SUPPLEMENTARY APPENDIX

# Genomic alterations in patients with somatic loss of the Y chromosome as the sole cytogenetic finding in bone marrow cells

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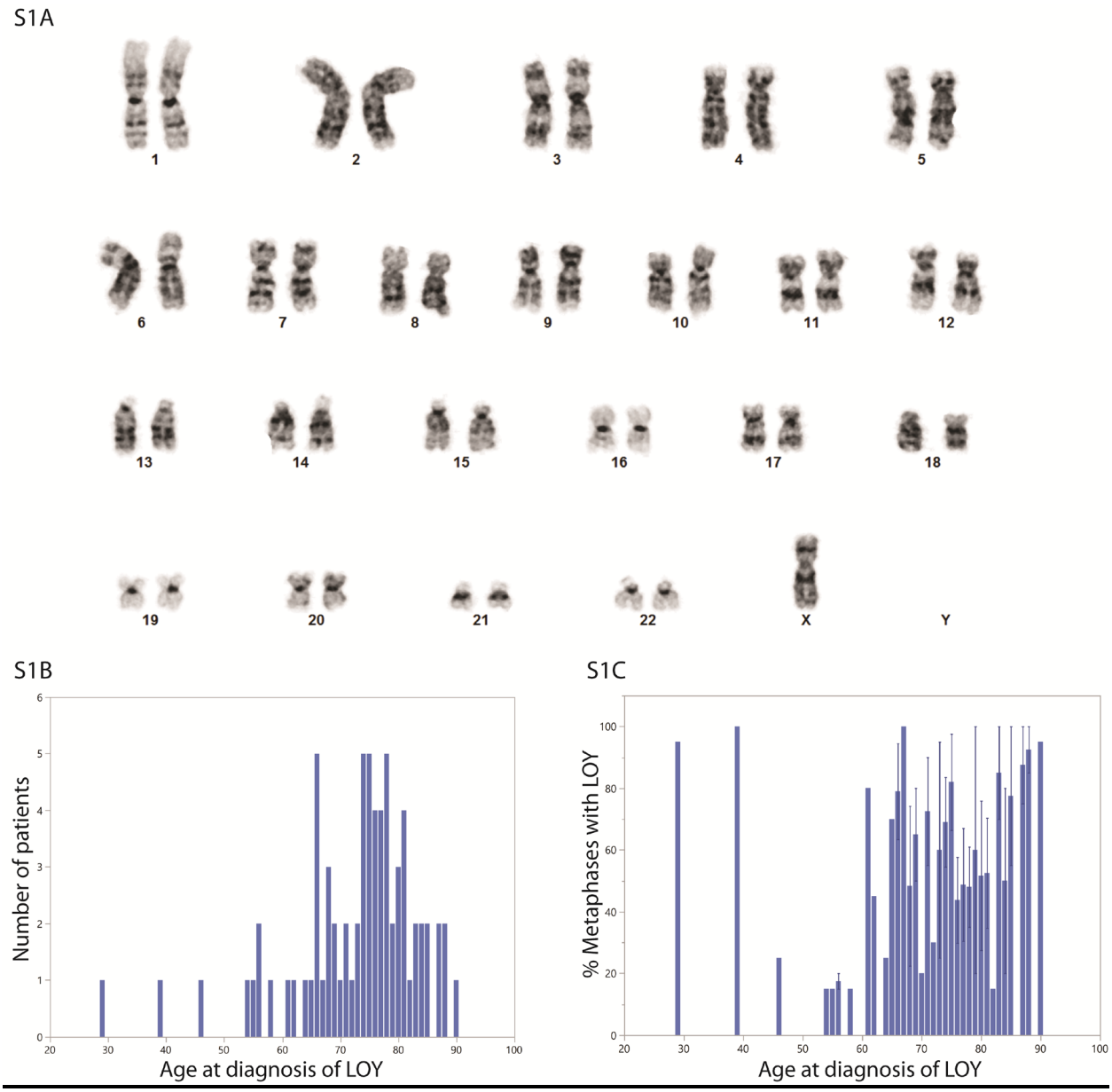
**Supplemental information:**

**Supplemental Figure 1:**




**Supplemental Figure 1: Age at diagnosis and percentage of metaphases involved by LOY:** Figure 1A: Karyogram of bone marrow demonstrating isolated LOY. Figure 1B: Bar diagram showing age at diagnosis of LOY in 73 patients evaluated in this study. Figure 1C: Bar diagram showing percentage of metaphases with LOY compared to age at diagnosis of LOY. %

metaphases with LOY is represented as mean (error bar represents standard error). Abbreviations: loss of Y chromosome (LOY).

**Supplemental Table 1**

| |
|---|
| BWH RapidHeme panel; The 95 gene targets covered by this test are as follows (exons): ABL1 (2-10), ASXL1 (1-13), ATM (2-63), BCL11B (4), BCOR (2-15), BCORL1 (1-12), BRAF (15), BRCC3 (3-11), CALR (9), CBL (7-8), CBLB (9-11), CD79B (5-6), CEBPA (1), CNOT3 (1-2), CREBBP (2-21, 23-31), CRLF2 (6), CSF1R (22), CSF3R (14-18), CTCF (3-12), CTNNB1 (2-4), CUX1 (1-21), CXCR4 (2), DNMT3A (2-23), DNMT3B (2-23), EED (1-12), EGFR (18-21), EP300 (18-27), ETV6 (1-8), FANCL (1-14), FBXW7 (8-12), EZH2 (2-8, 11-20), FLT3 (14-16, 20), GATA1 (2-6), GATA2 (2-6), GATA3 (4-6), GNAS (8-9), GNB1 (5-6), IDH1 (4), IDH2 (4), IKZF1 (2-8), IKZF2 (1-8), IKZF3 (1-8), IL7R (6), JAK1 (10-25), JAK2 (12, 14), JAK3 (11-24), KIT (8-9, 11, 17), KRAS (2-5), LUC7L2 (3-11), MAP2K1 (2-3), MEF2B (3), MPL (10), MYD88 (5), NOTCH1 (24-28), NOTCH1 (34), NOTCH2 (24-28, 34), NOTCH3 (25-26, 33), NPM1 (10-11), NRAS (2-5), PAX5 (3, 6-7), NT5C2 (9, 11, 13, 15, 17), PDS5B (3-35), PHF6 (2-10), PDGFRA (10-21, 23), PIGA (2-6), PIM1 (1-6), PRPF40B (2-26), PIK3CA (2, 10, 21), PRPF8 (2-43), PTEN (1-9), PTPN11 (1-15), RAD21 (2-14), RET (7), RIT1 (1-6), RPL10 (5), RUNX1 (2-9), SETBP1 (4), SF3B1 (12-16), SF1 (1-10, 13), SF3A1 (1-2, 5-16), SETD2 (1-4, 6-21), SH2B3 (2-8), SMC1A (1-25), SMC3 (2-29), SRSF2 (1), STAG2 (3-35), TET2 (3-11), STAT3 (2-17, 21-23), TLR2 (1), TP53 (2-11), U2AF1 (2, 6), U2AF2 (1-12), WHSC1 (17-18), WT1 (1-10), XPO1 (15-16), ZRSR2 (1-11). |
| MGH SnapShot panel; The 103 gene targets covered by this test are as follows (exons): ABL1 (4-10), ALK (22-25), ANKRD26 (1), ASXL1 (1-12), ATM (1-63), ATRX (8-11, 17-32), BCOR (2-15), BCORL1(1-12), BCR (1-5), BIRC3 (2-9), BRAF (3, 10-15), BTK (15), CALR (1-9), CARD11 (5-9), CBL (2-5,7-9,16), CBLB (3,9,10), CBLC (9,10), CD79A (4, 5), CD79B (5, 6), CDKN2A (1-3), CEBPA (1), CREBBP (1-31), CSF3R (10, 14-18), CUX1(1-24), CXCR4 (1,2), DCK(2,3), DDX41 (1-17), DNM2 (17,19), DNMT3A (1-23), EP300 (1-31), ETV6 (1-8), EZH2 (2-20), FBXW7(1-11), FLT3 (8-17, 19-21), GATA1 (2), GATA2 (2-6), GNAS (8-11), HRAS (2-4), IDH1 (3,4), IDH2 (4, 6), IKZF1 (2-6, del 1-3), IKZF3 (5,8), JAK1 (14-16), JAK2 (12-16, 19-25), JAK3 (3,11,13,15,18,19), KDM5A (8,11,13,14,18,21,23,25), KDM6A (1-29), KIT (1,2, 5, 8-15, 17, 18), KLF2 (1-3), KMT2A (1-36), KMT2C (14,25,27,36,38,43,44,55), KMT2D (8,11,15,31,34,39,44,53), KMT2E (14,15,21), KRAS (2-4), LUC7L2 (1-10), MAP2K1 |

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|---|
| (1-11), MEF2B (1, 2), MPL (10, 12), MYC (1-3), MYD88 (3-5), NF1 (1-57), NFKBIE (1), NOTCH1 (UTR,26-28,34), NOTCH2 (34), NPM1 (11), NRAS (2- 5), NT5C2 (9,11,13,15,17-19), PDGFRA (12,14,15,18), PHF6 (2-10), PLCG2 (19,24), PML (1-9), PPM1D (6), PRPF40B (1-26), PTEN (1-9), PTPN11 (3,4,7,8,11-13), RAD21 (2-14), RARA (5-7,9), RB1 (1-27), RBBP6 (16), RHOA (2), RPS15 (4), RUNX1 (2-9), SETBP1 (4), SETD2 (1-21), SF3B1 (13-21), SH2B3 (2-8), SLC29A1 (4,13), SMC1A (1-25), SMC3 (10,13,19,23,25,28), SRC (10), SRSF2 (1,2), STAG2 (2-33), STAT3 (2-24), STAT5B (15-17), TET2 (3-11), TNFAIP3 (1-9), TNFRSF14 (1-6), TP53 (2-11), U2AF1 (2,6,7), U2AF2 (1-12), WT1 (1-9), XPO1 (15,16,18), ZRSR2 (1-11). |

**Supplemental Table 1: Next generation based sequencing panels used in this study.**

**Supplemental Table 2**

| Number of somatic mutations (number of patients with mutations; mean VAF) | [%] LOY (total number of patients) | | | |
|---|---|---|---|---|
| | <25% (23) | 25-49% (10) | 50-74% (8) | ≥75% (32) |
| *TET2* | 2 (1; 0.33) | 0 | 5 (4; 0.36) | 12 (9; 0.40) |
| *SF3B1* | 1 (1; 0.23) | 0 | 1 (1; 0.41) | 10 (10; 0.36) |
| *U2AF1* | 0 | 0 | 0 | 4 (4; 0.25) |
| *ZRSR2* | 0 | 0 | 0 | 4 (3; 0.48) |
| *ASXL1* | 1 (1; 0.43) | 1 (1; 0.05) | 1 (1; 0.07) | 4 (3; 0.32) |
| *JAK2* | 0 | 1 (1; 0.46) | 0 | 3 (3; 0.37) |
| *SETBP1* | 0 | 0 | 0 | 2 (2; 0.41) |
| *CBL* | 0 | 0 | 1 (1; 0.49) | 2 (2; 0.27) |
| *SH2B3* | 0 | 0 | 0 | 2 (2; 0.27) |

| | | | | |
|---|---|---|---|---|
| *STAG2* | 0 | 0 | 0 | 2 (2; 0.31) |
| *EZH2* | 0 | 0 | 0 | 1 (1; 0.33) |
| *DNMT3A* | 1 (1; 0.10) | 2 (2; 0.21) | 0 | 1 (1; 0.32) |
| *IDH1* | 0 | 0 | 0 | 1 (1; 0.28) |
| *RUNX1* | 0 | 0 | 0 | 1 (1; 0.12) |
| *KRAS* | 0 | 0 | 0 | 1 (1; 0.20) |
| *NF1* | 0 | 0 | 0 | 1 (1; 0.49) |
| *SRSF2* | 1 (1; 0.43) | 0 | 0 | 1 (1; 0.57) |
| *ATRX* | 0 | 0 | 0 | 1 (1; 0.81) |
| *EP300* | 0 | 0 | 0 | 1 (1; 0.47) |
| *PPM1D* | 1 (1; 0.06) | 0 | 0 | 1 (1; 0.38) |
| *GNB1* | 0 | 0 | 0 | 1 (1; 0.46) |
| *TP53* | 1 (1; 0.05) | 0 | 0 | 1 (1; 0.83) |
| *BCOR* | 3 (2; 0.51) | 0 | 0 | 1 (1; 0.94) |
| *KIT* | 0 | 0 | 0 | 1 (1; 0.12) |
| *PHF6* | 0 | 0 | 0 | 1 (1; 0.89) |
| *SETD2* | 0 | 0 | 0 | 1 (1; 0.46) |
| *ATM* | 0 | 0 | 0 | 1 (1; 0.06) |
| *CUX1* | 0 | 0 | 0 | 1 (1; 0.14) |
| *NOTCH1* | 0 | 0 | 0 | 1 (1; 0.52) |
| *NOTCH2* | 0 | 0 | 0 | 1 (1; 0.40) |
| *PRPF40b* | 0 | 1 (1; 0.53) | 0 | 1 (1; 0.48) |
| *KMT2D* | 0 | 0 | 1 (1; 0.53) | 0 |

| | | | | |
|---|---|---|---|---|
| *KMT2C* | 0 | 1 (1; 0.19) | 0 | 0 |
| *TNFAIP3* | 0 | 1 (1; 0.18) | 0 | 0 |
| *CREBBP* | 1 (1; 0.37) | 0 | 0 | 0 |
| *GATA2* | 0 | 0 | 0 | 0 |
| *KDM5A* | 1 (1; 0.05) | 0 | 0 | 0 |
| Total number of somatic mutations (number of patients with mutations) | 13 (7) | 7 (5) | 9 (5) | 65 (25) |
| Number of patients with mutations in spliceosome components, *JAK2* and *RUNX1* (%) | 2 (8.7) | 1 (10) | 1 (12.5) | 16 (50) |
| Number of patients with ≥2 mutations (%) | 2 (8.7) | 1 (10) | 3 (37.5) | 16 (50) |
| Number of patients with combination with mutations in *TET2*, *DNMT3A* and/or *ASXL1* (%) | 1 (4.4) | 1 (10) | 2 (25) | 9 (28.1) |

**Supplemental Table 2: LOY is associated with somatic mutations associated with myeloid neoplasia**. Abbreviations: percentage of metaphases with loss of Y chromosome ([%] LOY), and variant allele fraction (VAF).

**Supplemental Table 3**

| |
|---|
| **ASXL1 NM_015338 c.1934dupG p.G646Wfs*12** |
| ASXL1 NM_015338 c.1471C>T p.Q491* |
| **ASXL1 NM_015338 c.1926_1927insG p.G642fs*** |
| **ASXL1 NM_015338 c.1636C>T p.Q546Ter** |
| **ASXL1 NM_015338 c.3514delG p.A1172LfsTer2** |

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|---|
| **ASXL1 NM_015338 c.1900_1922del p.E635RfsTer15** |
| **ATM NM_000051 c.5092A>T p.K1698*** |
| **ATRX NM_000489 c.1549A>C p.I517L** |
| BCOR NM_001123385 c.2064_2068delCCCAA p.N689fs* |
| **BCOR NM_001123385 c.2129G>A p.R710H** |
| BCOR NM_001123385 c.3810G>A p.W1270* |
| BCOR NM 017745 c.2032C>T p.P678S |
| **CBL NM_005188 c.1178T>A p.I393N** |
| **CBL NM_005188 c.1259G>T p.R420L** |
| **CBL NM_005188 c.643A>G p.I215V** |
| CREBBP NM_004380 c.4560+7G>C splice region variant |
| **CUX1 NM_181552 c.2475G>A p.W825Ter** |
| **DNMT3A NM_022552 c.2225G>A p.R742Q** |
| **DNMT3A NM_022552 c.2339T>C p.I780T** |
| DNMT3A NM_175629 c.1767_1767insA p.K590fs* |
| **DNMT3A NM_022552 c.1937-1G>A splice acceptor variant** |
| **EP300 NM_001429 c.2513G>C p.R838P** |
| **EZH2 NM_004456 c.1831_1840delGAACTGCAGT p.S610fs*** |
| **EZH2 NM_004456 c.2069G>A p.R690H** |
| **EZH2 NM_004456 c.1212_1216delGAAGA p.K405RfsTer2** |
| GATA2 NM_ 001145662 c.1054G>A p.G352R |
| **GNB1 NM_002074 c.169A>G p.K57E** |
| **JAK2 NM_004972 c.1849G>T p.V617F** |
| **JAK2 NM_004972 c.1849G>T p.V617F** |
| **JAK2 NM_004972 c.1849G>T p.V617F** |
| **JAK2 NM_004972 c.1711G>A p.G571S** |
| KDM5A NM_001042603 c.1468T>G p.Y490D |
| **KIT NM_000222 c.2447A>T p.D816V** |
| KMT2C NM_170606 :c.2532+6G>A splice region variant |
| KMT2D NM_003482 c.7018C>T p.P2340S |
| **KRAS NM_004985 c.351A>C p.K117N** |
| KRAS NM_004985 c.38G>A p.G13D |

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| **NF1 NM_001042492 c.7322-2A>C splice acceptor variant** |
| **NOTCH1 NM_017617 c.4985G>T p.R1662L** |
| **NOTCH2 NM_024408 c.6979A>G p.T2327A** |
| PHF6 NM_032458 c.522delG p.L175CfsTer43 |
| PPM1D NM_003620 c.1535dupA p.N512KfsTer16 |
| **PPM1D NM_003620 c.1709C>G p.S570Ter** |
| **PRPF40B NM_001031698 c.1390G>A p.D442N** |
| **SETBP1 NM_015559 c.2602G>A p.D868N** |
| **SETBP1 NM_015559 c.2608G>A p.G870S** |
| **SETD2 NM_014159 c.1903A>G p.I635V** |
| **SF3B1 NM_012433 p.R625L (ENST00000335508.6:c.1874G>T)** |
| **SF3B1 NM_012433 c.1998G>T p.K666N** |
| **SF3B1 NM_012433 c.1998G>T p.K666N** |
| **SF3B1 NM_012433 c.2098A>G p.K700E** |
| SF3B1 NM_012433 c.2098A>G p.K700E |
| **SF3B1 NM_012433 c.2422A>G p.T808A** |
| SF3B1 NM_012433 c.1774G>A p.E592K |
| **SF3B1 NM_012433 c.1998G>T p.K666N** |
| **SF3B1 NM_012433 c.2098A>G p.K700E** |
| **SH2B3 NM_005475 c.173_177delTGCGC p.L58RfsTer63** |
| **SH2B3 NM_005475 c.1605delG p.L535fs*** |
| **SRSF2** NM_003016 **c.284C>A p.P95H** |
| SRSF2 NM_003016 c.284C>T p.P95L |
| **STAG2 NM_001282418 c.1840C>T p.R614Ter** |
| **STAG2 NM_001282418 c.1254T>A p.Y418Ter** |
| **TET2 NM_001127208 c.3782G>A p.R1261H** |
| TET2 **NM_001127208** c.2859delG p.R953SfsTer54 |
| **TET2 NM_001127208 c.3578G>A p.C1193Y** |
| TET2 **NM_001127208** c.4804_4808delTCTCA p.Q1603CfsTer9 |
| **TET2 NM_001127208 c.2872_2875delCAGA p.E958SfsTer48** |
| **TET2 NM_001127208 c.5629A>T p.K1877Ter** |
| TET2 **NM_001127208** c.685dupA p.T229NfsTer25 |

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|---|
| **TET2 NM_001127208 c.1664dupC p.T556NfsTer11** |
| TET2 **NM_001127208** c.3308_3309insT p.N1103fs* |
| **TET2 NM_001127208 c.1581delC p.D527fs*** |
| TET2 NM_001127208 c.2050C>T p.Q684* |
| **TET2 NM_001127208 c.3348delA p.I1116fs*** |
| **TET2 NM_001127208 c.4075C>A p.R1359S** |
| **TET2 NM_001127208 c.4546C>T p.R1516*** |
| **TET2 NM_001127208 c.5353delA p.N1784fs*** |
| TET2 NM_001127208 c.5542delT p.W1847fs* |
| **TET2 NM_001127208 c.5618T>C p.I1873T** |
| **TET2 NM_001127208 c.949C>T p.Q317*** |
| TET2 **NM_001127208 c.2065T>G** p.S689A |
| **TP53** NM_000546 **c.783-1G>T splice acceptor variant** |
| **U2AF1 NM_006758 c.101C>T p.S34F** |
| **U2AF1 NM_006758 c.470A>C p.Q157P** |
| **U2AF1 NM_006758 c.470A>C p.Q157P** |
| **U2AF2 NM_001012478 c.38_49+20dupAGAATAAACAAGGTGAGGGCACCGGGGTCGCG** |
| ZRSR2 **NM_005089** c.676G>T p.E226Ter |
| **ZRSR2 NM_005089 c.802G>C p.G268R** |
| **ZRSR2 NM_005089 c.595A>C p.T199P** |
| **ZRSR2 NM_005089 c.112A>T p.R38*** |
| ZRSR2 NM_005089 c.367G>T p.E123* |

**Supplemental Table 3: LOY is associated with somatic mutations associated with myeloid neoplasia**. Pathogenic/likely pathogenic mutations identified in the study cohort. Genomic alterations in patients with ≥75% LOY in bold face.

**Supplemental Table 4**

| | Age | % LOY | Total number of mutations | Number of genes mutated |
|---|---|---|---|---|
| Age | 1.00 (<0.0001) | -0.05 (0.78) | -0.07 (0.66) | -0.09 (0.59) |
| % LOY | -0.05 (0.78) | 1.00 (<0.0001) | 0.49 (0.002) | 0.50 (0.001) |
| Total number of mutations | -0.07 (0.66) | 0.49 (0.002) | 1.00 (<0.0001) | 0.97 (<0.0001) |
| Number of genes mutated | -0.09 (0.59) | 0.50 (0.001) | 0.97 (<0.0001) | 1.00 (<0.0001) |

**Supplemental Table 4: % LOY demonstrates positive correlation with mutations in myeloid neoplasia related genes.** Correlation matrix (row-wise method) demonstrating correlation coefficients (correlation probability) total number of mutations as well as number of mutated genes and % LOY for diagnosis of myeloid neoplasia. Abbreviations: percentage of metaphases with loss of Y chromosome (% LOY).

**Supplemental Table 5:**

| Variable | by Variable | Correlation | Lower 95% | Upper 95% | Signif Prob |
|---|---|---|---|---|---|
| % LOY | Age | -0.05 | -0.36 | 0.27 | 0.78 |
| Total number of mutations | Age | -0.07 | -0.38 | 0.25 | 0.66 |
| Total number of mutations | % LOY | 0.49 | 0.21 | 0.70 | 0.002 |
| Number of genes mutated | Age | -0.09 | -0.39 | 0.23 | 0.59 |
| Number of genes mutated | % LOY | 0.50 | 0.22 | 0.71 | 0.001 |
| Number of genes mutated | Total number of mutations | 0.97 | 0.95 | 0.99 | <0.0001 |

**Supplemental Table 5: % LOY shows significant positive correlation with number of myeloid neoplasia related genes mutated as well as total number of mutations.** Pairwise correlation with correlation coefficients, 95% confidence intervals and *p*-value for important variables. Abbreviations: percentage of metaphases with loss of Y chromosome (% LOY).