VI. Discussion and Perspectives

VI.1 Section I: Effects of TKIs on normal hematopoiesis

As exposed earlier in section I.2.2, beneficial effects of allo-HSCT rely in a large part on elimination of malignant cells by donor T cells contained in the graft. This phenomenon is called the GvT effect. While allo-HSCT is a potentially curative treatment for Philadelphia-positive acute lymphocytic leukemia (ALL), the majority of older adults with ALL undergo allo-HSCT following RIC (reduced-intensity or non-myeloablative conditioning regimen). However, a 2-4 week TKI discontinuation may increase relapses in patients receiving RIC allo-HSCT for Philadelphia-positive CML and ALL. Administration of imatinib early after HSCT might thus provide an effective approach for preventing recurrent Philadelphia-positive leukemia, but the feasibility of this approach had not been systematically tested at the time of our study. We thus hypothesized that combining allo-HSCT (with or without RIC) with TKIs could maximize the antileukemic activity against Philadelphia-positive leukemias. Moreover, a careful evaluation of TKI administration in preclinical models was needed to ensure that reconstitution of a fully functional hematopoietic system is not disturbed, especially for TKIs administration early after transplant (corresponding to the engraftment period).

In this first study, we demonstrated that TKIs (both imatinib and nilotinib) did not impair *in vitro* two important features of human cord blood $CD34^+$ HSCs: migration (through a SDF gradient), and adhesion (to retronectin). However, cell-cycle entry and differentiation into hematopoietic progenitors were severely impaired upon treatment with both imatinib and nilotinib. Despite these important inhibitory effects, engraftment of human cord blood $CD34^+$ HSCs in NSG mice was not affected by a 42-day treatment with imatinib/nilotinib initiated the day of the transplantation, and no severe adverse effects were observed during the treatment administration period. This suggests that administration of imatinib or nilotinib early after transplantation could be safe.

These data confirmed our previous findings that imatinib, despite inhibition of hematopoietic progenitor cell growth *in vitro*, did not interfere with $CD133^+$ PBSC engraftment [403]. However, we found a significant decrease in VLA-4, VLA-5 expression on $CD34^+$ CB HSCs under imatinib and nilotinib treatment, in contrast to what was observed in our previous paper. This could be related to HSC origin since, in the current paper, $CD34^+$ CB HSCs were used, while in the publication by *Pirson* et al., $CD133^+$ cells isolated from peripheral blood of mobilized healthy volunteers were used.

Safety of imatinib prior to allo-HSCT has been largely investigated in retrospective studies. A CIBMTR study, including data from 1309 CML patients receiving or not imatinib before HSCT, provided interesting insight. Among patients in first chronic phase, imatinib therapy before HSCT was associated with better survival while no statistically significant differences were observed for other endpoints such as treatment-related mortality, relapse, and leukemia-free survival. Further, in patients transplanted for advanced CML, the use of imatinib before HSCT was not associated with transplantation outcomes. Furthermore, aGvHD incidences were similar between patients receiving imatinib or not regardless of leukemia phase [404]. A second smaller retrospective study assessed the impact dasatinib and nilotinib on 12 imatinib-resistant CML patients undergoing allo-HSCT. All patients engrafted and full donor chimerism was reached post-transplant. Only three patients experienced GvHD and no transplant-related mortality was recorded. Altogether, these data suggest that both imatinib and second-generation TKIs given before allo-HSCT do not negatively affect engraftment and response, nor increase transplant-related toxicity [405].

On the other hand, other studies have assessed the potential impact of TKI administration after allo-HSCT to maximize the anti-leukemic effect. Indeed, in 2007, a retrospective study on 22 (15 ALL and 7 CML) patients receiving myeloablative conditioning reported that administration of imatinib from the time of engraftment until 365 days after HSCT was safe. Nausea, emesis, and serum transaminase elevations were the most commonly observed adverse effects [406]. A second study by *Ram* et al. showed that patients with Philadelphia chromosome-positive ALL, receiving non-myeloablative conditioning regimen, and treated with post-grafting imatinib (started on D+15 post-transplant or when neutrophils reached 0.5×10^9/L to avoid imatinib interference with engraftment and given at a daily dose ranging from 200 mg to 600 mg) had an encouraging 3-year overall survival rate of 62%. Further, in the subgroup of patients without evidence of minimal residual disease at transplantation, OS was 73% [407]. In general, imatinib was well tolerated. Three patients (17%) discontinued imatinib because of adverse events, all of which were reversible (two cases of gastrointestinal toxicity and one of recurrent pleural effusion).

Few prospective studies are available. Among them, Wassmann et al. administered imatinib at the initial dose of 400 mg/day to patients with detectable minimal residual disease (MRD) after either allo-HSCT or auto-HSCT. In 52%, the BCR-ABL transcript became undetectable after a median of 1.5 months. Patients achieving molecular remission remained relapse-free during imatinib administration. Among patients who failed to achieve MRD negativity the relapse rate was 92%. The LFS rate at one year was 91% for patients with

early MRD negativity compared to 9% for those who remained MRD-positive. The authors concluded that continued detection of BCR-ABL transcripts after 2 to 3 months on imatinib identifies patients who will ultimately experience relapse and in whom additional or alternative antileukemic treatment should be initiated [408]. In a second clinical trial, imatinib was administered for 3-12 months after allo-HSCT, until MRD negativity was confirmed for three consecutive tests or sustained for at least three months. The initial dose of imatinib in adults was 400 mg/day. Median time of treatment initiation was 70 days post-transplant. Only 16% of patients had to discontinue the treatment due to cytopenias, edema and nausea/emesis. The probabilities of relapse and OS at five years for patients receiving imatinib maintenance were 10% and 87%, respectively, confirming feasibility and suggesting high efficacy of imatinib prophylaxis after allo-HSCT [409].

The only randomized trial using imatinib (600 mg/day) post-transplant was published in 2013 [410]. Two different strategies were studied: prophylactic (n=26) and minimal residual disease (MRD)-triggered therapy (pre-emptive approach, n=29). In the pre-emptive group, the drug was initiated only after detection of MRD using qPCR. The median times of treatment initiation were 48 and 70 days post-transplantation in prophylactic and pre-emptive patients, respectively. However, the rates of early treatment discontinuation were 67% and 71%, in prophylactic and pre-emptive patients, respectively, probably due to the high dose of imatinib administered (600 mg/day). Although there was a tendency towards longer duration of molecular remission in the prophylactic treatment arm, event-free survival and OS (80% versus 75% at five years, p=0.84) did not differ significantly between the 2 groups.

Further, one prospective study has been published on the use of the second-generation TKI nilotinib after transplantation. Twenty-two allo-HSCT recipients with CML or Ph-positive ALL were enrolled and 16 patients received prophylactic nilotinib maintenance. Nilotinib was initiated a median of 38 days after allo-HSCT at a dose ranging from 200 mg to 400 mg every 12 hours. Major adverse effects included liver toxicities, elevated lipase/amylase, neutropenia, allergy, skin reaction, stroke. Interestingly, following nilotinib maintenance 11 patients achieved (n=9) or maintained (n=2) a complete molecular response [411].

In conclusion, little information is available on the use of TKI, especially imatinib and nilotinib, right after allo-HSCT. Indeed, most of the published retrospective studies have investigated the use of TKIs after engraftment of HSCs (when neutrophil counts reached a normal level). However, as explained earlier, TKI discontinuation could increase relapses in ALL and CML patients undergoing

HSCT. Our study suggests that it should be safe to continue the TKI throughout the transplantation period.

VI.2 Section II: TKI for scl-cGvHD

In the first study, we have demonstrated that TKIs did not impair *in vitro* or *in vivo* migration, adhesion and engraftment of human cord blood $CD34^+$ HSCs in a xenogeneic model of bone marrow transplantation using NSG mice. Moreover, no severe side effects were observed during the treatment administration, making the use of imatinib and nilotinib safe after transplantation.

Scl-cGvHD develops in approximately 20% of cGvHD patients and shares features with systemic fibrosis and other autoimmune disorders. Despite recent progress in understanding aGvHD pathogenesis, scl-cGvHD pathogenesis is still unclear. However, it has been shown that activated donor T cells are largely involved. Specifically, data from murine models of cGvHD suggested that donor T cells involved in scl-cGvHD are mainly Th2 $CD4^+$ cells. These Th2 cells secrete IL-4, IL-5, IL-10, IL-11 and IL-13 that stimulate other cells to release fibrosing factors such as IL-13, PDGF and TGF-β. These fibrosing factors then induce fibrosis in the skin and other affected organs. As explained earlier in section I.3.2.2.4, PDGF and TGF-β signaling pathways play a significant role in the fibrosing processes occurring during scl-cGvHD. Imatinib is also a well-demonstrated inhibitor of PDGF and TGF-β signaling pathways (by inhibiting PDGF-r and c-Abl tyrosine kinases) and has been studied in patients with scl-cGvHD.

The work by *Oliveiri* and colleagues confirmed the safety of imatinib for cGvHD treatment, with limited (extra-) hematological toxicity and no toxic deaths. Best responses were achieved in patients with skin and gastro-intestinal involvement. However, lung cGvHD was not improved. In contrast to these encouraging results, several reports observed limited efficacy of imatinib with few patients responding to the treatment. These observations were also true for steroid-resistant scl-cGvHD patients, since imatinib failed to improve skin sclerosis and presented serious adverse events (fluid retention and cytopenias) in some patients [308]. These conflicting results underline the interest for re-assessing the impact of imatinib in scl-cGvHD in pre-clinical models. This prompted us to investigate the impact of imatinib in a well-studied murine model of scl-cGvHD. The main observation for this study was that imatinib failed to ameliorate scl-cGvHD in a severe murine model of scl-cGvHD despite the fact that it significantly inhibited PDGF-r.

We first found that administration of imatinib *in vivo* was able to inhibit both