# *TP53*, *ETV6* and *RUNX1* germline variants in a case series of patients developing secondary neoplasms after treatment for childhood acute lymphoblastic leukemia

*Stefanie V. Junk,[1] Norman Klein,[1] Sabine Schreek,[1] Martin Zimmermann,[1] Anja Möricke,[2] Kirsten Bleckmann,[2] Julia Alten,[2] Elif Dagdan,[1,3] Gunnar Cario,[2] Christian P. Kratz,[1] Martin Schrappe[2,*] and Martin Stanulla[1,*]*

*[*]These authors contributed equally to this work.*

*[1]Pediatric Hematology and Oncology, Hannover Medical School, Hannover; [2]Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel and [3]Department of Pathology, Ruhr-University Bochum, Bochum, Germany*

*Correspondence: MARTIN STANULLA - stanulla.martin@mh-hannover.de*
*doi:10.3324/haematol.2018.205849*

# *TP53*, *ETV6* and *RUNX1* germline variants in a case series of patients developing secondary neoplasms after treatment for childhood acute lymphoblastic leukemia

Stefanie V. Junk[1], Norman Klein[1], Sabine Schreek[1], Martin Zimmermann[1], Anja Möricke[2], Kirsten Bleckmann[2], Julia Alten[2], Elif Dagdan[1, 3], Gunnar Cario[2], Christian P. Kratz[1], Martin Schrappe[2, ‡] and Martin Stanulla[1, ‡, §]

[1] Pediatric Hematology and Oncology, Hannover Medical School, Hannover, Germany

[2] Department of Pediatrics, University Hospital Schleswig-Holstein, Kiel, Germany

[3] Department of Pathology, Ruhr-University Bochum, Bochum, Germany

‡ These authors contributed equally to this work.

§ corresponding author

**Supplementary Appendix Content**

**Supplementary Methods**

**Patients**

All childhood acute lymphoblastic leukemia (ALL) patients included in this current sequencing project developed a SMN after treatment on one of seven consecutive ALL-BFM protocols - ALL-BFM 79 to AIEOP-BFM ALL 2000. With the exception of ALL-BFM 79 (two branches), treatment was stratified into three branches (standard, intermediate, and high risk), mainly according to the initial leukemic cell load, adverse genetic aberrations such as t(9;22) and t(4;11), and treatment response. Treatment details are described elsewhere[1-3] and consisted of standard drugs (e.g., prednisone, vincristine, daunorubicin, doxorubicin, L-asparaginase, cyclophosphamide, ifosfamide, cytarabine, 6-mercaptopurine, 6-thioguanine, and methotrexate) supplemented in some of the patients by preventive or therapeutic cranial irradiation (CI) and/or allogeneic hematopoietic stem cell transplantation (HSCT) (high-risk patients, only). None of the mutation positive patients reported here received HSCT before developing the SMN. Specific treatment characteristics for the individual patients with potential relevance to SMN development are demonstrated in Supplementary Table 3.

Follow-up data for patients were maintained through regular submissions of reports from the respective treatment centers in Germany. For the first five to ten years of follow-up, reports were filed on an annual basis. After this period up to adulthood reports were filed on a biannual basis. For adolescents and adults no longer returning to their pediatric treatment centers, but who consented for being further contacted, the nationwide operating German Childhood Cancer Registry in Mainz conducts an extended follow-up based on 3 to 5-year intervals. In case of SMN identification through the latter procedure, the trial leaders are informed and asked to help to secure the validity of the information.

**Supplementary References** (also referring to references given in supplementary figures)


1. Schrappe M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. Berlin-Frankfurt-Munster. Leukemia. 2000;14(12):2205-2222.
2. Flohr T, Schrauder A, Cazzaniga G, et al. Minimal residual disease-directed risk stratification using real-time quantitative PCR analysis of immunoglobulin and T-cell receptor gene rearrangements in the international multicenter trial AIEOP-BFM ALL 2000 for childhood acute lymphoblastic leukemia. Leukemia. 2008;22(4):771-782.
3. Moricke A, Reiter A, Zimmermann M, et al. Risk-adjusted therapy of acute lymphoblastic leukemia can decrease treatment burden and improve survival: treatment results of 2169 unselected pediatric and adolescent patients enrolled in the trial ALL-BFM 95. Blood. 2008;111(9):4477-4489.
4. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536(7616):285-291.
5. Hof J, Krentz S, van Schewick C, et al. Mutations and deletions of the TP53 gene predict nonresponse to treatment and poor outcome in first relapse of childhood acute lymphoblastic leukemia. J Clin Oncol. 2011;29(23):3185-3193.
6. Holmfeldt L, Wei L, Diaz-Flores E, et al. The genomic landscape of hypodiploid acute lymphoblastic leukemia. Nat Genet. 2013;45(3):242-252.
7. Zhang J, Walsh MF, Wu G, et al. Germline Mutations in Predisposition Genes in Pediatric Cancer. N Engl J Med. 2015;373(24):2336-2346.
8. Powell BC, Jiang L, Muzny DM, et al. Identification of TP53 as an acute lymphocytic leukemia susceptibility gene through exome sequencing. Pediatr Blood Cancer. 2013;60(6):E1-3.
9. Qian M, Cao X, Devidas M, et al. TP53 Germline Variations Influence the Predisposition and Prognosis of B-Cell Acute Lymphoblastic Leukemia in Children. J Clin Oncol. 2018;JCO2017755215.
10. Biasini M, Bienert S, Waterhouse A, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res. 2014;42(Web Server issue):W252-258.

**Supplementary Table 1. Characteristics of patients identified in the observation period from 1984 to 2008 with secondary malignant neoplasms (SMN) after treatment on ALL-BFM trials 79 to 2000 and included in targeted sequencing analysis in comparison to SMN patients not included.**

| | SMN patients included in *TP53* analysis (n=49), n(%) | SMN patients not included in *TP53* analysis (n=119), n(%) | SMN patients included in *ETV6 and RUNX1* analysis (n=38), n(%) | SMN patients not included in *ETV6 and RUNX1* analysis (n=130), n(%) |
|---|---|---|---|---|
| **Sex** | | | | |
| male | 30 (61.2%) | 71 (59.7%) | 22 (57.9%) | 79 (60.8%) |
| female | 19 (38.8%) | 48 (40.3%) | 16 (42.1%) | 51 (39.2%) |
| **Age [y]** | | | | |
| 1-6 | 28 (57.1%) | 76 (63.9%) | 22 (57.9%) | 82 (63.1%) |
| >6 | 21 (42.9%) | 43 (36.1%) | 16 (42.1%) | 48 (36.9%) |
| **Initial white blood cell count [/µL]** | | | | |
| < 10000 | 15 (30.6%) | 60 (50.4%) | 10 (26.3%) | 65 (50.0%) |
| ≥10000 < 50000 | 16 (32.7%) | 38 (31.9%) | 14 (36.8%) | 40 (30.8%) |
| ≥50000 < 100000 | 8 (16.3%) | 10 (8.4%) | 5 (13.2%) | 13 (10.0%) |
| ≥100000 | 10 (20.4%) | 11 (9.2%) | 9 (23.7%) | 12 (9.2%) |
| **Immunophenotype** | | | | |
| B | 31 (63.3%) | 81 (68.1%) | 24 (63.2%) | 88 (67.7%) |
| T | 16 (32.7%) | 17 (14.3%) | 12 (31.6%) | 21 (16.2%) |
| Other/not characterized | 2 (4.1%) | 21 (17.6%) | 2 (5.3%) | 21 (16.2%) |
| **BFM study** | | | | |
| ALL-BFM 79 | 2 (4.1%) | 2 (1.7%) | 1 (2.6%) | 3 (2.3%) |
| ALL-BFM 81 | 6 (12.2%) | 13 (10.9%) | 2 (5.3%) | 17 (13.1%) |
| ALL-BFM 83 | 3 (6.1%) | 8 (6.7%) | 2 (5.3%) | 9 (6.9%) |
| ALL-BFM 86 | 7 (14.3%) | 11 (9.2%) | 6 (15.8%) | 12 (9.2%) |
| ALL-BFM 90 | 13 (26.5%) | 29 (24.4%) | 10 (26.3%) | 32 (24.6%) |
| ALL-BFM 95 | 8 (16.3%) | 33 (27.7%) | 8 (21.1%) | 33 (25.4%) |
| ALL-BFM 2000 | 10 (20.4%) | 23 (19.3%) | 9 (23.7%) | 24 (18.5%) |
| **Cranial irradiation**[1] | | | | |
| No | 13 (26.5%) | 54 (45.4%) | 11 (28.9%) | 56 (43.1%) |
| Yes | 36 (73.5%) | 65 (54.6%) | 27 (71.1%) | 74 (56.9%) |
| **Risk group**[2] | | | | |
| Standard | 11 (23.4%) | 41 (35.0%) | 8 (21.6%) | 44 (34.6%) |
| Intermediate | 28 (59.6%) | 63 (53.8%) | 21 (56.8%) | 70 (55.1%) |
| High | 8 (17.0%) | 13 (11.1%) | 8 (21.6%) | 13 (10.2%) |
| **SMN**[3] | | | | |
| Hematologic | 24 (49.0%) | 54 (45.4%) | 18 (47.4%) | 60 (46.2%) |
| Brain tumors | 9 (18.4%) | 32 (26.9%) | 8 (21.1%) | 33 (25.4%) |
| Other solid tumors | 16 (32.7%) | 33 (27.7%) | 12 (31.6%) | 37 (28.5%) |
| **Time to SMN [y]** | | | | |
| < 3 | 17 (34.7%) | 26 (21.8%) | 14 (36.8%) | 29 (22.3%) |
| ≥3<10 | 25 (51.0%) | 59 (49.6%) | 20 (52.6%) | 64 (49.2%) |
| ≥10 | 7 (14.3%) | 34 (28.6%) | 4 (10.5%) | 37 (28.5%) |

[1] Cranial irradiation (CI) ranged from 12 to 30 gray, depending on study, risk stratification of the patient, tumor load and CNS involvement. Since ALL-BFM 83 CI was eliminated for standard risk patients and since ALL-BFM 90 only ≤ 18 gray were applied. Starting with ALL-BFM 95 and ALL-BFM 2000, preventive CI at 12 Gy was only applied in T-cell ALL and high-risk patients; CNS-positive patients received 18 Gy (<2 years, 12 Gy; <1 year, no CI).

[2] Patients from ALL-BFM 79 (n=4) were not included, because there were only 2 risk groups in that study.

[3] Specific diagnosis of SMN included in *TP53* genotyping were as follows: hematologic SMN: Hodgkin lymphoma (n=1), non-Hodgkin lymphoma (n=1), malignant rhabdoid tumor (n=1), AML/MDS(n=22); brain tumors: astrocytoma (n=1), glioblastoma (n=4), meningeoma (n=1), oligodendroglioma (n=1), primitive neuroectodermal tumor of CNS (n=2); other solid tumors: Ewing sarcoma/peripheral primitive neuroectodermal tumor(n=4), osteosarcoma (n=1), synovial sarcoma (n=1), small round cell sarcoma (not specified) (n=9).

Specific diagnosis of SMN included in *ETV6* and *RUNX1* genotyping were as follows: hematologic SMN: AML/MDS (n=15), bilinear leukemia (n=1), Hodgkin lymphoma (n=1), non-Hodgkin lymphoma (n=1); brain tumors: astrocytoma (n=1), glioblastoma (n=4), meningeoma (n=1), oligodendroglioma (n=0), primitive neuroectodermal tumor of CNS (n=2); other solid tumors: Ewing sarcoma/peripheral primitive neuroectodermal tumor(n=4), malignant rhabdoid tumor (n=1), osteosarcoma (n=1), small round cell sarcoma (not specified) (n=6).

**Supplementary Table 2. Sequencing information of mutation-positive patients and their characteristics of secondary malignant neoplasm (SMN).**

| Patient N° | Gene | Physical position[a] | Transcript change[a] | Protein change[b] | dbSNP | Sift | Polyphen | Global MAF[c] | CEU MAF[c] | Type of SMN | Time to SMN [y] | Death |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | ***TP53*** | chr17:7577577 | c.704A>G | p.Asn235Ser | rs144340710 | tolerated | benign | 0.0002(C)[d,e] | 0.0003(C)[d,f] | small round cell sarcoma | 3.8 | yes |
| 2 | ***ETV6*** | chr12:11905490 | c.140_141insAGGAT | p.Ile48Glyfs*2 | - | - | - | - | - | acute myeloid leukemia | 1.3 | yes |
| 3 | | chr12:11992145 | c.235_236delTTinsCC | p.Leu79Pro | - | damaging | probably damaging | - | - | osteosarcoma | 8.2 | no |
| 4 | | chr12:12038902 | c.1195C>G | p.Arg399Gly | - | damaging | probably damaging | - | - | acute myeloid leukemia | 1.5 | yes |
| 5 | ***RUNX1*** | chr21:36206754 | c.757_758insGCACG | p.Leu253Argfs*3 | - | - | - | - | - | Myelodysplastic syndrome / acute myeloid leukemia | 2.1 | no |
| 6 | | chr21:36259324 | c.167T>C | p.Leu56Ser | rs111527738 | tolerated | probably damaging | - | - | acute myeloid leukemia / bilineage leukemia | 0.4 | yes |

[a] GRCh37.p13, hg19, NM_000546.5 (TP53), NM_001987.4 (ETV6), NM_001754.4 (RUNX1).

[b] UniprotK: P04637 (TP53), NP_001978 (ETV6), NP_001745 (RUNX1).

[c] Minor allele frequencies (MAF) obtained as indicated from the 1000Genomes project phase 1 or 3 or from ExAC database; "-" means there was no frequency reported in the databases.

[d] No global or CEU minor allele frequencies (MAF) available, within the 1000Genomes project phase 1 or 3.

[e] Global ExAC MAF: Minor allele frequency of the total (global) population analyzed by the Exome Aggregation Consortium (ExAC); (n=60,706 individuals).

[f] European ExAC MAF (Non-Finnish): Minor allele frequency of the analyzed European population, Finnish individuals not included; (n=33,367 individuals).

**Supplementary Table 3. Characteristics of mutation-positive patients at initial diagnosis of acute lymphoblastic leukemia and related to its primary treatment.**

| Patient N° | Sex | Age at diagnosis [y] | Initial WBC[a] [/µL] | Initial platelet counts [/µL] | Immuno-phenotype | CNS[b] status | ICP[c] DNA index | Protocol | Risk group | Cumulative anthracycline dose [/m$^2$] | Cumulative alkylating agent dose [/m$^2$] | Cumulative etoposide dose [/m$^2$] | Cranial irradiation dose [Gy] | Relapse |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | female | 9.2 | 9400 | 162000 | T-ALL | positive | - | ALL-BFM 81 | IR | 240 mg | 3000 mg CPH[d] | - | 30 | no |
| 2 | male | 8.0 | 3400 | 220000 | pre B-ALL | negative | 1.14 | ALL-BFM 90 | HR | 270 mg | 2000 mg IFO[e] | 600 mg | - | no |
| 3 | male | 10.8 | 192000 | 23000 | pre B-ALL | - | - | ALL-BFM 86 | IR | 280 mg | 3000 mg CPH | - | 18 | yes |
| 4 | male | 5.5 | 4200 | 128000 | pre B-ALL | negative | - | ALL-BFM 95 | SR | 180 mg | 3000 mg CPH | - | - | no |
| 5 | male | 11.2 | 47300 | 100000 | T-ALL | negative | - | ALL-BFM 2000 | IR | 240 mg | 3000 mg CPH | - | 12 | no |
| 6 | female | 8.6 | 64700 | 270000 | pre B-ALL | negative | 1.0 | ALL-BFM 2000 | IR | 240 mg | 3000 mg CPH | - | 12 | no |

[a] WBC: White blood cell count

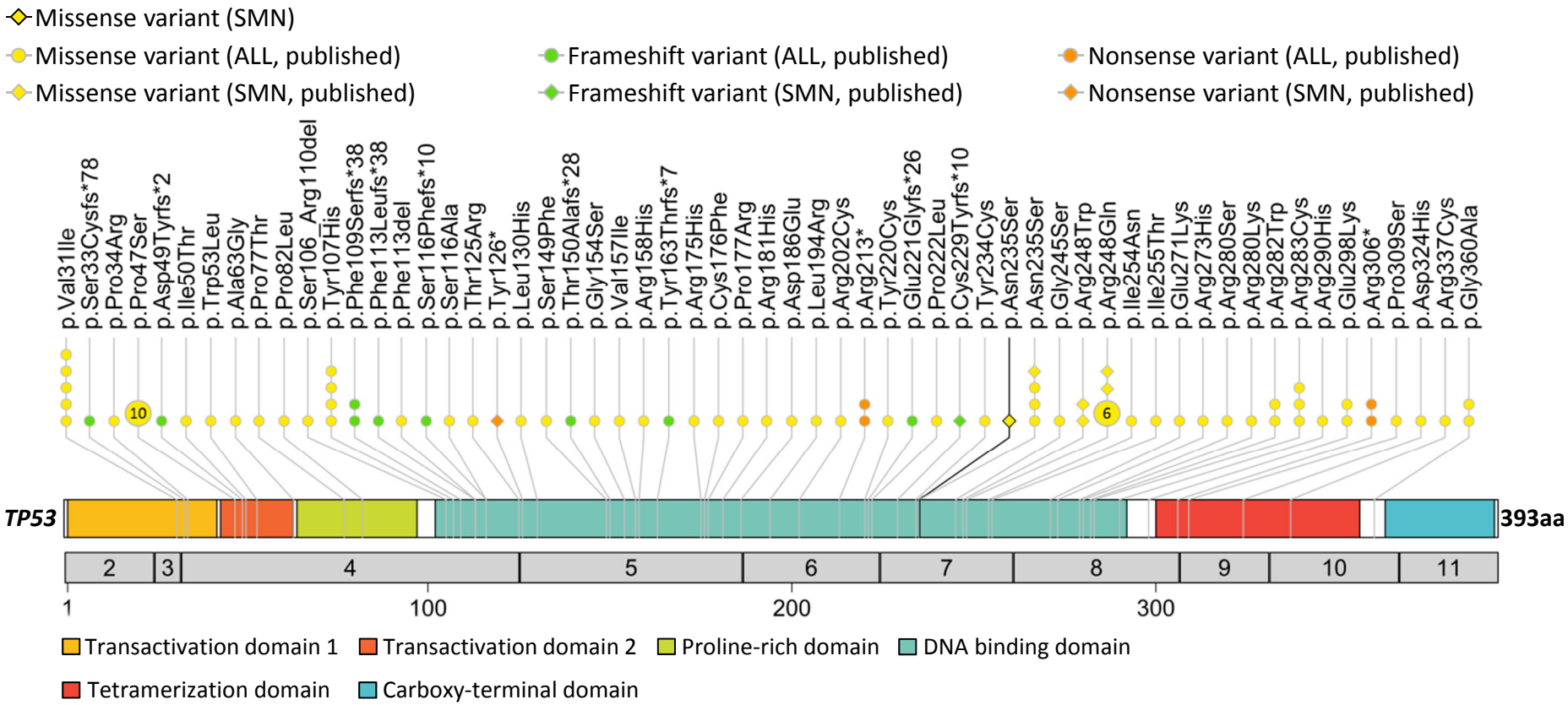
[b] CNS: Central nervous system

[c] ICP: Impulse cytophotometric results: hypodiploid: ≤ 0.8; normal karypotype: ≥ 1 and < 1.16; hyperdiploid: ≥ 1.16 and < 1.6

[d] CPH: Cyclophosphamide

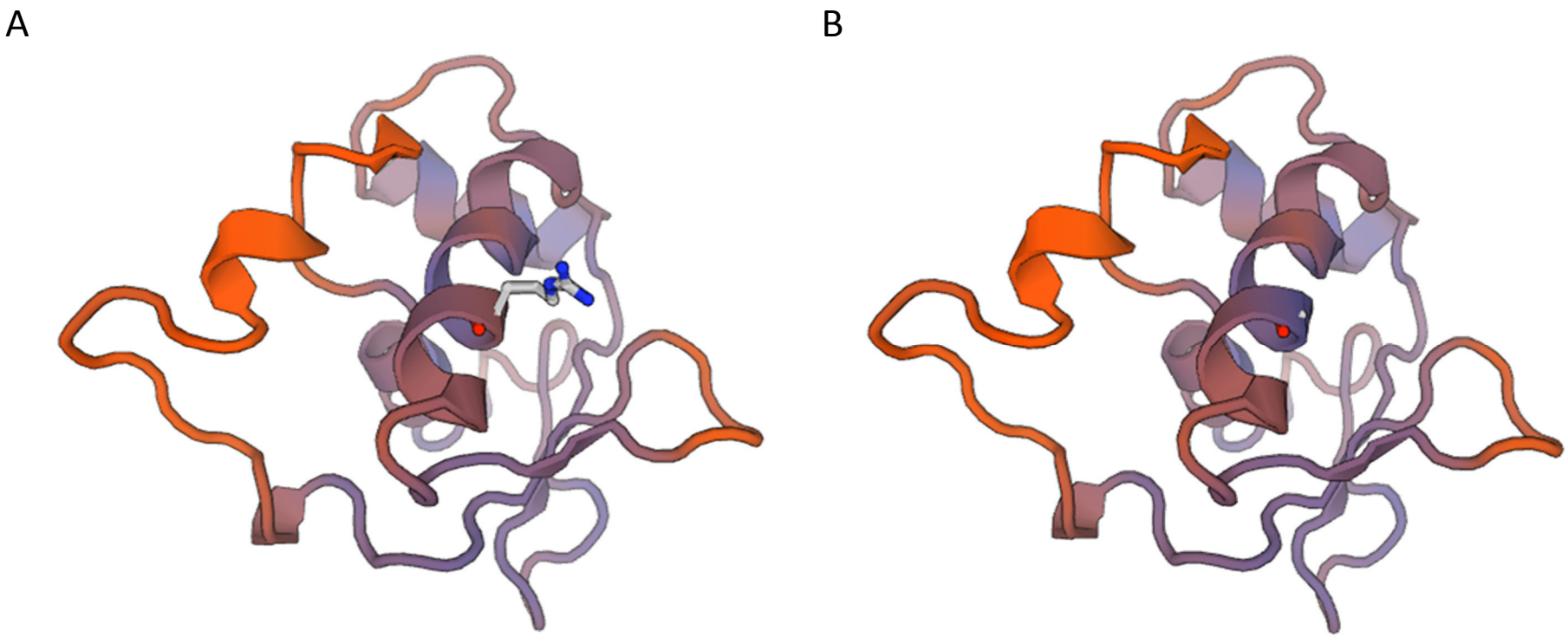
[e] IFO: Ifosfamide

## Supplementary Figure 1




***TP53* germline alterations detected in childhood ALL patients and ALL patients developing secondary malignant neoplasms.** Germline variants of the current study, observed in childhood ALL patients developing secondary malignant neoplasms (SMN), are indicated by rhombs and black lines. Germline variants, reported in the published for childhood ALL patients developing SMN (SMN, published)[5,9], are indicated by rhombs and grey lines. ALL-related germline alterations reported in the literature (ALL, published)[5-9] are represented by circles and grey lines; where some studies mainly focused on hypodiploid ALL[6-7], the latest included all kinds of B cell ALL patients[9].

**Supplementary Figure 2**




**Molecular modeling of *ETV6.*** Using SWISS-MODEL[10] based on the sequence information from NP_001978.1 and P41212 (UniprotK) combined with the human structural model 2DAO.A (Protein Data Bank) *in-silico* modeling was performed. The resulting protein structure of human (A) wildtype *ETV6* and (B) *ETV6* with missense substitution p.Arg399Gly is shown.