjustments were permitted in case of grade 3 or 4 hematologic AEs and in case of grade 2, 3 or 4 non-hematologic AEs.

Study endpoints

The proportion of patients with confirmed $MR^{4.0}$ at month 12 (a $BCR\text{-}ABL1^{IS}$ *level* ≤0.01% both at 12 and 15 months) was the primary endpoint for this study. To estimate the clinical impact of PegIFN in the combination treatment, we aimed to compare our results with

the historic ENESTcmr cohort, where patients with similar inclusion criteria were randomized to either continue imatinib monotherapy or switch from imatinib to nilotinib monotherapy.[20] The addition of PegIFN was considered of clinical interest if the rates of $MR^{4.0}$ in the present study were at least 25% higher than in the nilotinib arm of the historical cohort. Secondary endpoints included safety and tolerability, frequency and type of AEs/serious AEs. Other secondary endpoints were the proportion of patients with $MR^{4.0}$ and with $MR^{4.5}$ (*BCR-ABL1*^{IS} <0.0032%) at or by Month 3, 6, 12, 18 and 24, the proportion of patients who completed the planned 9 months of combination therapy with PegIFN, the rate of loss of complete cytogenetic response (CCyR, 0% Philadelphia positive chromosomes), major molecular response (MMR, *BCR-ABL1*^{IS}<0.1%) and $MR^{4.0}$ at month 12 and 18, the proportion of patients progressing to advanced disease phase, overall survival of the participating patients and quality of life changes during the trial.

Molecular response monitoring

The reverse transcription quantitative PCR (RT-qPCR) measurements of *BCR-ABL1* in peripheral blood samples were performed in 3 Nordic and 4 Dutch EUTOS-certified hospital laboratories (Oslo, Uppsala, Umeå, Amsterdam, Nijmegen, Enschede, Rotterdam). *BCR-ABL1* levels were expressed on the international scale (IS) and the *BCR-ABL1* copy numbers and control gene type and copy numbers were reported. Standardized definitions for deep molecular response were used.[11]

Quality of life

Quality of life was assessed using the EORTC QLQ-C30 questionnaire[12] in combination with the CML specific questionnaire EORTC QLQ-CML24, which was validated in a large international cohort of CML patients treated with various TKIs and proven to be consistent across languages and cultures.[13] Questionnaires were handed out to patients at baseline, 3, 6, 12 and 18 months.

Statistics

Molecular response and discontinuation rates were calculated for the intention-to-treat population. Both cumulative and non-cumulative molecular response rates were analyzed. Changes in quality of life were evaluated by calculating the mean change score between baseline (IM) and month 3 (NIL3m) and between month 3 (NIL3m) and month 6 (NIL6m+IFN3m) respectively month 12 (NIL12m+IFN9m). Only patients with questionnaires available at both specific time points were evaluated. A mean change score of ±10% or more was considered a clinically meaningful difference.[14] A *P*-value <0.05 was considered statistically significant. Analyses were performed with SPSS version 24 (IBM SPSS statistics).

Results

Patient population

Patient enrollment for this trial started in September 2013 and the study was prematurely closed in April 2015 because of slow recruitment. A total of 16 CP-CML patients were enrolled. *BCR-ABL1*^{IS} response results were evaluated until 24 months after inclusion. Because of preliminary closure of the study, exhaustive information regarding AEs was only evaluable until 12 months after inclusion. Patient characteristics of the 16 eligible patients are presented in table 1. All 16 patients received at least one dose of nilotinib after study start and were therefore eligible for both the safety and intention-to-treat analysis.

Table 1. Patient characteristics

	Median (range)	**N (%)**
Total		16 (100)
Patient characteristics at time of original diagnosis		
Male gender		10 (63)
Age, years	37 (20-63)	16 (100)
CML risk scores		
Sokal: low / intermediate / high		7 (44) / 5 (31) / 4 (25)
Hasford: low / intermediate / high		8 (50) / 5 (31) / 3 (19)
ELTS: low / intermediate / high		8 (50) / 4 (25) / 4 (25)
Cytogenetic abnormalities*		4 (27)
Patient characteristics at time of study inclusion		
Duration between diagnosis and inclusion, years	5.2 (2.5-15.1)	16 (100)
≤ 36 months		3 (19)
> 36 months		13 (81)
Charlson Comorbidity index: 2 / 3-4 / 5-6 / ≥7		12 (75) / 2 (13) / 1 (6) / 1 (6)
Time to first CCyR, years	0.8 (0.1-5.2)	15 (94)
Last two molecular tests before inclusion:		
BCR-ABL (IS) 1	0.028 (0.006-3.90)	16
Time between assessment and inclusion, months	7.2 (3.0-10.1)	
BCR-ABL (IS) 2	0.127 (0.0021-1.44)	16
Time between assessment and inclusion, months	1.7 (0-5.0)	
Responses at study start	0.033 (0.009-7.390)	16
No CCyR		1 (6)
CCyR but no MMR		3 (19)
MMR but no MR$^{4.0}$		11 (69)
MR4 but no MR$^{4.5}$**		1 (6)

* at diagnosis, 2x Variant translocation, 1x masked translocation, 1x additional t(8;17); simple t(9;22) in remaining patients.

** Two previous assessments at -7 and -3 months before inclusion were both MMR but no MR$^{4.0}$

Efficacy

The primary end point of confirmed $MR^{4.0}$ at 12 months was reached by 44% (7/16 patients, 95% confidence interval (CI: 23- 67%)) of patients. The unconfirmed rate of $MR^{4.0}$ (any single measurement of $BCR\text{-}ABL1^{IS}$ ≤0.01%) by 12 months was 81% (13/16 patients, 95% CI: 57-93%). Figure 1 shows the $BCR\text{-}ABL1^{IS}$ measurements during treatment for each individual patient (solid colours), and also as the median of the cohort (dotted line). The median $BCR\text{-}ABL1^{IS}$ level decreased by 0.03% in the first three months, increased by 0.001% between month 3 and month 6 and increased again by 0.001% between month 6 and month 12. Table 2 shows the proportion of patients with $MR^{4.0}$ and $MR^{4.5}$ at or by month 3, 6, 12, 18 and 24. No patient died, progressed to advanced disease phase or lost CCyR. One patient lost MMR at 24 months. Three patients did not reach a single $MR^{4.0}$ measurement during the 24 months trial duration. Six patients lost $MR^{4.0}$ over the course of the 24 months. All of these patients did not reach the primary endpoint.

Table 2. Proportion of patients with $MR^{4.0}$ and $MR^{4.5}$

	Baseline	**Month 3**	**Month 6**	**Month 12**	**Month 18**	**Month 24**
$BCR\text{-}ABL1^{IS}$						
Non-cumulative	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
No $MR^{4.0}$	15 (94)	6 (37)	5 (31)	6 (38)	5 (31)	4 (25)
$MR^{4.0}$	1 (6)	10 (63)	10 (63)	8 (50)	11 (69)	12 (75)
$MR^{4.0}$ but no $MR^{4.5}$	1 (6)	8 (50)	5 (31)	4 (25)	1 (6)	4 (25)
$MR^{4.5}$	0 (0)	2 (13)	5 (31)	4 (25)	10 (63)	8 (50)
Inevaluable		-	1 (6)	2 (13)	-	-
Cumulative	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
No $MR^{4.0}$	15 (94)	6 (37)	5 (31)	3 (19)	3 (19)	3 (19)
$MR^{4.0}$	1 (6)	10 (63)	11 (69)	13 (81)	13 (81)	13 (81)
$MR^{4.0}$ but no $MR^{4.5}$	1 (6)	8 (50)	5 (31)	6 (38)	2 (13)	1 (6)
$MR^{4.5}$	0 (0)	2 (13)	6 (38)	7 (44)	11 (69)	12 (75)
Inevaluable	-	-	-	-	-	-

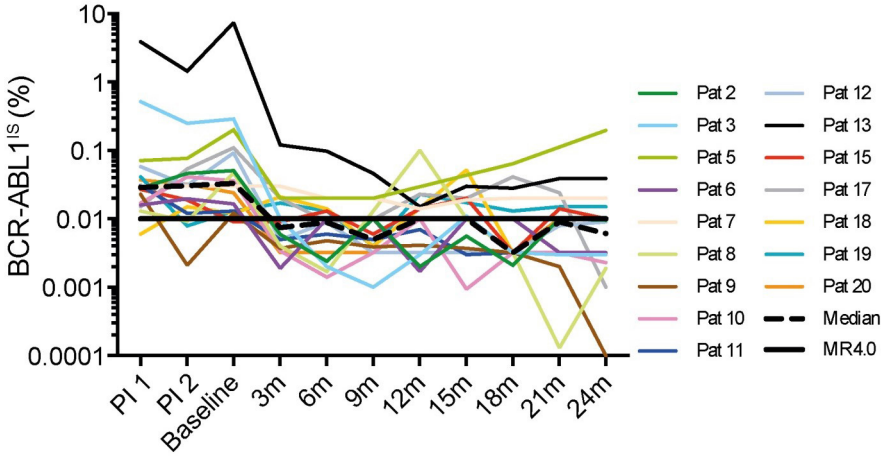
Abbr: MR, molecular response; $MR^{4.0}$, $BCR\text{-}ABL^{IS}$≤ 0.01%; $MR^{4.5}$, $BCR\text{-}ABL^{IS}$≤ 0.0032%.

Dosing and safety

During the first 12 months on study, the whole cohort received 86% of scheduled nilotinib, with a mean dose per patient of 515 mg/day, and 64% of scheduled pegIFN, with a mean dose per patient of 22μg/week. Only 9 out of 16 patients (56%, 95% CI: 33-77%) completed the scheduled combination treatment over the first 12 months. The reasons for drug discontinuations during the first 12 months are shown in figure 2. Data on reported AEs during the first 12 months are shown in table 3.

One patient experienced a serious AE (SAE) 4 months after inclusion consisting of a pneumonia (grade 3) requiring hospitalization. This SAE was designated as unlikely to be related to the study treatment. Another patient experienced two SAEs after the first twelve months of treatment. The first SAE was an ICU admission due to pneumosepsis in month 13 that required intubation and vasopressors. This SAE was considered as unlikely to be related to the study treatment. The second SAE concerned pericardial effusion in month 16 resulting in cardiac tamponade that required pericardiocentesis and an ICU admission. This SAE was assessed as possibly related to the nilotinib treatment.

Figure 1. Alterations in *BCR-ABL*^{IS} levels before and after switching to nilotinib and combination therapy with PegIFN.



The graph shows the changes in molecular response of each patient during therapy. The median of the cohort is shown in a dotted black line and MR$^{4.0}$ (*BCR-ABL1*^{IS} = 0.01%) is shown as a horizontal black line.

Quality of life

The EORTC QLQ-C30 questionnaire was available from 16, 10, 9 and 8 patients at baseline, 3 months, 6 months and 12 months, respectively. At the same time points during treatment, the EORTC QLQ-CML24 questionnaire was available from 13, 9, 9 and 8 patients, respectively. Of the 9 patients completing both questionnaires at 3 and 6 months, 1 patient had stopped IFN treatment before 6 months. Of the 8 patients completing both questionnaires at 3 and 12 months, 3 patients had stopped IFN treatment before 12 months.

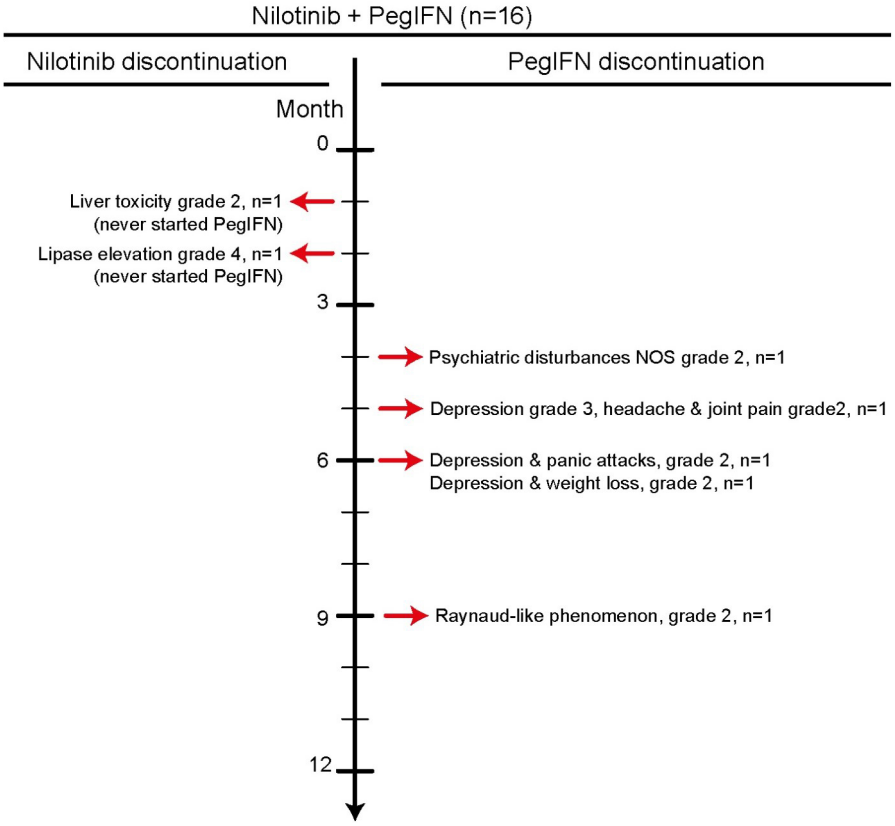
Three months after the switch to nilotinib, so just before the addition of PegIFN, patients self-reported an improvement in role functioning and satisfaction with social life, a

decrease in nausea and vomiting, less diarrhea, but higher pain, dyspnea and insomnia scores compared to baseline measurements (supplementary table S1), which were all clinically meaningful differences. After 6 months (3 months after the addition of PegIFN to NIL) clinically meaningful decreases in global health status, role functioning, social functioning and satisfaction with social life were observed, as well as increases in dyspnea, appetite loss and diarrhea. The decrease in social functioning and satisfaction with social life and the increase in dyspnea, appetite loss and diarrhea were still clinically meaningful differences at 12 months and therefore chronic, despite the decreased number of patients on NIL+PegIFN combination treatment at 12 months. New clinically relevant differences at 12 months compared to 3 months were a decreased emotional functioning and satisfaction with care and information, increased fatigue, nausea and vomiting, impact on worry mood and impact on daily life.

Table 3. Adverse events in the first 12 months

	Nilotinib (0-3 mo)		**Nilotinib + IFN (3-12 mo)**	
	Grade 2	*Grade 3-4*	*Grade 2*	*Grade 3-4*
	n (%)	*n (%)*	*n (%)*	*n (%)*
Non-haematological				
Headache	4 (25)	-	5 (31)	-
Depression/anxiety	-	-	3 (19)	2 (13)
Musculoskeletal	3 (19)	-	2 (13)	-
Rash/dermatological	3 (19)	-	1 (6)	-
Gastrointestinal	3 (19)	-	1 (6)	-
Flu-like symptoms	-	-	3 (19)	-
Fatigue	1 (6)	-	2 (13)	-
Non-cardiac chest pain	1 (6)	-	1 (6)	-
Infections	-	-	1 (6)	1 (6)
Weight loss	-	-	1 (6)	-
Insomnia	-	-	1 (6)	-
Injection site reaction	-	-	1 (6)	-
Alopecia	-	-	1 (6)	-
Miscellaneous	-	-	2 (13)	-
Haematological				
Leukopenia	-	-	4 (25)	-
Neutropenia	-	-	3 (19)	1 (6)
Biochemical				
Hypophosphatemia	-	-	6 (38)	1 (6)
Hypomagnesemia	-	-	1 (6)	-
Hepatotoxicity	3 (19)	-	1 (6)	-
Elevated Lipase	-	1 (6)	-	-

Figure 2. Drug discontinuations during the first 12 months of therapy.



An overview of the timing and reasons for drug discontinuations during the first 12 months of therapy is shown.

Abbr: PegIFN, pegylated Interferon-α2b; NOS, not otherwise specified.

Discussion

In this phase II single arm study, we evaluated whether switching to a combination of nilotinib and PegIFN in imatinib responders who had failed to achieve deep molecular responses (*BCR-ABL1*^{IS} <0.01%) after long-term treatment, would achieve improvement in molecular response. In an intention to treat analysis we found that 44% of the patients who had not achieved MR$^{4.0}$ on imatinib alone, attained confirmed MR$^{4.0}$ at 12 months by switching to combination therapy, which improvement was, however, already achieved by the majority of patients by nilotinib monotherapy during the first 3 months. The scheduled combination was only completed by 56% of the patients, with discontinuations mainly due to biochemical toxicity after switching to nilotinib and mood disturbances after the introduction of PegIFN. Moreover, a decline in several aspects of quality of life reflects the limited tolerability and feasibility of this treatment strategy for improving molecular responses.

The additive effect of PegIFN in our study may be compared with the 12-month follow-up data from the historical nilotinib arm of the ENESTcmr study.[15] The ENESTcmr study compared the effect of switching to nilotinib treatment (400 mg BID) with imatinib continuation in patients with detectable disease levels after a minimum of two years of imatinib treatment.[16] In a subgroup of patients with no MR$^{4.0}$ at baseline, an unconfirmed MR$^{4.0}$ rate of 48.6% was observed after 12 months in patients randomized for nilotinib, compared to 25.6% in the group who continued imatinib treatment (p=0.06).[15] Our study shows that in a comparable patient group, switching to nilotinib combined with PegIFN treatment resulted in an unconfirmed MR$^{4.0}$ rate of 81% (95% CI: 57-93%) at 12 months. The latter rate might suggest that the addition of PegIFN resulted in a higher rate of unconfirmed MR$^{4.0}$, but better molecular responses were already achieved during the first 3 months of nilotinib monotherapy, thereby questioning the additive effect of PegIFN. Although the difference clearly exceeded the limit that was deemed as clinically of interest, this effect of combination treatment should be interpreted with caution, as the size of our study cohort was small (n=16), there was no control arm consisting of patients who continued imatinib therapy, the levels of molecular response at inclusion could have influenced the attainment of MR$^{4.0}$ and, as shown in figure 1, the course of *BCR-ABL1* levels mainly showed a decline already in the first three months, when PegIFN was not yet added to the nilotinib therapy.

Improving the cytogenetic and molecular response by adding PegIFN to frontline imatinib therapy, has been reported before.[17-22] In a retrospective comparison, data from the Italian GIMEMA study group showed significantly higher CCyR (60% vs. 42%, p=0.003) and MMR (67% vs. 47%, p=0.001) rates after six months of combination treatment (n=76),

compared to monotherapy (n=419).[17-19] Similarly, a randomized controlled trial performed in the Nordic countries demonstrated higher MMR rates at 12 months in the imatinib plus PegIFN arm (n=56) than the monotherapy arm (n=56, 82% vs. 54%, p=0.002) and an effect of PegIFN treatment duration on response. The large French SPIRIT trial observed significantly higher $MR^{4.0}$ rates after 12 and 24 months in 159 patients who received combination treatment with 90μg PegIFN-α2a / week compared to 160 patients who received imatinib 400 mg monotherapy (30% vs. 14%, p=0.001 and 38% vs. 21%, p=0.001, respectively).[20] Again, longer duration of PegIFN affected response rates positively. Interestingly, the CML IV study did not show any benefit from the addition of non-pegylated IFN to imatinib 400 mg in a large cohort of patients.[21] Moreover, two small single arm studies combining frontline PegIFN with nilotinib[22] and dasatinib[23] have been published, showing relatively favourable, deep molecular response rates. Preliminary results from two large randomized controlled trials, evaluating the efficacy and safety of upfront nilotinib with nilotinib plus PegIFN will be expected soon.

Several immunological mechanisms of actions have been proposed, which, however, have never been substantially documented in *in vitro* and *in vivo* studies. Putative mechanisms of actions included activation of the autologous immune compartment, NK- and $CD8^+$ T cells in particular, that might prevent the escape of leukemic clones form such an immune response.[27] IFN has shown to restore adhesion of leukemic stem cells to stroma cells in the bone marrow microenvironment *in vitro*,[24] and β1-intergrin-mediated inhibition of leukemic stem cell (LSC) proliferation.[25] IFN has also been shown to induce cell cycle entry of dormant stem cells in vitro, which might sensitize the CML stem cells to imatinib.[26] More robust, a direct anti-proliferative effect of IFN has been well documented by several investigators.[28,29] An inhibition of outgrowth of primitive CML progenitor cells was shown, without eradication of normal or malignant early progenitor cells. Only the latter observation was supported by both *in vitro* and *in vivo* data and cell cultures of responders to IFN-alpha were characterized by a preferential inhibition of the outgrowth of malignant progenitor cells.

The high unconfirmed $MR^{4.0}$ rate after 12 months of nilotinib plus PegIFN (25-40 μg/week) combination treatment in our cohort (81%, 95% CI: 57-93%) was reached despite high PegIFN discontinuation rates (44%, 95%CI: 23-67%). This large number of premature PegIFN cessations is in line with the abovementioned studies combining higher dosages of PegIFN (50-150μg/week) with upfront imatinib (45-87%).[17-20,30] An amendment of the SPIRIT trial, lowering the PegIFN dose from 90 to 45μg/week showed similar efficacy but better tolerability, resulting in significantly lower discontinuation rates.[31] The discontinuation rates in our study seem higher than observed in the two studies combining upfront low dose (15-45 μg/week) PegIFN with dasatinib (27%) and nilotinib (22.5%).[22,23] It could be

hypothesized that in a second line setting, lower levels of toxicity are acceptable both for patients and physicians in view of the molecular responses already achieved.

The adverse events profile of the present study was fairly comparable with the NiloPeg trial, including the high incidence of depression/anxiety (32% vs. 34%).[22] We observed psychiatric disturbances more frequently than in previous combination trials of interferon and imatinib (2-13%)[17-20,30] and dasatinib (13%).[23] This is also reflected in the patient self-reported changes in quality of life during treatment, although inconsistent completion of the forms enabled only a descriptive analysis. We observed an initial and general improvement in QoL after switching from imatinib to nilotinib and prior to starting PegIFN (3 months). However, after starting on PegIFN we saw an expected decline in several QoL measures (6 months), some even persisting after PegIFN cessation (12 months), indicating chronic ailments. Collectively, both the premature cessation of IFN and the high percentage of side effects led to a conclusion of non-feasibility in our study.

In conclusion, this NordDutchCML009 study was the first study introducing a TKI plus PegIFN combination treatment as second line therapy in order to improve the molecular response. While the response showed some improvement, the most pronounced decline in *BCR-ABL1* levels was observed prior to the addition of PegIFN during nilotinib monotherapy, questioning the need for combination therapy. Moreover, the high rate of PegIFN discontinuation, primarily due to a substantial incidence of mood disturbances, indicated poor feasibility.

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Supplementary methods

Inclusion Criteria:

1. Patients ≥18 years of age.
2. At diagnosis, chronic myeloid leukemia in chronic phase.
3. Persistent disease demonstrated by two PCR positive tests (i.e. *BCR-ABL* level above 0.01% IS) which have been performed during the past 9 months and more than 10 weeks apart. One of these should be performed within 1 month of registration.
4. Treatment with imatinib ≤ 600 mg for at least 2 years. The dose must have been stable in the previous 6 months and, before that, may not have exceeded 600 mg because of pre-existent hematologic, cytogenetic or molecular resistance.
5. No other current or planned anti-leukemia therapies.
6. ECOG Performance status 0, 1, or 2.
7. Adequate organ function as defined by:
8. Total bilirubin <1.5 x ULN (ULN = local lab upper limit of normal). Does not apply to patients with isolated hyperbilirubinemia (e.g. Gilbert's disease) grade <3.
9. ASAT and ALAT <2.5 x ULN.
10. Serum amylase and lipase ≤1.5 x ULN.
11. Alkaline phosphatase ≤2.5 x ULN.
12. Creatinine clearance >30 ml/min.
13. Mg++, K+ ≥LLN.
14. Life expectancy of more than 12 months in the absence of any intervention
15. Patient has given written informed consent to participate in the study.

Exclusion criteria:

1. Prior accelerated phase or blast crisis.
2. Patient has received another investigational agent within last 6 months.
3. Previous treatment with nilotinib or dasatinib.
4. Prior stem cell transplantation.
5. Impaired cardiac function including any one of the following:
    a) Inability to monitor the QT/QTc interval on ECG.
    b) Long QT syndrome or a known family history of long QT syndrome.
    c) Clinically significant resting brachycardia (<50 beats per minute).
    d) QTc >450 msec on baseline ECG (using the QTcF formula). If QTcF >450 msec and electrolytes are not within normal ranges, electrolytes should be corrected and then the patient re-screened for QTc.
    e) Myocardial infarction within 12 months prior to starting study.
    f) Other clinically significant uncontrolled heart disease (e.g. unstable angina, congestive heart failure or uncontrolled hypertension).
    g) History of or presence of clinically significant ventricular or atrial tachyarrhythmias.

6. Known atypical *BCR-ABL* transcript not quantifiable by standard RQ-PCR
7. Presence of uncontrolled cardiovascular risk factors: history of cardiovascular events, like myocardial infarction, symptomatic vascular disease, stroke or transient ischemic attacks; untreated hypertension; untreated hypercholesterolemia, smoking, when patient refuses to quit; poorly controlled diabetes mellitus (i.e. HbA1c >9.0% (>75 mmol/mol)) or clinically relevant diabetic complications such as neuropathy, retinopathy, nephropathy, coronary or peripheral vascular disease.
8. History of active malignancy during the past 5 years with the exception of basal carcinoma of the skin or carcinoma in situ of cervix uteri or breast.
9. Acute liver disease or cirrhosis.
10. Previous or active acute or chronic pancreatic disease.
11. Another severe and/or life-threatening medical disease.
12. History of significant congenital or acquired bleeding disorder unrelated to cancer.
13. Impairment of gastrointestinal (GI) function or GI disease that may significantly alter the absorption of study drug.
14. Patients actively receiving therapy with strong CYP3A4 inhibitors and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug.
15. Patients who are currently receiving treatment with any medications that have the potential to prolong the QT interval and the treatment cannot be either discontinued or switched to a different medication prior to starting study drug.
16. Patients who are:
    a) pregnant.
    b) breast feeding.
    c) of childbearing potential without a negative pregnancy test prior to baseline.
    d) male or female of childbearing potential unwilling to use contraceptive precautions throughout the trial (post-menopausal women must be amenorrheic for at least 12 months to be considered of non-childbearing potential).
17. Interruption of imatinib therapy for a cumulative period in excess of 21 days in the preceding 3 months.
18. Major toxicity on imatinib in past 3 months.
19. History of non-compliance, or other inability to grant informed consent.
20. Past or present history of alcohol abuse, use of illicit drugs, or severe psychiatric disorders, including depression.
21. Known hypersensitivity to any interferon preparation
22. Autoimmune hepatitis or a history of autoimmune disease
23. Pre-existing thyroid disease unless it can be controlled with conventional treatment
24. Epilepsy and/or compromised central nervous system (CNS) function.
25. HCV/HIV patients.

Supplementary Table

Supplementary table S1. Quality of life scales EORTC QLQ-C30 and QLQ-CML24

		Baseline		3 months			
		N	Initial score (SD)	N	Initial score (SD)	3mo score (SD)	Change Score
EORTC QLQ-C30 Functional scales	Global health status / QoL	16	74,5 (16,2)	10	70,8 (18,1)	76,7 (19,6)	5,8
	Physical functioning	16	92,1 (8,9)	10	92,0 (9,8)	89,9 (10,0)	-2,7
	Role Functioning	16	82,3 (18,7)	10	78,3 (19,3)	88,3 (17,7)	10
	Emotional functioning	16	81,8 (16,4)	10	80,8 (19,3)	83,3 (11,8)	2,5
	Cognitive functioning	16	89,6 (14,8)	10	90,0 (16,1)	90,0 (17,9)	0
	Social functioning	16	90,6 (19,2)	10	90,0 (22,5)	96,7 (7,0)	6,6
EORTC QLQ-C30 Symptom scales	Fatigue	16	28,5 (16,7)	10	31,1 (18,7)	31,1 (23,3)	0
	Nausea and vomiting	16	11,5 (13,2)	10	15,0 (12,3)	3,3 (7,0)	-11,7
	Pain	16	7,3 (13,6)	10	6,7 (16,1)	25,0 (27,5)	18,3
	Dyspnoea	16	10,4 (16,0)	10	10,0 (16,1)	20,0 (23,3)	10
	Insomnia	16	16,7 (27,2)	10	13,3 (17,2)	26,7 (37,8)	13,3
	Appetite loss	16	4,2 (11,4)	10	6,7 (14,6)	0,0 (0,0)	-6,7
	Constipation	16	8,3 (14,9)	10	10,0 (16,1)	13,3 (28,1)	3,3
	Diarrhoea	16	14,6 (17,1)	10	13,3 (17,2)	3,3 (10,5)	-10
	Financial difficulties	16	10,4 (23,5)	10	13,3 (28,1)	6,7 (21,1)	-6,7
EORTC QLQ-CML24	Symptom Burden	13	17,9 (10,8)	9	21,1 (10,2)	19,3 (12,3)	-1,8
	Impact on worry mood	13	10,3 (7,7)	9	12,0 (8,4)	12,0 (7,3)	0
	Impact on daily life	13	14,5 (19,4)	9	17,3 (22,3)	11,1 (13,6)	-6,2
	Satisfaction with Care and Information	13	89,7 (27,7)	9	96,3 (7,3)	94,4 (11,8)	-1,9
	Body image problems	13	12,8 (16,9)	9	11,1 (16,7)	18,5 (24,2)	7,4
	Satisfaction with social life	13	70,5 (36,1)	9	75,9 (34,5)	94,4 (16,7)	18,5

All 16 patients provided a baseline QLQ-C30 and 13 patients completed a baseline QLQ-CML24. Since two patients terminated the study within 3 months, follow-up assessments of 14 patients at 3, 6 and 12 months were expected, but not reached.

	6 months				12 months		
N	3mo score (SD)	6mo score (SD)	Change Score	N	3mo score (SD)	12mo score (SD)	Change Score
9	74,1 (18,3)	62,0 (19,1)	-12,0	8	74,0 (21,1)	65,6 (22,5)	-8,3
9	88,1 (9,9)	85,9 (13,9)	-22,0	8	90,8 (10,7)	90,8 (11,8)	0
9	87,0 (18,2)	75,9 (23,7)	-11,1	8	87,5 (19,4)	81,3 (18,8)	-6,3
9	81,5 (10,8)	75,9 (25,8)	-5,6	8	83,3 (10,0)	71,5 (35,2)	-11,8
9	88,9 (18,6)	87,0 (16,2)	-1,9	8	91,7 (17,8)	91,7 (17,8)	0
9	96,3 (7,3)	79,6 (28,6)	-16,7	8	97,9 (5,9)	87,5 (23,1)	-10,4
9	34,6 (21,8)	37,0 (20,0)	2,5	8	31,9 (26,2)	45,8 (24,8)	13,9
9	3,7 (7,3)	13,0 (13,9)	9,3	8	4,2 (7,7)	14,6 (16,5)	10,4
9	27,8 (27,6)	24,1 (27,8)	-3,7	8	25,0 (30,9)	16,7 (17,8)	-8,3
9	22,2 (23,6)	37,0 (30,9)	14,8	8	16,7 (25,2)	29,2 (27,8)	12,5
9	29,6 (38,9)	25,9 (27,8)	-3,7	8	20,8 (39,6)	25,0 (34,5)	4,2
9	0,0 (0,0)	11,1 (16,7)	11,1	8	0,0 (0,0)	20,8 (30,5)	20,8
9	14,8 (29,4)	18,5 (29,4)	3,7	8	16,7 (30,9)	25,0 (29,5)	8,3
9	3,7 (11,1)	22,2 (16,7)	18,5	8	4,2 (11,8)	20,8 (17,3)	16,7
9	7,4 (22,2)	11,1 (33,3)	3,7	8	8,3 (23,6)	8,3 (23,6)	0
9	21,0 (10,2)	23,6 (12,9)	2,6	8	18,3 (12,7)	24,0 (13,9)	5,8
9	15,7 (8,8)	17,6 (15,3)	1,9	8	12,5 (7,7)	25,0 (18,9)	12,5
9	13,6 (13,4)	19,8 (23,4)	6,1	8	8,3 (11,5)	23,6 (28,8)	15,3
8	89,6 (15,3)	85,4 (13,9)	-4,2	8	93,8 (12,4)	75,0 (35,6)	-18,8
9	22,2 (23,6)	33,3 (33,3)	11,1	8	16,7 (25,2)	16,7 (30,9)	0
9	88,9 (22,0)	70,4 (35,1)	-18,5	8	93,8 (17,7)	70,8 (27,8)	-22,9

Since the whole study was terminated preliminary after 12 months, the number of QoL-assessments at 18 months was low and therefore QoL was not evaluated at this time point. The change in scores was compared between baseline and 3 months and 3 months and 6 / 12 months respectively.

CHAPTER 8

General discussion

General discussion

Outcome of patients with CML has improved impressively the last 1,5 decade since the introduction of tyrosine kinase inhibitor (TKI) therapy.[1,2] Nevertheless, TKI resistance and intolerance, persistence of leukemic stem cells, and inability to completely eradicate the malignant clone require continued experimental and clinical study. In addition to prospective randomized studies, population-based registry studies may provide important information as regards the extent of TKI resistance and intolerance and parameters of the quality of care in a 'real-world' setting. This thesis is predominantly based on an analysis of data from the Dutch population-based CML registry, (PHAROS, Population-based HAematological Registry for Observational Studies). Treatment responses and monitoring frequencies were assessed and the additional value of routine cytogenetic response monitoring was evaluated in a population of 459 CML patients diagnosed between January 2008 and April 2013 and treated in the twelve regions of the Netherlands, including 7 University Hospitals, 25 larger non-academic hospitals and 43 general hospitals. In addition, the predictive value of a new baseline risk score developed by researchers from the European Treatment Outcome Study (EUTOS), named the EUTOS long-term survival (ELTS) score, was validated in this Dutch cohort of CML patients in combination with a population-based registry cohort of 694 Swedish patients. It yielded important information with respect to the prognostic value of this novel score in predicting molecular response, death due to CML and overall survival. The prognostic value of additional (cyto) genetic abnormalities was reviewed based on existing literature, resulting in a practical guideline. Furthermore, a single arm, phase 2, clinical trial was performed to evaluate whether switching imatinib to a combination of nilotinib and pegylated interferon-alpha (PegIFN) would be tolerated and might result in deeper molecular response in patients with no MR$^{4.0}$ (*BCR-ABL1* <0.01%IS) on imatinib. Following the observations in the preceding chapters, here results are discussed in an integrated way by addressing 3 questions: 1.How to improve the quality of CML care and molecular monitoring? 2.Can TKI treatment be stopped in patients with a molecular response, when and in which subgroup? 3. Should we strive for definite or operational cure in CML?

1. Quality of CML care and molecular monitoring

Currently, 5 different TKI's are available for targeted inhibitory therapy of enhanced *BCR-ABL1* activity in CML, including imatinib, nilotinib, dasatinib, bosutinib and ponatinib. These inhibitors differ as regards binding affinity, pharmacokinetic properties, efficacy, adverse events, and preserved activity in the presence of specific *BCR-ABL1* kinase domain mutations.[3,4] Upfront treatment of newly diagnosed CML patients in first chronic phase is highly efficacious with either kind of TKI[5-9] and combined with close molecular moni-

toring, allowing TKI-switches before disease progression has resulted in a life expectancy, which approaches the life expectancy of the general population.[10-12] All 5 TKIs are available in the Netherlands and are reimbursed by insurance companies, if prescribed according to approved labels and indications. In principle, CML treatment according to current hematological guidelines is covered completely by the Dutch health care system. Bosutinib and ponatinib are not indicated for front-line therapy, but have an important role as second-line or third-line in case of TKI resistance of intolerance. The 20-year prevalence of CML in the Netherlands was 1738 patients in 2016[13] and with a raw incidence of 0.98 per 100 000/year[11,14] the Dutch CML population is growing with approximately 165 new CML patients per year. Although the costs of continued TKI treatment are considerable, ranging in between 20.000 and 80.000,- euro's per year of treatment, so far all costs are reimbursed for each insured patient. Collectively, approximately 30-40 million euro is spent each year in The Netherlands for TKI-treatment, indicating high costs for a relatively small group of patients. Fortunately, the patent of imatinib recently expired, making it available at only 5% of the original price, which may result in a considerable reduction of the financial CML-health care burden in the Netherlands.

Collectively, due to the availability of all these 5 effective TKI's, in terms of survival there is limited room for improvement left; all front line treatments result in similar survival rates.[5-9] Despite these excellent results in upfront treatment of patients in first chronic phase CML, there is still a relevant percentage of patients, who present in (6%), or progress to (5%) accelerate phase or blast crisis[15] with accompanying high mortality rates.[16] Adequate monitoring of the response to treatment is important, as early recognition of TKI failure and subsequent switch contributes to prevention of progression. Surprisingly, evaluations of response monitoring in the United States demonstrated low levels of physician adherence to CML monitoring guidelines, with negative consequences for TKI adherence[17], progression[18], survival[18] and treatment costs.[19] Surveys performed among haemato-oncologists in the US demonstrated that patient resource barriers were the most prominent reason for non-adherence to monitoring guidelines.[20] In **chapter 5** we observed that despite full reimbursement of care for CML by insurance companies, a significant proportion of Dutch patients did not receive adequate response monitoring in the first year of treatment. We even confirmed that inadequate monitoring independently affected overall survival, stressing the importance of education and guideline adherence.

One of our important findings was the association between hospital experience and the quality of response monitoring. In cancer care, both for cancers requiring surgical procedures and nonsurgical cancers the impact of hospital volume and/or physician experience on outcome has been wellestablished. A large review article showed that across studies, the absolute benefit from care at high-volume centers exceeds the benefit from break-

through treatments.[21] CML treatment is provided by 80 out of 90 Dutch hospitals, and with an annual rate of 165 newly diagnosed patients, on average only 2 new CML treatments per year are initiated per hospital. This illustrates the decentralized character of CML care in the Netherlands. In line with a previous definition of hospital experience[22], hospitals were categorized into three groups, with the number of CML treatment initiations over the 5 year inclusion period between 2008 and 2013 as an approximation of hospital CML treatment experience: less experienced (≤ 5 patients); medium experienced (6-10 patients) and most experienced (> 10 patients). We observed that slightly more than half (57%) of the patients were treated in one of the most experienced hospital; the remainder (26% and 17% respectively) in medium or less experienced hospitals (**chapter 5**). Furthermore, we observed that hospital CML treatment experience, rather than e.g. academic hospital status, was the strongest predictor for proper monitoring. In contrast, the use of *BCR-ABL1* kinase domain mutation testing in patients switching TKI therapy due to failure (34%) was poor across the patient cohort, independent of the hospital experience.

Improving the quality of response monitoring may be established at three different levels. First, the findings of our evaluation may be helpful in creating awareness among physicians about the importance of frequent response monitoring. Second, it may be beneficial to organizing the care for CML patients in the Netherlands in a more centralized way, by advising physicians to refer newly diagnosed patients to colleagues with affinity and confirmed experience with CML treatment. The Dutch cooperative study group HOVON ("hemato-oncologie voor volwassenen in Nederland") established a hospital-ranking system based on expertise as regards transplantation and hematological intensive care. In short, 4 levels ("echelons") were designated with level A hospitals providing care for recipients of allogeneic stem cell transplantation; level B for recipients of an autograft; level C for hematological intensive care such as needed for treatment of acute leukemia: and level D hospitals providing non-intensive care for hemato-oncological patients. Should care for CML-patients be centralized according to that HOVON-ranking system, for example to level A, B, and C hospitals? Restricting care to those levels may, however, not solve the problem of insufficient monitoring and insufficient experience, as we identified level D hospitals with established experience and affinity with CML care, capable to perform high quality treatment. In addition, we also observed suboptimal monitoring in some patients in level A-C hospitals. Furthermore, the HOVON-ranking system is based on hematological intensive care expertise, rather than on numbers of patients being treated, the latter which appeared the discriminating parameter in our study. Therefore, I would prefer to clearly identify those hospitals, in which both a sufficient number of patients are treated and in which local hematologists have demonstrated experience and expertise with all aspects of CML treatment, including the different phases of the disease as well as all the different types of treatment. The identification of such hospitals

may urge cooperating hematologists on a regional level to centralize care in a restricted number of hospitals regionally and, in addition, develop a consultation system in which all CML-patients are being discussed on a regular basis with established experts in the field. Moreover, involving patients themselves may contribute to transparent and better care, for example by interactive networks such as CMyLife[23], that teach patients about the recommended frequencies and importance of response monitoring, thereby empowering them to demand adequate monitoring from their physicians.

2. TKI discontinuation

Despite rapidly inducing cytogenetic and even molecular remissions by TKI therapy, persistence of leukemic stem cells was invariably demonstrated by a number of investigators reporting deep molecular remissions, even after years of treatment.[24-27] Several explanations have been suggested, including insufficient TKI susceptibility of malignant stem cells[28,29], the quiescent state of leukemic stem cells[30,31], enhanced P-glycoprotein-activity in stem cells, resulting in enhanced removal of TKI out of the cell[32,33], and a possible *BCR-ABL1* independent mechanism of survival.[34,35] It resulted in the notion that TKI therapy in CML patients should be continued lifelong in order to prevent relapse originating from relative insensitive stem cells. Surprisingly, in 2007, French investigators observed in a pilot-study that 6 out of 12 patients with undetectable minimal residual disease (UMRD) lasting already for a minimum of two years, maintained their UMRD after discontinuation of imatinib treatment. The preceding median duration of imatinib treatment and *BCR-ABL1* negativity were 45 and 32 months, respectively.[36] Thereafter, several larger studies, evaluating imatinib discontinuation, have confirmed these earlier results, although relapse percentages differed among these studies (table 1).

Whereas the achievement of complete cytogenetic response (CCyR) or major molecular response (MMR, BCR-ABL1 <0.1% on the international scale (IS)) is sufficient to attain a normal life expectancy,[37,38] achieving deep molecular responses has been established to be an essential requirement for TKI discontinuation. In general, deep molecular responses are defined as *BCR-ABL1* levels below 0.01%^{IS} ($MR^{4.0}$). The term undetectable minimal residual disease (UMRD), also incorrectly designated as complete molecular response (CMR) in the past, is currently used when no *BCR-ABL1* transcripts can be demonstrated. This level of response is obviously related to the sensitivity of the assay. Conventional quantitative polymerase chain reaction (qPCR) is currently the gold standard, widely used in clinical practice, and applied in all published TKI discontinuation trials for the determination of the level of deep molecular response prior to TKI cessation. It's sensitivity varies between 0.01%^{IS} and 0.001%^{IS} ($MR^{4.0}$ - $MR^{5.0}$) but in most discontinuation studies an assay sensitivity of at least 0.0032%^{IS} ($MR^{4.5}$) had to be achieved, in order for *BCR-ABL1*

negative results to be defined as UMRD. More sensitive PCR techniques, such as digital PCR with sensitivity levels between $MR^{5.0}$ - $MR^{7.0}$, demonstrated *BCR-ABL1* positivity in approximately 20% of patients with UMRD according to conventional qPCR.[39,40] The TKI discontinuation trials used different durations and levels of deep molecular response as eligibility criterion for stopping treatment, varying between $MR^{4.0}$ and UMRD. Moreover, they used different levels for definition of molecular relapse and different triggers to restart of TKI treatment, varying from loss of UMRD to loss of MMR. Furthermore, definitions of molecular relapse free survival (MRFS), treatment free remission (TFR) and event free survival (EFS) varied among them,, hampering the comparison of results between these non-randomized discontinuation trials.

The French Stop Imatinib (STIM) trial and the Australian Trial of Withdrawing Imatinib in STablE molecular Remission (TWISTER) both required as inclusion criteria at least 3 years of imatinib treatment and 24 months of UMRD. TKI treatment was restarted if two consecutive samples proved *BCR-ABL1* positive without loss of MMR, or when patients lost their MMR. These studies showed, respectively, a MRFS of 40% at 18 months (STIM) and a treatment-free remission of 47% at 24 months (TWISTER).[41,42] The long term results (median follow-up of 6.4 years) of the STIM trial demonstrated a plateau phase (of continued molecular remission) of approximately 40% within two years of TKI discontinuation and a continuing MRFS of 38% after 5 years.[43] In three subsequent imatinib discontinuation trials, loss of MMR was the only trigger to resume TKI treatment, resulting in considerably higher TFR rates in two out of three studies (61% at 36 months[44], 59% at 24 months[40] and 48% at 36 months[39], respectively). These trials taught us that using loss of MMR as a trigger for restarting TKI treatment is safe, as all relapsing patients regained MMR after TKI reinitiating. Inclusion in the large EURO-SKI trial required less stringent sustained deep molecular response levels (MR^4 for at least 12 months). In this trial, loss of MMR was also defined as the threshold level to reinitiate TKI treatment, resulting in a MRFS (no loss of MMR) of 52% after 24 months.[45] The less stringent EURO-SKI inclusion criteria regarding depth and duration of deep molecular response, increased the rate of patients becoming eligible for a TKI cessation attempt, but demonstrated TFR rates which were approximately 10% lower compared to the earlier French results.

Table 1. Large imatinib discontinuation trials

Trial	Size	Required duration TKI treatment, months	Median TKI duration, months	Required response level and duration	Median DMR duration, months
STIM[41,43] 2010	n=100	> 36	59	UMRD, >24	36
TWISTER[42] 2013	n=40	>36	70/72**	UMRD, >24	30/41**
A-STIM[44] 2014	n=80	> 36	79	UMRD, >24***	41
ISAV[39] 2015	n=108	>24	103	UMRD, >18	26
KID[40] 2016	n=90	>36	81	UMRD, >24	40
EUROSKI[45] 2016	n=772****	>36	91	<MR^4, >12	56

* Showing a significant increase (by 10 times; ie, one log), at two consecutive assessments.

** IM-only (n=19)/IFN-IM (n=21).

***patients with confirmed CMR with occasional weekly positive samples before study entry were also considered eligible.

****Imatinib 1st line, dasatinib or nilotinib 1st or subsequent line, excluding patients with resistance to any TKI.

Table 2. Large second generation TKI discontinuation trials

Trial	Size (% prior TKI resistance)	Investigational treatment and line	Required duration TKI treatment, months	Median TKI duration, Months*
DADI[48] 2015	63 (21%)	Dasatinib 2nd or subsequent after imatinib	> 12 mo = consolidation	17/82
DASFREE[49] 2017 *Abstract*	63 (NA)	Dasatinib, 1st or subsequent line	> 24 mo	NA/67-72
D-STOP[50] 2017	54 (NA)	Dasatinib, 2nd or subsequent after any TKI	>24 mo = consolidation	24/92
STOP-2GTKI[51] 2017	60 (22%)	Dasatinib or nilotinib 1st or susbsequent after imatinib	> 36 mo total TKI therapy	39/76
STAT2[52] 2016 *Abstract*	73 (NA)	Nilotinib, 1st or subsequent after imatinib	>24 mo = consolidation	24-31/93-110
NILSt[53] 2016 *Abstract*	87 (NA)	Nilotinib, 1st or subsequent after imatinib	>24 mo = consolidation	NA
ENESTop[54] 2016 *Abstract*	126 (24%)	Nilotinib, 2nd line after imatinib	>3y, total >4y 1y consolidation	53/88
ENEST freedom[55] 2017	190 (0%)	Nilotinib, 1st line	>36 months 1y consolidation	44

* Investigational TKI / total TKI treatment including previous line(s).

** MR$^{4.5}$ in the last assessment, no assessment worse than MR^4 and ≤2 assessments between MR^4 and MR$^{4.5}$.

Threshold restart TKI	Endpoint
2x pos tests* or 1x loss MMR	MRFS 43% at 6 months, 40% at 18 months and 38% at 60 months
2x pos tests or 1x loss MMR	TFR 47% at 24 months
Loss of MMR	TFR 64% at 12 and 24 months, 61% at 36 months
2x pos test of which 1x loss of MMR	RFS 48% at 36 months
Loss of MMR	Sustained MMR 62% at 12 months and 59% at 24 months
Loss of MMR	MRFS 62% at 6 months, 56% at 12 months and 52% at 24 months

Abbr: TKI, tyrosine kinase inhibitor; DMR, deep molecular response; MMR, major molecular response; UMRD, undetectable minimal residual disease; MRFS, molecular recurrence-free survival; TFR, treatment-free remission; RFS, relapse free survival.

Required response level and duration	Median DMR duration, months	Threshold restart TKI	Endpoint
<MR^4, >12 months	NA	*BCR-ABL1* of 0.0069% or higher	TFR 49% at 6 months and 48% at 12 months
< MR$^{4.5}$, >12 months	NA	Loss of MMR	EFS 49% at 12 months
<MR^4, >24 months	51	Confirmed *BCR-ABL1* of 0.0069% or higher	TFS 69% at 6 months, 63% at 12 months and 57% at 24 months
UMRD, > 24 months	29	Loss of MMR	TFR 63% at 12 months and 54% at 48 months
<MR$^{4.5}$, >24 months	NA	Confirmed loss of MR$^{4.5}$	TFR 68% at 12 months
<MR$^{4.5}$, >24 months	NA	Loss of MR$^{4.5}$	TFR 59% at 12 months
<MR$^{4.5}$, >12 months	NA	Confirmed loss of MR^4 or loss of MMR	TFR of 58% at 12 months
Sustained DMR**, >12 months	30	Loss of MMR	TFR 52% at 12 months

Abbr: TKI, tyrosine kinase inhibitor; DMR, deep molecular response; MMR, major molecular response; UMRD, undetectable minimal residual disease; TFR, treatment-free remission; EFS, event free survival.

In the above mentioned trials, a number of patient-, disease- and treatment-specific characteristics were tested as potential predictors of successful TFR. However, the majority of these features, such as Sokal risk score[41], duration of interferon therapy prior to TKI treatment[42], speed of initial UMRD achievement[42], age[39] and TKI withdrawal syndrome[40], were not consistently associated with TFR. Duration of TKI treatment prior to discontinuation was independently associated with successful TFR in three studies, including the large EURO-SKI trial which found that patients with more than 5.8 years of imatinib treatment achieved a MRFS rate of 66% compared to 43% of patients with less than 5.8 years imatinib pretreatment.[40,41,46] Moreover, a minimum of 3.1 years of MR^4 duration was observed as optimal in terms of MRFS proportions in the EURO-SKI trial, using the minimal p-value approach. Apart from the two cut-offs, an almost linear increase in MRFS probability was demonstrated per additional year on treatment and/or in MR^4.[47] Based on (preliminary) results of several second generation TKI discontinuation trials, all evaluated in a non-randomized uncontrolled manner (table 2), it might be suggested that treatment with nilotinib and dasatinib in various treatment lines may result in slightly higher TFR rates compared to imatinib pretreatment only, but these findings need conformation in randomized controlled trials before solid conclusions can be drawn.

Presently, patients with a second molecular remission following relapse after TKI discontinuation are being enrolled in the second TKI-stop studies. In a pilot study (n=16) a second attempt was performed to discontinue imatinib in CML patients with a second sustained complete molecular response and it was observed that 25% of patients remained in TFR, thereby showing that it seems possible to discontinue TKIs a second time.[56] The recently published results from the RE-STIM trial confirmed these findings by demonstrating TFR rates of 48%, 42% and 35% at 12, 24 and 36 months in a group of 70 patients who re-attempted TKI discontinuation after a first unsuccessful stop attempt.[57] Of note, 28 patients included in this trial had reinitiated their TKI during the first TFR attempt based on loss of UMRD, while loss of MMR was used as TKI reinitiating criterium for the second TFR attempt.

Meanwhile, more than 2000 CML patients have discontinued TKI therapy in multiple clinical trials, in the absence of major safety issues. Molecular relapse generally let to quick regain of MMR and often also deep molecular response after TKI re-initiation. One case of sudden blast crisis was described, 9 months after restarting imatinib, while the patient had achieved MMR again for six months. It is unclear whether this progression was related to the TKI discontinuation.[44] As treatment-free remission has major advantages in terms of elimination of (long-term) toxicity, increasing quality of life and cost reduction, we should no longer restrain TKI discontinuation from patients with sustained durable deep molecular responses in general clinical practice. It should however be emphasized that survival is still the main treatment goal in CML and stopping TKI-treament may

hold unknown risks for the long-term. Therefore, in order to guarantee safety outside of clinical trials, TKI cessation should only be performed in a highly selected patient population, in centers with a quick (2 week) molecular monitoring turnaround time and by physicians how gained experienced with TKI discontinuation from participation in TKI discontinuation trials. In order to achieve the highest chance of successful TFR, chronic phase CML patients should meet the following criteria, based on current knowledge from the TKI discontinuation trials: minimum of 6 years TKI treatment duration, at least 3 years in sustained MR[4] and no prior history of TKI failure. Patient characteristics such as TKI toxicity, quality of life, pregnancy wishes etc. should be taken into account in the decision when to stop TKI treatment, until more robust predictors of TFR will become available. Monthly molecular monitoring is of utmost importance in the first 6 months after TKI discontinuation, as the majority of relapses occur in the first 6 months after TKI discontinuation and follow a steep path of *BCR-ABL1* increase. Six-weekly molecular assessments in the second half of the first year and 3-monthly thereafter have proven to be sufficient and loss of MMR has shown to be a safe trigger for TKI re-initiation. The importance of referring patients eligible for TKI cessation to physicians with known experience with this novel treatment goal and molecular diagnostic facilities with a high turnaround time available, is underlined by our finding in **chapter 5** which demonstrated that molecular monitoring of at least 3 times in the first year of TKI therapy was already lacking in a quarter of the patients in the first year of TKI treatment and associated with lower hospital experience with the treatment of CML patients.

3. 'Operational cure' versus 'definite cure'

Currently, allogeneic hematopoietic stem cell transplantation (alloHSCT) is still the only curative treatment strategy for CML. Despite the introduction of reduced-intensity conditioning and a significant reduction of non-relapse mortality, transplant-related morbidity and mortality limit the application of this type of powerful immunotherapy. At present, alloHSCT is predominantly applied in a selected group of CML patients in case of (progression to) advanced disease phases, multi-resistance or multi-intolerance to TKI treatment. TKIs are capable of reducing the leukemic clone to a level of "minimal residual disease" (MRD), which can be defined by different quantitative levels of *BCR-ABL1* transcripts using conventional qPCR techniques.[58-60] Undetectable *BCR-ABL1* transcripts may also be interpreted as MRD in patients treated with TKI, because very sensitive techniques may still demonstrate residual disease[61,62] and upon TKI withdrawal many patients will relapse. As described before, those patients may benefit, at least temporarily, from a period of TKI withdrawal, but as TKIs are not able to eradicate leukemic stem cells (LSCs),[28-31,34,35,63] TKIs can only provide a so-called 'operational cure', defined by a state of persistent low level MRD.[60]

At the same time, several TKI discontinuation trials have demonstrated that a 'definite' cure is not necessary for patients in order to stop TKI treatment. The proportion of patients in chronic phase CML at diagnosis, who could obtain TFR with the current TKI treatment strategies, is however rather small. In **chapter 3** we demonstrated a 6-year TKI discontinuation eligibility rate of ± 30%, of which we know that only approximately 50% of these patients will obtain long-term TFR (table 1 and 2), resulting in an overall TFR-rate of approximately 15%. Driven by the major advantages of obtaining TFR as mentioned in the previous paragraph, a substantial part of the CML research focusses on how to increase sustained deep molecular response rates in order to facilitate stopping attempts, which research is thereby based on promoting 'operational cure' rather than a 'definite cure'.

Sustained MR$^{4.5}$ rates after 8 years of imatinib treatment have been observed in approximately 40% of patients.[64,65] Optimization of TKI treatment strategies could be one way to increase sustained deep molecular response rates and to enhance 'operational cures'. Nilotinib and dasatinib may result in higher rates of deep molecular response, compared to imatinib[6,7], but it remains unclear if longer duration of upfront, prior imatinib treatment may result in similar sustained deep molecular response rates, especially when subsequent treatment lines in case of intolerance of failure are taken into account, as observed in **chapter 3**. Treating all CML patients upfront with nilotinib or dasatinib is however discouraged, taking the less favorable toxicity profiles and higher costs into account (generic imatinib available in the Netherlands since 2017 at 5% of the original price). Preferably, a personalized approach might be applied, taking baseline risk scores and early response rates into consideration. In **chapter 4** we demonstrated that the EUTOS long term survival (ELTS) score, based on baseline characteristics, outperformed the Sokal, Hasford and EUTOS risk scores and was capable of identifying a relatively small high risk group with significantly lower rates of major molecular response on upfront imatinib therapy and higher rates of 'death due to CML'. Until better risk scores specifically developed for the prediction of sustained deep molecular responses are available, one treatment strategy could be to treat only the small ELTS high risk group upfront with the more potent nilotinib or dasatinib,[66] thereby assuming that this will lead to a higher rate of patients obtaining sustained deep molecular responses, but prospective trials are needed to confirm these assumptions.

Early molecular response rates have already proven to be strong predictors for sustained deep molecular response rates. Three independent studies observed significantly higher (sustained) deep molecular response rates if a *BCR-ABL1* level of respectively <0.61%, <0.10% or <1.56% was obtained at 3 months.[64,67,68] These findings support an approach in which all CML patients in chronic phase at diagnosis are treated upfront with imatinib,

and switched to nilotinib or dasatinib when optimal molecular milestones are not met during the first year of treatment (and if comorbidities allow for this). Improving the molecular response rate and depth by combination therapy has been pursued in a number of studies, but has so far not been shown to enhance definite cure. Only somewhat higher molecular response rates have been observed, for example by combining TKI-treatment with interferon-alpha.

Interferon-alpha (IFN) has proven to be effective as monotherapy in a subset of patients, in the pre-imatinib era.[69,70] From an immunological point of view, interferon-alpha has been suggested to exert its antileukemic effect via immunomodulation.[71] Several immunological mechanisms of actions have been proposed, which, however, have never been substantially documented in *in vitro* and *in vivo* studies. A direct anti-proliferative effect of IFN has been well documented by several investigators.[72,73] An inhibition of outgrowth of primitive CML progenitor cells was shown, without eradication of normal or malignant early progenitor cells. Only the latter observation was supported by both *in vitro* and *in vivo* data and cell cultures of responders to IFN-alpha were characterized by a preferential inhibition of the outgrowth of malignant progenitor cells.

Improving the cytogenetic and molecular response by adding PegIFN to frontline imatinib therapy, has been reported before.[74-79] In a retrospective comparison, data from the Italian GIMEMA study group showed significantly higher CCyR (60% vs. 42%, p=0.003) and MMR (67% vs. 47%, p=0.001) rates after six months of combination treatment (n=76), compared to monotherapy (n=419).[74-76] Similarly, a randomized controlled trial performed in the Nordic countries demonstrated higher MMR rates at 12 months in the imatinib plus PegIFN arm (n=56) than the monotherapy arm (n=56, 82% vs. 54%, p=0.002) and an effect of PegIFN treatment duration on response. The large French SPIRIT trial observed significantly higher $MR^{4.0}$ rates after 12 and 24 months in 159 patients who received combination treatment with 90μg PegIFN-α2a / week compared to 160 patients who received imatinib 400 mg monotherapy (30% vs. 14%, p=0.001 and 38% vs. 21%, p=0.001, respectively).[77] Collectively, these studies have suggested a somewhat better molecular response of combination therapy, but at the expense of significant toxicity and without the prospects of definite cure. Our results described in **chapter 7** underlines that general conclusion and emphasizes the considerable toxicity associated with IFN.

Treatment strategies with the potential to eradication of LSCs are expected not only to overcome resistance and increase the rate of treatment-free remissions, but could even provide a path towards a definite cure, at least in some patients. Another part of research in the CML field is currently focused on the identification of targets on the CML stem cells or in the bone marrow niche, which can be modulated by existing or novel

compounds, in order to eradicate LSCs. As patients with CML have a near to normal life-expectancy on TKI treatment with only limited levels of toxicity, manageable toxicity and no treatment-related mortality will be important requirements for novel LSC eradicating treatments in order to become applicable in the general CML population. For patients with multiple drug resistance or in advanced stages of disease, effective treatments for the eradication of LSCs are even more desirable. Higher toxicity and mortality levels are acceptable for this specific group of patients with high risk disease, as long as they do not exceed the risks of allogeneic stem cell transplantation.

Asciminib (ABL001), a novel allosteric inhibitor of *BCR-ABL1* targeting the myristoyl pocket of the *ABL1* kinase, is currently being tested as monotherapy or in combination with imatinib, nilotinib or dasatinib in a phase 1 clinical trial including heavily pretreated patients with CML. Preliminary data show that asciminib monotherapy is well tolerated and exhibits significant and durable activity in a heavily pretreated subgroup of TKI resistant CML patients.[80] Dual targeting of BCR-ABL with nilotinib and asciminib, has been able to eradicate CML in mice when combined with nilotinib, without recurrence after the cessation of treatment.[81] A phase 3 trial comparing asciminib to bosutinib in third line CML treatment is ongoing (https://clinicaltrials.gov/ct2/show/NCT03106779).

Peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists (also used in the treatment of diabetes) were capable of eroding the CML leukemia stem cell pool in biological assays and 3 anecdotal clinical cases[82] and in a small proof of concept study, addition of the PPAR-γ agonist pioglitazone induced $MR^{4.5}$ in 54% of patients not achieving $MR^{4.5}$ with imatinib alone.[83] A randomized trial (ACTIW) is currently recruiting to evaluate the effect of the addition of pioglitiazon to TKI therapy on deep molecular response and TFR rates in a larger cohort of patients who did not achieve $MR^{4.5}$ on TKI monotherapy.

CD26 (dipeptidylpeptidase IV) is a specific and pathogenetically relevant biomarker of $CD34^{+}/CD38^{-}$ leukemic stem cells in CML. Targeting CD26 by gliptins (used on a large-scale for the treatment of diabetes) suppressed the expansion of *BCR-ABL1*$^{+}$ cells in NSG mice. In 2 CML patients receiving a gliptin because of uncontrolled diabetes, gliptin treatment was followed by a substantial decrease of *BCR-ABL1* to undetectable or near undetectable levels.[84] A single arm phase I/II trial (Dolphin-STAR) will soon start recruiting patients in the Netherlands to evaluate safety, feasibility and efficacy of nilotinib plus vildagliptin.

In conclusion, as of today alloHSCT is still the only treatment modality associated with definite cure, but its inherent toxicities prevent the application in chronic phase CML patients. Therefore, 'operational' rather than 'definite cure' is currently the preferred goal

of treatment by aiming for a molecular response of at least 3 log reduction of *BCR-ABL1* transcript copies. Those patients may experience life expectancy that does no longer differ from a non-leukemic, matched control population. Although several interesting agents are being studied for effective anti-leukemic stem cell properties, no such therapies are currently available leaving the goal of definite cure still on the horizon for chronic phase CML patients.

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APPENDICES

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English summary

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder defined by the presence of a reciprocal translocation between chromosome 9 and 22, resulting in short derivative chromosome 22, also known as the Philadelphia chromosome. The translocation leads to the fusion of the Abelson1 (*ABL1)* gene originally located on chromosome 9 with the Breakpoint Cluster Region (*BCR*) gene on chromosome 22. The *BCR-ABL1* fusion gene encodes a constitutively active tyrosine kinase which activates numerous signal transduction pathways leading to increased proliferation and differentiation of the CML progenitors and decreased apoptosis and adhesion to the bone marrow stroma. CML is a rare disease, with an incidence of 165 new patients per year in the Netherlands. More than 90% of CML patients present in chronic phase. In untreated patients, within three to five years the disease progresses to a rapidly fatal myeloid or lymphoid blast crisis. Outcome of patients with CML has improved impressively the last 1,5 decade since the introduction of tyrosine kinase inhibitor (TKI) therapy, resulting in 5-year overall survival rates of more than 90%.

Monitoring of the response to TKI treatment is essential in order to recognize TKI-resistance at an early stage. Cytogenetic analysis and molecular diagnostics are generally applied to monitor response in CML. Cytogenetic analysis requires a bone marrow aspiration and uses karyotyping to determine the percentage of Philadelphia chromosome positive metaphases out of at least 20 metaphases analysed. At diagnosis this percentage is usually 100% and within 6 months after the start of treatment a decrease to 0% (complete cytogenetic response, CCyR) is aimed for. Molecular diagnostics involves a quantitative PCR (qPCR) technique to express the amount of *BCR-ABL1* mRNA transcripts in peripheral blood as a percentage relative to a standardized baseline, allowing quantification according to the international scale (IS). A complete cytogentic response equals approximately a reduction to 1% *BCR-ABL* transcripts according to the international scale. This technique, however, can measure at a much lower level, up to 0.001-0.0001% *BCR-ABL1* transcripts. The achievement of a major molecular response (MMR, <0.1% *BCR-ABL1*^{IS}) within 1 year after the start of the treatment is an important treatment goal, as it associated with an excellent prognosis.

Currently 5 TKIs are available for the treatment of CML: imatinib, nilotinib, dasatinib, bosutinib and ponatinib. Unfortunately, TKIs do not have the ability to eradicate all leukemic stem cells and therefore they cannot provide a definite cure. Interestingly, recent clinical trials in patients with durable deep molecular responses (MR^4, <0.01% *BCR-ABL1*^{IS} of MR$^{4.5}$, <0.0032% *BCR-ABL1*^{IS}), have demonstrated that approximately half of the patients meeting strict inclusion criteria can discontinue their TKI treatment and maintain their

molecular remission, or a so-called treatment-free remission. Nevertheless, TKI resistance and intolerance, persistence of leukemic stem cells, and inability to completely eradicate the malignant clone require continued experimental and clinical study.

In addition to prospective randomized studies, population-based registry studies may provide important information as regards the extent of TKI resistance and intolerance and parameters of the quality of care in a 'real-world' setting. This thesis is predominantly based on evaluations of treatment response and monitoring data from the Dutch population-based CML registry, (Pharos, Population-based HAematological Registry for Observational Studies), a cohort of 459 CML patients diagnosed between January 2008 and April 2013 and treated in the twelve regions of the Netherlands, including 7 University Hospitals, 25 larger non-academic hospitals and 43 general hospitals. These observational evaluations are complemented by a literature review and a small phase 2 clinical trial.

In **chapter 2**, we provided a practical guide on cytogenetic and molecular variants and abnormalities in CML treatment, based on an extensive review of the most recent literature. This information is complemented by knowledge on the underlying pathophysiology, current and future diagnostic possibilities in CML. Several cytogenetic and molecular variations and abnormalities can influence the clinical course, monitoring and response to treatment of individual CML patients. It is therefore important for the physician to have knowledge of these variations, abnormalities and their consequences. Understanding the pitfalls of the underlying laboratory techniques helps prevent misinterpretations of results. Molecular monitoring is becoming the preferred monitoring method in CML, due to superior accuracy, less need for invasive bone marrow sampling and the ability to measure deep molecular responses. Cytogenetic assessments are still relevant for the recognition of additional cytogenetic abnormalities and variant translocations. Additional genetic abnormalities may be signs of genomic instability that can be associated with progression to accelerated and blast phase CML. The assessment of mutations in case of TKI resistance is essential for good clinical practice to rationally select a next-line TKI. New techniques, such as digital PCR and next generation sequencing, are entering clinical practice and show promise to further improve molecular monitoring in CML treatment.

Multiple randomized controlled trials have provided solid evidence for the efficacy and safety of TKIs as treatment for CML, but analyses from observational studies, gathered in patients who did not participate in clinical trials ('the real world') are scarce. We used data from the Dutch population-based CML registry to evaluate (deep) response rates to first and subsequent treatment lines and eligibility for a treatment cessation attempt. The results of this evaluation are presented and discussed in **chapter 3**. At 3 years 44% of patients had discontinued their first line treatment, mainly due to intolerance (21%) or

treatment failure (19%). At 18 months 73% of patients had achieved a complete cytogenetic response and 63% a major molecular response. Deep molecular responses ($MR^{4.0}$ and $MR^{4.5}$) were achieved in 69% and 56% of patients at 48 months. All response milestones were achieved faster in patients treated upfront with a second generation TKI, but ultimately also patients initially treated with imatinib reached similar levels of responses. The 6-year cumulative incidence of stop attempt eligibility according to EURO-SKI criteria was 31%. Overall, our findings showed that also in a 'real-world' setting the long-term outcome of patients treated with tyrosine kinase inhibitors was excellent.

Since the major cause of death in patients with CML is no longer CML-related, it has become important to use baseline risk prediction models that predict disease-specific mortality rather than overall survival. In **chapter 4** we combined data from the Swedish and Dutch population-based CML registry to perform an independent validation the recently introduced EUTOS long-term survival (ELTS) score, which was developed to predict 'Death due to CML'. The ELTS score was capable of differentiating risk groups with significantly different time to achieve major molecular response in both imatinib and 2GTKI treated patients. The 8-year probability of death due to CML in patients treated upfront with imatinib was 8%, 5% and 1% in the ELTS high, intermediate and low risk group respectively (high vs. low, p=0.001). For patients treated upfront with a second generation TKI (2GTKI) the 8-year cumulative incidence of CML-related death was 6%, 0% and 0% respectively (high vs. low, p=0.010; high vs intermediate, p=0.030). The ELTS risk score also correlated with overall survival. The ELTS performed better than the earlier Sokal, Hasford and EUTOS risk scores on all three endpoints in both imatinib and 2GTKI treatment groups. Because of its superior predictive value on clinically relevant outcome parameters, our data support the preferred use of the ELTS-score in clinical practice for baseline risk stratification in CML.

The importance of adequate response monitoring during the treatment of CML with TKIs and testing for *BCR-ABL1* kinase domain mutations in case of TKI failure is generally acknowledged and clearly outlined in guidelines and recommendations on CML management. Recent studies from the US have indicated that TKI response monitoring may be suboptimal in clinical practice. Since no population-based European data have been published on the quality of response monitoring in CML so far, in **chapter 5** we conducted an evaluation of response monitoring in the Dutch population-based CML registry during the first year after diagnosis. In our study, we observed suboptimal monitoring of response to TKI treatment in a quarter of the patients (<3 molecular response assessments in the first year). Inadequate monitoring was associated with impaired overall survival and hospital CML treatment experience was the strongest predictor for proper monitoring. Also, we found that *BCR-ABL1* kinase domain mutation testing was performed in only

34% of patients switching TKI therapy due to TKI failure. Collectively, it was concluded that molecular monitoring needs to be improved in the Netherlands and several recommendations were suggested, including centralization of care in experienced centers.

As already mentioned above, cytogenetic monitoring has a lower sensitivity than molecular monitoring and requires invasive bone marrow sampling. Moreover, the analysis is expensive. Nevertheless, CML guidelines continue to recommend performing routine cytogenetic response assessments, even when adequate molecular diagnostics are available. In **chapter 6,** we used Dutch population-based CML registry data to identify all simultaneous cytogenetic and molecular assessments performed at 3, 6 and 12 months and classified the response of these matched assessments according to European Leukemia Net (ELN) recommendations into optimal, warning and failure categories. The impact of discrepant cytogenetic and molecular response classifications and course of patients with additional chromosomal abnormalities were evaluated. The overall agreement of 200 matched assessments was 78%. In case of discordant responses, response at 24 months was consistently better predicted by the molecular outcome. Cytogenetic response assessments provided relevant additional clinical information only in some cases of molecular 'warning'. The development of additional cytogenetic abnormalities was always accompanied with molecular 'failure'. We concluded that it is safe to omit routine cytogenetics for response assessment during treatment and to only use molecular monitoring in order to prevent ambiguous classifications, reduce costs and reduce the need for invasive bone marrow sampling. Cytogenetic re-assessment should still be performed when molecular response is classified as 'warning' of 'failure'.

Confirmed deep molecular response rates ($MR^{4.5}$) in CML are currently achieved by 37-46% of CML patients after 8 years of imatinib treatment. For the majority of patients treated with imatinib, a stopping attempt is therefore not feasible. In order to improve molecular response depth, enabling a treatment cessation trial, in **chapter 7** we aimed at establishing whether CML patients, who did not achieve at least a $MR^{4.0}$ after long-term imatinib therapy, could attain $MR^{4.0}$ after a switch to nilotinib combined with pegylated interferon-α2b (PegIFN). In this NordDutchCML009 study, the primary endpoint of confirmed $MR^{4.0}$ was reached by 44% (95% CI: 23-67%) of the patients (n=16), with 81% (95% CI: 57-93%) of patients achieving an unconfirmed $MR^{4.0}$. The scheduled combination was only completed by 56% of the patients, with discontinuations mainly due to mood disturbances after the introduction of PegIFN. While the response showed some improvement, the most pronounced decline in *BCR-ABL1* levels was observed prior to the addition of PegIFN during nilotinib monotherapy, questioning the need for combination therapy. Moreover, the high rate of PegIFN discontinuation, primarily due to a substantial incidence of mood disturbances, indicated poor feasibility.

Finally, in **chapter 8** the main findings of all previous chapters are presented and discussed by addressing 3 important questions, relating to quality of CML care, molecular monitoring, TKI discontinuation and 'operational cure' versus 'definite cure'. Furthermore, the current implications of our findings for clinical practice and future perspectives are provided.

Nederlandse samenvatting

Chronische myeloïde leukemie (CML) is een ziekte van de hematopoëtische stamcel, gekenmerkt door de aanwezigheid van een gebalanceerde translocatie tussen chromosoom 9 en chromosoom 22, hetgeen resulteert in een kort chromosoom 22, ook bekend als het Philadelphia chromosoom. De translocatie leidt tot een fusie van het Abelson1 (*ABL1*) gen, oorspronkelijk gelokaliseerd op chromosoom 9, met het Breakpoint Cluster Region (*BCR*) gen op chromosoom 22. Het *BCR-ABL1* fusiegen codeert voor een constitutioneel actief tyrosine kinase dat diverse signaaltransductiepaden activeert, die leiden tot toegenomen proliferatie en differentiatie van de CML voorlopercellen en remming van apoptose en adhesie aan het beenmergstroma. CML is een zeldzame ziekte met een incidentie van 165 nieuwe patiënten per jaar in Nederland. Meer dan 90% van de CML patiënten presenteren zich in chronische fase. In onbehandelde patiënten ontwikkelt de ziekte zich binnen 3-5 jaar tot een snel fatale myeloïde of lymfoïde blastencrisis. Door de introductie van tyrosine kinase remmers (tyrosine kinase inhibitors, TKIs) zijn de uitkomsten van patiënten met CML zijn in de afgelopen 15 jaar sterk verbeterd. Dit heeft ertoe geleid dat de overleving van CML patiënten 5 jaar na diagnose momenteel al meer dan 90% is.

Nauwgezette monitoring van het behandelingsresultaat (hierna te noemen: 'respons') is essentieel om TKI-resistentie in een vroeg stadium te herkennen. De twee meest gebruikte technieken om behandelingsresultaten te meten zijn cytogenetisch onderzoek en moleculaire diagnostiek. Bij cytogenetisch onderzoek is het noodzakelijk dat bij de patiënt middels een beenmergpunctie materiaal wordt afgenomen, waarvan de cellen in deling gebracht worden om vervolgens door middel van karyotypering het percentage Philadelphia chromosoom positieve metafasen uit ten minste 20 onderzochte metafasen vast te stellen. Bij diagnose is dit percentage 100% en het is wenselijk dat dit percentage binnen een half jaar na behandeling met een TKI naar 0% is gedaald, hetgeen ook wel een complete cytogenetische respons wordt genoemd. Moleculaire diagnostiek maakt gebruik van een kwantitatieve PCR (qPCR) techniek om de hoeveelheid *BCR-ABL1* mRNA transcripten in perifeer bloed uit te drukken als percentage ten opzichte van een internationaal gedefinieerde standaard (de internationale schaal (IS)-methode). Een complete cytogenetische 'respons' is ongeveer gelijk aan 1% *BCR-ABL1*^{IS} transcripten. Door middel van deze techniek kunnen ook veel kleinere hoeveelheden leukemie gemeten worden. Er wordt gestreefd naar een majeure moleculaire respons (MMR, <0.1% *BCR-ABL1*^{IS}) binnen 1 jaar na de start van TKI behandeling, daar deze geassocieerd is met een vrijwel normale levensverwachting.

Er zijn op dit moment 5 verschillende TKIs beschikbaar voor de behandeling van CML: imatinib, nilotinib, dasatinib, bosutinib en ponatinib. TKIs zijn uitstekend in staat

om CML-cellen te remmen en uit te schakelen, maar ze beschikken helaas niet over de eigenschap om álle leukemische stamcellen te doden en daarom kunnen ze geen definitieve genezing bewerkstelligen. Interessant is dat recente klinische studies hebben aangetoond dat in patiënten met een langdurige en diepe moleculaire respons ongeveer de helft van de patiënten die voldeden aan de strenge studiecriteria hun TKI behandeling konden staken en in moleculaire remissie bleven, hetgeen ook wel een behandelingsvrije remissie wordt genoemd. Experimenteel en klinisch onderzoek naar CML blijft echter van groot belang vanwege het optreden van TKI resistentie en intolerantie, het persisteren van de leukemische stamcellen en het onvermogen van TKIs om de gehele maligne kloon te verwijderen.

Als aanvulling op prospectieve gerandomiseerde studies bevatten 'population based registry' studies belangrijke informatie met betrekking tot de mate van TKI resistentie, intolerantie en parameters voor de kwaliteit van zorg in een 'real-world' setting. Dit proefschrift is hoofdzakelijk gebaseerd op evaluaties van behandelingsrespons en monitoringsdata afkomstig uit het Nederlandse 'population based' CML register, (Pharos, Population-based HAematological Registry for Observational Studies), een cohort van 459 CML patiënten gediagnosticeerd tussen januari 2008 en april 2013 en behandeld in de twaalf Nederlandse provincies, inclusief 7 universitair medische centra, 25 grote niet-academische ziekenhuizen en 43 algemene ziekenhuizen. Naast deze observationele evaluaties hebben we ook een literatuurbeschouwing verricht en een (kleine) klinische fase 2 studie geanalyseerd.

In **hoofdstuk 2** hebben we een praktische leidraad geschreven met betrekking tot cytogenetische en moleculaire variaties en afwijkingen in de behandeling van CML, gebaseerd op een uitgebreide beschouwing van de meest recente literatuur. Deze informatie is aangevuld met kennis over de onderliggende pathofysiologie, huidige en toekomstige diagnostische mogelijkheden in CML. Diverse cytogenetische en moleculaire variaties en afwijkingen kunnen het klinisch beloop, de monitoring en respons op behandeling van individuele CML patiënten beïnvloeden. Het is daarom belangrijk voor de clinicus om kennis te nemen van deze variaties, afwijkingen en hun consequenties voor de klinische praktijk. Het begrijpen van de valkuilen van de onderliggende laboratorium technieken helpt in het voorkomen van misinterpretaties van uitslagen. Bij de monitoring van CML gaat steeds meer de voorkeur uit naar moleculaire monitoring vanwege de superieure accuratesse, het niet hoeven afnemen van invasieve beenmergpuncties en de mogelijkheid om diepe moleculaire responsen te meten. Voor de herkenning van additionele cytogenetische afwijkingen en variant translocaties zijn cytogenetische testen nog steeds relevant. Additionele genetische afwijkingen kunnen een teken zijn van genomische instabiliteit die in specifieke gevallen zelfs beschouwd moet worden als kenmerk van progressie naar acceleratie- of blastenfase.

Mutatie analyse in geval van TKI resistentie is ook essentieel voor adequate CML-zorg zodat rationeel een volgende behandelingslijn gekozen kan worden. Nieuwe technieken zoals digitale PCR en 'next generation sequencing' worden steeds vaker toegepast in de klinische praktijk en zullen leiden tot een verdere verbetering van moleculaire diagnostiek in de behandeling van CML.

Diverse gerandomiseerde gecontroleerde klinische studies hebben solide bewijs geleverd voor de effectiviteit en veiligheid van TKIs voor de behandeling van CML, maar analyses van observationele data, verzameld in patiënten die niet participeerden in klinische onderzoeken ('the real world') zijn zeldzaam. Wij hebben data gebruikt van het Nederlandse 'population based' CML register om (diepe) respons percentages op eerste en latere behandelingslijnen te evalueren en te inventariseren welk percentage patiënten uiteindelijk zal voldoen aan de criteria voor het verrichten van een TKI stop poging. De resultaten van deze evaluatie zijn gepresenteerd en bediscussieerd in **hoofdstuk 3**. Na 3 jaar is in 44% van de patiënten de eerste lijn behandeling gestaakt, hoofdzakelijk vanwege intolerantie (21%) of falen van de behandeling (19%). Na 18 maanden heeft 73% van de patiënten een complete cytogenetische respons bereikt en 63% een majeure moleculaire respons. Diepe moleculaire responsen ($MR^{4.0}$ en $MR^{4.5}$) werden bereikt in 69% en 56% van de patiënten na 48 maanden. Alle respons mijlpalen werden sneller bereikt in patiënten die met een tweede generatie TKI (nilotinib of dasatinib) als eerste lijn werden behandeld, maar uiteindelijk bereikten patiënten die met eerste lijn imatinib behandeld werden vergelijkbare respons niveaus. De cumulatieve incidentie van patiënten die na 6 jaar voldeden aan de EURO-SKI criteria voor een TKI stop poging was 31%. Over het geheel genomen toonde onze bevindingen dat ook in een 'real-world' setting de behandeling van CML met TKIs leidt tot excellente uitkomsten op de lange-termijn.

Als een positief gevolg van de effectieve behandeling van CML met TKIs, overlijdt nog slechts een enkele CML patiënt aan de gevolgen van de ziekte en is het merendeel van de doodsoorzaak van patiënten met CML niet meer CML-gerelateerd. Om die reden is er tegenwoordig meer behoefte aan predictiemodellen die bij diagnose het risico op ziekte-specifieke mortaliteit kunnen voorspellen in plaats van totale sterfte. In **hoofdstuk 4** hebben we data van de Zweedse en Nederlandse 'population based' CML registers gecombineerd om een onafhankelijke validatie uit te voeren van de recent geïntroduceerde EUTOS long-term survival (ELTS) score, die is ontwikkeld voor het voorspellen van 'dood door CML'. De ELTS score was in staat om risico groepen te differentiëren met significant verschillende tijden tot het bereiken van majeure moleculaire respons in zowel patiënten behandeld met imatinib als tweede generatie TKIs. De 8-jaars cumulatieve incidentie van overlijden ten gevolge van CML in patiënten behandeld met eerste lijn imatinib was 8%, 5% en 1% in de ELTS hoog, intermediair en laag risico groepen respectievelijk (hoog vs.

laag, p=0.001). Voor patiënten behandeld met een tweede generatie TKI in de eerste lijn was de 8-jaars cumulatieve incidentie van 'dood door CML' 6%, 0% en 0% respectievelijk (hoog vs. laag, p=0.010; hoog vs. intermediair, p=0.030). De ELTS risico score correleerde ook met de totale overleving. Op alle drie de eindpunten presteerde de ELTS score beter dan de eerder ontwikkelde Sokal, Hasford en EUTOS risico scores, zowel in patiënten die imatinib als tweede generatie TKIs ontvingen bij de start van de behandeling. Vanwege zijn superieure voorspellende waarde op klinisch relevante uitkomstmaten, ondersteunen onze bevindingen de voorkeur om de ELTS-score in de klinische praktijk te gebruiken voor de risico stratificatie van patiënten met chronische fase CML bij diagnose.

Het belang van adequate respons monitoring tijdens de behandeling van CML met TKIs en het testen van *BCR-ABL1* kinase domein mutaties in geval van TKI falen wordt breed gedragen en is dan ook vertaalt in diverse richtlijnen en aanbevelingen met betrekking tot CML. Recente studies uit de Verenigde Staten hebben aangetoond dat de respons monitoring van behandeling met TKIs suboptimaal wordt uitgevoerd in de klinische praktijk. Aangezien er niet eerder 'population based' data zijn gepubliceerd over de kwaliteit van respons monitoring in Europa, hebben we in **hoofdstuk 5** een evaluatie verricht van respons monitoringsfrequenties met data uit de Nederlandse 'population based' CML registratie tijdens het eerste jaar na diagnose. In deze studie hebben we geobserveerd dat suboptimale monitoring van de moleculaire respons optrad in een kwart van de patiënten (<3 moleculaire testen in het eerste jaar). Inadequate monitoring was geassocieerd met een verminderde totale overleving. Ziekenhuis ervaring met CML behandeling was de sterkste voorspeller voor adequate monitoring. Ook vonden we dat in slechts 34% van de patiënten die een nieuwe behandelingslijn ontvingen vanwege TKI falen, een kinase domein mutatie test was verricht. Geconcludeerd werd, dat de kwaliteit van monitoring in Nederland verbetering behoeft, waarvoor aanbevelingen worden gedaan zoals o.a. de aanbeveling om de behandeling te centraliseren in expertise-centra.

Zoals boven reeds beschreven heeft cytogenetische monitoring een lagere sensitiviteit dan moleculaire monitoring en vereist het invasieve beenmergpuncties. Bovendien is het een kostbare analyse. Desondanks blijven CML richtlijnen het routinematig verrichten van cytogenetische respons testen aanbevelen, zelfs wanneer adequate moleculaire diagnostiek beschikbaar is. In **hoofdstuk 6** hebben we gebruik gemaakt van de Nederlandse 'population based' CML register data om alle cytogenetische en moleculaire testen die 3, 6 en 12 maanden na de start van de behandeling tegelijkertijd verricht werden te identificeren en de respons van deze gepaarde testen volgens de European Leukemia Net (ELN) aanbevelingen te classificeren in de categorie optimaal, waarschuwing of falen. De impact van discrepanties in cytogenetische en moleculaire respons classificaties en het beloop van de patiënten met additionele chromosomale afwijkingen werd geëvalueerd.

Van de 200 gepaarde testen kwamen de respons classificaties van 78% overeen. In geval van een discordante respons werd de respons op 24 maanden consistent beter voorspeld door de moleculaire uitkomst. Cytogenetische respons testen verschaften in sommige gevallen van moleculaire 'waarschuwing' relevante additionele klinische informatie. De ontwikkeling van additionele cytogenetische afwijkingen ging altijd gepaard met moleculair 'falen'. We concludeerden dat het veilig is om routinematig cytogenetisch testen voor de monitoring van respons te laten vervallen en alleen moleculaire monitoring te gebruiken om tegenstrijdige respons classificaties te voorkomen, kosten te reduceren en het verrichten van invasieve beenmerg puncties te verminderen. Indien er sprake is van een moleculair respons in de categorie 'waarschuwing' of 'falen', dan is het wel raadzaam om een cytogenetische evaluatie te verrichten.

In 37-46% van de CML patiënten wordt tegenwoordig na 8 jaar behandeling met imatinib stabiele diepe moleculaire respons percentages ($MR^{4.5}$) bereikt. Voor de meerderheid van de patiënten behandeld met imatinib is een TKI stop poging daarom niet haalbaar. Om het percentage patiënten met een diepe moleculaire respons te verbeteren hebben we ons in **hoofdstuk 7** gericht op het vaststellen of CML patiënten die geen $MR^{4.0}$ hebben bereikten na langdurig imatinib behandeling, een $MR^{4.0}$ konden bereiken door over te stappen naar behandeling met nilotinib gecombineerd met gepegyleerd interferon-alfa2b (PegIFN). In deze NordDutchCML009 studie werd het primaire eindpunt van bevestigde $MR^{4.0}$ bereikt door 44% van de patiënten (n=16). De geplande combinatiebehandeling werd slechts door 56% van de patiënten volbracht, hoofdzakelijk vanwege stemmingsstoornissen die optraden na de introductie van PegIFN. De toegevoegde waarde van de combinatie PegIFN en nilotinib kan op basis van onze resultaten in twijfel getrokken worden, omdat de meest uitgesproken afname in moleculaire response werd geobserveerd na de start van nilotinib en voorafgaand aan het toevoegen van PegIFN. Bovendien concludeerden wij dat de onderzochte behandelingsstrategie niet haalbaar is in deze specifieke setting vanwege de hoge stakingspercentage door een substantiële incidentie van stemmingsstoornissen.

Tenslotte worden in **hoofdstuk 8** de hoofdbevindingen van alle voorafgaande hoofdstukken gepresenteerd en bediscussieerd in het licht van enkele belangrijke, resterende vragen met betrekking tot de kwaliteit van CML zorg, moleculaire monitoring, het staken van TKI behandeling en de 'operationele genezing' versus 'definitieve genezing'. Bovendien worden de huidige implicaties van onze bevindingen voor de klinische praktijk beschreven en een toekomstperspectief geschetst.

List of abbreviations

ABC	ATP-binding cassette
ABL001	Asciminib
ABL1	Abelson1
ACA	Additional cytogenetic abnormalities
AE	Adverse event
AP	Accelerated phase
BC	Blast crisis
BCR	Breakpoint Cluster Region
BCR-ABL1	Fusion gene
BID	Bis in die, twice a day
CBA	Chromosome banding analysis
CCA/Ph$^+$	Clonal cytogenetic abnormalities in the Philadelphia positive clone
CCA/Ph$^-$	Clonal cytogenetic abnormalities in the Philadelphia negative clone
CCyR	Complete cytogenetic response, 0% Philadelphia positive metaphases
CD26	Dipeptidylpeptidase IV
cDNA	Complementary deoxyribonucleic acid
CHR	Complete hematologic response
CI	Confidence interval
c-KIT	Receptor for stem-cell factor
CML	Chronic myeloid leukemia
CMR	Complete molecular response
CP	Chronic phase
CT	Cycle threshold
CTCAE	Common Toxicity Criteria for Adverse Events
CyR	Cytogenetic response
DAS	Dasatinib
DLI	Donor lymphocyte infusions
dPCR	Digital polymerase chain reaction
EFS	Event free survival
ELN	European LeukemiaNet
ELTS	EUTOS Long Term Survival
ESMO	European Society of Medical Oncology
EUTOS	European Treatment Outcome Study
FISH	Fluorescence in situ hybridization
G-banding	Giemsa banding
GI	Gastrointestinal
HLA	Human Leukocyte Antigen

HR	Hazard ratio
HSCT	Hematopoietic allogeneic stem cell transplantation
IFN	Interferon
IFN-α	Interferon-alpha
IKNL	Netherlands Comprehensive Cancer Organisation
IM	Imatinib
IQR	Interquartile range
IRIS	International Randomized study for Interferon and STI571
IS	International scale
KD	Kinase domain
LSC	Leukemic stem cell
M-bcr	Major breakpoint
m-bcr	Minor breakpoint
MCyR	Minor cytogenetic response
MinCyR	Minimal cytogenetic response
MMR	Major molecular response, 0.1% *BCR-ABL1* transcripts on the international scale
$MR^{4.0}$	Deep molecular response, 0.01% *BCR-ABL1* transcripts on the international scale
$MR^{4.5}$	Deep molecular response, 0,032% *BCR-ABL1* transcripts on the international scale
$MR^{5.0}$	Deep molecular response, 0,001% *BCR-ABL1* transcripts on the international scale
MRD	Minimal residual disease
MRFS	Molecular relapse-free survival
mRNA	Messenger ribonucleic acids
NGS	Next-generation sequencing
NIL	Nilotinib
NoCyR	No cytogenetic response
OCT-1	Organic-cation transporter-1
OR	Odds ratio
PCYR	Partial cytogenetic response
PDGF	Platelet Derived Growth Factor
PegIFN	Pegylated-interferon
Ph	Philadelphia chromosome
Ph^+ B-ALL	*BCR-ABL1* positive B-cell acute lymphoblastic leukemia
PHAROS	Population-based HAematological Registry for Observational Studies
PPAR-γ	Peroxisome proliferator-activated receptor gamma
QD	Quaque die, once daily
qPCR	Quantitative polymerase chain reaction
RCT	Randomized controlled trial
RT-PCR	Reverse transcriptase polymerase chain reaction

RT-qPCR	Real-time quantitative polymerase chain reaction
SAE	Serious adverse event
SD	Standard deviation
STI571	Imatinib
TFR	Treatment-free remission
TKI	Tyrosine kinase inhibitor
ULN	Upper limit of normal
UMRD	Undetectable minimal residual disease
WBC	White blood cell count
2GTKI	Second generation TKI

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List of publications

Geelen IGP, Gullaksen SE, Ilander MM, Olsson-Strömberg U, Mustjoki S, Richter J, Blijlevens NMA, Smit WM, Gjertsen BT, Gedde-Dahl T, Markevärn B, Koppes MMA, Cornelissen JJ, Westerweel PE, Hjorth-Hansen H, Janssen JJWM. Improving molecular response by switching imatinib to nilotinib combined with pegylated interferon-α2b in chronic phase CML. Submitted.

Geelen IGP, Valk PJM, Beverloo HB, Alikian M, Janssen JJWM, Cornelissen JJ, Westerweel PE. Cytogenetic and molecular abnormalities in chronic myeloid leukemia: pathophysiology and implications for clinical practice. Submitted.

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Summary of PhD training and teaching activities

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1. PhD TRAINING	**Year**	**Workload (ECTS)**
General academic/research skills		
Good Clinical Practice (Albert Schweitzer Hospital)	2015	1
Scientific writing in English (Albert Schweitzer Hospital)	2016	2
Biostatistics (Albert Schweitzer Hospital)	2016	1
Research Integrity (Erasmus MC)	2017	0.3
Conferences / seminars / symposia		
17th Annual John Goldman Conference on CML: Biology and Therapy, Estoril, Portugal	2015	1
Oral presentation: Omitting cytogenetic response assessment and using only molecular response assessment to TKI treatment in CML is safe and prevents misclassification as treatment failure		1
18th Annual John Goldman Conference on CML: Biology and Therapy, Houston, USA	2016	1
Oral presentation: Second generation TKIs provide a more effective treatment with higher persistence rates than imatinib in "Real-World" newly diagnosed CML patients		1
Oral presentation: CML treatment in Dutch hospitals with low patient volumes is associated with a substandard quality of molecular response monitoring		1
19th Annual John Goldman Conference on CML: Biology and Therapy, Estoril, Portugal	2017	1
Poster presentation: Validation of the EUTOS long term survival (ELTS) score in Dutch CML-patients		1
Poster presentation: Clinical and Immunological Effects of Nilotinib in Combination with Pegylated Interferon-α2b in Patients with Suboptimal Molecular Response on Imatinib (NordDutchCML009)		1

58th American Society of Hematology Meeting and Exposition, San Diego, USA	2016	1
Poster Presentation: CML treatment in Dutch hospitals with low patient volumes is associated with a substandard quality of molecular response monitoring (Travel grant winner)		1
Trainee day, 58th ASH Meeting and Exposition, San Diego, USA	2016	0.3
17th Congress of EHA, Madrid, Spain	2017	1
Poster presentation: Validation of the EUTOS long term survival (ELTS) score in Dutch CML-patients		1
ePoster presentation: Clinical and immunological effects of nilotinib in combination with pegylated interferon-α2b in patients with suboptimal molecular response on imatinib (NordDutchCML009)		1
CML-Summit Choice Means Life, Stockholm, Sweden	2017	0.6
Annual Dutch Hematology Congress, Arnhem, the Netherlands	2016	1
Oral presentation: CML treatment in hospitals with low patient volumes is associated with a substandard quality of molecular response monitoring		1
Oral presentation: Second generation TKI treatment is better tolerated and is more effective than imatinib in "Real-World" first line CML treatment		1
Annual Dutch Hematology Congress, Arnhem, the Netherlands	2017	1
Oral presentation: Validation of the EUTOS long term survival (ELTS) score in Dutch CML-patients		1
Klinische dag NVVH, Utrecht, the Netherlands	2015	0.3
Regionale nascholing Hematologie, Rotterdam, the Netherlands	2015	0.3
Regionale nascholing Hematologie, Rotterdam, the Netherlands	2016	0.3
Wetenschapsdag ASZ, Dordrecht, the Netherlands	2015	0.3
Wetenschapsdag ASZ, Dordrecht, the Netherlands	2016	0.3
Oral presentation: Omitting Cytogenetic Response Assessment and Using Only Molecular Response Assessment to TKI Treatment in CML Is Safe and Prevents Misclassification As Treatment Failure		1
Wetenschapsdag ASZ, Dordrecht, the Netherlands	2017	0.3
Oral presentation: CML treatment in Dutch hospitals with low patient volumes is associated with a substandard quality of molecular response monitoring (Prijs 'Beste mondelinge presentatie')		1

In-depth courses		
Raindance ddPCR platform (Hammersmith Hospital, London)	2016	0.6
Digital Droplet PCR preceptorship (Næstved Sygehus, Denmark)	2017	1
Next Generation Sequencing, library preparation (Imperial College, London)	2017	1

Scientific meetings		
Weekly research meetings, dept. of hematology, ASZ, Dordrecht, NL	2015-2017	4
Regiobijeenkomst hematologie, Erasmus MC	2016	0.3
NCLMSG group meeting, Stockholm, Sweden	2017	0.3

2. TEACHING ACTIVITIES		
Educational talks about CML for nurses, medical students and residents	2016	1
Wetenschapslunch ASZ	2015-2017	1

3. OTHER ACTIVITIES		
Editor WASz (scientific journal of Albert Schweitzer Hospital), Dordrecht, NL	2014-2017	6
Total		**42.2**

Curriculum Vitae

Inge Geelen was born on September 1st, 1988 in Hunsel, Limburg, the Netherlands. In 2006 she obtained her Atheneum diploma (cum laude) at the Philips van Horne SG in Weert and went to Maastricht University to study Medicine. As part of her clinical rotations she visited South-Africa in 2010. She carried out her senior clinical rotation in the internal medicine & intensive care department at Laurentius hospital in Roermond and wrote her master thesis in the department of hematology and clinical chemistry at Maastricht University Medical Center (MUMC+). In 2012, she obtained her Master's degree in Medicine and started working as a resident 'not in training' (ANIOS) at the department of Internal Medicine at Sint Jans Gasthuis in Weert and Albert Schweitzer Hospital in Dordrecht. In February 2015, she started her PhD trajectory at the Albert Schweitzer Hospital, under supervision of prof. dr. Jan J. Cornelissen (Erasmus University Medical Center, Rotterdam), dr. Jeroen J.W.M. Janssen (VU University Medical Center, Amsterdam) and dr. Peter E. Westerweel (Albert Schweitzer Hospital, Dordrecht). During her PhD trajectory, she carried out several visits to the department of Molecular Biology of the Imperial College in London to perform laboratory work. The last months of her research period were completed with a weekly out-patient clinic for patients with chronic myeloid leukemia, under supervision of dr. Jeroen J.W.M. Janssen at the VU University Medical Center in Amsterdam. Inge got accepted into the Internal Medicine Residency training program (program director: Dr. Adrienne A.M. Zandbergen). As of January 2018, she started working as a resident in training (AIOS) at the department of Internal Medicine of the Albert Schweitzer Hospital (program director: Dr. Peter J.H. Smak Gregoor).

Dankwoord

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