**Clonal hematopoiesis, myeloid disorders and BAX-mutated myelopoiesis in patients receiving venetoclax for CLL**

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**Supplementary Material**

**Supplementary Table 1 – Scoring system (adapted from Drake *et al. British Journal of Haematology* 1997[1]) for cumulative exposure to prior lines of myelotoxic therapy**

| Toxicity score | Chemotherapeutic agent |
|---|---|
| 0 | Prednisolone, Dexamethasone |
| 1 | Vincristine, Vinblastine, Bleomycin, Alpha interferon, **Mercaptopurine** |
| 2 | Cyclophosphamide, Anthracyclines, Cisplatin, Etoposide, **Fludarabine, Radiotherapy, Cladribine, Mitoxantrone** |
| 3 | Chlorambucil, Procarbazine |
| 4 | Melphalan, Carmustine, Mechlorethamine, Lomustine, **Bendamustine** |

Chemotherapeutic agents in bold were added, as they did not appear in the original scoring system
Each line of therapy was assigned as score by adding the component agents *i.e.* fludarabine-cyclophosphamide = 2 + 2 = 4

**Supplementary Table 2 – Genes and regions targeted with unique molecular index-based next generation sequencing**

| Gene | Transcript | Targeted exons | Gene | Transcript | Targeted exons | Gene | Transcript | Targeted exons |
|---|---|---|---|---|---|---|---|---|
| *ABL1* | NM_007313.2 | 4-10 | *FOXO1* | NM_002015.3 | 1 | *PIGA* | NM_002641.3 | All coding |
| *ANKRD26* | NM_014915.2 | 5'UTR | *FLT3* | NM_004119.2 | 14, 15, 17, 20 | *PHF6* | NM_001015877.1 | 7-10 |
| *ARAF* | NM_001654.4 | 7,10,15 | *FYN* | NM_002037.5 | 7 | *PLCG1* | NM_002660.2 | 11 |
| *ASXL1* | NM_015338.5 | 10,11,12 | *GATA1* | NM_002049.3 | 2-6 | *PLCG2* | NM_002661.3 | 16,19,20,24 |
| *BAK1* | NM_001188.3 | All coding | *GATA2* | NM_032638.4 | All coding | *RHOA* | NM_001664.2 | 2 |
| *BAX* | NM_138761.3 | All coding | *HAVCR2* | NM_032782.3 | All coding | *RUNX1* | NM_001754.4 | All coding |
| *BCL2* | NM_000633.2 | All coding | *ID3* | NM_002167.4 | 1,2 | *SETBP1* | NM_015559.2 | 4 |
| *BCL2L1* | NM_001191.2 | All coding | *IDH1* | NM_005896.2 | 4, 7 | *SF3B1* | NM_012433.2 | 14-16 |
| *BIRC3* | NM_001165.4 | 6-9 | *IDH2* | NM_002168.2 | 4, 7 | *SH2B3* | NM_005475.2 | All coding |
| *BRAF* | NM_004333.4 | 15 | *IKZF1* | NM_006060.4 | All coding | *SRSF2* | NM_003016.4 | 1 |
| *BTK* | NM_000061.2 | 11, 15, 16 | *IRF8* | NM_002163.2 | 3 | *STAT3* | NM_139276.2 | 6,13,15,18-21 |
| *CALR* | NM_004343.3 | 9 | *JAK2* | NM_004972.3 | 12, 13, 14, 16 | *STAT5B* | NM_012448.3 | 16 |
| *CARD11* | NM_032415.4 | 4-9, 15, 20 | *JAK3* | NM_000215.3 | 11, 13, 15 | *STAT6* | NM_001178078.1 | 10, 13, 16 |
| *CBL* | NM_005188.3 | 8, 9 | *KIT* | NM_000222.2 | 8, 10, 11, 17 | *TCF3* | NM_001136139.2 | 17 |
| *CD274* | NM_014143.3 | All coding, 3'UTR | *KRAS* | NM_033360.2 | 2-4 | *TET2* | NM_001127208.2 | All coding |
| *CD79B* | NM_000626.2 | 5,6 | *MAP2K1* | NM_002755.3 | 2,3 | *TP53* | NM_000546.5 | All coding |
| *CEBPA* | NM_004364.3 | 1 | *MCL1* | NM_021960.4 | All coding | *U2AF1* | NM_006758.2 | 2, 6 |
| *CSF3R* | NM_156039.3 | 14, 17 | *MPL* | NM_005373.2 | All coding | *XPO1* | NM_003400.3 | 15,16 |
| *CXCR4* | NM_003467.2 | 1 | *MYD88* | NM_002468.4 | 4,5 | *ZRSR2* | NM_005089.3 | All coding |
| *DDX41* | NM_016222.2 | All coding | *NOTCH1* | NM_017617.3 | 26-28,34,3'UTR | | | |
| *DNMT3A* | NM_022552.4 | All coding | *NPM1* | NM_002520.6 | 11 | | | |
| *ETNK1* | NM_018638.4 | 3 | *NRAS* | NM_002524.4 | 2-4 | | | |
| *EZH2* | NM_004456.4 | All coding | *PDCD1LG2* | NM_025239.3 | All coding, 5'UTR | | | |

**Supplementary Table 3 – Clinical characteristics of patients with therapy-related myeloid neoplasm emergent on venetoclax (diagnosed during venetoclax therapy or after venetoclax discontinuation if cytopenias emerged during venetoclax therapy)**

| Patient | Sex | Age at VEN initiation | Prior treatments | Time from FCT to tMN diagnosis (years) | VEN dose | Time on VEN (months) | Time from VEN initiation to tMN (months) | Cytogenetics at tMN diagnosis* | Effect of VEN cessation | tMN treatment | Follow up post tMN (months) | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLL69 | M | 66 | CLB; FCR; ofatumumab | 7 | 400 mg | 14 | 6 | NA | No improvement in cytopenias | Supportive care | 8 | Dead (sudden death) |
| CLL7 | F | 63 | FCR | 10 | 300 mg | 58 | 15 | Monosomy 2, monosomy 10, t(1;2), derived (1;9) chromosome, abn(5q), abn(6q), abn(8p), abn(12q), marker chromosome | NA (did not cease VEN) Spontaneous resolution | No directed MDS therapy | 73 | Alive |
| CLL30 | M | 66 | CLB + R, FCR; CVP + R, R monotherapy | 7 | 400 mg | 11 | 22 | #1: **del(5q), t(5;15), # 2: del(5q), abn(8p), abn(17p)** | NA (ceased VEN prior to tMN diagnosis) | Supportive care | 4 | Dead (sepsis) |
| CLL80 | M | 73 | CLB; CLB; CLB; FCR; FCR; duvelisib; ibrutinib | 9 | 400 mg | 29 | 29 | #1: -1, **-4, -21, abn(X)**, abn(9p), **abn(10q)**, **abn(17p)**, t(?;1q) #2: Hypotetraploid with stemline doubling, abn(1q), abn(6q), abn(9p), add(1), -15. #3: t(X;1), dic(6;17), del(6q), del(17p), -18, ring chromosome | No improvement in cytopenias | Azacitidine | 3 | Dead (cardiac arrest) |
| CLL57 | M | 47 | FCRx6; R-CHOP; navitoclax. **After VEN: autoSCT; zanubrutinib** | 9 | 400 mg | 15 | 33 | #1: del(6q), del(9q), abn(5q), abn(10q) #2: del(11q) #3: del(7q) | NA (ceased VEN prior to tMN diagnosis) | No tMN therapy. AlloSCT 3 years after tMN diagnosis for progressive CLL | 60 | Alive |
| CLL45 | M | 68 | CVP; FC; F | 16 | 400 mg | 61 | 42 | -7 | Worsening thrombocytopenia New diagnosis plasmacytoid dendritic neoplasm, likely tMN related | Supportive care (planned for azacitidine) | 19 | Alive |

**Supplementary Table 3 – Clinical characteristics of patients with therapy-related myeloid neoplasm emergent on venetoclax (cont'd)**

| Patient | Sex | Age at VEN initiation | Prior treatments | Time from FCT to tMN diagnosis (years) | VEN dose | Time on VEN (months) | Time from VEN initiation to tMN (months) | Cytogenetics at tMN diagnosis* | Effect of VEN cessation | tMN treatment | Follow up post tMN (months) | Status |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CLL23 | M | 78 | FCR | 5 | 150 mg | 37 | 43 | abn(1q), abn(5q), 6-, two marker chromosomes | NA (ceased VEN prior to tMN diagnosis) | Supportive care (planned for azacitidine) | 2 | Dead (intracranial hemorrhage) |
| CLL16 | M | 73 | CLB; R monotherapy; FCR | 11 | 400 mg (+ rituximab) | 76 | 68 | -7, -18, del(5q), abn(1p) | Complete resolution of cytopenias, loss of excess blasts (azacitidine commenced at VEN cessation) | Azacitidine | 14 | Alive |
| CLL9 | F | 62 | CLB; FCR; FC; MP+R | 9 | 300 mg (+ rituximab) | 87 | 73 | t(3;12;21), abn(3q) abn(12q), abn(21p) | No improvement in cytopenias | Supportive care | 17 | Dead (tMN) |
| CLL12 | M | 69 | C + R; FCR; alemtuzumab; MP + R | 11 | 400 mg | 31 | 86 | del(5q), del(7q), del(13q), -Y, -10, -13, -14, -15, -17, t(4;10), abn(1q), abn(9p), abn(8q), abn(18q), three marker chromosomes | NA (ceased VEN prior to tMN diagnosis) | Supportive care | 2 | Alive |

FCR = Fludarabine, cyclophosphamide, rituximab, R = rituximab, C = cyclophosphamide, MP = methylprednisolone, CVP = cyclophosphamide, vincristine, prednisolone, CLB = chlorambucil, VEN = venetoclax, M = male; F = female, tMN = therapy-related myeloid neoplasm

*Cytogenetics were performed on the diagnostic bone marrow sample, except for patient CLL9 (cytogenetics preceded WHO MDS diagnosis by 6 months) and CLL30(cytogenetics precede WHO MDS diagnosis by 18 months). Where multiple subclonal cell populations are identified, these are distinguished by #. Cytogenetic lesions that had been detected prior to VEN initiation are bolded.

**Supplementary Table 4 – Univariate analysis of variables associated with development of therapy-related myeloid neoplasm in patients receiving venetoclax**

| Variable | | *n* = | tMN cases | HR | 95%CI | *p* value |
|---|---|---|---|---|---|---|
| Age ≥ 65 | Yes | 53 | 7 (13%) | 1.45 | 0.37-5.6 | 0.583 |
| | No | 36 | 3 (8%) | | | |
| ≥ 4 prior therapy lines | Yes | 35 | 4 (11%) | 1.10 | 0.31-3.9 | 0.889 |
| | No | 54 | 6 (11%) | | | |
| F-combination exposed | Yes | 72 | 10 (14%) | Undefined | Undefined | **0.05** |
| | No | 17 | 0 (0%) | | | |
| VEN < 400 mg/day | Yes | 22 | 3 (14%) | 0.93 | 0.24-3.7 | 0.914 |
| | Np | 67 | 7 (10%) | | | |
| VEN ≥ 24 months | Yes | 52 | 7 (13%) | 0.55 | 0.13-2.2 | 0.418 |
| | No | 37 | 3 (8%) | | | |
| Adapted Drake score ≥ 8* | Yes | 46 | 5 (11%) | 1.13 | 0.32-3.93 | 0.851 |
| | No | 43 | 5 (12%) | | | |

*The median adapted Drake score for the cohort was 8

F = Fludarabine, VEN = venetoclax, tMN = therapy-related myeloid neoplasm

**Supplementary Table 5 – Clinical characteristics of patient cohort with relapsed/refractory chronic lymphocytic leukaemia (CLL) treated with venetoclax (n=41) with adequate samples for molecular assessment and low CLL burden**

| Clinical characteristic | Cohort (*n*=41) |
|---|---|
| Age at venetoclax initiation, years | 67 (46-86) |
| Male:Female | 35:6 |
| Median number of prior therapies | 2 (0-8) |
| Prior fludarabine-combination therapy exposure | 29 (72%) |
| Del(17p) and/or TP53 mutation prior to venetoclax initiation (n/N, %) | 18/37 (49%) |
| Median duration on venetoclax, months | 34 (9-90) |
| Median survivor follow-up from venetoclax initiation, months | 66 (21-93) |

**Supplementary Table 6 – Mutations detected in genes associated with age-related clonal hematopoiesis (ARCH)/myeloid neoplasia in patients with chronic lymphocytic leukemia treated with long-term venetoclax**

| Patient | Gene | HGVSc/HGVSp | Variant allele frequency (%)* |
|---|---|---|---|
| **CLL3** | *ASXL1* | NM_015338.5:c.2338C>T; p.(Gln780*) | 39 |
| | *DNMT3A* | NM_022552.4:c.1144A>T; p.(Lys382*) | 1.0 |
| | *DNMT3A* | NM_022552.4:c.923del; p.(Gly308Alafs*8) | 1.0 |
| | *DNMT3A* | NM_022552.4:c.2257T>A; p.(Trp753Arg) | 1.5 |
| **CLL5** | *TET2* | NM_001127208.2:c.2050C>T; p.(Gln684*) | 13.6 |
| **CLL8** | *TP53* | NM_000546.5:c.743G>A; p.(Arg248Gln) | 6.7 |
| | *ASXL1* | NM_015338.5:c.1585C>T; p.(Gln529*) | 27.2 |
| | *EZH2* | NM_004456.4:c.403G>A; p.(Gly135Arg) | 1.8 |
| **CLL11** | Nil | | |
| **CLL12** | *DNMT3A* | NM_022552.4:c.2635A>G; p.(Asn879Asp) | 4.1 |
| **CLL16** | *DNMT3A* | NM_022552.4:c.2644C>T; p.(Arg882Cys) | 26.1 |
| | *SF3B1* | NM_012433.2:c.2098A>G; p.(Lys700Glu) | 7.4 |
| | *U2AF1* | NM_006758.2:c.101C>A; p.(Ser34Tyr) | 6.9 |
| | *DNMT3A* | NM_022552.4:c.2663T>C; p.(Leu888Pro) | 8.1 |
| | *TP53* | NM_000546.5:c.817C>T; p.(Arg273Cys) | 4.5 |
| | *TP53* | NM_000546.5:c.916del; p.(Arg306Glufs*39) | 5.4 |
| **CLL20** | *TET2* | NM_001127208.2:c.4073G>A; p.(Cys1358Tyr) | 1.2 |
| | *ZRSR2* | NM_005089.3:c.407T>A; p.(Leu136*) | 2.5 |
| | *TET2* | NM_001127208.2:c.3501-2A>T; p.? | 1.6 |
| | *DNMT3A* | NM_022552.4:c.2579G>A; p.(Trp860*) | 1.4 |
| | *DNMT3A* | NM_022552.4:c.1532dup; p.(Gly512Argfs*34) | 1.1 |
| | *DNMT3A* | NM_022552.4:c.2159G>C; p.(Arg720Pro) | 0.8 |
| **CLL21** | *TET2* | NM_001127208.2:c.4210C>T; p.(Arg1404*) | 1.3 |
| | *TP53* | NM_000546.5:c.826G>C; p.(Ala276Pro) | 0.8 |
| | *TP53* | NM_000546.5:c.743G>A; p.(Arg248Gln) | 1.3 |
| | *TP53* | NM_000546.5:c.422G>A; p.(Cys141Tyr) | 1.9 |
| **CLL26** | *DNMT3A* | NM_022552.4:c.2663T>C; p.(Leu888Pro) | 31.9 |
| | *DNMT3A* | NM_022552.4:c.855+1G>A; p.? | 4.6 |
| | *U2AF1* | NM_006758.2:c.101C>A; p.(Ser34Tyr) | 14.6 |
| **CLL27** | *DNMT3A* | NM_022552.4:c.2478+1G>A; p.? | 3.7 |
| | *DNMT3A* | NM_022552.4:c.1851+1G>T; p.? | 3.0 |
| | *DNMT3A* | NM_022552.4:c.1610G>C; p.(Cys537Ser) | 1.4 |
| **CLL32** | *TET2* | NM_001127208.2:c.3796A>C; p.(Asn1266His) | 13.8 |
| | *ZRSR2* | NM_005089.3:c.896G>T; p.(Cys299Phe) | 1.8 |
| | *DNMT3A* | NM_022552.4:c.1430-2A>G; p.? | 1.5 |
| | *ZRSR2* | NM_005089.3:c.827+1G>A; p.? | 6.0 |
| **CLL34** | *TP53* | NM_000546.5:c.416A>C; p.(Lys139Thr) | 2.8 |
| **CLL35** | *ASXL1* | NM_015338.5:c.1934dup; p.(Gly646Trpfs*12) | 2.8 |
| | *ZRSR2* | NM_005089.3:c.716T>G; p.(Phe239Cys) | 1.4 |
| **CLL36** | *DNMT3A* | NM_022552.4:c.1015-2A>T; p.? | 22.3 |

| | | | |
|---|---|---|---|
| **CLL38** | *U2AF1* | NM_006758.2:c.101C>A; p.(Ser34Tyr) | 1.9 |
| | *DNMT3A* | NM_022552.4:c.2204A>C; p.(Tyr735Ser) | 1.1 |
| | *DNMT3A* | NM_022552.4:c.2383T>A; p.(Trp795Arg) | 1.6 |
| **CLL39** | *TP53* | NM_000546.5:c.376T>C; p.(Tyr126His) | 2.7 |
| | *DNMT3A* | NM_022552.4:c.2645G>A; p.(Arg882His) | 22.4 |
| | *DNMT3A* | NM_022552.4:c.2104del; p.(Asp702Ilefs*3) | 2.5 |
| | *TET2* | NM_001127208.2:c.1A>T; p.? | 1.8 |
| **CLL40** | *TP53* | NM_000546.5:c.818G>A; p.(Arg273His) | 1.7 |
| | *DNMT3A* | NM_022552.4:c.2302G>T; p.(Asp768Tyr) | 2.6 |
| | *DNMT3A* | NM_022552.4:c.1628_1629delinsTG; p.(Gly543Val) | 16.8 |
| | *DNMT3A* | NM_022552.4:c.2204A>G; p.(Tyr735Cys) | 12.1 |
| | *DNMT3A* | NM_022552.4:c.2645G>A; p.(Arg882His) | 1.1 |
| **CLL41** | Nil | | |
| **CLL42** | *STAT3* | NM_139276.2:c.1940A>T; p.(Asn647Ile) | 1.6 |
| | *TET2* | NM_001127208.2:c.4393C>T; p.(Arg1465*) | 3.7 |
| | *PHF6* | NM_001015877.1:c.1024C>T; p.(Arg342*) | 19.4 |
| | *DNMT3A* | NM_022552.4:c.892G>A; p.(Gly298Arg) | 2.4 |
| | *TET2* | NM_001127208.2:c.236C>T; p.(Thr79Ile) | 1.6 |
| | *PHF6* | NM_001015877.1:c.719A>G; p.(Tyr240Cys) | 7.2 |
| **CLL43** | *TET2* | NM_001127208.2:c.5618T>C; p.(Ile1873Thr) | 41.7 |
| | *DNMT3A* | NM_022552.4:c.2257T>C; p.(Trp753Arg) | 47.6 |
| **CLL45** | *TET2* | NM_001127208.2:c.2756dup; p.(Tyr919*) | 42.6 |
| | *DNMT3A* | NM_022552.4:c.2645G>A; p.(Arg882His) | 44.5 |
| | *U2AF1* | NM_006758.2:c.101C>T; p.(Ser34Phe) | 34.7 |
| **CLL46** | *ZRSR2* | NM_005089.3:c.605T>C; p.(Ile202Thr) | 5.3 |
| **CLL49** | *TET2* | NM_001127208.2:c.4075C>T; p.(Arg1359Cys) | 1.1 |
| | *DNMT3A* | NM_022552.4:c.912_928del; p.(Trp305Cysfs*13) | 0.9 |
| **CLL53** | *DNMT3A* | NM_022552.4:c.2666T>C; p.(Leu889Pro) | 10.5 |
| | *DNMT3A* | NM_022552.4:c.1851+5G>A; p.? | 5.9 |
| **CLL69** | *TP53* | NM_000546.5:c.524G>C; p.(Arg175Pro) | 29.2 |
| | *TP53* | NM_000546.5:c.524G>A; p.(Arg175His) | 33.7 |
| | *DNMT3A* | NM_022552.4:c.2063G>A; p.(Arg688His) | 34.0 |
| **CLL70** | *DNMT3A* | NM_022552.4:c.1668G>C; p.(Arg556Ser) | 3.3 |
| **CLL73** | Nil | | |
| **CLL74** | Nil | | |
| **CLL75** | *TET2* | NM_001127208.2:c.3379C>T; p.(Gln1127*) | 0.94 |
| **CLL76** | *TP53* | NM_000546.5:c.713G>A; p.(Cys238Tyr) | 2.2 |
| | *DNMT3A* | NM_022552.4:c.895A>T; p.(Lys299*) | 4.5 |
| **CLL78** | *DNMT3A* | NM_022552.4:c.2478+1G>A; p.? | 40.2 |
| | *DNMT3A* | NM_022552.4:c.941G>A; p.(Trp314*) | 0.57 |
| **CLL79** | *ASXL1* | NM_015338.5:c.1934dup; p.(Gly646Trpfs*12) | 6.6 |
| | *DNMT3A* | NM_022552.4:c.2257T>C; p.(Trp753Arg) | 3.0 |
| **CLL80** | *DNMT3A* | NM_022552.4:c.1628G>T; p.(Gly543Val) | 35.7 |
| **CLL81** | *U2AF1* | NM_006758.2:c.101C>T; p.(Ser34Phe) | 8.5 |
| | *DNMT3A* | NM_022552.4:c.1660T>G; p.(Cys554Gly) | 1.4 |
| | *TET2* | NM_001127208.2:c.2770C>T; p.(His924Tyr) | 2.4 |
| | *U2AF1* | NM_006758.2:c.101C>A; p.(Ser34Tyr) | 4.7 |
| **CLL83** | Nil | | |
| **CLL85** | Nil | | |
| **CLL86** | *DNMT3A* | NM_022552.4:c.2408+5G>A; p.? | 1.7 |

| | | | |
|---|---|---|---|
| | *DNMT3A* | NM_022552.4:c.2359G>A; p.(Ala787Thr) | 1.7 |
| | *TET2* | NM_001127208.2:c.3819T>G; p.(Cys1273Trp) | 5.7 |
| **CLL87** | *U2AF1* | NM_006758.2:c.101C>A; p.(Ser34Tyr) | 22.0 |
| | *ZRSR2* | NM_005089.3:c.1384C>T; p.(Arg462*) | 2.0 |
| **CLL88** | Nil | | |
| **CLL90** | *KRAS* | NM_033360.2:c.351A>T; p.(Lys117Asn) | 3.8 |
| | *TET2* | NM_001127208.2:c.649dup; p.(Ser217Phefs*8) | 0.6 |
| **CLL91** | *DNMT3A* | NM_022552.4:c.1793_1809del; p.(Arg598Profs*8) | 0.82 |

*highest variant allele frequency detected if detected in multiple samples

**Supplementary Table 7 – *BAX* mutations detected in the non-CLL hematopoietic compartment. Samples with minimal or no residual CLL from patients treated with long-term venetoclax for CLL were analyzed to minimize the chance that the mutations were in CLL cells**

| Patient | HGVSc/HGVSp | Predicted consequence for BAX function | Variant allele frequency (%) | Sample type |
|---|---|---|---|---|
| **CLL3** | c.475-1G>A; p.? | Splice site mutation | 39.7 | BM |
| | c.280del; p.(Arg94Glufs*39) | Truncation/NMD | 3.8 | BM |
| | c.554_557del; p.(Leu185Profs*55) | Truncation | 1.5 | BM |
| **CLL16** | c.121del; p.(Glu41Argfs*19) | Truncation/NMD | 24.2 | BM |
| | c.547G>C; p.(Ala183Pro) | Missense (α9) | 8.1 | BM |
| | c.368A>T; p.(Lys123Met) | Missense (α5) | 1.3 | BM |
| | c.100C>T; p.(Arg34*) | Truncation/NMD | 1.1 | BM |
| | c.265C>T; p.(Arg89*) | Truncation/NMD | 1.1 | BM |
| | c.519_526del; p.(Thr174Cysfs*30) | Truncation | 1.1 | BM |
| | c.109C>T; p.(Arg37*) | Truncation/NMD | 0.6 | BM |
| **CLL20** | c.90C>G; p.(Phe30Leu) | Missense (α1) | 0.8 | BM |
| **CLL21** | c.475-32_475-19del; p.? | Splice site mutation | 37.3 | BM |
| | c.511C>T; p.(Gln171*) | Truncation | 0.9 | BM |
| **CLL32** | c.82C>T; p.(Gln28*) | Truncation/NMD | 1.6 | BM |
| **CLL34** | c.554_557del; p.(Leu185Profs*55) | Truncation | 0.5 | BM |
| **CLL35** | c.121del; p.(Glu41Argfs*19) | Truncation/NMD | 3.6 | BM |
| | c.509G>C; p.(Trp170Ser) | Missense (α9) | 0.6 | BM |
| **CLL39** | c.87-2A>G; p.? | Splice site mutation | 3.9 | BM |
| | c.547G>C; p.(Ala183Pro) | Missense (α9) | 1.7 | BM |
| | c.100C>T; p.(Arg34*) | Truncation/NMD | 1.0 | BM |
| **CLL43** | c.551C>G; p.(Ser184*) | Truncation | 52.6 | BM |
| **CLL46** | c.564del; p.(Trp188*) | Truncation | 11.8 | BM |
| **CLL49** | c.121del; p.(Glu41Argfs*19) | Truncation/NMD | 3.8 | BM |
| **CLL78** | c.121del; p.(Glu41Argfs*19) | Truncation/NMD | 4.5 | PB |
| | c.536_538delinsTCTTTGACCATCTT; p.(Gly179Valfs*66) | Truncation | 0.7 | PB |
| **CLL81** | c.121del; p.(Glu41Argfs*19) | Truncation/NMD | 19.0 | BM |
| | c.547G>A; p.(Ala183Thr) | Missense (α9) | 0.9 | BM |
| | c.109C>T; p.(Arg37*) | Truncation/NMD | 0.7 | BM |

NMD = nonsense mediated decay, BM = bone marrow, PB = peripheral blood

NCBI RefSeq transcripts – NM_138761.3 (*BAX*)

**Supplementary Table 8 – Comparison of characteristics of patients with *BAX* mutations developing in non-CLL compartment on venetoclax**

| Characteristic | *BAX* mutation detected (*n* = 13) | No *BAX* mutation detected (*n* = 28) | *p* value |
|---|---|---|---|
| Age | 67 (54-86) | 67 (46-84) | 0.556 |
| Lines of Rx pre-VEN | 2 (0-8) | 3 (1-8) | 0.627 |
| Prior FCT | 9/13 (69%) | 20/28 (71%) | >0.999 |
| Adapted Drake score | 10 (4-23) | 9 (4-26) | 0.725 |
| del(17p)/TP53 pre VEN | 5/11 (45%) | 13/26 (50%) | >0.999 |
| CK pre-VEN | 0/8 (0%) | 7/23 (30%) | 0.146 |
| tMN | 1/13 (8%) | 4/28 (14%) | >0.999 |

Rx = treatment; VEN = venetoclax, FCT = fludarabine-cyclophosphamide combination therapy; CK = complex karyotype; tMN = therapy-related myeloid neoplasm

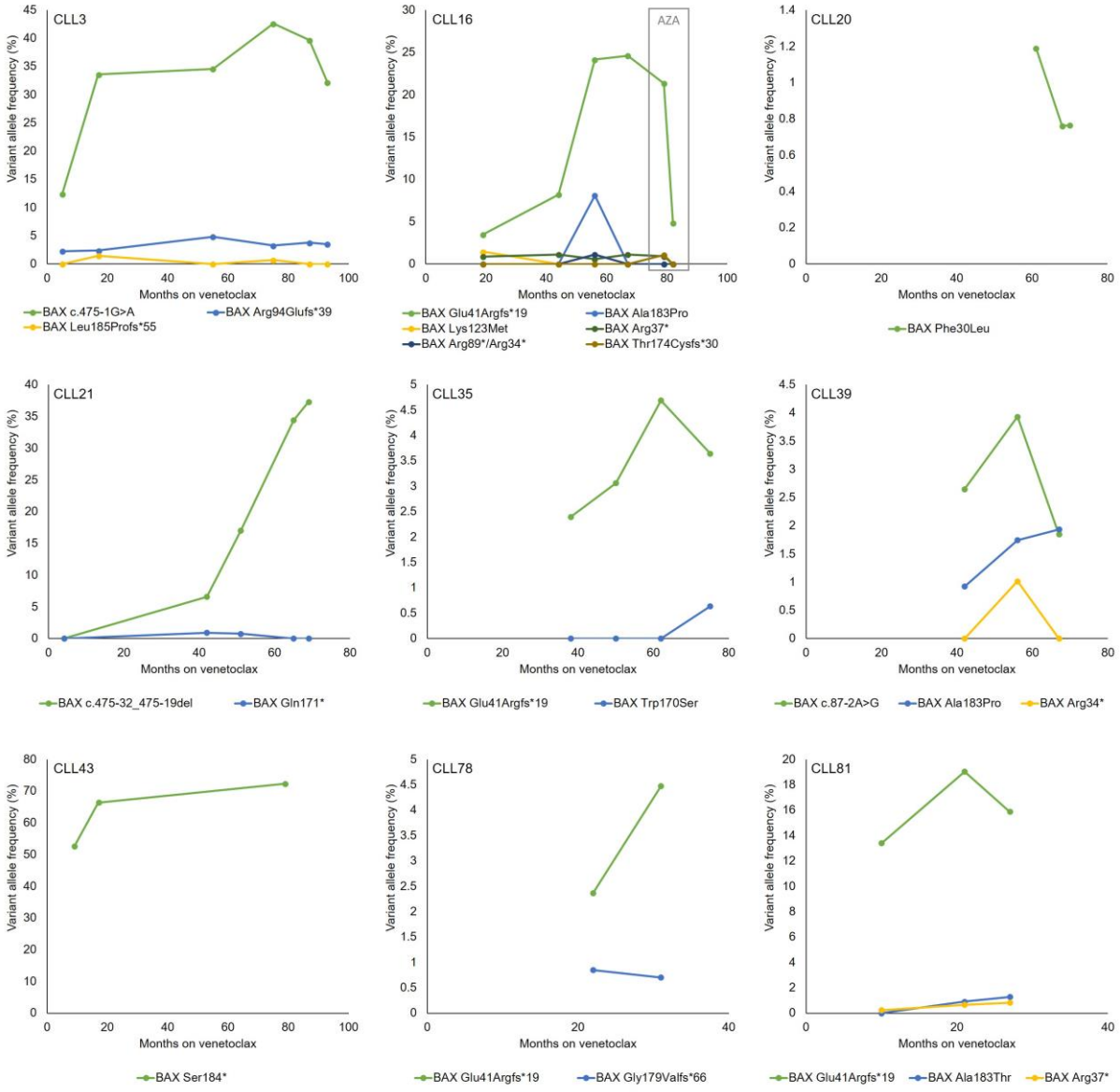
**Supplementary Table 9 – Variants detected in flow cytometry sorted myeloid compartment cells (CD3-/CD19-), T-cells (CD3+/CD19-) and CLL cells (CD3-/CD19+)**

| Patient | Myeloid compartment - CD3-/CD19- (VAF) | T-cell compartment - CD3+, CD19- (VAF) | CLL compartment - CD3-/CD19+ (VAF) |
|---|---|---|---|
| **CLL3** | *ASXL1* Gln780* (38.7%)<br>*TET2* Leu1637Tyrfs*58 (1.3%)<br>*BAX* c.475-1G>A (45.5%)<br>*BAX* Arg94Glufs*39 (4.6%) | *STAT3* Asp661Val (3.2%)<br>*ASXL1* Gln780* (1.0%)<br>*DNMT3A* Lys382* (2.9%)<br>*DNMT3A* Gly308Alafs*8) (6.2%)<br>*DNMT3A* Ile670Leu (1.2%)<br>*DNMT3A* Trp753Arg (9.9%)<br>*BAX* c.475-1G>A (1.4%) | *TP53* Arg273His (96.0%)<br>*SF3B1* Lys700Glu (49.6%)<br>*BCL2* Gly101Val (27.3%)<br>*BCL2* c.326_327insGCGCCGCTACCG Arg107_Arg110dup (8.4%)<br>*BCL2* c.319_330dup Arg107_Arg110dup (6.4%) |
| **CLL16** | *TP53* Arg306Glufs*39 (4.7%)<br>*TP53* Arg273Cys (7.0%)<br>*DNMT3A* Arg882Cys (28.3%)<br>*BAX* Arg37* (2.6%)<br>*BAX* Glu41Argfs*19 (24.0%) | *TP53* Arg273Cys (1.3%)<br>*DNMT3A* Trp860* (0.9%) | *KRAS* Ala146Thr (33.9%)<br>*BCL2* Gly101Val (1.3%)<br>*TP53* c.993+1_993+20del (3.8%)<br>*TP53* c.783-2A>C (4.8%)<br>*BCL2* Ala113Gly (10.0%) |
| **CLL81** | *U2AF1* Ser34Tyr (9.4%)<br>*U2AF1* Ser34Phe (6.9%)<br>*BAX* Arg37* (0.9%)<br>*BAX* Glu41Argfs*19 (15.3%) | Nil | *KRAS* Gln22Lys (17.0%)<br>*KRAS* Gly12Asp (23.4%)<br>*TP53* Leu252_Ile254del (28.2%)<br>*TP53* Ser241Tyr (18.0%)<br>*TP53* Cys176Ser (16.9%)<br>*BCL2* Asp103Glu (20.0%) |

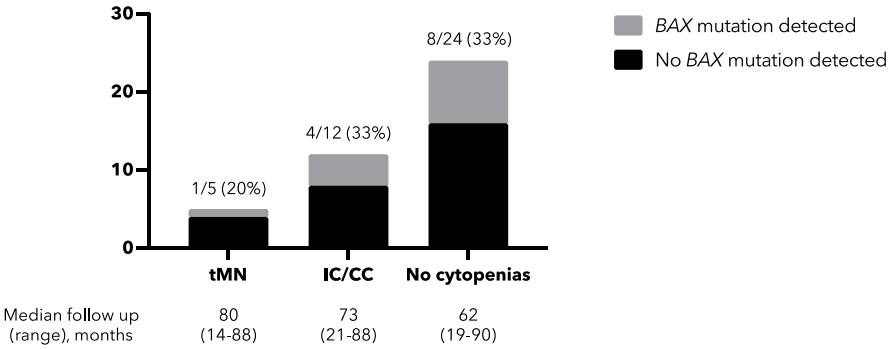
VAF = variant allele frequency

NCBI RefSeq transcripts – NM_015338.5 (*ASXL1*), NM_138761.3 (*BAX*), NM_000633.2 (*BCL2*), NM_022552.4 (*DNMT3A*), NM_033360.2 (*KRAS*), NM_012433.2 (*SF3B1*), NM_139276.2 (*STAT3*), NM_001127208.2 (*TET2*), NM_000546.5 (*TP53*), NM_006758.2 (*U2AF1*)

**Supplementary Figure 1 – Longitudinal changes in *BAX* mutations in non-CLL compartment over time for 9 patients with serial samples during treatment for CLL.**



**Supplementary Figure 2 – Distribution of *BAX* mutations across groups of myeloid dysfunction (tMN, IC/CC or no persistent cytopenias)**




tMN = therapy-related myeloid neoplasm; IC/CC = idiopathic cytopenias/clonal cytopenias

**Supplementary Methods**

*Genomic analyses*

UMI-based libraries were prepared using the standard protocol for QIAseq targeted DNA panel as per manufacturer's specifications. Pooled libraries were sequenced on a NextSeq 500 instrument (Illumina, California). Alignment and variant calling was performed using the QIAGEN CLC Genomic Workbench (v12.0.2). All variant calls were manually inspected in Integrated Genome Viewer (Broad Institute, USA). Copy number was analysed using CNspector[2]. For Sanger sequencing of the *BAX* homopolymer mutant (CLL16) genomic DNA was amplified using AmpliTaq Gold 360 Master Mix (Applied Biosystems) and a primer pair flanking BAX c.114_c.121 (homopolymer guanine) (forward primer: 5'-CCCGTTCTGATTCTGC-3', reverse primer: 5'-ACTGTCCAGTTCGTCC-3', both primers were CS tagged). The cycling conditions were: 95°C for 5 minutes; followed by a program of 94°C for 30 seconds, 60°C for 45 seconds, and 72°C for 45 seconds for 40 cycles; and ending with a final extension at 72°C for 10 minutes. Amplicon product was purified using ExoSAP-IT (Applied Biosystems) and bidirectionally sequenced using ABI Big Dye v.3.1 Terminator Kit on an ABI3730 DNA Analyzer (Applied Biosystems). Data analysis was performed using Mutation Surveyor version 4.0.5 (SoftGenetics). For

hybridization-based next generation sequencing analysis (CLL43) indexed libraries were sequenced on an Illumina NextSeq (paired-end 75 bp reads). After base calling and de-multiplexing, a Seqliner-framework analysis pipeline was used to align reads to the human reference genome (GRCh37 assembly) using BWA-MEM, followed by marking of duplicate reads, base quality score recalibration, local indel realignment and variant calling using GATK Haplotype Caller (https://software.broadinstitute.org/gatk/). Copy number and B-allele frequency analysis was performed using on and off target reads from this hybridization-based NGS panel as described previously[2,3].

*Targeted amplicon single cell sequencing*

Cryopreserved cells were prepared and underwent unique barcoding and amplification with 70 custom primer pairs targeting 17 genes including *BCL2*, *BCL2L1*, *BAX*, *MCL1*, *DNMT3A* and *ASXL1*. The products were subsequently sequenced on a NextSeq 500 instrument (Illumina, California). FASTQ files were analyzed using the cloud-based Tapestri bioinformatics pipeline to perform adapter trimming, barcode correction, cell identification, read alignment to the human hg19 genome, and variant calling using a GATK-based algorithm.

*Carbonate extraction of BAX*

Carbonate extraction can be used to differentiate membrane-integrated BAX from that which is peripherally associated to the mitochondrial outer membrane (MOM). Treatment of membranes with sodium carbonate (high pH) disrupts protein-protein interactions, whereas protein-lipid interactions are largely retained. For carbonate extraction of BAX, treated cells were harvested and permeabilized with 0.025% w/v digitonin in fractionation buffer (20 mM HEPES KOH pH 7.5, 100 mM sucrose, 100 mM KCl, 2.5 mM $MgCl_2$) for 10 min on ice. Successful permeabilization of the cells was confirmed by trypan blue uptake and light microscopy. Cytosol and mitochondria-enriched heavy membrane were then separated by centrifugation. Membrane fractions were then re-suspended in sodium carbonate (0.1M, pH 11.5) and incubated on ice for 20 min before addition of an equal volume of 0.1 M HCL.

After treating the samples with DNase I (5 Units/50 µl), the supernatant fraction containing peripheral proteins and pellet fraction containing membrane-integrated proteins were separated by centrifugation. Cytosol, peripheral and integrated fractions were then run on SDS-PAGE and immunoblotted for BAX.

*Immunoblotting*

Protein lysates were prepared in Onyx buffer (20 mM Tris-HCl pH 7.4, 135 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1% (v/v) Triton X-100, 10% (v/v) glycerol) containing protease inhibitor cocktail (Roche, USA). Proteins were gel electrophoresed and transferred onto nitrocellulose membranes (Life Technologies, USA). Primary antibodies used were rabbit polyclonal anti-BAX NT (#06-499, Merck Millipore, Germany), mouse monoclonal anti-HSP70 (WEHI, in-house). Secondary antibodies were (HRP)-conjugated anti-rabbit (#4010-05, Southern Biotech, Birmingham, USA) and anti-mouse (#1010-05, Southern Biotech, Birmingham, USA). Proteins were visualized by Luminata Forte Western HRP substrate (#WBLUF0500, Merck Millipore, Germany).

*Statistical analysis*

Comparison of characteristics between groups were performed using Mann-Whitney U test and Fisher's exact test as appropriate. The Kaplan-Meier method was used to estimate overall survival from tMN diagnosis (censored at last follow-up or allogeneic stem cell transplant [alloSCT]). Estimates of the proportion of patients developing tMN were expressed as cumulative incidence, with death or alloSCT considered competing risks. Associations between clinicopathological variables and tMN diagnosis were analyzed using the Cox proportional hazards model to calculate hazard ratios with a significance level set at 0.05, with death or alloSCT considered as competing risks and treated as censorship events. Data were analyzed using Stata 14.1 for Mac (StataCorp, College Station, TX), GraphPad Prism 9 for Mac (GraphPad Software, La Jolla, CA), and in R v4.04 for Mac.

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3. Ryland GL, Jones K, Chin M, et al. Novel genomic findings in multiple myeloma identified through routine diagnostic sequencing. *J Clin Pathol*. 2018;71(10):895-899.