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Scientific Letters

Older Adults with Ph Negative Acute Lymphoblastic Leukemia: A Monocentric Experience on 57 Patients Focusing on Treatment Intensity and Age-Related Prognosis

Appendix 1

A) Treatment program

Before 2013, the patients were treated according to NILG protocols (NCT 00358072 and NCT 00795756, ClinicalTrial.gov). The scheme of therapy in older patients was similar and it was summarized below.
The protocol plan consisted of chemotherapy blocks administered over 25 weeks in association with central nervous system (CNS) prophylaxis Collection of autologous blood stem cells was performed after cycle 3 (**Figure S1**).

Risk stratification and measurable residual disease evaluation. At diagnosis, patients were stratified in standard risk (SR) and high risk (HR) according to molecular and clinical patient's features. The HR group included patients with the Ph translocation [ie, t(9;22)], or t(4;11), or with corresponding gene rearrangements (BCR::ABL1, KMT2A::AFF1), or adverse cytogenetics (monosomy 7, trisomy 8, 6q deletion, 7p deletion, t(8;14), low-hypodiploidy with 30 to 39 chromosomes, near-triploidy with 60 to 78 chromosomes, complex karyotype with ≥5 unrelated clonal abnormalities) or patients with white blood cell count (WBC) higher than 30 x 10^9/L in B-ALL or 100 x 10^9/L in T-ALL, respectively, patients achieving late complete remission (CR) after cycle 2 of chemotherapy, and those with an adverse EGIL immunophenotype (pro-B or pre-/mature-T). Patients with MRD ≥ 10^{-4} at week 10 were reclassified as HR independently from their initial risk group. The SR group included all patients without any of the HR features
For risk-oriented therapy, the consolidation program was considered concluded for MRD^{neg} cases. These patients began 2-year continuous maintenance therapy, reinforced by pairs of drugs alternated monthly.
In contrast, all MRD^{pos} patients with an available HLA-identical related or unrelated volunteer donor would undergo allogeneic SCT.
For MRD^{pos} patients who were unable to undergo an allogeneic SCT, an intensification treatment supported by autologous blood stem cells was proposed, followed by the maintenance program
When the MRD risk class was unknown ($MRD^{u/k}$), maintenance was the therapy in clinical SR subsets and allograft in HR patients, respectively. If an allogeneic SCT was not possible, the autologous SCT plus maintenance option was indicated.
The cytotoxic humanized monoclonal antibody rituximab was given to patients with CD20+ ALL after 2020.
Induction/Consolidation Therapy
Including subset-specific elements for patients, aged >55 years (y) and aged>65y. CR evaluation bone marrow is checked on days 28 and/or 56. Consolidation cycles are administered at 21-28 day intervals.

Induction/early consolidation therapy for patients aged more than 55 y
Pre-induction: prednisone 20 mg/m^2/bd per os (PO) on days -5 to -1, cyclophosphamide 200 mg/m^2/d intravenously (IV) on days -3 to –1
Cycle 1: idarubicin 9 mg/m^2/d IV on days 1 and 2, vincristine 1.4 mg/m^2/d (max. 2 mg) on days 1, 8, 15 and 22, L-asparaginase (E.Coli) 3.000 U/m^2 IV on days 8, 10, 12, 15, 17 and 19, dexamethasone 5 mg/m^2/bd IV on days 1-5, 15-19, G-CSF from day 5 (induction).

Cycle 2: idarubicin 9 mg/m^2/d IV on day 1, cyclophosphamide 1000 mg/m^2 IV on day 1, dexamethasone 5 mg/m^2/bd IV/PO on days 1-5, cytarabine 75 mg/m^2/d IV/subcutaneous (SC) on days 2-5, 6-mercaptopurine 60 mg/m^2/d PO on days 1-10, G-CSF from day 8 to resolution of absolute neutropenia <1 x10^9/L.
Cycles 3,7: methotrexate 1.5 g/m^2/d IV on day 1 (24-h infusion, folinic acid rescue), cytarabine 2 g/m^2/bd IV on days 3 and 4, G-CSF from day 8 (collection/cryopreservation of autologous blood stem cells at cycle 3).
Cycles 4,6: idarubicin 9 mg/m^2/d IV on day 1, cyclophosphamide 1000 mg/m^2 IV on day 1, vincristine 1.4 mg/m^2/d (max. 2 mg) IV on days 1 and 8, dexamethasone 5 mg/m^2/bd IV/PO on days 1-5, cytarabine 75 mg/m^2/d IV/SC on days 2-5, 6-mercaptopurine 60 mg/m^2/d PO on days 1-10, G-CSF from day 8 to resolution of absolute neutropenia <1 x10^9/L.
Cycle 5: methotrexate 1.5 g/m^2/d IV on day 1 (24-h infusion, folinic acid rescue), L-asparaginase (E. Coli) 10.000 U/m^2 IV on days 3 and 8.
Cycle 8: idarubicin 7.5 mg/m^2/d IV on days 1 and 8, vincristine 1.4 mg/m^2/d (max. 2 mg) IV on days 1 and 8, cyclophosphamide 200 mg/m^2/d IV on days 1-3, dexamethasone 5 mg/m^2/bd IV/PO on days 1-5, prednisone 20 mg/m^2/bd PO on days 8-12, G-CSF from neutropenia <0.5 microl to its resolution.

- CNS prophylaxis: Intrathecal MTX 12.5 mg, Ara-C 50 mg, and PDN 40 mg or dexa 4 mg during induction/consolidation and maintenance for a total of 12 IT

Variations for age >65 y:
Pre-induction: cyclophosphamide 100 mg/m^2/d IV on days -3 to –1
Cycle 1: idarubicin 10 mg/d IV on days 1 and 2, vincristine 1 mg/m^2/d (max. 2 mg) on days 1, 8, 15 and 22, dexamethasone 5 mg/m^2/d IV on days 1-5, 15-19, G-CSF from day 5 (induction).
Cycle 2: idarubicin 10 mg/d IV on day 1, cyclophosphamide 500 mg/m^2 IV on day 1, dexamethasone 5 mg/m^2/d IV/PO on days 1-5, cytarabine 60 mg/m^2/d IV/SC on days 2-5, 6-mercaptopurine 40 mg/m^2/d PO on days 6-10, G-CSF from day 8 to resolution of absolute neutropenia <1 x10^9/L
Cycles 3: methotrexate 0.5 g/m^2, cytarabine 1 g/m^2/bd IV on days 3 and 4, G-CSF from day 8 (collection/cryopreservation of autologous blood stem cells at cycle 3).
Cycles 4,6: idarubicin 10 mg/d IV on day 1, cyclophosphamide 500 mg/m^2 IV on day 1, vincristine 1 mg/m^2/d (max. 2 mg) IV on days 1 and 8, dexamethasone 5 mg/m^2/d IV/PO on days 1-5, cytarabine 60 mg/m^2/d IV/SC on days 2-5, 6-mercaptopurine 40 mg/m^2/d PO on days 6-10, G-CSF from day 8 to resolution of absolute neutropenia <1 x10^9/L.
Cycle 5: methotrexate 0.5 g/m^2, L-asparaginase (E. Coli) 5.000 U/m^2 IV on days 3
Cycle 7-8: omitted

- CNS prophylaxis: Intrathecal MTX 10 mg, Ara-C 40 mg, and PDN 40 mg or dexa 4 mg during induction/consolidation and maintenance for a total of 12 IT

MRD/Risk-Oriented Therapy

MRD-NEG/SR patients: Maintenance (24 4-week cycles)
Aged >55y
Cycles 1, 3, 5, 7, 9, 11: cyclophosphamide 50 mg/m^2/d PO on days 1-4 (100mg or 50 mg total dose day 1 cycles 1-3), 6-mercaptopurine 75 mg/m^2/d PO on days 8-28, methotrexate 15 mg/m^2/d PO/intramuscular (IM) on days 8, 15 and 22.
Cycles 2, 4, 6, 8, 10, 12: vincristine 1 mg/m^2 IV on day 1, prednisone 40 mg/m^2/d PO on days 1-5, 6-mercaptopurine 75 mg/m^2/d PO on days 8-28, methotrexate 15 mg/m^2/d PO/IM on days 8, 15 and 22.
Cycles 13-24: 6-mercaptopurine 75 mg/m^2/d PO on days 1-28, methotrexate 15 mg/m^2/d PO/IM on days 1, 8, 15 and 22.

Aged >65y
Cycles 1, 3, 5, 7, 9, 11: cyclophosphamide 50 mg/d PO on days 1-4, 6-mercaptopurine 60 mg/m^2/d PO on days 8-28, methotrexate 10 mg/m^2/d PO/IM on days 8, 15 and 22.

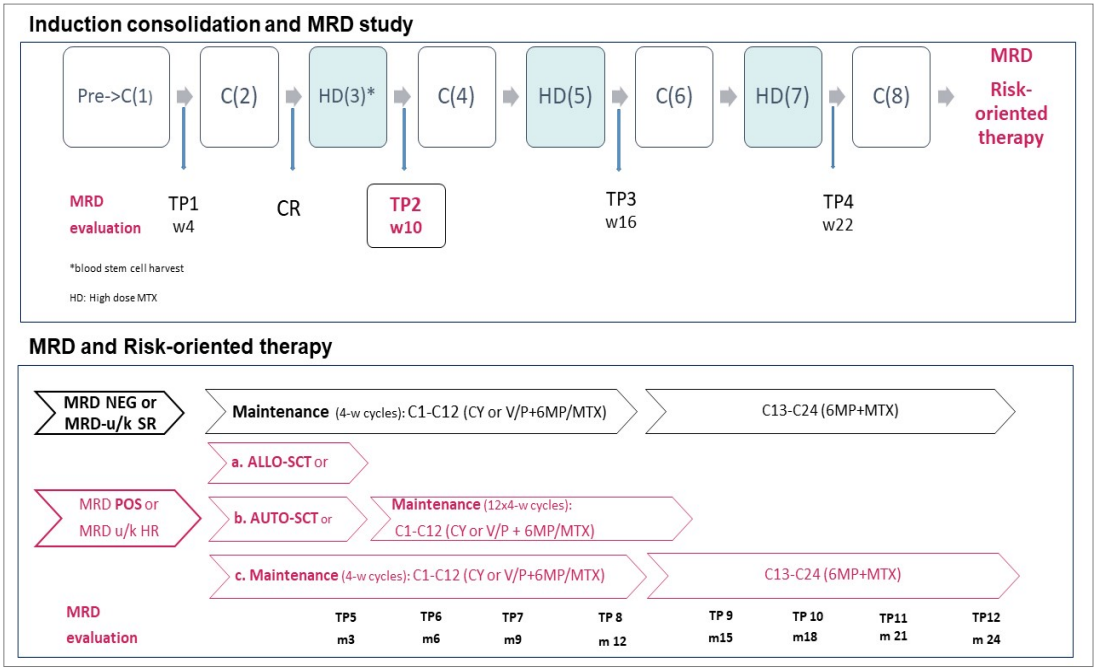
Cycles 2, 4, 6, 8, 10, 12: vincristine 1 mg IV on day 1, prednisone 20 mg/m^2/d PO on days 1-5, 6-mercaptopurine 50 mg/m^2/d PO on days 8-28, methotrexate 10 mg/m^2/d PO/IM on days 8, 15 and 22.
Cycles 13-24: 6-mercaptopurine 50 mg/m^2/d PO on days 1-28, methotrexate 10 mg/m^2/d PO/IM on days 1, 8, 15 and 22.

MRD-POS/HR patients: 1st option Allogeneic SCT
Allogeneic SCT: first choice option, from sibling/unrelated donor or cord blood. SCT procedure. SCT timing is by risk class and MRD study results (positive timepoint 2: early; others: at end of consolidation, with interim maintenance).

MRD-POS/HR: 2nd option Autologous SCT with Maintenance (12 4-week cycles)
Autologous SCT: second choice option if allogeneic SCT not possible (maintenance only if autologous SCT not feasible), with melphalan 100 mg/m^2/d IV on days 1 and 2 (100mg/mq days 1 if aged>65y), plus autologous CD34+ blood cells (2-6x10^6/kg) on day 4, and G-CSF.

MRD-POS/HR patients excluded from SCT: 3rd option Maintenance (24 4-week cycles)

Figure S1. Treatment plan and time points to MRD evaluation**.**



Abbreviations: C: cycle; MRD: measurable residual disease; TP: time point; CR: complete remission; w: week; HD: High-dose; POS: positive; NEG: negative; u/k: unknown; SR: Standard Risk; HR: High Risk; Allo: allogeneic; Auto: Autologous; SCT; stem cell transplantation; Cy: Cyclophosphamide; V/P: vincristine/prednisone;; m:months; MTX: methotrexate; 6MP: 6-mercaptopurine.

B) Generation of patient-specific probes for MRD study

The molecular evaluation of MRD was performed. DNA was extracted from mononuclear marrow cells using commercially available kits [QIAamp DNA Blood Kit (QIAGEN, Hilden, Germany)].
Leukemia-specific forward oligonucleotides were generated after genomic amplification and sequencing of the Leukemia-specific junctional regions of the rearranged IG heavy chain (H) or/and kappa light chain (K), and TCR gamma (G), delta (D), and beta (B) genes [27-29]. MRD quantification was performed by amplification of 500 ng sample DNA and the 10-fold dilution series of the the diagnostic DNA specimen in DNA obtained from mononuclear cells from a pool of five to 10 healthy donors. All samples were amplified in triplicate, and the MRD level was expressed as the logarithmic reduction of the leukemic burden detected at diagnosis, after correction for DNA quantity by amplification of a control gene.