cancers

MDPI

# Supplementary Materials: Prognostic Value of the Immunological Subtypes of Adolescent and Adult T-Cell Lymphoblastic Lymphoma, an Ultra-High-Risk Pro-T/CD2(–) Subtype

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**Table S1A.** A minimal panel of anti-human monoclonal antibodies (mAbs) used for the flow cytometry (FCM) analysis.

| mAb | Fluorochrome | Clone | Source |
|---|---|---|---|
| CD1a | PE | SK9 | BD[1] |
| CD2 | FITC, PE, APC | S5.2 | BD |
| CD3 | FITC, PE, PerCP, APC, APC-H7, APC-Cy7 | SK7 | BD |
| cCD3/CD79α/MPO * | FITC/PE/PerCP | UCHT1/5B8/HM47 | BD Oncomark |
| CD4 | FITC, APC | SK3 | BD |
| CD5 | FITC, PE, APC | L17F12 | BD |
| CD7 | FITC, PE, APC | M-T701 | BD |
| CD8 | FITC, PE, PE-Cy7, APC | SK1 | BD |
| CD13 | PE | L138 | BD |
| CD15 | FITC | MMA | BD |
| CD33 | APC | P67.6 | BD |
| CD34 | FITC, PE | 8G12 | BD |
| TdT * | FITC | E17-1519 | BD |
| HLADR | FITC, PerCP | L243 | BD |

**Table S1B.** Additional mAbs used for extended FCM diagnostics.

| | | | |
|---|---|---|---|
| CD3/HLADR | FITC/PE | SK7/L243 | BD Simultest |
| CD45/CD14 | FITC/PE | 2D1,24/MφP9 | BD Simultest |
| CD3/CD16&56 | FITC/PE | SK7/B73.1/MY31 | BD Simultest |
| CD3/CD19 | FITC/PE | SK7/4G7 | BD Simultest |
| CD4/CD8/CD3 | FITC/PE/PerCP | SK3/SK1/SK7 | BD Tritest |
| CD3/CD8/CD45/CD4 | FITC/PE/PerCP/APC | SK7/SK1/2D1/SK3 | BD Multitest |
| CD5/CD19 | FITC/PE | L17F12/SJ25C1 | BD Simultest |
| CD5/CD10/CD19 | FITC/PE/PerCP-Cy5 | L17F12/HI10a/SJ25C1 | BD Oncomark |
| CD10 | PE, PE-Cy7 | HI10a | BD |
| CD25 | PE, PE-Cy7, APC | 2A3 | BD |
| CD26 | FITC | L272 | BD |
| CD27 | APC | L128 | BD |
| CD28 | APC, APC-H7 | CD28.2 | BD Pharmingen |
| CD38 | FITC, PE, APC | HB-7 | BD |
| CD43 | APC | L10 | ThermoFisher Scientific[2] |
| CD44 | PE | MEM-85 | ThermoFisher Scientific |
| CD45 | FITC, Per-CP | 2D1 | BD |
| CD45RA | FITC | L48 | BD |
| CD45RO | PE | UCHL1 | BD |
| CD49d | APC | 9F10 | BD Pharmingen |
| CD52 | PE | 4C8 | BD Pharmingen |
| CD54 | APC | HA58 | BD Pharmingen |
| CD56 | FITC, PE, APC | NCAM16.2 | BD |

| | | | |
|---|---|---|---|
| CD62L | PE | SK11 | BD |
| CD71 | FITC, APC | L01.1 | BD |
| CD81 | APC | JS-81 | BD Pharmingen |
| CD94 | APC | HP-3D9 | BD |
| CD200 | PE | MRC OX-104 | BD Pharmingen |
| CD305 | PE | DX26 | BD Pharmingen |
| TCR$\alpha/\beta$ | FITC | WT31 | BD |
| TCR$\gamma/\delta$ | PE | 11F2 | BD |
| IOTest BetaMark TCRVb Repertoire Kit | PE, PE+FITC, FITC | 24 different clones | Beckman Coulter[3] |
| IgG1/IgG2a | FITC/PE | X40/X39 | BD Simultest |
| IgG1 | APC | MOPC-21 | BD |

Abbreviations: mAb, monoclonal antibodies; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin-chlorophyll protein; PerCP-Cy5.5, PerCP with a cyanine dye (Cy5.5); PE-Cy7, PE with a cyanine dye (Cy7); APC, allophycocyanin; IgG1/IgG2a and IgG1, antibodies of isotype control conjugated with FITC/PE and APC, respectively. *The intracytoplasmic staining of CD3, CD79$\alpha$ and MPO, and intranuclear staining of TdT was performed following permeabilization of cells with PermeaFix/Perm2 (BD—Becton Dickinson, San Jose, CA, USA.). [1] BD, Becton Dickinson, San Jose, CA, USA; [2] ThermoFisher Scientific, Waltham, MA, USA; [3] Beckman Coulter, manufactured by Immunotech SA, Marseille, Cedex.

**Table S2.** Details of chemotherapy GMALL 05/93 (**A**) and GMALL T-LBL 1/2004 (**B**). **(A)** GMALL 05/93 Total treatment duration in T-ALL was 2.5 years (with maintenance therapy), in T-LBL at least 6 months (including reinduction) and up to 12 months was recommended. Central nervous system irradiation (24 Gy), after CR confirmation and mediastinal irradiation (36 Gy) was recommended during the second induction.

| Drug | Dose | Weeks | Days of Administration |
|---|---|---|---|
| **Induction I** | | | |
| Prednisone | 60 mg/m²/p.o.[1] | 1–4 | d.[4]1–28 (than taper over few days) |
| Vincristine | 2 mg total/i.v.[2] | | d. 1, 8, 15, 22 |
| Daunorubicin | 45 mg/m²/i.v. | | d. 1, 8, 15, 22 |
| Methotrexate (MTX)/ Dexamethasone (Dx) | 15/4 mg i.th.[3] | | d. 1 |
| L-asparaginase | 5000 IU/m²/i.v. | | d.15–28 (every other day) |
| **Induction II** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 5–8 | d. 29, 43, 57 |
| Cytarabine (Ara-C) | 75 mg/m²/i.v. | | d. 31–34, 38–41, 45–48, 52–55 |
| 6-Mercaptopurine | 60 mg/m²/p.o. | | d. 29–57 |
| MTX/Dx | 15/4 mg i.th. | | d. 31, 38, 45, 52 |
| Mediastinal irradiation (24 Gy) during phase II of induction (all patients) and prophylactic cranial irradiation (24 Gy) after achievement of CR | | | |
| **Consolidation I/II** | | | |
| Cytarabine | 1000 mg/m²/i.v. (every 12 hours) | 13–14 | d. 1–4 |
| Mitoxantrone | 10 mg/m²/i.v. | | d. 3–5 |
| Methotrexate | 1500 mg/m²/i.v. 24 h infusion | 17–20 | d. 1, 15 |
| L-asparaginase | 10000 IU/m² | | d. 2, 16 |
| 6-Mercaptopurine | 25 mg/m² | | d. 1–5, 15–19 |

| | | | |
|---|---|---|---|
| **Reinduction I** | | | |
| Prednisone | 60 mg/m²/p.o. | 21–24 | d. 1–28 |
| Vincristine | 2 mg total/i.v. | | d. 1, 8, 15, 22 |
| Adriamycine | 25 mg/m²/i.v. | | d. 1, 8, 15, 22 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 1 |
| **Reinduction II** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 25–27 | d. 29 |
| Cytarabine | 75 mg/m²/i.v. | | d. 31–34, 38–41 |
| 6-Thioguanine | 60 mg/m²/p.o. | | d. 29–42 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 29 |
| **Consolidation III/IV/V/VI** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 33–52 | d. 1, 84 |
| Cytarabine | 500 mg/m²/i.v. 24 h | | d. 1, 84 |
| Teniposide | 100 mg/m²/i.v. | | d. 1–5 (28–32), (56–60) |
| Cytarabina | 150 mg/m²/i.v. | | d. 1–5 (28–32), (56–60) |
| **Maintenance Therapy** | | | |
| 6-Mercaptopurine | 60 mg/m²/p.o. | 54–130 | daily |
| Methotrexate | 20 mg/m²/p.o. | | once weekly |

Abbreviations: [1] p.o., per os; [2] i.v., intravenously; [3] i.th., intrathecal; [4] d., day

**Table S2.** (**B**) GMALL 1/2004/T-LBL Total treatment duration in T-ALL was 2,5 years (with maintenance therapy), in T-LBL—52 months. After I consolidation (13 week), for patients who did not achieve CR/CRun., salvage therapy was applied (Nelarabine, FLAM-scheme), and allo-SCT in consolidation. In case of blood/bone marrow involvement, minimal residual disease (MRD) was monitored after induction I, induction II and consolidation. Patients with persistent MRD positivity (MRD > $10^{-4}$ after induction) and those with early and mature immunophenotype were transplanted at first complete remission. For T-ALL strategy based on monitoring MRD close to GMALL 07/2003. Central nervous system irradiation (24 Gy), after CR confirmation was recommended during second induction. Mediastinal irradiation (36 Gy) was not routinely recommended, it was considered only for patients with positive PET-CT after consolidation.

| Drug | Dose | Week | Days of Administration |
|---|---|---|---|
| **Pre-Phase** | | | |
| Dexamethasone (Dx) | 10 mg/m²/p.o.[1] | 1 | d.[4] 1–5 |
| Cyclophosphamide | 200 mg/m²/i.v.[2] | | d. 3–5 |
| MTX/Dx | 15/4 mg i.th.[3] | | d. 1 |
| **Induction I** | | | |
| Dexamethasone | 10 mg/m²/p.o. | 2–4 | d. 6–7, 13–16 |
| Vincristine | 2 mg/i.v./total | | d. 6, 13, 20 |
| Daunorubicin | 45mg/m² (30 mg > 55 ys[5]) | | d. 6–7, 13–14 |
| Peg-asparaginase | 1000 U/m² (500 U > 55 ys) | | d. 20 |
| **Induction II** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 5–8 | d. 26, 46 |
| Cytarabine | 75 mg/m²/i.v. | | d. 28–31, 35–38, 42–45 |
| 6-Mercaptopurine | 60 mg/m²/p.o. | | d. 26–46 |
| MTX/Dx | 15/4 mg i.th. | | d. 28, 35, 42 |
| Mediastinal irradiation (36 Gy) after phase II of induction, only for patients with PET-CT positive evaluation (individ- | | | |

| | | | |
|---|---|---|---|
| ual decision) and prophylactic cranial irradiation (24 Gy) after achievement of CR | | | |
| **Consolidation I** | | | |
| Dexamethasone | 10 mg/m²/p.o. | 11–12 | d. 1–5 |
| Vindesine | 3 mg/m²/i.v. (max 5 mg) | | d. 1 |
| Methotrexate | 1500 mg/m² i.v. 24 h (1000 mg/m² > 55 ys) | | d. 1 |
| Etoposide | 250 mg/m²/i.v. | | d. 4–5 |
| Cytarabine | 2000 mg/m² (every 12 h) | | d. 5 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 12 |
| **Consolidation II** | | | |
| Methotrexate | 1500 mg/m²/i.v. 24 h (1000 mg > 55 ys) | 16–19 | d. 1, 15 |
| Peg-asparaginase | 500 U/m²/ i.v. | | d. 2, 16 |
| 6-Mercaptopurine | 60 mg/m²/p.o. | | d. 1–7, 15–21 |
| **Reinduction I** | | | |
| Prednisone | 60 mg/m²/p.o. | 24–26 | d. 1–14 |
| Vindesine | 3 mg/m²/i.v. (max 5 mg) | | d. 1,7 |
| Adriamycine | 50 mg/m²/i.v. | | d. 1,7 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 1,15 |
| **Reinduction II** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 24–26 | d. 15 |
| Cytarabine | 75 mg/m²/i.v. | | d. 17–20, 24–27 |
| 6-Thioguanine | 60 mg/m²/p.o. | | d. 15–28 |
| **Consolidation III** | | | |
| Methotrexate | 1500 mg/m²/i.v. 24 h (1000 mg > 55 ys) | 30–33 | d. 1, 15 |
| Peg-asparginase | 500 U/m²/i.v. | | d. 2, 16 |
| 6-Mercaptopurine | 60 mg/m²/p.o. | | d. 1–7, 15–21 |
| **Consolidation IV** | | | |
| Teniposide | 100 mg/m²/i.v. | 36–37 | d. 1–5 |
| Cytarabine | 150 mg/m²/i.v. | | d. 1–5 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 1 |
| **Consolidation V** | | | |
| Cyclophosphamide | 1000 mg/m²/i.v. | 41 | d. 1 |
| Cytarabine | 500 mg/m²/i.v. 24 h | | d. 1 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | | d. 1 |
| **Consolidation VI** | | | |
| Methotrexate | 1500 mg/m²/i.v. 24 h (1000 mg >55 ys) | 46–48 | d. 1, 15 |
| Peg-asparginase | 500 U/m²/i.v. | | d. 2, 16 |
| 6-Mercaptopurine | 60 mg/m²/p.o. | | d. 1–7, 15–21 |
| MTX/Ara-C/Dx | 15/40/4 mg i.th. | 52 | d. 1 |
| **Maintenance Therapy** | | | |
| 6-Mercaptopurine | 60 mg/m²/p.o. | 54–130 | daily |
| Methotrexate | 20 mg/m²/p.o. | | once weekly |

Abbreviations: [1] p.o.,per os; [2] i.v., intravenously; [3] i.th., intrathecal; [4] d., day; [5] ys, years old

**Table S3.** Baseline diagnostic characteristics of T-LBL patients (49 patients, including 2 with CD2 expression detected by immunohistochemistry).

| | **Total** ***n*** **= 49 (100%)** |
|---|---|
| Gender, male | 37 (75.5%) |
| Age, median (range) | 28 (17–56) |
| <35 year, *n* (%) | 35 (71.4%) |
| mediastinal involvement | 45 (91%) |
| pleural effusion | 23 (47%) |
| pericardial effusion | 17 (34.7%) |
| abdominal/pelvis lymph nodes | 8 (16.3%) |
| nasopharynx | 4 (8%) |
| lung | 1 (2%) |
| kidneys | 2 (4%) |
| testis | 1 (2%) |
| breast | 1 (2%) |
| spinal canal infiltration | 1 (2%) |
| Bone marrow involvement | |
| T-LBL (<25%) | 11 (22%) |
| CNS involvement | 3 (6%) |
| Immunophenotype WHO 2008* [1] | |
| Pro-T | 9 (19%) |
| Pre-T | 7 (15%) |
| Cortical/thymic | 28 (59.5%) |
| Mature/medullary | 3 (6.5%) |
| ETP** | 9 (20%) |
| Median WBC (G/L)/(range) | 9.9 (2.5–21.2) |
| HGB (g/dl) | 13.7 (9.1–17.7) |
| PLT (G/L) | 313 (151–788) |
| Treatment GMALL 05/93 | 20 (41%) |
| GMALL 01/2004 | 29 (59%) |

Abbreviations: LBL, lymphoblastic lymphoma; CNS, central nervous system; ETP, early-T precursor; WBC, white blood count; HGB, hemoglobin; PLT, platelets; GMALL, German Multicenter Study Group for Adult ALL. * *n* = 47 patients, ** *n* = 45 patients.

**Table S4.** Clinical and immunophenotype characteristics, and outcome for T-LBL CD2(−) patients.

| **No.** | **G/A** | **WHO2008/ WHO2017** | **CR** | **Clinical Outcomes** | **Allo-SCT** | **Status/Last FU (Years)** |
|---|---|---|---|---|---|---|
| 1. | M/40 | pro-T/ ETP | yes | R 15 mo after CR with massive BMinv. | no | DOD |
| 2. | M/38 | pro-T/ ETP | no | PD, mediastinal mass | no | DOD |
| 3. | M/21 | medullary/ non-ETP | yes | R 10 mo after CR with massive BMinv. | no | DOD |
| 4. | M/38 | medullary/ non-ETP | no | PD, mediastinal mass | no | DOD |

| | | | | | | |
|---|---|---|---|---|---|---|
| 5. | M/18 | pro-T/ ETP | yes | R 11 ys after CR1 with BMinv., allo-SCT in CR2 | yes CR2 | ANED, CCR2 (5 ys) |
| 6. | M/57 | pro-T/ non-ETP | no | PD, CNSinv. | no | DOD |
| 7. | M/30 | pro-T/ ETP | yes | R 12 mo after CR with massive BMinv. | no | DOD |
| 8. | F/30 | pro-T/ ETP | yes | PD 2 mo after allo-SCT(CR1) with massive BMinv. | yes CR1 | DOD |
| 9. | M/39 | pro-T/ non-ETP | no | PR, sepsis | no | TRM in PR |
| 10. | M/29 | pro-T/ ETP | yes | R 12 mo after CR, BMinv. with secondary AML | no | DOD |
| 11. | M/19 | cortical/ non-ETP | yes | R 18 mo after CR1 with soft tissue infiltration | yes CR2 | ANED, CCR2 (11 ys) |
| 12. | M/23 | pro-T/ non-ETP | yes | CR, allo-SCT in CR1 | yes CR1 | ANED, CCR1 (3 ys) |
| 13. | M/24 | cortical/non-non-ETP | yes | R 2 mo after CR, CNSinv with PMRinv in FCM | Yes CR2 | ANED CCR2 (3 ys) |

Abbreviations: No., case number; G/A, gender/age (y), M-male, F-female; R, relapse; CR, complete remission; CCR, continuous complete remission; PR, partial remission; PRpd, Partial Remission followed by progressive disease; PD, progressive disease; BMinv, bone marrow involvement; CNSinv, central nervous system involvement; TBI, total body irradiation; SCT, stem cell transplantation; * autologous bone marrow transplantation; mo, months; ys, years; FU, follow up years after the end of treatment; ANED Alive, no evidence of disease; DOD, Died of Disease progression; TRM, Treatment Related Mortality; FCM, flow cytometry.

**Table S5.** Immunophenotype details (WHO 2008 [1] T-ALL/LBL subtypes).

| **Subtype (WHO 2008)** | **Total** ***n*** **= 71 (100%)** | **T-ALL** ***n*** **= 26 (37%)** | **T-LBL** ***n*** **= 45 (63%)** | ***p*** |
|---|---|---|---|---|
| Early pro-T | 15 (21%) | 6 (23%) | 9 (20%) | 0.76 |
| Early pre-T | 13 (18%) | 6 (23%) | 7 (16%) | 0.43 |
| Cortical/thymic | 38 (54%) | 12 (46%) | 26 (58%) | 0.344 |
| Medullary/mature | 5 (7%) | 2 (8%) | 3 (7%) | 0.871 |
| ETP (WHO 2017) | 13 (18%) | 4 (15%) | 9 (20%) | 0.598 |

Abbreviations: ALL-acute lymphoblastic leukemia; LBL-lymphoblastic lymphoma, WHO-World Health Organization, ETP-early T-cell precursor.

Reference:

1. Borowitz, M.J.; Chan, J.K.C. T lymphoblastic leukaemia/lymphoma. In *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, 4th ed.; Swerdlow, S.H., Campo, E., Harris, N.L., Eds.; IARC: Lyon, France, 2008; pp. 176–178