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The Value of Minimal Residual Disease (and Diamonds)

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When each us decided to wear a white coat daily, as a doctor, we all made a clear choice. Our beloveds usually did not; nevertheless, they had to endure it. Sometimes, when they pretend to show some interest in our activities, they are actually trying to estimate how much of our time, potentially shared with them, will be taken away in the near future.

So happened when I started investigating the impact of minimal residual disease (MRD) and chimerism on outcome in the transplantation setting of pediatric acute lymphoblastic leukemia (ALL). When my husband cautiously asked me what I was dealing with, my answer was, "It's like when you gave me the engagement ring, you wondered 'How could such a small thing being so expensive?' [By the way: most of the magic vanished upon that statement!] It is basically the same here: a residual disease, so small that you could hardly assess it, but that seems to make the difference between cure and disease. It's indeed a matter of life or death." It was clear to me that MRD would have had a deep impact on my patients' lives. And it was suddenly clear to my husband that such "a small thing" (smaller than a diamond!) would have had a big impact on our family's daily life, too.

During the last decade, MRD has been used to stratify patients and tailor risk-adapted therapy; moreover, MRD before and in the post-transplantation course has become an important prognostic factor to identify patients at highest risk of post-transplantation relapse. Also, chimerism analysis might be used not only to monitor engraftment, but also to predict the recurrence of the underlying disease.

In this issue of *Biology of Blood and Marrow Transplantation*, Rettinger et al. report a series of 89 consecutive patients who underwent first transplantation for ALL, in whom immunological interventions were planned in case of post-transplantation mixed chimerism or MRD positivity [1].

Chimerism analyses were performed in all patients weekly and then monthly, after the first semester, in peripheral blood (PB) or bone marrow (BM), while MRD was monitored in the BM monthly and then every 3 months, after the first quarter, in the 58 patients who had disease samples available [1].

The intervention consisted of immunosuppression (IS) tapering or discontinuation, for patients who were still receiving it, or donor lymphocyte infusion (DLI) upfront, for patients who had already discontinued IS or as a second-line treatment, in case recipient/disease cells persisted after IS discontinuation. Absence of grade II or higher graft versus host disease (GVHD) was required as an eligibility criterion for any intervention. In HLA-identical, matched, or haploidentical donor recipients, the CD3 content of the starting DLI doses were 1×10^6, 5×10^5, 1×10^5, respectively, per kilogram of recipient body weight. Subsequent DLIs were scheduled until MRD negativity or full donor chimerism were achieved; dose escalation was planned in case the donor was fully matched [1].

Of the 28 patients who experienced mixed chimerism or MRD positivity, 23 patients were treated; 9 with early IS withdrawal, 11 with DLI, and 3 with both. Of 23 patients, 15 are in continuous complete remission, with an event-free survival (EFS) at 3 years of 69% and a cumulative incidence of relapse (CIR) of 19%. These outcome estimates in the group at risk who underwent interventions were similar to the 69% EFS and 20% CIR achieved by the group overall [1].

The paper is interesting; not surprisingly, given the authorship.

In my opinion, it raises 4 crucial issues.

First, does chimerism contribute to MRD monitoring in the early prediction of relapse? The issue is still controversial and has been previously discussed in this journal [2].

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In this report from Frankfurt, 24 patients experienced mixed chimerism and 23 MRD positivity at any time after transplantation; the latter mostly overlaps with the former subgroup, since a total of 28 patients overall qualified for immune interventions [1]. It is not directly stated how many patients could not have been identified, if MRD only had been monitored. Apparently, all of the 23 treated patients had experienced MRD positivity and 19 of them had also experienced some mixed chimerism, but no MRD negativity was reported in patients with mixed chimerism [1]. One could conclude that chimerism did not identify additional patients. Nevertheless, chimerism in PB was checked much more frequently than MRD in BM, and the timing between the 2 techniques in each positive patient is not stated. MRD-positive results in BM might have followed a previous mixed chimerism detection in PB, without which no BM analysis would have been performed.

Chimerism is likely to be less sensitive and specific than PCR-based MRD tests. In our hands, chimerism never preceded MRD positivity, whereas the opposite is often the case; however, I must admit that we monitor both MRD and chimerism in BM at the same time points [3]. Increased frequency of sampling, allowed by PB accessibility compared with that of BM, might play a role. Although, the sensitivity cut-off of chimerism analyses is often closer to 10^{-3}, which would limit the efficacy of any intervention [3,4].

Second, does any intervention based on a molecular clue of residual disease ultimately improve survival? There are clues that it is the case, but to which extent, still must be assessed.

It is well demonstrated that ALL patients experiencing any MRD positivity after transplantation have a worse outcome compared with patients for whom no MRD could be detected in the post-transplantation course [3-9]. Nevertheless, whether the dismal prognosis of MRD-positive patients could be reset by an immunological intervention is still to be assessed.

A gap ranging from 25% and 46% in terms of EFS and CIR between patients who experience any positivity anytime and patients who never do so was consistent throughout groups [3-9]. The fact that the subgroup of the patients who received an immunotherapy, because of molecular positivity, reported an outcome which was similar to the outcome overall of the Frankfurt series would strongly suggest the efficacy of the immunotherapy applied [1].

In our previous report, relapse occurred in 7 of the 17 who received an immunological intervention and in 5 of the 8 who did not [3]. DLI were given only upon or after an MRD level of 10^{-3} was detected, when the disease load was too high to allow DLI alloreactivity to be effective [3]. Since 2014, in our institution, any post-transplantation MRD level prompted donor lymphocyte apheresis for DLI, whenever it is feasible.

In a French study, Pochon reported 133 pediatric patients who underwent transplantation for ALL in whom early discontinuation of IS or DLI were prospectively applied if $>10^{-3}$ MRD were detected before or after transplantation [5]. Cyclosporine treatment duration was independently associated with relapse, which would suggest again that IS discontinuation would have played a role in relapse prevention. The first 90 days were reported as the best window to intervene [5].

Pulsipher and colleagues identified the timing +55 to +200 days after transplantation as the optimal window to initiate intervention to prevent relapse. Their Children Oncology Group – Pediatric Blood and Marrow Trial Consortium report highlighted strong interactions between the occurrence of aGVHD and MRD before and after HCT in determining relapse risk and survival in children with ALL after transplantation by showing that aGVHD was protective against relapse and identifying patients who experienced no aGVHD as the best candidates for immune interventions to mimic or replace alloreactivity [6].

Lankester explored the protective potential of alloreactivity, deliberately induced early after transplantation, by early cyclosporine tapering and escalating DLI in 18 ALL patients with MRD level $>10^3$ before transplantation. The intervention was associated with GVHD grade II or higher in 23% of the patients and a 4-year EFS of 19% and CIR of 69% because of delayed relapse, often in extra medullary sites [7].

Third, does any intervention cause more harm than the biological course of mixed chimerism or MRD positivity?

The observation that not all patients becoming MRD positive or, even more, developing mixed chimerism, necessarily relapse poses the issue whether all of them should be treated upon those results. Any treatment would bring some toxicity and, besides potentially preventing relapse, anticipated tapering/discontinuation of IS and/or DLI may jeopardize the course of the transplantation by enhancing GVHD.

Horn and colleagues treated 26 patients with mixed chimerism with fast withdrawal of IS, at a median time of 49 days (range, 35 to 85), out of a series 43 pediatric patients who underwent transplantation with ALL in complete remission. Their EFS at 2 years was 73%, similar to the 83% EFS achieved overall, even though 3 patients developed GVHD, which was fatal in 1 of them [8].

Nevertheless, GVHD incidence and severity were not increased in the group who underwent interventions by Rettinger and colleagues. DLI starting doses were cautious and carefully targeted to the donor/recipient pair compatibility and the majority of the patients (11 of 14) received 1 dose only [1,10].

GVHD after immune interventions can be harmful, sometime fatal, even if this wasn't the case in this Frankfurt series. Nevertheless, interventions seem justified upon post-transplantation MRD detection. *Ex duobus malis, minimum est eligendum* (the lesser between 2 evils should be chosen). One might consider to intervene when MRD levels reached the level of 1×10^{-4}; however, the lower the disease load, the higher the impact would be expected. Ideally, a more sensitive and specific method for identification of patients with impending relapse is desirable; next-generation sequencing might unravel the conundrum in a near future [11].

Fourth, which methodology might help to answer the previous questions?

The outcome of MRD-positive versus MRD-negative patients is an estimate not only of the predictive value of MRD, measured before or after transplantation, but also of the effect of any intervention, which, in turn, might play a different role, according to the disease biology and the immunology of each donor-recipient pair. Whenever we do react to MRD detection, in a certain way, we dilute the predictive role of the MRD itself.

The comparison between patients who were at risk, defined by MRD, and did undergo some interventions, with those who did not, would not be fair, as the 2 subgroups represent different populations. Rettinger et al. wisely did not compare them. Nor did they calculate a *P* value, accordingly.

Patients who experienced any molecular positivity and who therefore would have been eligible but could not be

treated, for any cause, mostly because they had experienced an event before the intervention could apply, should have been accounted for in the treatment group, under the intention-to-treat principle. Otherwise, patients who might experience early events or be too sick to receive DLI would end up being allocated in the no-DLI arm, so that the comparison would be biased by the "waiting time to DLI" effect and by a selection bias.

A randomized trial would hardly be feasible in this setting, as immunological interventions are strongly perceived as effective and the no-intervention arm would not be acceptable for most investigators (and for patients, in the internet era). *Nemo ad impossibilia tenetur*… (nobody has to do what is impossible).

One proposal, which is under discussion within the International Berlin-Frankfurt-Münster Study Group and beyond, would be to join efforts through cooperative groups; agree on reproducible interventions (IS tapering modalities, DLI doses and timing, and so on); register interventions prospectively, besides GVHD and other reasons for no interventions; and report outcome.

Hopefully, tools to foresee impending relapse will gain better sensitivity and specificity, so that identified patients will be suitable candidates for MRD-driven targeted therapy, such as chimeric antigen receptor T cells, bispecific T cell engagers, and advanced conjugated immunotoxins [12,13]. Exciting strategies are in front of us.

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