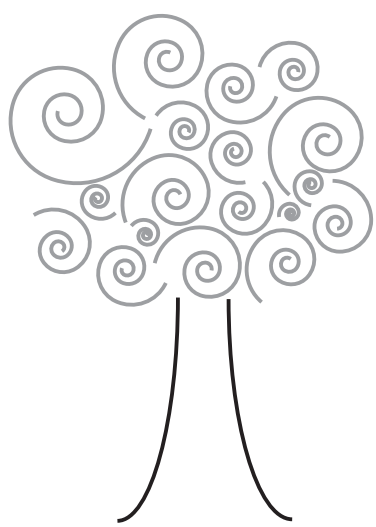
Detection and characterization of leukemic stem cells

van Rhenen, A.

2008

document version
Publisher's PDF, also known as Version of record

Summary and discussion



Presuming that acute myeloid leukemia is a malignant stem cell disease, we studied the cell fraction in which those stem cells are most likely present: the CD34-positive and CD38-negative (further referred to as "CD34+CD38-") compartment. We did so in samples of patients with newly diagnosed acute myeloid leukemia (AML) and in samples taken from patients who were in complete remission after induction chemotherapy with or without minimal residual disease (MRD). This thesis contains the reports of these studies. We addressed different questions on the impact of quantities of leukemic stem cells and on their specific features.

First, we discuss the prognostic impact of the size of the stem cell compartment in the AML bone marrow. Next, we describe the possible options for leukemic stem cell targeted antibody therapy by defining AML associated cell surface marker aberrancies. Since such therapies might preferentially be used after debulking therapy we addressed whether leukemic stem cells can be detected after chemotherapy and how this is related to prognosis. Also, we studied gene expression of leukemic CD34+CD38- cells with the aim to find new targets for therapy. Finally, we report on marker expression of the side population (SP) in AML, which might represent the leukemic stem cell population in the absence of a CD34+CD38- compartment.

Chapter 1 offers an overview of the literature regarding the topics of this thesis. Normal haematopoiesis, diagnosis of and therapy for AML, MRD and the different characteristics of leukemic stem cells are discussed.

In chapter 2 the direct clinical relevance of the stem cell compartment is presented. A high stem cell frequency at diagnosis significantly correlated with poor survival. Presumably, this is mediated by high levels of MRD, since a high stem cell frequency at diagnosis is significantly correlated with a high MRD frequency, which in turn directly correlated with poor survival and a high chance of relapse.

In chapter 3 the expression of the recently discovered antigen CLL-1 is reported. This antigen CLL-1 proved to be expressed on the CD34+CD38- population of AML patients at diagnosis but was completely absent on normal CD34+CD38- cells. Using the leukemia-relevant NOD/SCID mouse model, we found that the CD34+CLL-1+ population of AML patients did contain leukemia-initiating cells and therefore it can be concluded that CLL-1 is expressed on true stem cells in AML. Moreover, CLL-1 was significantly higher expressed on residual CD34+CD38- cells after chemotherapy in AML patients who subsequently relapsed. This is in contrast to patients in whom such a population (CD34+CD38-CLL-1+) could not be detected and who enjoyed continuous complete remission.

In **chapter 4** it is shown that leukemic CD34+CD38- cells of AML patients at diagnosis not only express CLL-1, but also many different lineage markers/marker combinations not present on normal stem. Taken together these results with that of chapter 3, at present normal and leukemic CD34+CD38- cells can be discriminated in the majority of AML patients, not only at diagnosis but also after chemotherapy. After chemotherapy, these immunophenotypic differences may be used to study the frequency of residual malignant CD34+CD38- cells, which are after all responsible for relapse.

In **chapter 5** we discuss the search for putative new targets on leukemic CD34+CD38- cells that are associated with the malignant behaviour of the leukemic stem cells. Since the focus was on stem cell associated targets we compared the gene expression profiles

of the leukemic stem cell compartment (CD34+CD38-) with the corresponding more mature progenitor compartment (CD34+CD38+). In total, 247 genes were found to be significantly differentially expressed between these leukemic cell populations. Of these genes, 191 were functionally characterized genes.

In **chapter 6** we describe the phenotypic characteristics of the side population (SP) in AML patients. The SP might represent the stem cell population in samples of AML patients without a detectable CD34+CD38- compartment. The SP compartment was studied in 40 AML patients and was found to be a highly heterogeneous population. Both lymphocyte precursors, malignant myeloid precursors and a mixture of normal and primitive cells all defined by marker expression and light scatter properties were present. Similar to the leukemic CD34+CD38- population, SP cells express the aberrant markers CLL-1 and the different lineage markers, all with complete absence on the normal bone marrow SP cells.

Future perspectives

It is generally thought that future strategies to improve clinical outcome of AML patients, AML research should be focussed on these malignant stem cells with the ultimate goal of cure for every AML patient with minimal morbidity and mortality.

The first requirement is the ability to detect malignant stem cells in every AML patient at every time point. The combined use of CLL-1 and different lineage markers fulfils the criteria required for stem cell markers. However, not all AML patients express CLL-1 and/or lineage markers and therefore new markers need to be found.

We have shown that detection of malignant stem cells in AML patients after therapy enable prognostication. It needs to be established whether this might result in the identification of patient groups that require more intensive or novel treatment strategies. Hopefully this would result in higher relapse-free survival with reduced mortality and/or morbidity.

Apart from the use as prognostic markers, the possibility to detect malignant stem cells enables the search for new treatment modalities that specifically target the leukemic stem cell population. CLL-1 might be an interesting target for monoclonal antibody therapy. To that end, anti-CLL-1 production should be performed under GMP conditions and conjugation with a (toxic) compound is necessary, followed by tissue distribution and toxicity studies with the final goal of performing phase I trials in AML patients. Apart from CLL-1 such modalities may include other monoclonal antibodies, modifications in chemotherapy, novel drugs like signal transduction inhibitors, or the use of vaccination programs. Most probably, combinations of these new developments are necessary. Moreover, specific detection of leukemic stem cells after chemotherapy might enable to investigate the effects of new treatment regimens in an individual patient.

This thesis helps to reach the ultimate goal by defining more precisely the leukemic stem cell population using sophisticated immunophenotypical technology.