University of Medicine and Pharmacy Tîrgu Mureş
Doctoral School
Abstract of the PhD thesis

The impact of the new diagnostic and therapeutic methods on the prognosis and survival of the patients with Chronic Myeloid Leukemia.

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Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder that arises in the stem cell compartment. The molecular hallmark of the disease is the *BCR-ABL* gene rearrangement, which usually occurs as the result of a reciprocal translocation between chromosomes 9 and 22. The molecular consequence of this translocation is the generation of a BCR-ABL fusion oncogene, which in turn translates into a BCR-ABL oncoprotein. This most frequently has a molecular weight of 210 kD ($p210^{Bcr/Abl}$) and has increased tyrosine kinase activity, which is essential to its transforming capability. Imatinib (previously STI571), a small molecule tyrosine kinase inhibitor (TKI), was the first drug that targeted BCR-ABL and it has become the standard frontline therapy for CML. The majority of patients will do well on standard therapy. However, some patients will fail on imatinib and require alternative therapies. Currently, the main tool to identify high-risk patients is close monitoring of their in vivo response to therapy. Real-time quantitative polymerase chain reaction (RQ-PCR) provides an accurate measure of the total leukemia cell mass and the degree to which BCR-ABL transcripts are reduced by therapy correlates with progression-free survival.

At the Clinical Hematology and BMT Unit Tg-Mures between 2008-2018 we performed the molecular monitoring the bcr-abl transcript levels with RQ-PCR at 59 patients diagnosed with CML. We collected a complete set of baseline data: a clinical exam with documentation of spleen size, complete blood count (CBC) with white blood cell differential, and bone marrow biopsy with metaphase karyotyping, RQ- PCR for BCR-ABL rearrangement. For the patients in advanced phase of the disease flow-cytometry was performed from bone marrow to determine the percent and the phenotype of the blast cells. At dose patients who have resistance to the treatment we performed the mutational analysis T351I, M351T. The statistical analysis was performed with Medcalc version 18.9.

We performed three studies with the aim of demonstrating the importance of molecular analysis performed regularly with RQ-PCR at the chronic myeloid leukemia patients and to determine the role of molecular analysis on the decision of treatment change and the impact of the tyrosine kinase inhibitors on the overall survival and progression free survival of the patients.

In the first study we analised the impact of new therapeutic methods on the prognosis of the patients with CML. We calculated the overall survival and progression free survival according to disease phase and Sokal score.

Due to the new therapeutical methods the prognosis of the pacients with CML regarding the long therm survival is very good. The progression free survival at 5 years is 85-95% and the overall survival at 5 years is 85-95% in the literature wich is slightly diffrent from our patients (PFS 5 year 75%, OS 80%).One of the possible causes of this diffrence is the longer time from diagnosis to TKI therapy before 2017. Median survival of the patients before the TKI treatment was 8.9 years in chronic phase, 4.8 years in accelerated phase and 6 months in blastic transformation. In our study the cumulative overall survival at 5 years was 85% and 76% at 8 years. The curve is presenting a plateau phase maintaining the response after 15 years.

There are significant diffrences when we are looking for progression free survivals according to Sokal score (low risc PFS 91%, intermediary 66% and high 51%) (Logrank test (P = 0.0029) The CML patients in chronic phase presenting a 90% OS at 5 years The OS at 5 years in accelerated phase is 83 and the patients in blastic

phase rarely survive over 5 years. (Logrank test (P = 0.0001)) The Sokal high risk patients presenting progression in the first 3 years from the diagnosis progression rate at 5 years is 76%. The intermediary risk patients presenting progression in 54% in the first 5 years and only 8% of the low risk patients.

In our set of patients 59% reached the MR with a median time of 11 months. The majority of patients reaching MR is in chronic phase at the diagnosis (28/31), 5 patients in accelerated phase. None of the patient in blastic transformation at the diagnosis reached MR. The response was durable. The cumulative probability of maintaining MMR on TKI is 100% at 5 years and 91% at 10 years and only 53% at 15 years.
Only 6 patients loosed the MR and the median time was 5 years after TKI initiation. Four patients regain MR under second line TKI but the median duration to reach MR was longer (33 month). The median time for follow up was 5 years and 9 months. (one month to 17 years). 19 patients presenting elevated BCR-ABL transcript levels. We performed the mutational analysis. We have possibility to perform only the T351I and M351T mutations. Six patients were positive to M351T mutation which is conferring resistance to imatinib. These patients responded to the second generation TKI. The extension of the mutational panel probably would identificate more mutations.

In the second study we presenting the impact of the molecular methods in management of patients with intolerance or resistance to TKI who undervent allogeneic hematopoietic stem cell transplantation.
The identification of the possible donors at the high risk patients setting is essential. The allogeneic transplant can cure up to 80-85% of CML patients but is associated with high rate of morbidity and mortality. The disease status at the transplant is a powerful predictor of the results. The advanced phase disease has a very poor prognosis. Most of the patients transplanted in our unit had advanced phase of the disease and with high EBMT score. The donors were 100% compatible siblings. The graft was constituted of peripheral blood stem cells obtained with apheresis procedure. The conditioning regimen was BuCy except in one case with reduced intensity conditioning due to the poor general condition. The immunosuppression was performed with cyclosporine and methotrexate and in two cases tacrolimus. All of the patients presented complications after the HSCT. The most of the complications was infectious (bacterial, fungal, viral). We had two cases with hemorrhagic complications and was manifested in bleeding from central venous line. We had one case of supraventricular extra systole. Three patients after a period of disease free survival died due to infectious complications. Five patients presented GVHD. Four cases of acute GVHD with grade I or II. Two of them had progression to cGVHD. One patient presenting extensive GVHDc after 5 years from transplantation unresponsive to the treatment (bullosus epidermiolysis, skin sclerosis). One patient is presenting progression free survival with the follow up of 12 years. The patient had intolerance to TKI and low EBMT score at diagnosis. We had two cases of early relapse both cases were Sokal high risk with elevated EBMT scores.

In the third study we analysed the efects of molecular monitoring with RQ-PCR in CML on the therapeutical decision.

Those patients who reached the complete cytogenetic response can be monitored with RQ-PCR for the MRD detection. The guidelines recommend monitoring every 3 months but due to high cost we could only performed every 6 months until 2015. The standardization process lead to the development of a conversion factor which allows to give results in IS comparable between laboratories. In our cases we had no standardization of the method but the serial determination in the same laboratory permits the comparison of the results. Increasing levels of transcript permit the treatment changes before the hematological changes. Those patients who presenting increasing transcript levels can benefit most of the treatment change and permits the search for a compatible donor before the progression of the disease to accelerated and blastic phase. The RQ-PCR although a reliable method of detecting minimal residual disease post-transplant coming in help of the physician with the identification of the high risk patients for relapse.