Immature Immunoglobulin Gene Rearrangements Are Recurrent in B Precursor Adult Acute Lymphoblastic Leukemia Carrying TP53 Molecular Alterations

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Abstract: Here, we describe the immunoglobulin and T cell receptor (Ig/TCR) molecular rearrangements identified as a leukemic clone hallmark for minimal residual disease assessment in relation to TP53 mutational status in 171 Ph-negative Acute Lymphoblastic Leukemia (ALL) adult patients at diagnosis. The presence of a TP53 alterations, which represents a marker of poor prognosis, was strictly correlated with an immature DH/JH rearrangement of the immunoglobulin receptor (p < 0.0001). Furthermore, TP53-mutated patients were classified as pro-B ALL more frequently than their wild-type counterpart (46% vs. 25%, p = 0.05). Although the reasons for the co-presence of immature Ig rearrangements and TP53 mutation need to be clarified, this can suggest that the alteration in TP53 is acquired at an early stage of B-cell maturation or even at the level of pre-leukemic transformation.

Keywords: acute lymphoblastic leukemia; TP53; immunoglobulin rearrangements

1. Introduction

Rearrangements of immunoglobulin (Ig) and T-cell receptors (TCR) genes are physiologic mechanisms starting in the early stage of lymphopoiesis and following a hierarchical order in which the DH-JH Ig rearrangement is one of the earliest events. The V, (D), J elements of these genes are juxtaposed in different combinations to generate a diverse set of receptors capable to recognize different antigens. [1,2] Therefore, Ig and TCR rearrangements represent fingerprint-like DNA regions
of each distinct lymphoid cell and its descendants, making this peculiar characteristic a suitable marker of clonality when cellular regulatory mechanisms fail. The identification of clonal Ig and TCR rearrangements found clinical application in Minimal Residual Disease (MRD) detection, particularly in Acute Lymphoblastic Leukemia (ALL). [3] Nowadays, it is established that MRD evaluation represents the most important factor for predicting clinical outcome for ALL patients [4–6]. Despite the high standardization level reached within European cooperative groups [7], clonality definition and MRD assessment is time-consuming and is not feasible for all the patients, even when it is performed by specialized laboratories. As a consequence, even if conventional risk factors defined at diagnosis still maintain a crucial role, in recent years great efforts were made to identify new additional molecular features useful not only for predicting outcome but also for the most appropriate treatment allocation. The aberrations involving TP53 gene are detectable in about 10% of adult ALL patients at diagnosis and represent a marker of poor prognosis [8–10]. Since the use of intensified treatments could potentially overcome the dismal outcome usually reported in these patients [11], the identification of TP53 mutations at diagnosis is now mandatory.

Here, we present a study conducted on 171 adult Philadelphia negative-ALL patients in which we analyzed the Ig/TCR molecular rearrangements identified as a leukemic clone hallmark in relation to TP53 mutational status. All the patients were enrolled in the NILG-ALL 09/00 clinical trial, based on the evaluation of MRD as a decisional tool for the post-consolidation program [5].

2. Patients and Methods

We analyzed the DNA samples obtained from the bone marrow or peripheral blood at diagnosis containing at least 30% of blasts of 171 unsellected ALL patients enrolled in the NILG-ALL 09/00 clinical trial (ClinicalTrials.gov identifier: NCT00358072). Our cohort of patients included 114 B precursor ALL negative for t(9;22) or BCR/ABL1 transcripts and 57 T-lineage adult ALL. DNA was extracted with commercially available kit (Gentra System, Germany) from mononuclear cells separated on Ficoll gradient. DNA samples were studied to identify clonal rearrangements amplifying and sequencing the VDJ/VJ regions of immunoglobulin heavy chain (IgH) or the kappa light chain (IgK), and the T-cell receptor (TCR) gamma (G), delta (D) and beta (B) genes by conventional method [5] or by an integrated NGS-based approach. Libraries for NGS were prepared by a custom NimbleGen SeqCap Target Enrichment assay (Roche) targeting coding V, D and J genes in the Ig/TCR loci for the identification of D-J and V-D-J rearrangements. Libraries were then sequenced on Illumina MiSeq platform (2 × 300 bp). [12] Clone-specific oligonucleotides were designed based on the unique junction region of each single rearrangement and used to perform MRD evaluation experiments by quantitative PCR, as previously described. [5] The TP53 gene (exons 4–11) was sequenced by 454 Roche Sequencing and data generated by this procedure were analyzed by GS Run Browser and GS Amplicon Variant Analyzer software as previously described. [9] Based on the platform detection limits, we considered only variants with a variant allele fraction (VAF) above 4%, which were checked in dbSNP, COSMIC and IARC databases to investigate their biological significance. The TP53 mutations identified with an allele burden >20% were also validated by PCR amplification and Sanger sequencing. Moreover, TP53 copy number analysis was performed in 158 DNA samples by quantitative PCR method using hTERT as reference gene. [9] Statistical tests used in the analysis are Chi-squared or Fisher-exact, as appropriate.

3. Results and Discussion

The median age of the 171 ALL patients analyzed in this study was 34.6 (range, 15.6–64.8) and male patients were 57%. Hepatosplenomegaly was present in 53% and CNS involvement in 6%. The cytogenetic profile was normal/not adverse in 56%, not evaluable in 26% and adverse in 18%. The complete remission achieved after induction chemotherapy was 91%. Within the TP53 exons investigated, 16 mutations (single nucleotide variations and small insertion/deletion) were recognized in 14 patients, accounting for the 8% of the cohort. In particular, the incidence of TP53 alteration
was higher in B-ALL than in T-ALL (10% vs. 5%). We previously demonstrated that these molecular aberrations were associated with the presence of one copy of TP53 gene and increasing age. Moreover, all patients carrying a TP53 alteration reached complete remission (CR) after induction therapy but 13 out of 14 relapsed in a short time (within 15 months from CR achievement), showing that the relapse rate was significantly higher in TP53 mutated than in wild-type subjects. By univariate and multivariate analysis [9], the overall survival was strikingly affected by age, WBC at diagnosis >30 × 10^9/L and CNS involvement. In addition, patients with a mutated TP53 gene showed a Leukemia Free Survival (LFS) and Overall Survival (OS) which was dramatically shorter than for wild-type patients, as previously described. [9] The identification of Ig/TCR clonal rearrangements for MRD probe design by conventional PCR was unsuccessful in the majority of the TP53 mutated cases. Therefore, we retrospectively applied an NGS approach [12] that allows the detection of a larger number of molecular rearrangements. Thanks to this method, the identification of Ig/TCR clonal rearrangements was feasible in all TP53-positive patients within the available diagnostic samples (13 out of 14). Interestingly, all the B lineage-ALL patients mutated in the TP53 gene presented a DH/JH clonal rearrangement (10/10), whereas no T-ALL (0/3) with an aberrant TP53 presented this type of rearrangement (Table 1).

The presence of a TP53 alteration was strictly correlated with an incomplete IgH rearrangement, indeed, the 100% of B precursor ALL mutated for TP53 carried the DH/JH rearrangement, whereas only the 36% of the wild-type B precursor-ALL showed the same types of rearrangement (p < 0.0001). The DH/JH locus recombination is the first event that occurs in the IgH genetic recombination mechanism, followed by the recombination of the VH genes with the rearranged DH-JH portion. As previously described, the detection of an incomplete rearrangement denotes an early stage of maturation of the leukemic cells, often associated with a less mature immunophenotype [13]. Conversely, the complete IgH V-D-J rearrangement was mostly associated with Bp-ALL wild type for TP53 gene (p = 0.02).

Considering this, we investigated the surface antigen asset of our molecularly characterized B cell derived-ALL cohort, and we found that all the TP53-mutated patients were defined as a B-I or B-II ALL according to European Group for the Immunological Characterization of Leukemias (EGIL) classification [14], which reflects the sequential stages of B-cell maturation. Furthermore, TP53-mutated patients were classified as pro-B ALL more frequently than their wild-type counterpart (46% vs. 25%, p = 0.05). Unfortunately, the number of T-ALL mutated for TP53 analyzed in this research was too small to draw any conclusion about the co-occurrence TP53 mutation and DH/JH rearrangements, which usually involves about 20% of the T-ALL [15]. In addition, 5 of the 10 TP53 mutated B-ALL patients which presented a DH/JH clonal rearrangement were evaluable for MRD assessment in at least two consecutive timepoints collected within the NILG protocol (Table 2). At least one of the samples evaluated for each of these patients proved positive for leukemia persistence, suggesting a linkage between TP53 mutation and MRD persistence. As previously specified, all these patients went toward a clinical relapse.
Table 1. Biological and molecular characteristics of TP53 mutated ALL NA = Not Available.

| Patient ID | Phenotype (EGIL) | Cytogenetics | Genetics | Risk Class | CR | Relapse | CR Duration (Months) | Allo SCT (Outcome) | Survival (Months) | DH/JH | Sanger Seq | Validation |
|------------|------------------|--------------|----------|------------|----|---------|----------------------|---------------------|-------------------|-------|------------|------------|
| BG_4205    | B-common         | Normal       | Negative | SR-B       | Y  | N       | 71.3+                | N                   | 71.3+             | NA    | NA         |            |
| BG_8345    | B-common         | Normal       | Negative | SR-B       | Y  | Y       | 6.6                  | N                   | 17.2              | DH3-22/JH6; DH1-26/JH4 | Y, Y |            |            |
| BG_2873    | pro-B            | Unknown      | Negative | HR-B       | Y  | Y       | 13.8                 | N                   | 17.4              | DH6-6/JH6        | Y       |            |            |
| BG_11584   | pro-B            | Unknown      | KMT2A-AFF1 | HR-B       | Y  | Y       | 14.7                 | Y, with disease (dead due to persistent disease) | 18.6 | DH1-26/JH3 | Y       |            |            |
| BG_9445    | B-common         | Hyperdiploid | Negative | HR-B       | Y  | Y       | 10.2                 | Y, CR2 (Dead due to relapse) | 12.9 | DH2-2/JH6   | Y       |            |            |
| BG_5702    | pro-B            | Hyperdiploid | Negative | HR-B       | Y  | Y       | 5.8                  | N                   | 8.5               | DH2-2/JH5        | Y       |            |            |
| BG_4254    | pro-B            | Hyperdiploid | Negative | HR-B       | Y  | Y       | 5.0                  | N                   | 16.4              | DH3-22/JH6; DH6-6/JH5 | Y, Y |            |            |
| BG_10112   | pro-B            | Normal       | KMT2A-AFF1 | HR-B       | Y  | Y       | 1.8                  | N                   | 3.7               | DH6-6/JH4; DH1-7/JH4 | Y, Y |            |            |
| BG_11543   | B-common         | Normal       | Negative | SR-B       | Y  | Y       | 1.8                  | N                   | 6.6               | DH2-21/JH6       | Y       |            |            |
| BG_2097    | B-common         | Complex      | Negative | HR-B       | Y  | Y       | 3.7                  | N                   | 7.3               | DH4-23/JH2       | Y       |            |            |
| BG_6490    | pro-B            | Hyperdiploid | Negative | HR-B       | Y  | Y       | 2.5                  | N                   | 7.5               | DH2-21/JH6; DH2-8/JH3 | Y, Y |            |            |
| BG_10442   | cortical-T        | Hyperdiploid | Negative | SR-T       | Y  | Y       | 4.6                  | Y, CR2 (Dead due to relapse) | 11.1 | NO DH/JH    | Y       |            |            |
| BG_8142    | cortical-T        | Hyperdiploid | Negative | SR-T       | Y  | Y       | 5.0                  | N                   | 16.2              | NO DH/JH        | Y       |            |            |
| BG_8646    | cortical-T        | Complex      | Negative | HR-T       | Y  | Y       | 4.0                  | N                   | 10.2              | NO DH/JH        | N       |            |            |
Table 2. MRD evaluation in the 5 TP53 mutated patients for whom a molecular probe was available.

| Patient ID | Phenotype (EGIL) | Available Probe/s | Rearrangement Used for MRD Evaluation | Method for Probe Identification | MRD Evaluation at PRE4 | MRD Evaluation at PRE6 | MRD Evaluation at PRE8 |
|------------|------------------|-------------------|--------------------------------------|-------------------------------|------------------------|------------------------|------------------------|
| BG_9445    | B-common         | 1                 | DH2JH6A                              | NGS                           | - (\(*)                  | -                      | + (10^-3)              |
| BG_5702    | pro-B            | 1                 | DH2JH5A                              | NGS                           | + (NQ)                  | -                      | + (NQ)                 |
| BG_11543   | B-common         | 1                 | DD2DD3                               | Conventional                  | + (10^-3)               | + (10^-3)              | + (10^-3)              |
| BG_2097    | B-common         | 1                 | DH4JH2B                              | NGS                           | -                      | +NQ                    | + (10^-1)              |
| BG_6490    | pro-B            | 2                 | DH2JH6A                              | NGS                           | +NQ                    | -                      | -                      |

NA = Not Available, NQ = Not Quantifiable. Reproducible sensitivity was at least 10^-4. * Inhibition of the tested sample which can alter the MRD evaluation.

4. Conclusions

In conclusion, although in this study the number of B precursor ALL cases characterized by both the presence of a mutation in the TP53 gene and an incomplete Ig rearrangement was relatively small, these events were very strictly associated. This observation suggests that the presence of TP53 mutation should be investigated in all B-precursor ALL at diagnosis in parallel with the search for specific incomplete DH/JH rearrangements for MRD evaluation. At this time, the reasons for the co-presence of TP53 mutation and this type of incomplete DH/JH rearrangement remain to be clarified. Nevertheless, this co-occurrence can suggest that the alteration of TP53 is acquired at the early stage of the B-cell maturation, or even at the level of pre-leukemic transformation, as already described for acute myeloblastic leukemia. [16] A comprehensive molecular characterization, now feasible by ad hoc designed NGS panels, allows the identification of TP53 gene mutations and other prognostically significant genes along with the Ig/TCR rearrangements for MRD evaluation. [12] This combined risk stratification based on molecular genetics at diagnosis and MRD evaluation during treatment remains feasible only at referral laboratories. Nonetheless, the clinical readout is so important that each ALL patient should benefit from such a definition of the risk profile.

Author Contributions: S.S. performed TP53 analysis, interpreted data and wrote the manuscript, R.C. and M.L.G.M. performed the NGS evaluation, G.U. contributed to TP53 analysis, M.T. and B.P. performed MRD analysis, T.I. followed patients and provided clinical data, E.O. collected clinical data, C.P. performed statistical analyses, U.G. performed cytogenetic analysis, A.S. took care of sample preparation, R.B. designed the clinical protocol and followed patients, A.R. designed clinical protocol and interpreted data, O.S. designed the study, interpreted results and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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References

1. Davi, F.; Faili, A.; Gritti, C.; Blanc, C.; Laurent, C.; Sutton, L.; Schmitt, C.; Merle-Beral, H. Early onset of immunoglobulin heavy chain gene rearrangements in normal human bone marrow CD34+ cells. Blood 1997, 90, 4014–4021. [CrossRef] [PubMed]
2. Nemazee, D. Receptor selection in B and T lymphocytes. Annu. Rev. Immunol. 2000, 18, 19–51. [CrossRef] [PubMed]
3. Bruggemann, M.; Raff, T.; Kneba, M. Has MRD monitoring superseded other prognostic factors in adult ALL? Blood 2012, 120, 4470–4481. [CrossRef] [PubMed]
4. Cave, H.; van der Werff ten Bosch, J.;Suciu, S.; Guidal, C.; Waterkeyn, C.; Otten, J.; Bakkus, M.; Thielemans, K.; Grandchamp, B.; Vilmer, E. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer–Childhood Leukemia Cooperative Group. N. Engl. J. Med. 1998, 339, 591–598. [CrossRef] [PubMed]
5. Bassan, R.; Spinelli, O.; Oldani, E.; Intermesoli, T.; Tosi, M.; Peruta, B.; Rossi, G.; Borlenghi, E.; Pogliani, E.M.; Terruzzi, E.; et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). Blood 2009, 113, 4153–4162. [CrossRef] [PubMed]

6. Bruggemann, M.; Raff, T.; Flohr, T.; Gokbuget, N.; Nakao, M.; Droese, J.; Luschen, S.; Pott, C.; Ritgen, M.; Scheuring, U.; et al. Clinical significance of minimal residual disease quantification in adult patients with standard-risk acute lymphoblastic leukemia. Blood 2006, 107, 1116–1123. [CrossRef] [PubMed]

7. Bruggemann, M.; Schrauder, A.; Raff, T.; Pfeifer, H.; Dworzak, M.; Ottmann, O.G.; Asnafi, V.; Baruchel, A.; Bassan, R.; Beneit, Y.; et al. Standardized MRD quantification in European ALL trials: Proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18–20 September 2008. Leukemia 2010, 24, 521–535. [CrossRef] [PubMed]

8. Stengel, A.; Schnittger, S.; Weissmann, S.; Kuznia, S.; Kern, W.; Kohlmann, A.; Haferlach, T.; Haferlach, C. TP53 mutations occur in 15.7% of ALL and are associated with MYC-rearrangement, low hypodiploidy, and a poor prognosis. Blood 2014, 124, 251–258. [CrossRef] [PubMed]

9. Salmoiraghi, S.; Montalvo, M.L.; Ubiali, G.; Tosi, M.; Peruta, B.; Zanghi, P.; Oldani, E.; Boschini, C.; Kohlmann, A.; Bungaro, S.; et al. Mutations of TP53 gene in adult acute lymphoblastic leukemia at diagnosis do not affect the achievement of hematologic response but correlate with early relapse and very poor survival. Haematologica 2016, 101, e245–e248. [CrossRef] [PubMed]

10. Salmoiraghi, S.; Rambaldi, A.; Spinelli, O. TP53 in adult acute lymphoblastic leukemia. Leuk Lymphoma 2018, 59, 778–789. [CrossRef] [PubMed]

11. Kanagal-Shamanna, R.; Jain, P.; Takahashi, K.; Short, N.J.; Tang, G.; Issa, G.C.; Ravandi, F.; Garcia-Manero, G.; Yin, C.C.; Luthra, R.; et al. TP53 mutation does not confer a poor outcome in adult patients with acute lymphoblastic leukemia who are treated with frontline hyper-CVAD-based regimens. Cancer 2017, 123, 3717–3724. [CrossRef] [PubMed]

12. Cavagna, R.; Guinea Montalvo, M.L.; Tosi, M.; Paris, M.; Pavoni, C.; Intermesoli, T.; Bassan, R.; Mosca, A.; Rambaldi, A.; Spinelli, O. Capture-based Next-Generation Sequencing Improves the Identification of Immunoglobulin/T-Cell Receptor Clonal Markers and Gene Mutations in Adult Acute Lymphoblastic Leukemia Patients Lacking Molecular Probes. Cancers (Basel) 2020, 12, 1505. [CrossRef] [PubMed]

13. Szczepanski, T.; Willems, M.J.; van Wering, E.R.; van Weerden, J.F.; Kamps, W.A.; van Dongen, J.J. Precursor-B-ALL with D(H)-J(H) gene rearrangements have an immature immunogenotype with a high frequency of oligoclonality and hyperdiploidy of chromosome 14. Leukemia 2001, 15, 1415–1423. [CrossRef] [PubMed]

14. Chiaretti, S.; Zini, G.; Bassan, R. Diagnosis and subclassification of acute lymphoblastic leukemia. Mediterr. J. Hematol. Infect. Dis. 2014, 6, e2014073. [CrossRef] [PubMed]

15. Szczepanski, T.; Pongers-Willems, M.J.; Langerak, A.W.; van Dongen, J.J. Unusual immunoglobulin and T-cell receptor gene rearrangement patterns in acute lymphoblastic leukemias. Curr. Top Microbiol. Immunol. 1999, 246, 205–213; discussion 214–215. [PubMed]

16. Lal, R.; Lind, K.; Heitzer, E.; Ulz, P.; Aubell, K.; Kashofer, K.; Middeke, J.M.; Thiede, C.; Schulz, E.; Rosenberger, A.; et al. Somatic TP53 mutations characterize preleukemic stem cells in acute myeloid leukemia. Blood 2017, 129, 2587–2591. [CrossRef] [PubMed]

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