SUPPLEMENTARY APPENDIX

Sequential therapy for patients with primary refractory acute myeloid leukemia: a historical prospective analysis of the German and Israeli experience

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 doi:10.3324/haematol.2018.203869

Received: August 5, 2018.

Accepted: January 28, 2019.

Pre-published: February 7, 2019.

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Donor Search

Blood for HLA typing was obtained from each patient upon starting induction chemotherapy (or starting hypomethylating agent treatment in the ambulatory day care unit). High-resolution molecular typing using polymerase chain reaction (PCR) in the sampled DNA with sequence-specific primers was performed for HLA A, B, and C as well as for class II alleles (HLA DRB1 and DQB1). In the case of potential matched siblings, the goal was to achieve HLA results by day 14 after starting induction chemotherapy and at least low/ intermediate resolution for unrelated donor search results by day 21. In addition, the "Donor Search Centre" was notified once the patient was identified as having a "probably refractory disease" according to suggestive findings in the day 14 marrow, unrecovered blood count by day 28, or evidence of progression early during treatment. This notification was done to shorten donor identification time.

Treatment

The FLAMSA/ TBI protocol had been previously described (1) and contains a fludarabine, amsacrine, a cytarabine block and a TBI, cyclophosphamide, ATG block. The FLAMSA/ treosulfan protocol (2, 3) is based on a substitution of the 4 Gy TBI with treosulfan. The FITCy protocol is a modified version of the FLAMSA protocol, essentially omitting amsacrine. In detail, patients were initially treated with fludarabine (30 mg/m^2/d) and cytarabine (2 g/m^2/d <65 years or 1 g/m^2/d if ≥ 65 years) for 5 consecutive days from day −13 to day −9. This block was followed by a 3-day break. The RIC part was based on 4 Gy TBI (day -5) and cyclophosphamide (40 mg/kg/d in case of a matched related donor and 60 mg/kg/d in case of a

matched unrelated donor) from day −4 to day −3. For further details, see **supplemental figure 1**.

Anti-thymocyte globulin, ATG (Grafalon, Neovii) (10 mg/kg BW/d until 2013 with related donors, and 10-20 mg/kg BW/d with unrelated or mismatched donors) was used in the FLAMSA/TBI or the FLAMSA/treosulfan from day −3 to day −1. Since 2013, ATG was omitted from the protocol in patients given allografts from siblings. In the FITCy protocol, the same product was used but at a lower dose (5 mg/kg BW/d) and only for unrelated donors. As a graft source, G-CSF mobilized peripheral blood stem cells (PBMC) were preferred and bone marrow (BM) was accepted at the donor's preference. No graft manipulation was performed.

In the two patients with haploidentical donors, no ATG was given and cyclophosphamide was given on day +3 and +4 instead of day -4 and -3.

Supportive care

All patients were hospitalized in a designated ward in single-bed rooms equipped with HEPA filters. Patients given the FLAMSA/TBI or FLAMSA/treosulfan protocol were not routinely treated with antibiotic prophylaxis, while all patients receiving FITCy were given prophylaxis with ciprofloxacin. Antifungal prophylaxis consisted of posaconazole (300 mg/d), micafungin (100 mg/d) or fluconazole (400 mg/d), and HSV/VZV prophylaxis of valaciclovir (1000 mg/d) or acyclovir (1600 mg/d). Pneumocystis jirovecii prophylaxis consisted of either co-trimoxazole 3 days per week or monthly pentamidine inhalation. Weekly monitoring of peripheral blood CMV-DNA by PCR was performed. In case of CMV reactivation, valganciclovir or intravenous

ganciclovir treatment was initiated. In patients who were not given posaconazole prophylaxis, a weekly monitoring of galactomannan antigen in the peripheral blood was performed. Prophylaxis of graft versus host disease (GvHD) consisted of cyclosporine A (CsA, given from day −1 to +100 adjusted to serum level (200–350 ng/ml), tapered from day +100 and discontinued from day +180, if no signs of GvHD were present and mycophenolate mofetil (MMF, 2 g/day in matched related donors and 2-3 gr/day in unrelated donors given from day 0 to +30, tapered from day +30 and discontinued from day +50). In case of CsA side effects or non-tolerability, CsA was replaced by tacrolimus adjusted to serum level (8–12 ng/ml) or sirolimus adjusted to serum levels (5–10 ng/ml). During hospitalization, clinical status, adverse events, hematological as well as biochemistry parameters were monitored on a daily basis. After discharge, patients were seen in the outpatient clinic at least twice per week until day +100 with gradually longer intervals thereafter. Regimen-related toxicities were graded according to the Bearman criteria (4). Acute and chronic GvHD were graded and staged by standard criteria.

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Supplemental figure 1 - Preparative regimens