# Somatic mutations of cell-free circulating DNA detected by Next Generation Sequencing reflect the genetic changes in both Germinal Center B-Cell like and Activated B-Cell like Diffuse Large B-Cell Lymphoma tumors at the time of diagnosis

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**Letter to the Editor,**

**Somatic mutations of cell-free circulating DNA detected by Next Generation Sequencing reflect the genetic changes in both Germinal Center B-Cell like and Activated B-Cell like Diffuse Large B-Cell Lymphoma tumors at the time of diagnosis.**

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Diffuse Large B-cell Lymphoma (DLBCL) is the most common form of lymphoma, accounting for 30-40% of newly diagnosed Non-Hodgkin Lymphomas (NHL). The molecular heterogeneity of DLBCLs has been deciphered by gene expression profiling, and DLBCLs have been divided into three main molecular subtypes: the Germinal Center B-cell like (GCB) subtype, the Activated B-Cell like (ABC) subtype, and the Primary Mediastinal B-cell Lymphoma (PMBL) subtype with distinct clinical outcomes and responses to immunochemotherapy. Next Generation Sequencing (NGS) technologies, which allow for massively parallel, high-throughput DNA sequencing, have emerged over the past decade and have provided new insights into the genomic characterization of DLBCL. Recurrent Single Nucleotide Variants (SNVs) are now well defined and provide new therapeutic opportunities for the three molecular subtypes. These SNVs target genes that play a crucial role in several pathways including BCR signaling (*CD79A/CD79B*), NFκ-B (*CARD11*), Toll-like receptor signaling (*MYD88*), immunity (*CD58, TNFSRF14, B2M*), cell cycle/apoptosis (*TP53, BCL2*) or epigenetic regulation (*EZH2,CREBBP, MLL2*)(1, 2) .

Recently, whole exome sequencing in breast cancer has shown that mutations observed in the tumor could also be detected in circulating cell-free DNA (cfDNA) and could be used to detect genetic changes during treatments and relapse, defining the concept of "liquid biopsy"(3). In DLBCL, whereas tumoral circulating cells or leukemic phase are not usually detectable, clonotypic sequences have been constantly detected in cfDNA extracted from serum/plasma or PBMC(4-8).

In this study we sought to determine by routinely applicable NGS technology whether the pattern of acquired SNVs observed in tumor DNA could also be detected in cfDNA in DLBCL patients at the time of diagnosis. For this purpose, we analyzed twelve DLBCL cases with available matched tumor DNA and plasma collected at the time of diagnosis. Patients harboring typical GCB/ABC-related mutations targeting CD79A*/B, EZH2, CARD11* or *MYD88* genes previously identified by Sanger Method were selected(9). This study was approved by the regional ethical committee (numbered as CPP N°01/006/2014).

The main clinical features of the patients are summarized in **Table 1**. None of the selected cases harbored detectable circulating lymphoma cells by routine blood smear examination. Of note, no peripheral blood cytometry was performed, in accordance with our center's initial staging procedures for DLBCL patients. Tumor DNA was extracted from frozen lymph node samples by standard methods. cfDNA was extracted from archived EDTA-anticoagulated plasma aliquots (1mL) stored at -80°C using the QIAamp® Circulating Nucleic Acid Kit (Qiagen) (with the QIAvac 24 Plus vacuum manifold, following the manufacturer's instructions), and concentrations were measured using Fluorometric assay (Qubit® dsDNA HS Assay Kit, Life Technologies). The mean cfDNA concentration in plasma was 1.65 ng/µl (range 0.46-11.2) (**Table 1**). The Cell of origin (COO) signature was determined by cDNA-mediated annealing, Selection, extension, and Ligation (DASL) technology based on the expression of 19 genes as previously reported(10). Among the 12 cases analyzed, 5 belonged to the ABC subgroup, 6 to the GCB subgroup and one case was unclassified (Table 1).

Sequencing of tumor DNA was performed using Ion Torrent Personal Genome Machine (PGM, Life technologies). Ten ng of genomic DNA were submitted to NGS using a laboratory-developed Lymphopanel set, designed to identify mutations in 34 genes relevant to lymphomagenesis (**Supplementary file 1A**). This design covers 87 703 bases and generates 872 amplicons. Amplified

libraries (Ion AmpliSeq™ Library Kit 2.0) were submitted to emulsion PCR with the Ion OneTouch™ 200 Template Kit (Life Technologies) using the Ion OneTouch™ System (Life Technologies) according to the manufacturer's instructions. The templated Ion Sphere™ Particles (ISPs) were enriched with the Ion OneTouch™ Enrichment System and loaded and sequenced on an Ion 316™ v2 Chip (Life Technologies).

After alignment to a reference genome sequence (hg19) and variant calling procedure, variants were filtered through a dedicated bioinformatic pipeline that eliminated synonymous variants and variants with a Variant Allele Frequency greater than 1% in the 1000 genome database (considered as polymorphisms). Only non-synonymous SNV/In/Del with a quality score > 22 and/or confirmed by Sanger experiment were retained as acquired somatic mutations in lymphoma cases and were used for the subsequent sequencing of the matched cfDNA (for the detailed pipeline see **supplementary file 1B**). Somatic mutations identified in tumor DNA were used to build a hot-spot file enabling higher sensibility tracking of somatic mutations in circulating DNA.

In order to increase variant detection sensitivity and specificity in cfDNA, we exclusively amplified amplicons targeting mutations detected in the corresponding tumor DNA by the complete Lymphopanel by performing a dedicated sequencing procedure with a pool of oligonucleotide primers selected among the 872 pairs provided by Life Technologies. The procedure to make librairies and sequence amplicons was the same as for tumor DNA but used a 314™ v2 Chip. When possible, circulating DNA was extracted and sequenced from 2 different aliquots of plasma.

The SNV/In/Del detected in both tumor and circulating DNA are indicated in **Table 1** with their corresponding variant allele frequency (VAF). As expected, we identified a typical SNV pattern in the five ABC DLBCL cases including mutations targeting *MYD88, CD79A/B, PIM1, PRDM1, CARD11* or *IRF4*, whereas *EZH2, BCL2, GNA13,* or *TNFSRF14* were mutated in the unclassified and GCB DLBCL cases. *MLL2 (KMT2D), CREBBP* or *ITPKB* were targeted by somatic mutations shared in the two COO subtypes. The sequencing depths obtained for each sample and targets are indicated in Table 1.

The mean number of reads targeting the mutated regions for tumor DNA was 241 (range 23-741) as compared to 5,987 (range 6-22,541) for plasma DNA, indicating a 24-fold mean depth sequencing increase. The mean VAF in the tumor DNA was 35% (range 17-64%) as compared to a mean of 11% for plasma DNA (range 2-89%) (**Table 1**).

In 11/12 DLBCL cases, we observed somatic mutations in cfDNA, similar or partially similar to those observed in the tumor (**Figure 1, supplementary figure 1 and table 1**). We defined the concordance rate as the ratio of the number of mutated cfDNA genes and the number of mutated tumor DNA genes. This rate ranges from 33% to 100% (5 cases) in the 11/12 cases with detected mutated cfDNA. Overall, the median concordance rate between tumor DNA and cfDNA is 85%.

In one case (#1251), SNVs observed in the tumor DNA were not detected in plasma DNA. In an additional case (#1559), SNVs were barely detectable (VAF of 0.5% for two variants). Of note, both cases displayed a limited disease (stage I or II) and normal LDH level, indicating that tumor specific circulating cfDNA amount is at least partially related to tumor burden. Despite a low amount of circulating DNA extracted from plasma for cases #1251 and #1559, we obtained adequate sequencing quality and depth (overall number of reads sequenced with mutated target = 4,685 and

51,195 respectively, Table 1), indicating that in some rare cases, the tumor specific cfDNA is absent or beneath the level of sensitivity of the NGS sequencing method used. Of note, in case #1631 characterized by a limited stage I disease, SNVs were detected with a mean VAF of 5.2% in plasma DNA, as compared to a mean VAF of 34.6% in the tumor DNA (**Table 1**). In contrast, cases #1639 and #1768 (both with stage IV disease and elevated LDH level) displayed a high proportion of tumor specific circulating DNA as indicated by the high VAF observed (**Table 1**). Interestingly, in these two cases, the sub-clonal distribution of certain mutations, as indicated by the VAF distribution of each individual variant, was also observed in cfDNA, suggesting that sequencing cfDNA can reflect the SNV pattern observed in tumor cells in some instances (**Figure 1** and **supplementary figure 1**). In case # 1524, despite a sufficient number of relevant reads (> 4,000) we failed to detect the B2M SNV present in the lymph node biopsy. This result was confirmed by manual Integrative Genomics Viewer (IGV) checking, suggesting that the B2M SNV is present only in a subclone caught in the biopsy sample but not highlighted by the cfDNA that reflects the entire tumor burden.

By contrast, in some cases, the number of target reads is clearly insufficient for adequate SNV detection (# case 964, CARD11; case 1003, MYD88 see table 1), most likely reflecting the low amount of cfDNA available rather than a true clonal divergence between tumor DNA and cfDNA. This was not observed in cases with higher cfDNA amount.

Failure to detect SNVs in cfDNA appears related more to the proportion of tumor specific DNA quantity than to the total amount or quality of total cfDNA. Of note, we failed to find any tumor-related SNV in DNA extracted from 11/12 PBMC using this approach (data not shown), indicating that serum or plasma is preferable to detect mutated circulating DNA. In a large cohort of Hodgkin lymphoma, mantle cell lymphoma and DLBCL, increased levels of plasma DNA (determined using quantitative PCR for the β-globin gene) were associated with advanced stage disease, presence of B-symptoms, elevated lactate dehydrogenase levels, and age > 60 years also indicating that the amount of circulating DNA is partially related to tumor burden(8). Furthermore, it has been shown in a cohort of EBV-positive lymphoma that serum and plasma were equivalent to detect Lymphoma-specific DNA but that only the lymphoma specific DNA could be used to monitor disease response in lymphoma(7).

To our knowledge this is the first report of the detection of non-immunoglobulin somatic mutations in DLBCL from circulating DNA by routine NGS sequencing, enabling the identification of lymphoma-specific cfDNA. Other quantitative approaches, including digital PCR, are also suitable and could be used in this setting for detecting recurrent translocations or mutations(11). More recently the LymphoSIGHT®, a high-throughput DNA sequencing method, was developed to detect and quantify circulating tumor DNA as minimal residual disease (MRD) and was able to predict both early treatment failure and relapse in newly diagnosed DLBCL patients, chronic lymphocytic leukemia or acute lymphoblastic leukemia(12-15). This approach is based on tumor DNA amplification using locus-specific primer sets for the immunoglobulin heavy/light-chain which failed in a substantial number of DLBCL cases. Importantly the Lymphopanel used in this study is able to detect at least one acquired SNV in 95% of DLBCL cases at initial diagnosis (manuscript in preparation) and may therefore constitute a simple routinely applicable test to provide the COO subtype or to detect targetable mutations at the time of diagnosis or relapse. However, its capacity to detect MRD with a high level of sensitivity remains to be determined and we can hypothesize that at least 5 to 10% of

DLBCL cases will not display any SNVs detectable by our Lymphopanel. Furthermore, cfDNA sequencing was successfully performed using the entire Lymphopanel (including the 34 targeted genes) in one case (data not shown), indicating that this approach is feasible without the knowledge of the tumor variant calling. However this requires an increase in the sequencing depth capacity and entails a substantial cost increase.

To conclude, our results indicate that cfDNA can also be used in DLBCL to detect somatic variants, validating the concept of "liquid biopsy" in this type of tumor(3). These preliminary results have prompted us to start a prospective study in the aim of serial sequencing cfDNA during DLBCL treatment and follow-up (registered on clinicaltrials.gov as NCT02339805). If these preliminary results are confirmed by a prospective study, new strategies should be proposed for both diagnosis and treatment tailoring based on the simple detection and quantification of SNV in plasma.

**Conflicts of interest**: The authors have no conflicts of interest to declare

**Contributions**: EB and CM performed PGM and Sanger sequencing experiments; FJ and EB designed the study; FJ, HT and CB supervised the experiments and provided clinical data; PJV, SM and PB performed bioinformatics and designed pipeline analysis; JMP performed histopathological review; PR performed Sanger analysis; FJ, KL, TF, EB, PB and PR designed the lymphopanel. All authors were involved in the manuscript writing and approved the final manuscript.

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| | | | | | | | | | Tumor DNA | | | Circulating DNA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UPN | Sex | Age | Stage | IPI | LDH (xUNL) | Bone marrow involvement | Phenotype | Gene | VAF (%) | read number | mean VAF (%) | VAF (%) | read number | mean VAF (%) | Allele Call | concentration (ng/µl) |
| | | | | | | | | *ITPKB (1)* | 40.9 | 79/193 | | 2.5 | 158/6313 | | Heterozygous | |
| | | | | | | | | *ITPKB (2)* | 40.6 | 78/192 | | 2.4 | 152/6260 | | Heterozygous | |
| 964 | F | 17 | IV | 3 | 5 | 0 | GCB | *CARD11* | 17.1 | 13/76 | 40 | 0.0 | 0/54 | 2 | Absent | 2.02 |
| | | | | | | | | *EZH2* | 26.3 | 76/289 | | 2.8 | 126/4435 | | Heterozygous | |
| | | | | | | | | *B2M* | 75.6 | 102/135 | | 4.0 | 130/4016 | | Heterozygous | |
| | | | | | | | | *ITPKB* | 35.3 | 36/102 | | 1.4 | 22/1540 | | Heterozygous | |
| 1003 | F | 75 | III | 3 | 2.5 | 0 | ABC | *MYD88* | 26.5 | 67/253 | 40 | 0.0 | 0/21 | 5 | Absent | 1.94 |
| | | | | | | | | *CIITA* | 51.9 | 14/27 | | 16.7 | 1/6 | | Heterozygous | |
| | | | | | | | | *CD79B* | 46.3 | 93/201 | | 2.1 | 369/17260 | | Heterozygous | |
| | | | | | | | | *TNFRSF14* | 52.5 | 74/141 | | 0.0 | 0/1113 | | Absent | |
| 1251 | M | # | IE | 0 | 0.8 | 0 | GCB | *EZH2* | 9.8 | 38/389 | 31 | 0.0 | 0/2519 | 0 | Absent | 0.46 |
| | | | | | | | | *SOCS1* | 30.4 | 7/23 | | 0.0 | 0/1053 | | Absent | |
| 1437 | M | 76 | III | 4 | 5.85 | 0 | ABC | *CD79A (1)* | 17.0 | 53/318 | 17 | 89.1 | 344/386 | 89 | Heterozygous | 11.2 |
| | | | | | | | | *CD79A (2)* | 17.0 | 53/318 | | 89.1 | 344/386 | | Heterozygous | |
| | | | | | | | | *TNFRSF14* | 45.3 | 77/170 | | 17.6 | 539/3064 | | Heterozygous | |
| | | | | | | | | *EZH2* | 22.3 | 106/475 | | 7.0 | 818/11751 | | Heterozygous | |
| | | | | | | | | *KMT2D (1)* | 24.1 | 64/266 | | 12.1 | 1269/10509 | | Heterozygous | |
| | | | | | | | | *KMT2D (2)* | 30.6 | 33/108 | | 14.9 | 1015/6818 | | Heterozygous | |
| 1524 | F | 45 | III | 2 | 1.52 | 0 | GCB | *B2M* | 33.3 | 44/132 | 34 | 0.0 | 0/4555 | 14 | Absent | 1.48 |
| | | | | | | | | *CREBBP* | 34.4 | 45/131 | | 14.2 | 47/331 | | Heterozygous | |
| | | | | | | | | *GNA13 (1)* | 43.7 | 52/119 | | 23.1 | 1909/8254 | | Heterozygous | |
| | | | | | | | | *GNA13 (2)* | 43.7 | 52/120 | | 23.1 | 1920/8314 | | Heterozygous | |
| | | | | | | | | *GNA13 (3)* | 44.2 | 53/120 | | 23.4 | 1946/8312 | | Heterozygous | |
| | | | | | | | | *BCL2* | 17.1 | 42/245 | | 4.8 | 409/8558 | | Heterozygous | |
| | | | | | | | | *ITPKB (1)* | 9.3 | 14/151 | | 0.0 | 0/1713 | | Absent | |
| | | | | | | | | *ITPKB (2)* | 15.2 | 19/125 | | 0.0 | 0/3582 | | Absent | |
| | | | | | | | | *ITPKB (3)* | 15.2 | 19/125 | | 0.0 | 0/3577 | | Absent | |
| | | | | | | | | *ITPKB (4)* | 15.5 | 19/123 | | 0.0 | 0/3570 | | Absent | |
| | | | | | | | | *MYD88* | 37.8 | 74/196 | | 8.5 | 161/1888 | | Heterozygous | |
| | | | | | | | | *TNFAIP3* | 45.7 | 32/70 | | 5.6 | 177/3152 | | Heterozygous | |
| | | | | | | | | *EZH2* | 5.9 | 44/741 | | 2.1 | 152/7401 | | Heterozygous | |
| | | | | | | | | *KMT2D* | 36.1 | 122/338 | | 5.7 | 207/3620 | | Heterozygous | |
| 1528 | F | # | III | 4 | 1.39 | 0 | GCB | *B2M* | 50.0 | 63/126 | 31 | 3.2 | 78/2465 | 3 | Heterozygous | 1.94 |
| | | | | | | | | *CIITA (1)* | 39.0 | 30/77 | | 0.0 | 0/2130 | | Absent | |
| | | | | | | | | *CIITA (2)* | 7.7 | 14/183 | | 0.0 | 0/9150 | | Absent | |
| | | | | | | | | *CIITA (3)* | 7.6 | 14/184 | | 0.0 | 0/9162 | | Absent | |
| | | | | | | | | *GNA13 (1)* | 71.0 | 105/148 | | 0.0 | 0/2695 | | Absent | |
| | | | | | | | | *GNA13 (2)* | 71.0 | 105/148 | | 3.1 | 82/2672 | | Heterozygous | |
| | | | | | | | | *BCL2(1)* | 34.1 | 47/138 | | 4.3 | 214/4929 | | Heterozygous | |
| | | | | | | | | *BCL2 (2)* | 32.9 | 45/137 | | 4.2 | 209/5013 | | Heterozygous | |
| | | | | | | | | *MEF2B* | 33.3 | 137/411 | | 5.9 | 654/11153 | | Heterozygous | |
| | | | | | | | | *CD58* | 92.9 | 92/99 | | 0.0 | 0/13231 | | Absent | |
| | | | | | | | | *MYD88* | 51.8 | 187/361 | | 0.0 | 0/11427 | | Absent | |
| 1559 | F | # | IE | 1 | 0.8 | 0 | ABC | *CREBBP* | 46.3 | 154/333 | 64 | 0.0 | 0/5663 | <1 | Absent | 0.618 |
| | | | | | | | | *TP53* | 89.0 | 105/118 | | 0.0 | 0/13012 | | Absent | |
| | | | | | | | | *CD79A (1)* | 52.0 | 122/235 | | 0.5 | 20/3930 | | Absent | |
| | | | | | | | | *CD79A (2)* | 52.0 | 122/235 | | 0.5 | 20/3932 | | Absent | |
| | | | | | | | | *TNFRSF14* | 13.8 | 44/318 | | 2.1 | 6/284 | | Heterozygous | |
| | | | | | | | | *EZH2* | 13.8 | 41/298 | | 0.0 | 0/6575 | | Absent | |
| 1586 | F | # | III | 2 | 0.85 | 0 | NA | *STAT6* | 18.9 | 83/439 | 20 | 0.0 | 2/5517 | <1 | Absent | 1.31 |
| | | | | | | | | *CREBBP* | 33.6 | 49/146 | | 1.3 | 65/4894 | | Absent | |
| | | | | | | | | *GNA13* | 17.7 | 11/62 | | 0.0 | 0/4391 | | Absent | |
| | | | | | | | | *TNFRSF14* | 34.8 | 146/419 | | 19.3 | 694/3587 | | Heterozygous | |
| | | | | | | | | *EZH2* | 9.9 | 57/575 | | 1.8 | 62/3480 | | Heterozygous | |
| | | | | | | | | *STAT6* | 15.4 | 79/514 | | 5.1 | 200/3889 | | Heterozygous | |
| 1623 | M | 53 | IV | 2 | 1.03 | 0 | GCB | *CREBBP (1)* | 23.2 | 166/717 | 18 | 11.6 | 489/4222 | 11 | Heterozygous | 0.95 |
| | | | | | | | | *CREBBP (2)* | 14.0 | 54/387 | | 10.1 | 328/3239 | | Heterozygous | |
| | | | | | | | | *CD79B* | 17.1 | 83/486 | | 18.0 | 839/4651 | | Heterozygous | |
| | | | | | | | | *MEF2B* | 11.8 | 74/626 | | 9.9 | 1283/12969 | | Heterozygous | |

**Table 1** (to be continued)

| | | | | | | | | | Tumor DNA | | | Circulating DNA | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UPN | Sex | Age | Stage | IPI | LDH (xULN) | Bone marrow involvement | Phenotype | Gene | VAF (%) | read number | mean VAF (%) | VAF (%) | read number | mean VAF (%) | Allele Call | concentration (ng/µl) |
| | | | | | | | | *MYD88* | 49.8 | 220/442 | | 11.7 | 655/5586 | | Heterozygous | |
| | | | | | | | | *PIM1* | 20.6 | 14/68 | | 2.7 | 130/4797 | | Heterozygous | |
| | | | | | | | | *CARD11 (1)* | 47.1 | 99/210 | | 5.1 | 154/3028 | | Heterozygous | |
| 1631 | M | # | I | 2 | 1.54 | 0 | ABC | *CARD11 (2)* | 47.9 | 68/142 | 35 | 5.5 | 344/6300 | 5 | Heterozygous | 1.1 |
| | | | | | | | | *STAT6* | 18.1 | 96/532 | | 4.3 | 570/13250 | | Heterozygous | |
| | | | | | | | | *TP53* | 32.4 | 72/222 | | 3.9 | 522/13317 | | Heterozygous | |
| | | | | | | | | *CD79B* | 26.7 | 52/195 | | 3.3 | 386/11853 | | Heterozygous | |
| | | | | | | | | *TNFRSF14* | 75.8 | 47/62 | | 82.3 | 2400/2915 | | Heterozygous | |
| | | | | | | | | *MYD88* | 27.5 | 133/483 | | 7.1 | 1616/22541 | | Heterozygous | |
| | | | | | | | | *EZH2* | 24.0 | 126/526 | | 32.8 | 3259/9939 | | Heterozygous | |
| | | | | | | | | *KMT2D (1)* | 33.8 | 187/554 | | 35.0 | 5878/16784 | | Heterozygous | |
| 1639 | M | # | IV | 2 | 2.4 | 0 | GCB | *KMT2D (2)* | 28.3 | 13/46 | 39 | 22.9 | 671/2929 | 34 | Heterozygous | 10.2 |
| | | | | | | | | *CREBBP* | 37.0 | 17/46 | | 30.6 | 2918/9530 | | Heterozygous | |
| | | | | | | | | *BCL2 (1)* | 45.8 | 11/24 | | 31.5 | 223/709 | | Heterozygous | |
| | | | | | | | | *BCL2 (2)* | 53.0 | 98/185 | | 28.2 | 2267/8032 | | Heterozygous | |
| | | | | | | | | *EP300* | 29.6 | 34/115 | | 38.3 | 4597/11990 | | Heterozygous | |
| | | | | | | | | *MYD88* | 62.1 | 216/348 | | 45.0 | 5936/13194 | | Heterozygous | |
| | | | | | | | | *IRF4* | 28.5 | 75/263 | | 21.2 | 929/4374 | | Heterozygous | |
| | | | | | | | | *PIM1 (1)* | 61.7 | 145/235 | | 38.2 | 1541/4030 | | Heterozygous | |
| 1768 | M | # | IV | 3 | 4.2 | 0 | ABC | *PIM1 (2)* | 29.8 | 34/114 | 52 | 25.9 | 987/3812 | 34 | Heterozygous | 1.82 |
| | | | | | | | | *PRDM1(1)* | 81.9 | 149/182 | | 34.2 | 1273/3724 | | Heterozygous | |
| | | | | | | | | *PRDM1(2)* | 80.7 | 221/274 | | 43.4 | 5076/11689 | | Heterozygous | |
| | | | | | | | | *MYC* | 36.4 | 102/280 | | 26.8 | 262/979 | | Heterozygous | |
| | | | | | | | | *CD79B* | 36.5 | 57/156 | | 33.6 | 3041/9060 | | Heterozygous | |

**Table 1.** Clinical characteristics and list of somatic variants (insertion/deletion/ single nucleotide variant) detected by sequencing in tumor DNA and cell-free plasma circulating DNA. Details of the locations of the mutations are indicated in the **Supplementary table 1**.

ABC: activated B-cell like; GCB: germinal center B-cell like; In/del: insertion/deletion; LDH: Lactate dehydrogenase; IPI: international prognosis index; SNV: single nucleotide variant; UPN: unique personal number; ULN: upper limit value; VAF: variant allele frequency

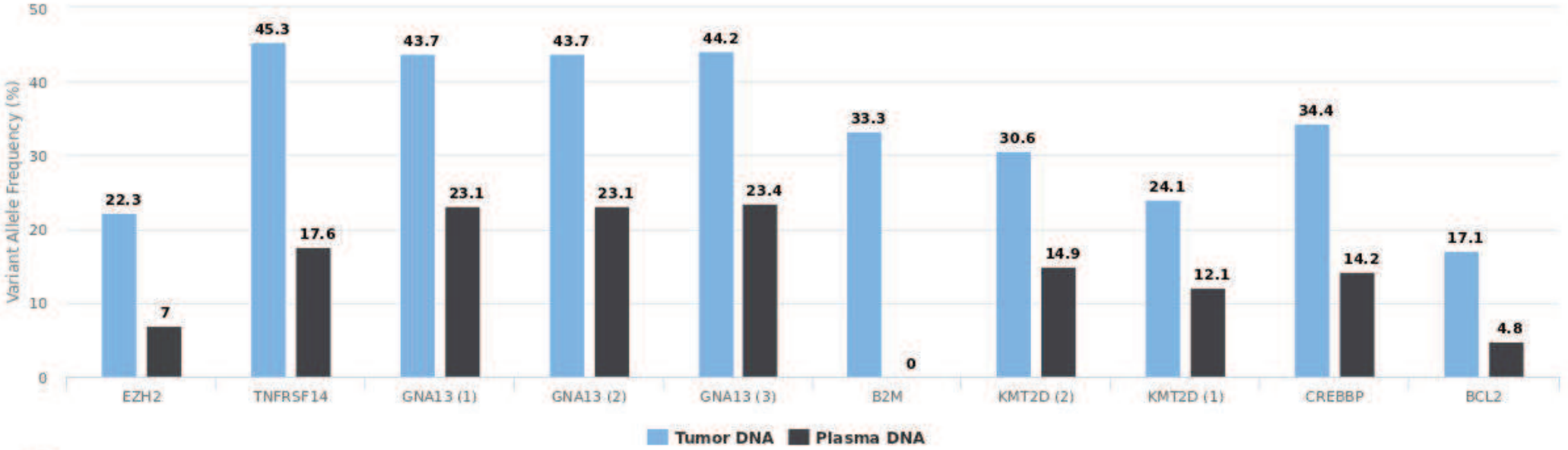
Figure legends

**Figure 1. Representative examples of Variant allele frequency observed in tumor DNA and matched circulating cell-free DNA at the time of diagnosis.**

A

**#UPN1524**

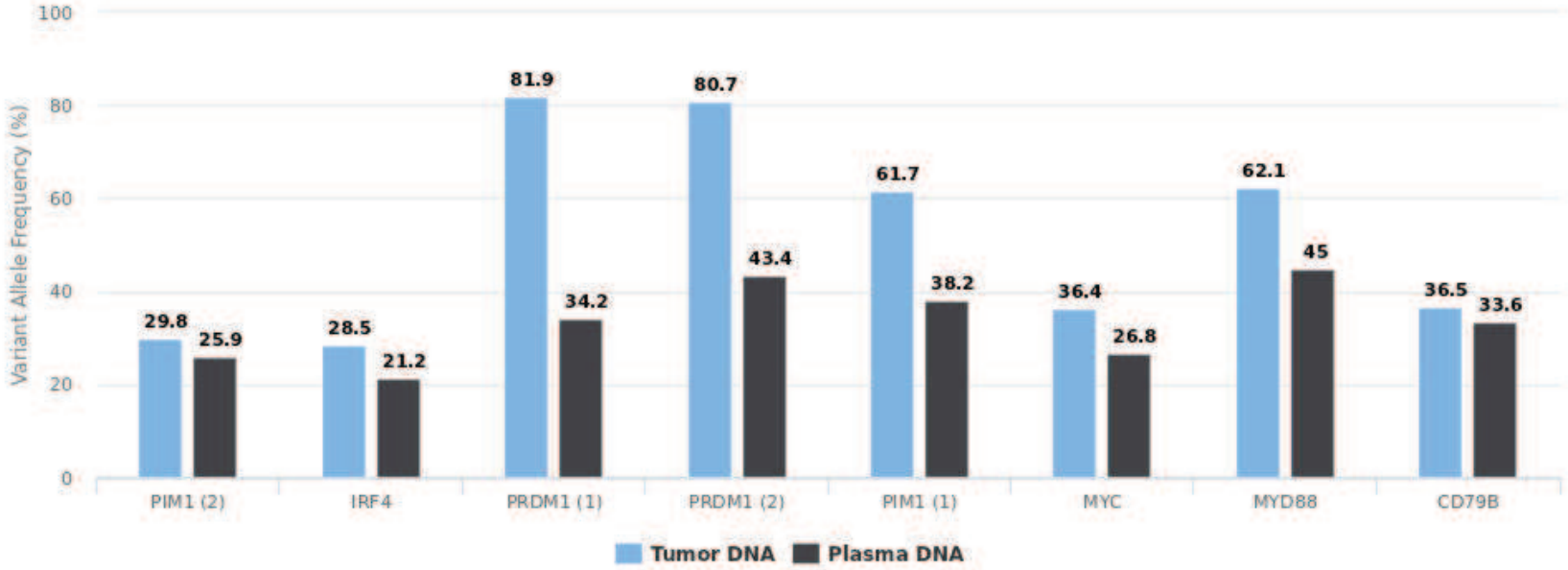
Relevant reads sequenced
Tumor: 1,886
Plasma: 70,466




B

**#UPN1768**

Relevant reads sequenced
Tumor: 1,852
Plasma: 50,862



*Supplementary file 1. Lymphopanel and PGM data analysis*

**A. Lymphopanel set used for NGS experiments**

| Gene | Transcript Reference | Hotspot / Exons partially sequenced | Chromosomal location | Size sequenced (bp) |
|---|---|---|---|---|
| **B2M** | NM_004048 | Hotspots exons 1 & 2, exon 3 | 15q21.1 | 360 |
| ***BCL2*** | NM_000633 | Hotspot exon 2 | 18q21.33 | 585 |
| ***BRAF*** | NM_004333 | Exon 15 | 7q34 | 119 |
| ***CARD11*** | NM_032415 | Coiled-coil domain exons 4-9 | 7p22.2 | 1121 |
| ***CD58*** | NM_001779 | Exons 1-6 | 1p13.1 | 753 |
| ***CD79A*** | NM_001783 | ITAM domain exons 4 & 5 | 19q13.2 | 183 |
| ***CD79B*** | NM_000626 | ITAM domain exons 5 & 6 | 17q23.3 | 141 |
| ***CDKN2A*** | NM_000077+NM_058195+ NM_058197+NM_001195132 | Exons 1, 2A, 2B, 3, 4 & 5 | 9p21.3 | 1,737 |
| ***CDKN2B*** | NM_004936 + NM_0078487 | Exons 1A, 1B & 2 | 9p21.3 | 1289 |
| ***CIITA*** | NM_000246 | Exons 1-19 | 16p13.13 | 3393 |
| ***CREBBP*** | NM_004380 | Exons 1-31 | 16p13.3 | 7323 |
| ***EP300*** | NM_001429 | Exons 1-31 | 22q13.2 | 7245 |
| ***EZH2*** | NM_004456 | SET domain, hotspots exon 16 & 18 | 7q36.1 | 177 |
| ***FOXO1*** | NM_002015 | Hotspots exon 1 & FH domain exon 2 | 13q14.11 | 780 |
| ***GNA13*** | NM_006572 | Exons 1-4 | 17q24.1 | 1134 |
| ***ID3*** | NM_002167 | Exons 1 & 2 | 1p36.12 | 360 |
| ***IRF4/MUM1*** | NM_002460 | Exons 2-9 | 6p25.3 | 1356 |
| ***ITPKB*** | NM_002221 | Exons 2-8 | 1q42.12 | 2830 |
| **KMT2D/MLL2** | NM_003482 | Exons 1-54 | 12q13.12 | 16614 |
| ***MEF2B*** | NM_001145785 | Exons 2-9 | 19p13.11 | 1107 |
| ***MFHAS1*** | NM_004225 | Exons 1-3 | 8p23.1 | 3159 |
| ***MYC*** | NM_002467 | Exons 1-3 | 8q24.21 | 1365 |
| ***MYD88*** | NM_001172567 | Exons 2-5 | 3p22.2 | 587 |
| ***NOTCH1*** | NM_017617 | PEST domain exon 34 | 9q34.3 | 1488 |
| ***NOTCH2*** | NM_024408 | Exons 26-28 & 34 (HD/PEST domains) | 1p12-p11.2 | 2091 |
| ***PIM1*** | NM_002648 | Exons 1-6 | 6p21.2 | 942 |
| ***PRDM1/BLIMP1*** | NM_001198 | Exons 1-7 | 6q21 | 2478 |
| ***SOCS1*** | NM_003745 | Exon 2 | 16p13.13 | 636 |
| ***STAT6*** | NM_001178078 | Exons 9-14 (DNA binding domain hotspot) | 12q13.3 | 795 |
| ***TCF3*** | NM_001136139 | B-HLH domain of E47 isoform exons 17 & 18 | 19p13.3 | 370 |
| ***TNFAIP3*** | NM_006290 | Exons 2-9 | 6q23.3 | 2373 |
| ***TNFRSF14*** | NM_003820 | Exons 1-8 | 1p36.32 | 852 |
| ***TP53*** | NM_000546 | Mutation hotspots exons 4-10 | 17p13.1 | 1004 |
| ***XPO1*** | NM_003400 | Exons 15-18 | 2p15 | 640 |

***B. PGM Data analysis***

Torrent Suite™ version 4.0 (Life Technologies) software was used to perform primary analysis, including signal processing, base calling, sequence alignment to the reference genome (hg19) and generation of Binary Alignment/Map (BAM) files. BAM files were used by Torrent Suite™'s Variant Caller to detect point mutations as well as short insertions and deletions using the "Somatic" and "Low Stringency" default parameters. VCF files generated by Variant Caller were annotated by ANNOVAR(1).

Data generated from tumor DNA samples were considered of sufficient quality when more than 90% of targeted bases were read at least 20 times with sequencing and mapping precisions of at least Q20. Only frameshift deletions and insertions, nonframeshift deletions and substitutions, splicing, nonsynonymous, stopgain or stoploss Single Nucleotide Variations (SNVs) were kept. Variants with a minimal Variant Allele Frequency (MAF) greater than 1% in the 1000 genome database were considered as polymorphisms and were discarded(2). A normal probability plot defined thresholds separating true positives [confirmed by Sanger sequencing, TVC (Torrent variant calling) quality score ≥ 22] from true negatives (discredited by Sanger sequencing, TVC score < 9.5) and highlighted a gray zone (9.5 ≤ TVC score < 22) in which variants must be confirmed by Sanger sequencing or pyrosequencing..

Further verification by Sanger sequencing was performed using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies) and an ABI PRISM 3130 analyzer (Life Technologies). Further verification by pyrosequencing was performed using the PyroMark PCR kit (Qiagen, France) with internal and sequencing primers designed using PyroMark software (Qiagen).

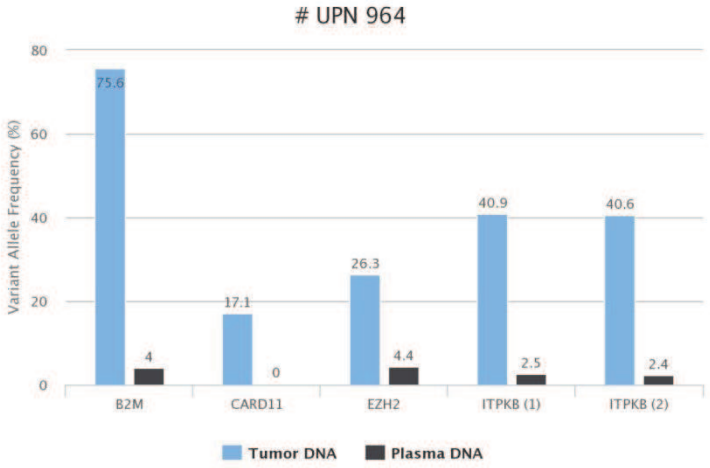
For circulating DNA variant calling, the parameter file was modified as follows: hotspot_min_variant_score: 3; hotspot_strand_bias: 1; downsample_to_coverage: 30 000. In addition, a dedicated hotspot VCF file generated from variants found in tumor DNA was used. This file instructs the Variant Caller to include these positions in its output files, including evidence for a variant and the filtering thresholds that disqualify a variant candidate.

References

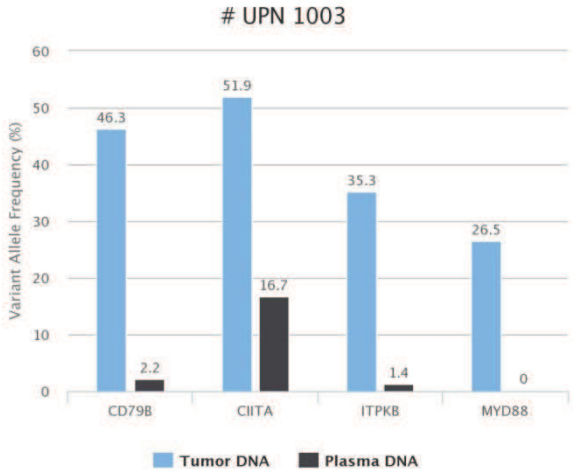
1. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic acids research. 2010 Sep;38(16):e164.
2. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. Nature. 2012 Nov 1;491(7422):56-65.

Figure S1

Relevant reads sequenced
Tumor: 885
Plasma: 21,078




Relevant reads sequenced
Tumor: 582
Plasma: 18,827




Relevant reads sequenced
Tumor: 553
Plasma: 4,685

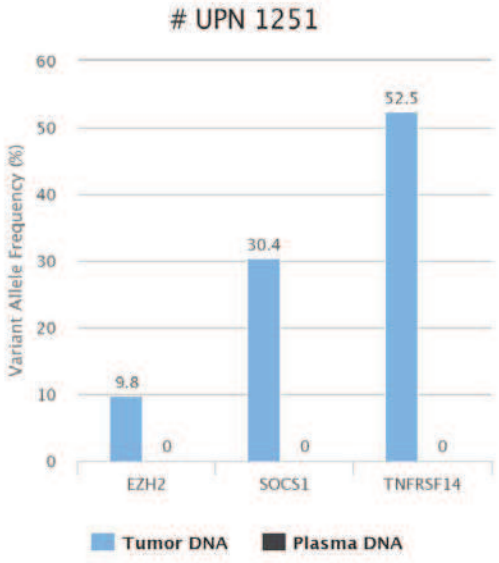
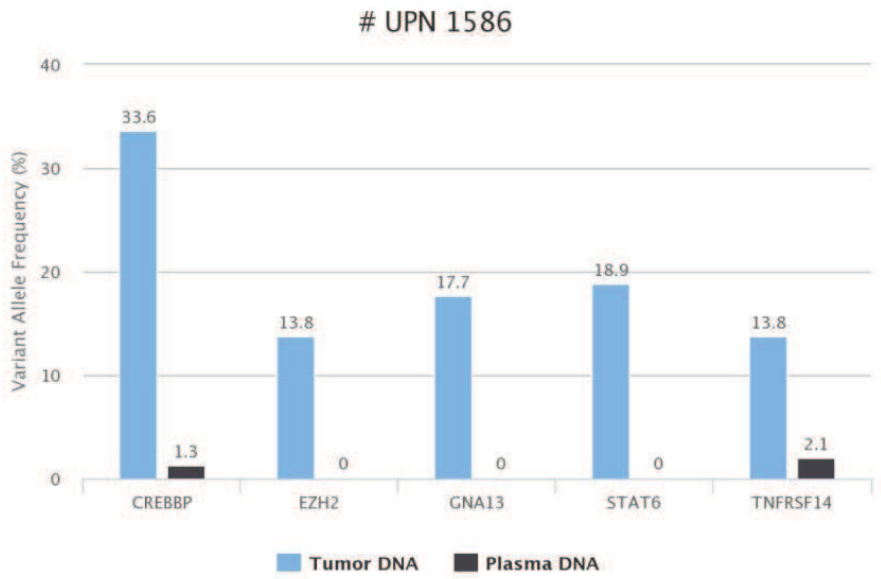


Figure S1

Relevant reads sequenced
Tumor: 1,263
Plasma: 21,661




Relevant reads sequenced
Tumor: 1,381
Plasma: 51,195

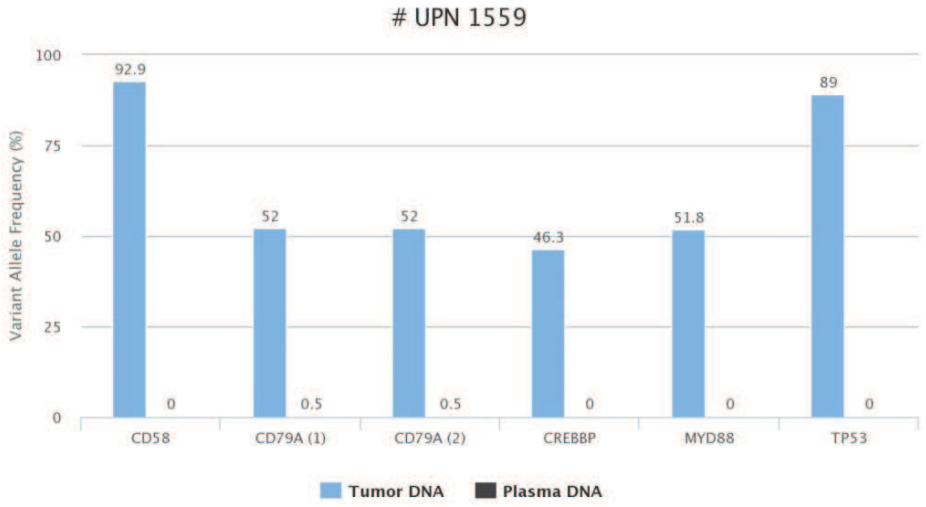


Figure S1

Relevant reads sequence
Tumor: 1,886
Plasma: 70,466

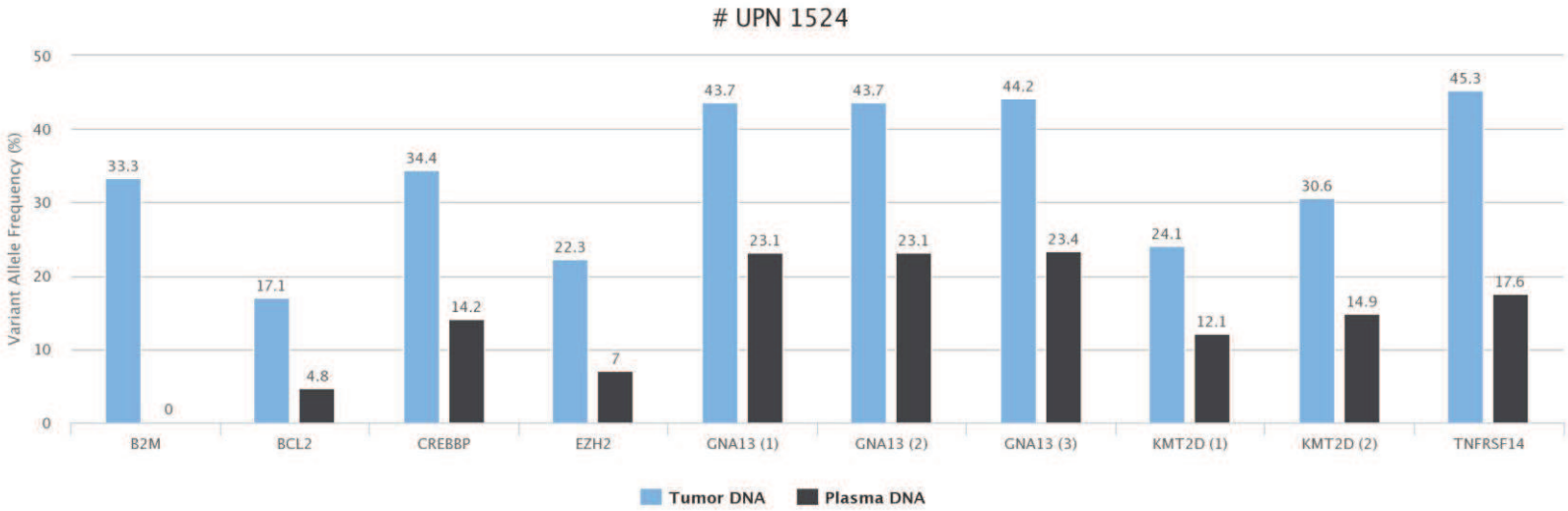
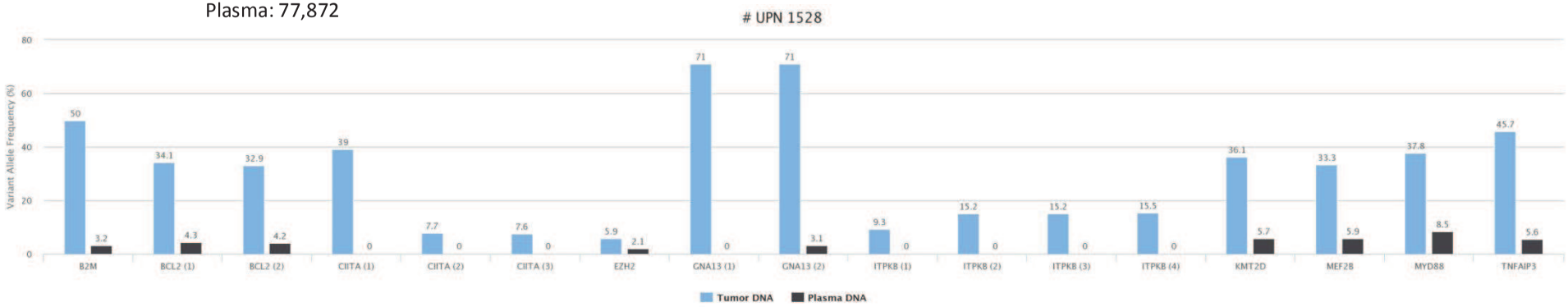


Figure S1

Relevant reads sequenced
Tumor: 3421
Plasma: 77,872




Relevant reads sequenced
Tumor: 636
Plasma: 386

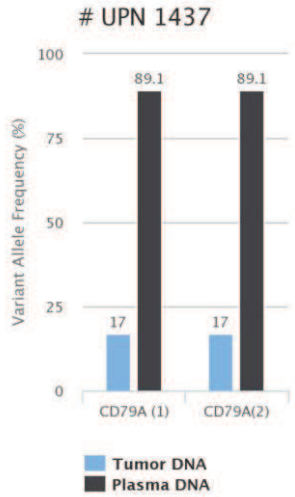
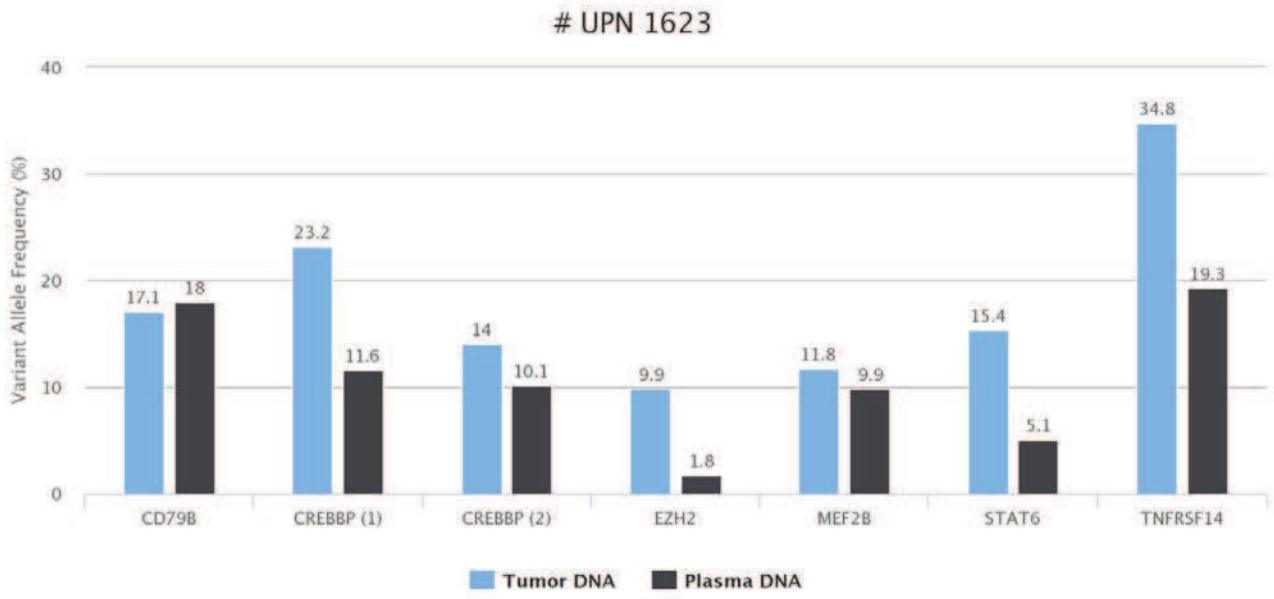


Figure S1

Relevant reads sequenced
Tumor: 3,724
Plasma: 36,037




Relevant reads sequenced
Tumor: 1,811
Plasma: 58,131

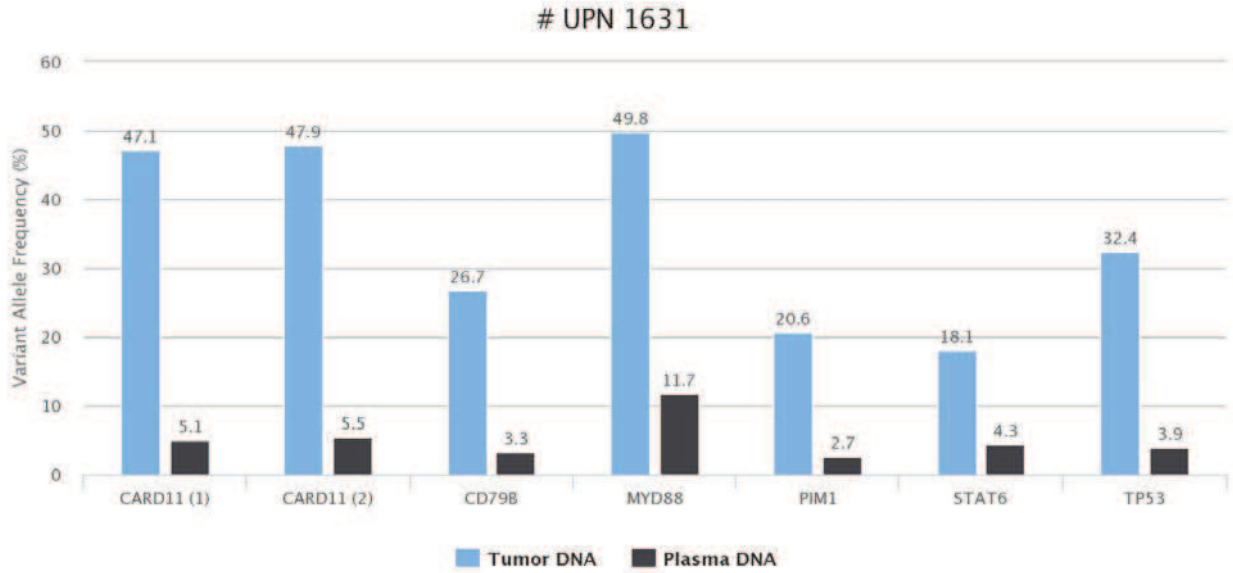
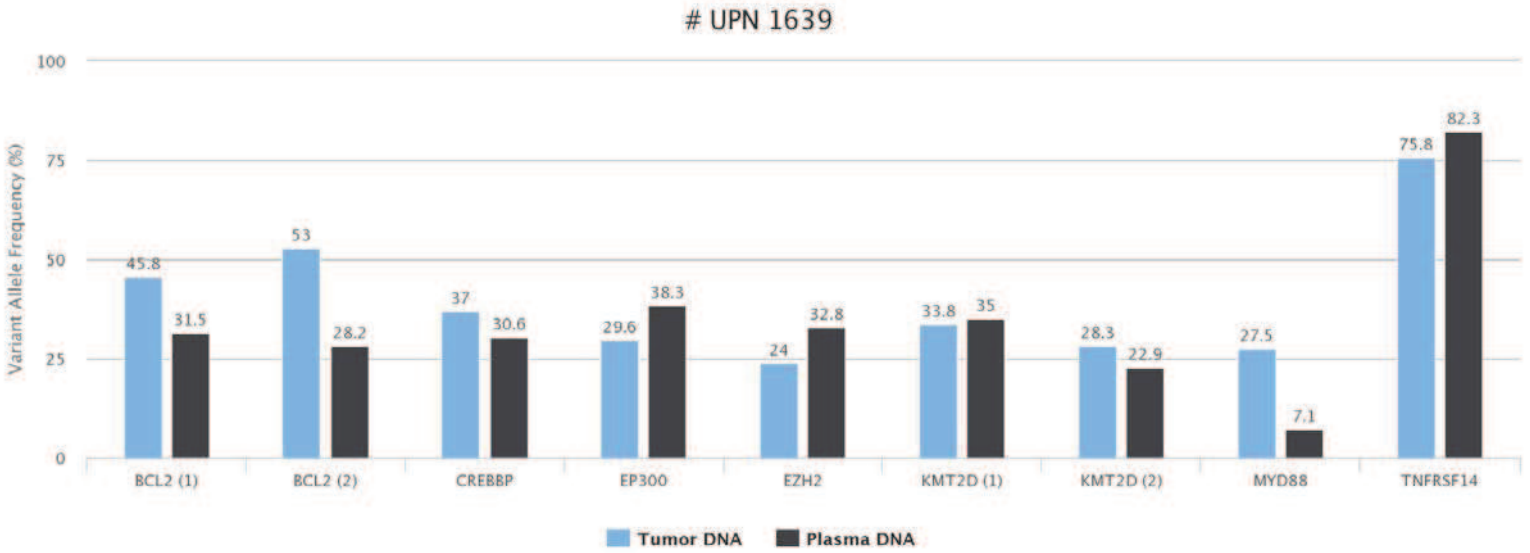
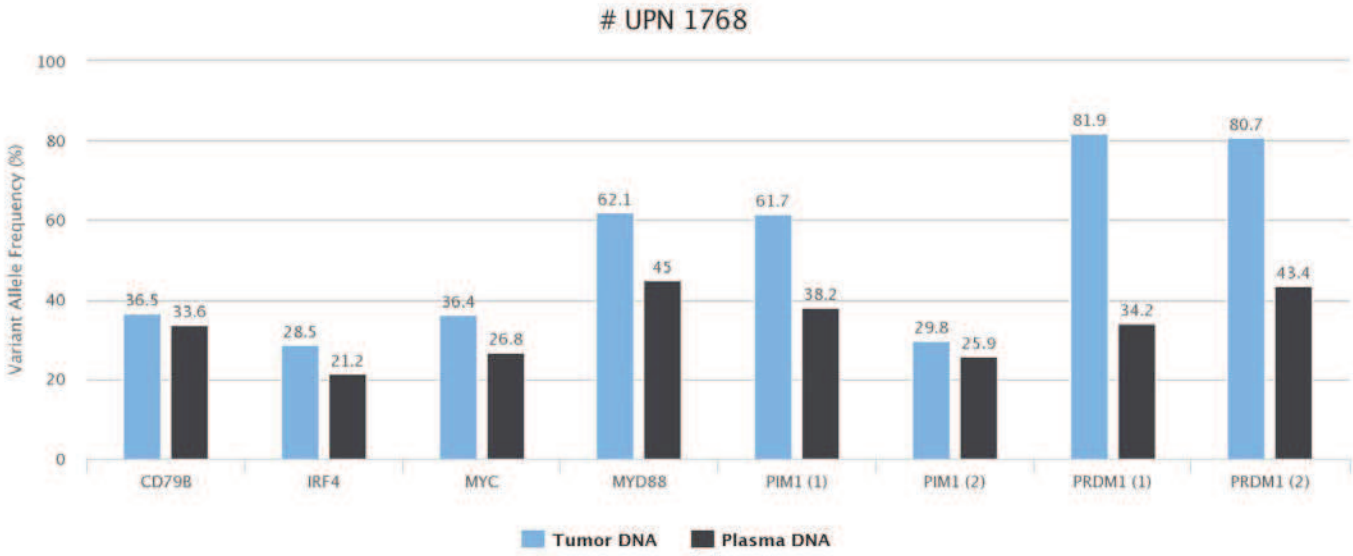


Figure S1

Relevant reads sequenced
Tumor: 2,041
Plasma: 85,369




Relevant reads sequenced
Tumor: 1,852
Plasma: 50,862



# Supplementary table 1

| UPN | Sex | Age | Stage | IPI | LDH (x UNL) | Bone marrow involvement | Phenotype | Gene | SNV | Tumor DNA | | | circulating DNA | | | | | Duration of Tumor/plasma storage (y) before sequencing |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | VAF (%) | read number | mean VAF (%) | VAF (%) | read number | mean VAF (%) | Allele Call | concentration (ng/µl) | |
| 964 | F | 17 | IV | 3 | 5 | 0 | GCB | *ITPKB* | NM_002221:exon2:c.C731T-(p.T244I) | 40.9 | 79/193 | 40.1 | 2.5 | 158/6313 | 2.3 | Heterozygous | 2.02 | 14 |
| | | | | | | | | *ITPKB* | NM_002221:exon2:c.726_728del-(p.242_243del) | 40.6 | 78/192 | | 2.4 | 152/6260 | | Heterozygous | | |
| | | | | | | | | *CARD11* | NM_032415:exon7:c.G1010A-(p.R337Q) | 17.1 | 13/76 | | 0.0 | 0/54 | | Absent | | |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.A1937T-(p.Y646F) | 26.3 | 76/289 | | 2.8 | 126/4435 | | Heterozygous | | |
| | | | | | | | | *B2M* | NM_004048:exon1:c.T2A-(p.M1K) | 75.6 | 102/135 | | 4.0 | 130/4016 | | Heterozygous | | |
| 1003 | F | 75 | III | 3 | 2.5 | 0 | ABC | *ITPKB* | NM_002221:exon2:c.541delC-(p.R181fs) | 35.3 | 36/102 | 40.0 | 1.4 | 22/1540 | 5.1 | Heterozygous | 1.94 | 13 |
| | | | | | | | | *MYD88* | NM_002468:exon4:c.T695C-(p.M232T) | 26.5 | 67/253 | | 0.0 | 0/21 | | Absent | | |
| | | | | | | | | *CIITA* | NM_000246:exon13:c.C2819A-(p.T940K) | 51.9 | 14/27 | | 16.7 | 1/6 | | Heterozygous | | |
| | | | | | | | | *CD79B* | NM_000626:exon6:c.596delT-(p.L199fs) | 46.3 | 93/201 | | 2.1 | 369/17260 | | Heterozygous | | |
| 1251 | M | 42 | IE | 0 | 0.8 | 0 | GCB | *TNFRSF14* | NM_003820:exon1:c.G3A-(p.M1I) | 52.5 | 74/141 | 30.9 | 0.0 | 0/1113 | 0.0 | Absent | 0.46 | 10 |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.A1937T-(p.Y646F) | 9.8 | 38/389 | | 0.0 | 0/2519 | | Absent | | |
| | | | | | | | | *SOCS1* | NM_003745:exon2:c.C174A-(p.F58L) | 30.4 | 7/23 | | 0.0 | 0/1053 | | Absent | | |
| 1437 | M | 76 | III | 4 | 5.85 | 0 | ABC | *CD79A* | NM_001783:c.568-2A>G | 17.0 | 53/318 | 17.0 | 89.1 | 344/386 | 89.1 | Heterozygous | 11.2 | 8 |
| | | | | | | | | *CD79A* | NM_001783:exon5:c573_617del-(p.191_206del) | 17.0 | 53/318 | | 89.1 | 344/386 | | Heterozygous | | |
| 1524 | F | 45 | III | 2 | 1.52 | 0 | GCB | *TNFRSF14* | NM_003820:exon3:c.T269C-(p.L90P) | 45.3 | 77/170 | 33.9 | 17.6 | 539/3064 | 14.0 | Heterozygous | 1.48 | 7 |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.T1936A-(p.Y646N) | 22.3 | 106/475 | | 7.0 | 818/11751 | | Heterozygous | | |
| | | | | | | | | *KMT2D* | NM_003482:exon40:c.G13671C-(p.Q4557H) | 24.1 | 64/266 | | 12.1 | 1269/10509 | | Heterozygous | | |
| | | | | | | | | *KMT2D* | NM_003482:exon34:c.C8401T-(p.R2801X) | 30.6 | 33/108 | | 14.9 | 1015/6818 | | Heterozygous | | |
| | | | | | | | | *B2M* | NM_004048:exon1:c.T35C-(p.L12P) | 33.3 | 44/132 | | 0.0 | 0/4555 | | Absent | | |
| | | | | | | | | *CREBBP* | NM_004380:c.4394+2A>T | 34.4 | 45/131 | | 14.2 | 47/331 | | Heterozygous | | |
| | | | | | | | | *GNA13* | NM_006572:exon3:c.T533A-(p.L178Q) | 43.7 | 52/119 | | 23.1 | 1909/8254 | | Heterozygous | | |
| | | | | | | | | *GNA13* | NM_006572:exon3:c.T530C-(p.F177S) | 43.7 | 52/120 | | 23.1 | 1920/8314 | | Heterozygous | | |
| | | | | | | | | *GNA13* | NM_006572:exon3:c.T526A-(p.Y176N) | 44.2 | 53/120 | | 23.4 | 1946/8312 | | Heterozygous | | |
| | | | | | | | | *BCL2* | NM_000633:exon2:c.G17C-(p.R6T) | 17.1 | 42/245 | | 4.8 | 409/8558 | | Heterozygous | | |
| 1528 | F | 66 | III | 4 | 1.39 | 0 | GCB | *ITPKB* | NM_002221:exon2:c.C1583T-(p.S528L) | 9.3 | 14/151 | 31.0 | 0.0 | 0/1713 | 2.5 | Absent | 1.94 | 7 |
| | | | | | | | | *ITPKB* | NM_002221:exon2:c.C1471G-(p.P491A) | 15.2 | 19/125 | | 0.0 | 0/3582 | | Absent | | |
| | | | | | | | | *ITPKB* | NM_002221:exon2:c.C1454A-(p.A485E) | 15.2 | 19/125 | | 0.0 | 0/3577 | | Absent | | |
| | | | | | | | | *ITPKB* | NM_002221:exon2:c.C1438G-(p.P480A) | 15.5 | 19/123 | | 0.0 | 0/3570 | | Absent | | |
| | | | | | | | | *MYD88* | NM_002468:exon4:c.G728A-(p.S243N) | 37.8 | 74/196 | | 8.5 | 161/1888 | | Heterozygous | | |
| | | | | | | | | *TNFAIP3* | NM_006290:exon9:c.T2335C-(p.C779R) | 45.7 | 32/70 | | 5.6 | 177/3152 | | Heterozygous | | |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.T1936A-(p.Y646N) | 5.9 | 44/741 | | 2.1 | 152/7401 | | Heterozygous | | |
| | | | | | | | | *KMT2D* | NM_003482:exon48:c.C15142T-(p.R5048C) | 36.1 | 122/338 | | 5.7 | 207/3620 | | Heterozygous | | |
| | | | | | | | | *B2M* | NM_004048:exon1:c.G3A-(p.M1I) | 50.0 | 63/126 | | 3.2 | 78/2465 | | Heterozygous | | |
| | | | | | | | | *CIITA* | NM_000246:exon11:c.2239delT-(p.Y747fs) | 39.0 | 30/77 | | 0.0 | 0/2130 | | Absent | | |
| | | | | | | | | *CIITA* | NM_000246:exon18:c.T3236A-(p.V1079D) | 7.7 | 14/183 | | 0.0 | 0/9150 | | Absent | | |
| | | | | | | | | *CIITA* | NM_000246:exon18:c.T3241A-(p.Y1081N) | 7.6 | 14/184 | | 0.0 | 0/9162 | | Absent | | |
| | | | | | | | | *GNA13* | NM_006572:exon1:c.G201C-(p.Q67H) | 71.0 | 105/148 | | 0.0 | 0/2695 | | Absent | | |
| | | | | | | | | *GNA13* | NM_006572:exon1:c.T158C-(p.L53P) | 71.0 | 105/148 | | 3.1 | 82/2672 | | Heterozygous | | |
| | | | | | | | | *BCL2* | NM_000657:exon2:c.G589A-(p.G197S) | 34.1 | 47/138 | | 4.3 | 214/4929 | | Heterozygous | | |
| | | | | | | | | *BCL2* | NM_000633:exon2:c.C502G-(p.P168A) | 32.9 | 45/137 | | 4.2 | 209/5013 | | Heterozygous | | |
| | | | | | | | | *MEF2B* | NM_001145785:exon3:c.G229C-(p.E77Q) | 33.3 | 137/411 | | 5.9 | 654/11153 | | Heterozygous | | |

Supplementary table 1

| | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1559 | F | 83 | IE | 1 | 0.8 | 0 | ABC | *CD58* | NM_001779:exon3:c.C454T-(p.R152X) | 92.9 | 92/99 | 64.0 | 0.0 | 0/13231 | <1 | Absent | 0.618 | 7 |
| | | | | | | | | *MYD88* | NM_002468:exon5:c.T778C-(p.L265P) | 51.8 | 187/361 | | 0.0 | 0/11427 | | Absent | | |
| | | | | | | | | *CREBBP* | NM_004380:exon30:c.G5105T-(p.R1702L) | 46.3 | 154/333 | | 0.0 | 0/5663 | | Absent | | |
| | | | | | | | | *TP53* | NM_000546:exon5:c.G524A-(p.R175H) | 89.0 | 105/118 | | 0.0 | 0/13012 | | Absent | | |
| | | | | | | | | *CD79A* | NM_001783:c.568-2A>G | 52.0 | 122/235 | | 0.5 | 20/3930 | | Absent | | |
| | | | | | | | | *CD79A* | NM_001783:exon5:c573_617del-(p.191_206del) | 52.0 | 122/235 | | 0.5 | 20/3932 | | Absent | | |
| 1586 | F | 62 | III | 2 | 0.85 | 0 | NA | *TNFRSF14* | NM_003820:exon2:c.C164T-(p.P55L) | 13.8 | 44/318 | 20 | 2.1 | 6/284 | <1 | Heterozygous | 1.31 | 6 |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.A1937T-(p.Y646F) | 13.8 | 41/298 | | 0.0 | 0/6575 | | Absent | | |
| | | | | | | | | *STAT6* | NM_001178078:exon12:c.A1256G-(p.D419G) | 18.9 | 83/439 | | 0.0 | 2/5517 | | Absent | | |
| | | | | | | | | *CREBBP* | NM_004380:exon26:c.G4283C-(p.R1428P) | 33.6 | 49/146 | | 1.3 | 65/4894 | | Absent | | |
| | | | | | | | | *GNA13* | NM_006572:c.561+2A>G | 17.7 | 11/62 | | 0.0 | 0/4391 | | Absent | | |
| 1623 | M | 53 | IV | 2 | 1.03 | 0 | GCB | *TNFRSF14* | NM_003820:c.179-1G>C | 34.8 | 146/419 | 18 | 19.3 | 694/3587 | 11 | Heterozygous | 0.95 | 6 |
| | | | | | | | | *EZH2* | NM_004456:exon16:c.A1937T-(p.Y646F) | 9.9 | 57/575 | | 1.8 | 62/3480 | | Heterozygous | | |
| | | | | | | | | *STAT6* | NM_001178078:exon12:c.A1256G-(p.D419G) | 15.4 | 79/514 | | 5.1 | 200/3889 | | Heterozygous | | |
| | | | | | | | | *CREBBP* | NM_004380:exon28:c.A4628T-(p.D1543V) | 23.2 | 166/717 | | 11.6 | 489/4222 | | Heterozygous | | |
| | | | | | | | | *CREBBP* | NM_004380:exon27:c.T4504C-(p.W1502R) | 14.0 | 54/387 | | 10.1 | 328/3239 | | Heterozygous | | |
| | | | | | | | | *CD79B* | NM_000626:exon6:c.611dupC-(p.T204fs) | 17.1 | 83/486 | | 18.0 | 839/4651 | | Heterozygous | | |
| | | | | | | | | *MEF2B* | NM_001145785:exon3:c.G229A-(p.E77K) | 11.8 | 74/626 | | 9.9 | 1283/12969 | | Heterozygous | | |
| 1631 | M | 64 | I | 2 | 1.54 | 0 | ABC | *MYD88* | NM_002468:exon4:c.G728A-(p.S243K) | 49.8 | 220/442 | 35 | 11.7 | 655/5586 | 5 | Heterozygous | 1.1 | 6 |
| | | | | | | | | *PIM1* | NM_002648:exon4:c.G290A-(p.S97N) | 20.6 | 14/68 | | 2.7 | 130/4797 | | Heterozygous | | |
| | | | | | | | | *CARD11* | NM_032415:exon5:c.645_647del-(p.215_216del) | 47.1 | 99/210 | | 5.1 | 154/3028 | | Heterozygous | | |
| | | | | | | | | *CARD11* | NM_032415:exon4:c.T343C-(p.F115L) | 47.9 | 68/142 | | 5.5 | 344/6300 | | Heterozygous | | |
| | | | | | | | | *STAT6* | NM_001178078:exon12:c.A1249G-(p.N417D) | 18.1 | 96/532 | | 4.3 | 570/13250 | | Heterozygous | | |
| | | | | | | | | *TP53* | NM_000546:exon5:c.T518A-(p.V173E) | 32.4 | 72/222 | | 3.9 | 522/13317 | | Heterozygous | | |
| | | | | | | | | *CD79B* | NM_000626:exon5:c.A587T-(p.Y196F) | 26.7 | 52/195 | | 3.3 | 386/11853 | | Heterozygous | | |
| 1639 | M | 69 | IV | 2 | 2.4 | 0 | GCB | *TNFRSF14* | NM_003820:exon6:c.C620A-(p.S207X) | 75.8 | 47/62 | 39 | 82.3 | 2400/2915 | 34 | Heterozygous | 10.2 | 6 |
| | | | | | | | | *MYD88* | NM_002468:exon3:c.C840G-(p.S219C) | 27.5 | 133/483 | | 7.1 | 1616/22541 | | Heterozygous | | |
| | | | | | | | | *EZH2* | M_004456:exon16:c.A1937T-(p.Y646F) | 24.0 | 126/526 | | 32.8 | 3259/9939 | | Heterozygous | | |
| | | | | | | | | *KMT2D* | NM_003482:exon34:c.C9838T-(p.Q3280X) | 33.8 | 187/554 | | 35.0 | 5878/16784 | | Heterozygous | | |
| | | | | | | | | *KMT2D* | NM_003482:exon22:c.5296_5297insGAGG-(p.D1766fs) | 28.3 | 13/46 | | 22.9 | 671/2929 | | Heterozygous | | |
| | | | | | | | | *CREBBP* | NM_004380:c.4560+2A>G | 37.0 | 17/46 | | 30.6 | 2918/9530 | | Heterozygous | | |
| | | | | | | | | *BCL2* | NM_000633:exon2:c.C176A-(p.P59Q) | 45.8 | 11/24 | | 31.5 | 223/709 | | Heterozygous | | |
| | | | | | | | | *BCL2* | NM_000633:exon2:C20T-(p.T7I) | 53.0 | 98/185 | | 28.2 | 2267/8032 | | Heterozygous | | |
| | | | | | | | | *EP300* | NM_001429:exon27:c.4373_4375del-(p.1458_1459del) | 29.6 | 34/115 | | 38.3 | 4597/11990 | | Heterozygous | | |
| 1768 | M | 83 | IV | 3 | 4.2 | 0 | ABC | *MYD88* | NM_002468:exon5:c.T778C-(p.L265P) | 62.1 | 216/348 | 52 | 45.0 | 5936/13194 | 34 | Heterozygous | 1.82 | 4 |
| | | | | | | | | *IRF4* | NM_002460:exon2:c.G53A-(p.S18N) | 28.5 | 75/263 | | 21.2 | 929/4374 | | Heterozygous | | |
| | | | | | | | | *PIM1* | NM_002648:exon3:c.237delG-(p.E79fs) | 61.7 | 145/235 | | 38.2 | 1541/4030 | | Heterozygous | | |
| | | | | | | | | *PIM1* | NM_002648:exon4:c.G382C-(p.D128H) | 29.8 | 34/114 | | 25.9 | 987/3812 | | Heterozygous | | |
| | | | | | | | | *PRDM1* | NM_001198:exon2:c.G284A-(p.S95N) | 81.9 | 149/182 | | 34.2 | 1273/3724 | | Heterozygous | | |
| | | | | | | | | *PRDM1* | NM_001198:exon5:c.1103_1112del-(p.368_371del) | 80.7 | 221/274 | | 43.4 | 5076/11689 | | Heterozygous | | |
| | | | | | | | | *MYC* | NM_002467:exon2:c.C145T-(p.Q49X) | 36.4 | 102/280 | | 26.8 | 262/979 | | Heterozygous | | |
| | | | | | | | | *CD79B* | NM_000626:exon5:c.A587C-(p.Y196S) | 36.5 | 57/156 | | 33.6 | 3041/9060 | | Heterozygous | | |

**Supplementary Table 1**: Clinical characteristics and list of somatic variants (insertion/deletion/ single nucleotide variant) detected by sequencing in tumor DNA and cell-free plasma circulating DNA.

ABC: Activated B-Cell like; GCB: Germinal Center B-cell like; In/del: insertion/deletion; LDH: Lactate dehydrogenase; IPI: International Prognosis Index; SNV: single nucleotide variant; UPN: Unique Personal Number; ULN: upper limit value; VAF: variant allele frequency