

in silico hematology

Application of mathematical modeling to predict the outcome of leukemia treatment

by Ingmar Glauche and Ingo Röder

Chronic Myeloid Leukemia (CML) accounts for about 20 % of all leukemias in adults. The malignant cells, which express the BCR-ABL fusion protein, can be targeted efficiently by tyrosine kinase inhibitors (TKI). Due to the specificity of TKI treatment, CML has developed into a show-case example for an efficient, targeted tumor therapy. Applying a single-cell based mathematical model, which describes CML as a clonal competition between normal and leukemic hematopoietic stem cells, we suggest different approaches to further optimize CML therapy. With our model predictions, we particularly address combination therapies and patient-specific treatment cessation protocols.

Chronic Myeloid Leukemia as a model disease

Chronic Myeloid Leukemia (CML) is a fatal disease of the blood forming system accounting for about 20 % of all leukemias in adults. Unlike other leukemia types, the initiating event of CML is known for the majority of patients; about 95 % of them show a characteristic translocation of chromosomes 9 and 22. This translocation results in the formation of the BCR-ABL fusion gene coded on the shortened chromosome 22, which is called “Philadelphia chromosome”. Harboring this mutation (which leads to the production of the BCR-ABL protein, a constitutively activated tyrosine kinase) in a hematopoietic stem cell-like cell can lead to an initially slow but sustained expansion of a leukemic cell population. This expansion comes along with a repression of normal hematopoiesis, which is progressively outcompeted, finally leading to the manifestation of a CML (Figure 1A). The primary, mostly symptom-free chronic phase of the disease eventually transforms into an acute blast crisis, in which a majority of undifferentiated peripheral blood cells severely constrain normal blood function and lead to the patient’s death in the untreated situation. The abundance of

immature “white” blood cells was name-giving for a whole family of blood cancers (greek *leuchaimia*, from *leukós* – white and *haima* – blood).

Since the turn of the last century, treatment and prognosis of CML underwent significant changes, and the disease can now be controlled in many cases. The availability of a specific class of drugs, named tyrosine kinase inhibitors (TKI), allows specific targeting of cells carrying the BCR-ABL oncoprotein. Due to this specificity, TKI treatment – in contrast to classical chemotherapies – widely spares healthy cells (Figure 1B). Already the introduction of the first-generation TKI *Imatinib* significantly improved the treatment prognosis compared to previous therapeutic options, such as the treatment with Hydroxyurea, Interferon- α (IFN α) or bone marrow transplantation. Five-year survival levels increased to values above 90 %. The availability of second-generation (*Dasatinib*, *Nilotinib*) and third-generation TKIs (*Bosutinib*, *Ponatinib*) currently further increases therapy effectiveness, especially regarding the successful treatment of a broad spectrum of secondary resistance mutations. Because of the availability and the success of a therapy specifically targeting the tumor cells (“targeted therapy”), CML has developed in a show-case example for the treatment of many tumor entities.

Molecular monitoring of tumor load in peripheral blood, using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), revealed that *Imatinib* monotherapy induces a biphasic decline of BCR-ABL transcript levels in most patients (Figure 2A). It is characterized by an initially steep decline, followed by a second moderate decline. A sensible explanation for the first decline is the rapid depletion of actively cycling BCR-ABL positive, leukemic cells. In contrast, the second decline most likely represents the slow elimination of quiescent residual leukemic stem cells (LSC) owing to their comparatively low turnover.

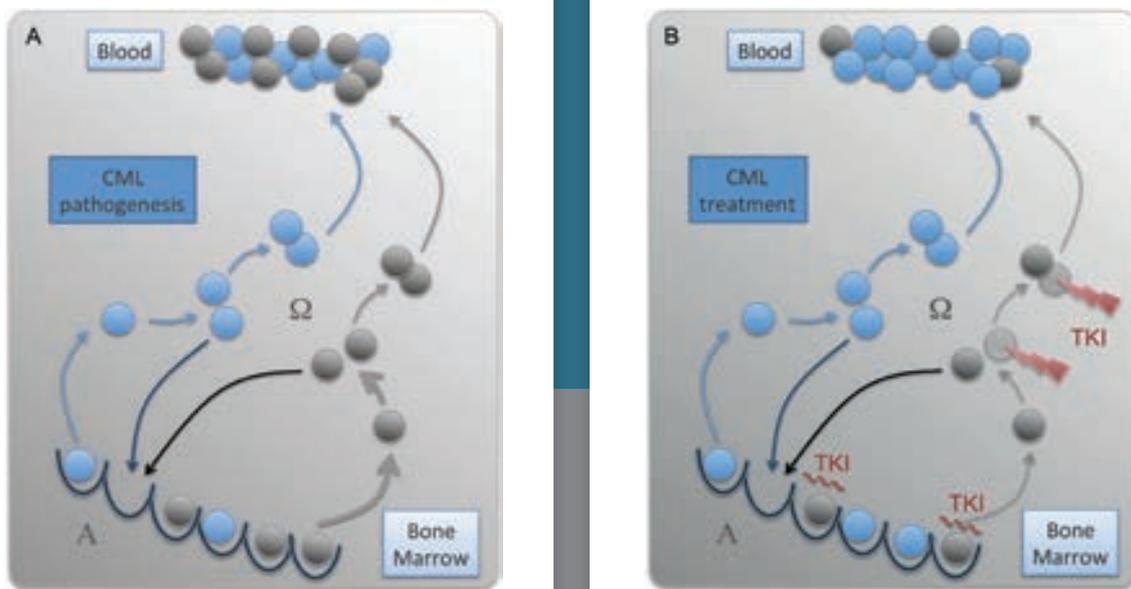


Figure 1: Chronic Myeloid Leukemia

- A)** Normal (blue) and leukemic (grey) stem cells are regularly activated from their bone marrow niches (bottom, indicated in the model as *signaling context A*) and subsequently divide (indicated in the model as *signaling context Ω*). Some cells return to the niches while others undergo further proliferation and differentiation, and contribute to peripheral blood. Due to an increased activation of the leukemic cells compared to normal cells the leukemic pool slowly outcompetes normal hematopoiesis.
- B)** TKIs preferentially target activated leukemic cells (indicated by red arrows), thus leading to a significant reduction of tumor load. Furthermore it appears that leukemic stem cells remain largely quiescent under TKI treatment (indicated by the red lines). Therefore, a residual pool of leukemic cells persists over long time scales (Source: adapted from Glauche *et al.*, 2012)

Although most patients respond well to TKI treatment and often reach complete cytogenetic or even complete molecular remission (i.e. hardly any leukemic cell can be detected in the peripheral blood), it appears that even after a massive and persisting reduction of tumor load over many years of treatment a residual disease is retained in the majority of patients. While these patients often relapse after cessation of TKI treatment, a sustained molecular remission after treatment stop has been observed in some patients (e.g. Mahon *et al.*, 2010). Although these cases are not the rule, they suggest that an eradication of the leukemia might in principle be possible and support the view that CML stem cells are not always found in a treatment-protected (potentially quiescent) state, but can in principle be successfully targeted over time.

Although CML has developed into a controllable disease, side effects of TKI therapy persist and especially for younger patients the impact of long-term TKI therapy is not well understood. Furthermore, high costs of TKI treatment exert also economic pressure on the health care system and pose the question, whether drug combinations can further increase treatment efficiency of the disease (with cure as the ultimate goal) or whether discontinuation of TKI therapy at a certain

time point is a safe option for CML patients with good treatment response.

Mathematical modeling of CML

Taking a theoretical, systems-biological view, we perceive CML as a clonal competition phenomenon between normal hematopoietic and leukemic stem cells, which can be simulated in the computer using an agent-based modeling approach. This concept and its mathematical representation has originally been developed by Ingo Roeder and Markus Loeffler at the University of Leipzig to describe different phenomena in animal and in vitro models (Roeder and Loeffler, 2002). Later on, this model has successfully been applied to humans, namely to describe CML (Roeder *et al.*, 2006; Glauche *et al.*, 2012; Horn *et al.*, 2013). We could show that small differences in cell-specific parameters of leukemic and normal cells can lead to a slow but sustained outcompetition of normal cells, thus mimicking the clinically observed chronic phase in human CML. Treatment of CML patients with the TKI *Imatinib* is assumed to induce first a cytotoxic effect and second an inhibition of the proliferative activity of leukemic stem cells. Technically, the cytotoxic effect is modeled by a selective kill of a fixed percentage of leukemic cells per time step, while

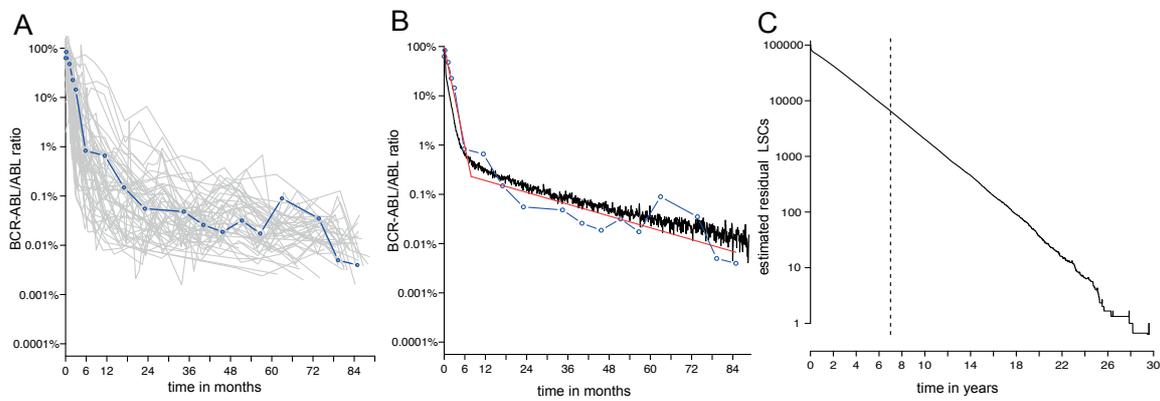


Figure 2: Kinetics of CML under continuous TKI treatment

- A)** Individual time courses of BCR-ABL transcripts levels for CML patients under *Imatinib* treatment with typical biphasic decline. Time course data for a single patient is highlighted in blue.
- B)** For the individual patient, a biphasic regression (red lines) provides the necessary input for a simulated predicted time course (black curve).
- C)** Based on our mathematical modeling approach we provide an estimate of the number of residual leukemic stem cells for this patient, which appears as a major predictor of relapse risk upon treatment cessation. The dashed line corresponds to the time frame of subfigures A and B (Source: adapted from Horn *et al.*, 2013).

the proliferation inhibition is modeled by a reduction of the activation of leukemic cells into cell cycle (compare Figure 1B). We showed that these assumptions are sufficient to reproduce the typical biphasic decline of BCR-ABL transcript levels in TKI-treated CML patients (Figure 2 A,B).

Treatment combinations to increase the long-term success of TKI therapy

Combination of TKI treatment with a cell cycle stimulating drug represents one potential way to increase efficacy of therapy. This idea is based upon the assumption that a cell cycle activation of leukemic stem cells makes them more susceptible to the cytotoxic effect of TKIs, which proved more efficient for cycling compared to quiescent cells, and could, therefore, lead to a faster reduction of the residual clone. Marieke Essers and Andreas Trumpp at the HI-STEM / DKFZ in Heidelberg (Essers *et al.*, 2009) reported about the activating effect of IFN α that directly acts on murine HSCs and induces increased cell cycle activity. Although the findings were obtained in mice, these results again fostered the discussion on enhancing the TKI treatment in CML patients by cell cycle stimulating drugs.

Together with the biological partners in Heidelberg we took up the idea of a combination treatment of TKI and a secondary drug, stimulating the cell cycle activity of hematopoietic stem cells, exemplified for IFN α (Glauche *et al.*, 2012). Specifically, we used our mathematical model to study the potential overall treatment benefit for different activation effects of IFN α on human leukemic stem cells as well as for different treatment schedules. Our model suggests that a successful

combination therapy of TKIs with IFN α in CML patients requires the simultaneous application of both drugs in overlapping time intervals. In addition, a less frequent application of IFN α reduces the speed of tumor reduction but might also decrease potential side effects and risks of the combination therapy. We demonstrated that a weekly or biweekly administration of IFN α under optimal conditions still shows a significant advantage compared to *Imatinib* monotherapy and can reduce the predicted time to tumor eradication considerably. Even in a less favorable situation (i.e. IFN α does not induce activation of leukemic stem cells in humans), a pulsed IFN α therapy under continuous TKI administration is predicted to show no adverse effects compared to standard TKI monotherapy. These findings support recent clinical results that argue in favor of lower doses/longer cycles of IFN α administration in combination therapies to reduce severe side effects while retaining the curative intent.

Estimating residual disease levels and predicting optimal timing for treatment cessation

The question whether TKI therapy can safely be withdrawn after sustained molecular remission, is still controversial. Recent clinical trials report that about 40% of patients being in complete molecular remission for at least two years, retain their previously achieved molecular responses after *Imatinib* treatment cessation, while a molecular recurrence of BCR-ABL transcript levels is observed in the remaining 60%. Relapses can even be observed in patients lacking any measurable BCR-ABL transcripts in peripheral blood. Together with Markus Loeffler and Matthias Horn (Universität Leipzig), we applied our mathematical modeling framework to study whether individual

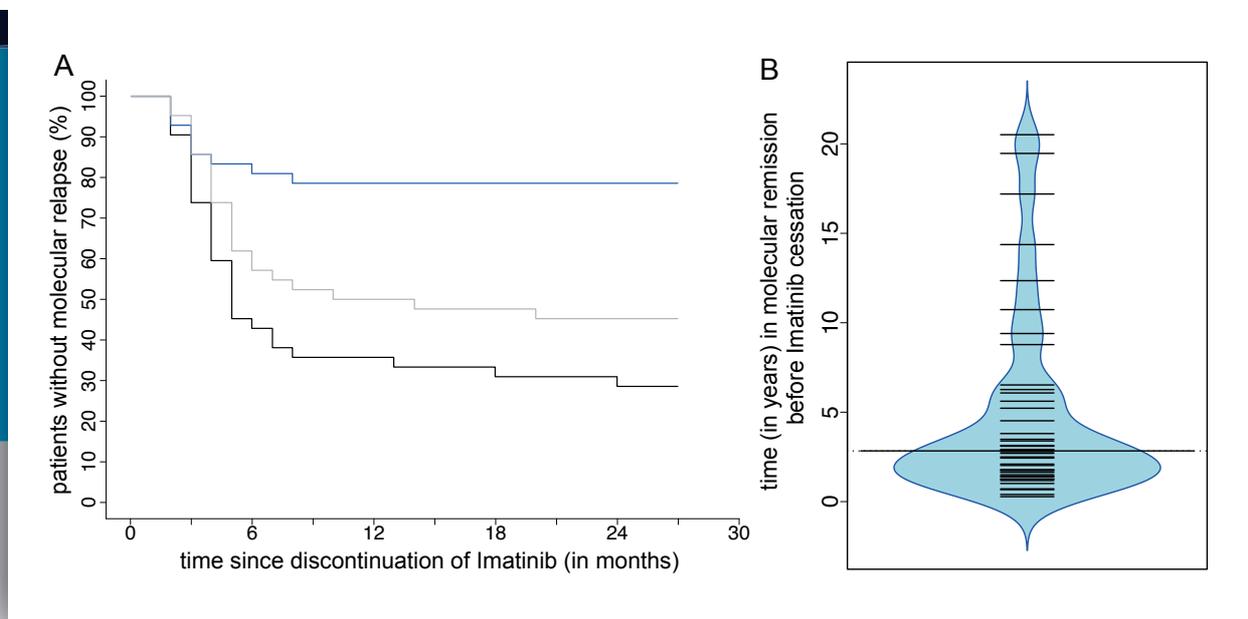


Figure 3:

- A)** Model predictions for the relapse free survival of *Imatinib* treated CML patients after therapy discontinuation: **black** (standard strategy) – therapy cessation after 2 years in major molecular remission; **grey** – therapy cessation after 2.8 years in major molecular remission (compare median in subfigure 3B); **blue** (individualized strategy) – therapy cessation for sufficiently low LSC numbers based on model predictions.
- B)** Estimated individual waiting times in major molecular remission until treatment discontinuation is recommended for the individualized, model-based cessation strategy. Median treatment time: 2.8 (range, 0.3-20.5) years (Source: adapted from Horn *et al.*, 2013).

treatment response kinetics are predictive for the relapse risk after treatment cessation (Horn *et al.*, 2013). In particular, we aimed at a sufficiently precise estimate of residual LSC numbers, which are a critical determinant of relapse after therapy discontinuation. Based on seven-year follow-up data of BCR-ABL transcript dynamics from the German cohort of the IRIS trial, provided by Andreas Hochhaus (Universitätsklinikum Jena) and Martin Müller (Medizinische Fakultät Mannheim der Universität Heidelberg), we determined model parameters, which quantitatively characterize the inter-individual heterogeneity of the molecular treatment response. Given a patient's BCR-ABL transcript kinetic, the adapted model generates predictions for patient-specific long-term response to *Imatinib* (Figure 2 B,C) as well as individual times to complete eradication of residual leukemic stem cells. Based thereon, we derived a model-based predictor for the individual risk of molecular relapse upon treatment cessation. A simulation-based comparison of overall relapse-free survival demonstrates that our proposed patient-specific predictor results in a superior clinical decision rule to decide on potential discontinuation of therapy as compared to relying on a fixed (e.g. two years) time in sustained deep molecular remission. Figure 3A

indicates that relapse-free survival can possibly be achieved for up to 80% of patients. Furthermore, our results suggested that there is a high patient heterogeneity with respect to the time in complete molecular remission, which is required to guarantee a sustained remission in case of treatment cessation (Figure 3B). Whereas for some patients a safe treatment stop is predicted to be feasible already after one year in complete molecular remission, for others 10 or even more years appear necessary.

Outlook

Ongoing clinical trials on combination therapies (e.g. CML-V trial) and controlled TKI cessation will yield major insights on a potential operational cure of CML, but will also generate important data to refine the computational disease models. Based on these models, adaptations to individual patient data will allow predicting disease development and thus estimating the risks of clinical interventions. Together with our clinical and experimental collaborators, we plan to develop the current mathematical models to a stage where they can eventually assist clinical decision-making.

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Further information:

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www.haematosys.de

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