

Molecular and immunophenotypic features of chronic myeloid leukemia in an era of evolving therapeutic strategies

Until only a decade ago, treatment of CML was very unsatisfactory. The only curative treatment option was allogeneic stem cell transplantation, however, due to donor availability or age restrictions, only around a third of patients was eligible for this procedure that was associated with considerable mortality and morbidity. The alternative, interferon alpha with or without cytosine-arabioside, was moderately active in only a subset of patients, and was poorly tolerated by almost all patients. In the majority of patients, acceptable treatment results were therefore not attained. It is against this background that we started the studies that are described in this thesis. In view of the large number of patients that were ineligible for allogeneic stem cell transplantation, we first set out a study that tried to assess the feasibility and efficacy of autologous stem cell transplants. Although cure of the disease was not what we expected by performing this procedure, we aimed at slowing progression of this otherwise fatal disease. Remarkably, we detected less contaminating malignant cells after one induction chemotherapy cycle than after the second, a finding that is interesting, but cannot easily be explained (Chapter 8). Due to the advent of the very effective tyrosine kinase inhibitor imatinib, treatment results have improved dramatically since then. Still, a substantial number of patients shows an unsatisfactory response and even after the introduction of the second generation tyrosine kinase inhibitors, like dasatinib, nilotinib and bosutinib, blast crisis of CML remains a problem, as is illustrated by the two case reports that are presented in this thesis (Chapter 10 and 11).

In a preliminary effort to unravel the genetic background of disease progression in CML, we identified differentially expressed genes during the course of the disease in a large number of patients by suppression subtractive hybridization (Chapter 2). Several candidate genes that potentially play a role in development of blast crisis were found. Unfortunately, one of the genes that was expected to be quite promising in this respect, interleukin-8, seemed to be irrelevant after closer examination. Other candidate genes like GAS2 are currently being investigated, with the hope of finding new therapeutic targets.

In the meantime, new treatment strategies for blast crisis are urgently needed. Demethylating agents like decitabine and 5-azacytidine have recently become available in clinical practice. We investigated their potential value in blast crisis CML by performing methylation specific multiplex ligation dependent amplification in order to assess methylation patterns of tumor suppressor genes (Chapter 7). Indeed we could demonstrate extensive methylation in blast crisis CD34⁺ progenitor cells, which makes demethylating treatment a promising therapeutic option that deserves further clinical and preclinical investigations.

Even better than improving outcome of blast crisis is preventing its development. This can only be achieved after optimizing chronic phase treatment results. As we show in Chapter 9, the addition of cytarabine to imatinib seems to have enhanced antileukemic activity over imatinib alone. The current randomized HOVON-78 study comparing imatinib with imatinib plus two courses of intermediate dose cytarabine will assess the value of combination therapy in a prospective way.

An important barrier in attaining cure is the inherent resistance of CML stem cells against a multitude of agents, including tyrosine kinase inhibitors. Moreover, these persistent stem cells are a potential source of blast crisis initiating cells. Leukemic stem cells make use of several mechanisms to defy antileukemic agents, as reviewed in Chapter 1. Unfortunately, many of these mechanisms are shared by normal hematopoietic stem cells, which leaves pharmacologic manipulation of these mechanisms non-leukemia specific and thereby possibly too toxic. Immunotherapeutic approaches may be able to specifically kill CML stem cells without addition of toxicity, most probably in concert with tyrosine kinase inhibitors. In Chapter 4, we demonstrate that imatinib does not reduce the T-cell stimulating properties of CML derived dendritic cells, implicating that concomitant tyrosine kinase inhibition and immunotherapy is indeed possible. However, vaccination strategies have thus far demonstrated only limited clinical efficacy. Therefore, identification of new leukemia and stem cell specific targets is still needed. With that aim, we developed a new flowcytometric strategy that enabled us to distinguish malignant from normal stem cells in chronic phase CML with high fidelity (Chapter 5). Moreover, we could demonstrate that CD90 is a leukemia specific stem cell marker at diagnosis (Chapter 6), possibly pointing at CD90 as a future leukemia target.

Never before, it has been possible to select malignant and benign stem cells within single patients in such a reliable and prospective way. Currently, by exploiting this technique, we are collecting benign and malignant stem cells from newly diagnosed CML patients to perform comparative assays. In this way, we anticipate to identify new CML stem cell specific therapeutic targets which will hopefully pave the way to new specific signal transduction inhibitor strategies or innovative immunologic treatments.