STAT3 mutations in natural killer cells are associated with cytopenia in patients with chronic lymphoproliferative disorder of natural killer cells

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Abstract

Chronic lymphoproliferative disorder of natural killer (NK) cells (CLPD-NK) is a rare disease with an indolent clinical course, which is characterized by persistent increase in large granular lymphocytes of NK cell type. A somatic mutation in s*ignal transducer and activator transcription 3* (*STAT3)* has been reported in patients with CLPD-NK; however, the details of the mutational profiles and their clinical significance remain unclear. We performed mutation analyses of the *STAT3*, *STAT5B* and *TNF-alpha-induced-protein 3* (*TNFAIP3)* genes for mononuclear cells-derived DNA in 17 CLPD-NK patients using allele-specific polymerase chain reaction and amplicon sequencing. Mutations in *STAT3* and *TNFAIP3* were found in 29% (5/17) and 6% (1/17) of cases, respectively. All patients were negative for *STAT5B* mutations. In all three *STAT3*-mutation(+) patients studied, *STAT3* mutations were restricted to sorted NK cells. *STAT3* mutation(+) patients had a lower hemoglobin level (6.6 g/dL vs. 13.9 g/dL, P= 0.0044) and showed a trend toward reduced neutrophil counts (1.22×10^9/L vs. 3.10×10^9/L, P= 0.070) compared with the *STAT3* mutation(-) patients. No mutations in these genes were found in patients with neuropathy. These results suggest that heterogeneity of CLPD-NK and *STAT3*-mutated NK cells may play a significant role in cytopenia in CLPD-NK patients.

Key words: STAT3, TNFAIP3, CLPD-NK, cytopenia

Introduction

Chronic lymphoproliferative disorder of natural killer (NK) cells (CLPD-NK) is a rare lymphatic disease defined by persistent clonal increase in large granular lymphocytes (LGLs) of NK cell lineage [1]. Patients with CLPD-NK show an indolent course, and in some cases, they are associated with complications such as cytopenia, neuropathy or autoimmune diseases. Somatic mutations in s*ignal transducer and activator transcription 3* (*STAT3)*, a STAT signaling molecule, are a prominent molecular finding characteristic of LGL leukemia, including T cell LGL leukemia (T-LGLL) and CLPD-NK [2-4]. Activating mutations in the Src-homology 2 (SH-2) domain of *STAT3* that lead to the constitutional phosphorylation of STAT3 are considered to enhance the transcriptional activity by the JAK/STAT signaling pathways. The frequencies of *STAT3* mutations in CLPD-NK reportedly range from 30%-64% [3-5]. It has been suggested that the *STAT3* mutation is associated with cytopenia in CLPD-NK; however, the details remain unclear [5, 16].

Although whole-exome sequencing for CLPD-NK was performed in a previous study [6], the genetic background of the subjects, including their *STAT3* status, are still unclear. Somatic mutations in the SH2 domain of the *STAT5B* gene have also been recognized in T-LGLL and CLPD-NK [7]. In addition, the *tumor necrosis*

factor-alpha-induced protein 3 (*TNFAIP3)* gene is reported to be mutated in 8% of patients with T-LGLL [8]. *TNFAIP3* is a tumor suppressor gene that encodes A20, negative regulator of nuclear factor kappa B (NF-κB).

In this study, we analyzed the *STAT3, STAT5B* and *TNFAIP3* genes in order to evaluate their genetic profiles and their clinical significance concerning CLPD-NK.

Materials and Methods

Patients

A total of 17 patients with CLPD-NK, including previously reported cases [4, 9, 10], were enrolled in this study. The diagnoses were based on the 2008 WHO classification [1]. In this study, CLPD-NK was characterized by an LGL count over 0.5×10^9/L with a phenotype of $CD2^+CD3^-CD16^+$ or $CD56^+$ and T cell receptor $(TCR)^-$ for more than 6 months' duration [11]. The clinical data, including the age, sex, underlying conditions, laboratory data, therapeutic medications and their outcomes, were collected from medical records. Peripheral blood or bone marrow samples collected at the time of the diagnosis, and if possible, during and after therapy, were obtained.

This study was conducted in accordance with the Declaration of Helsinki and was approved by each of the institutional review boards of Shinshu University School of Medicine and Matsumoto Medical Center Matsumoto Hospital. Written informed consent was obtained from the patients.

DNA extraction

Mononuclear cells (MNCs) were isolated from peripheral blood or bone marrow using Ficoll gradient separation (GE Healthcare, Little Chalfont, Buckinghamshire, UK) and

stored at -80 °C until DNA extraction. DNA was extracted using a QIAamp DNA blood mini-kit (QIAGEN, GmbH Hilden, Germany) according to the manufacturer's instructions.

Allele-specific polymerase chain reaction (AsPCR)

AsPCR to detect *STAT3* Y640F and D661Y mutations was performed using previously described primers [3, 4]. The primers for AsPCR to detect *STAT5B* N642H and Y665F mutations are described in Supplemental Table 1.

Amplicon sequencing of the STAT3, STAT5B and TNAIP3 gene

Amplicon sequencing was performed using Ion AmpliSeq technology. The primers were designed to cover 98% of the *STAT3* and 96% of the *STAT5B* and *TNFAIP3* gene coding sequences with the Ampliseq Designer system (Supplemental Table S2) (Thermo Fisher Scientific, Waltham, MA, USA). The libraries were made using the Ion AmpliSeq Kit for Chef DL8 or Ion Ampliseq Library Kit 2.0 according to the protocol for preparing Ion Ampliseq libraries (Thermo Fisher Scientific).

Other primers covering the residual 2% of the *STAT3* and 4% of the *STAT5B* and *TNFAIP3* gene coding sequences were designed, and 10 amplicons were made by

multiplex PCRs (Supplemental Table S3). These mixed amplicons were quantitated using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific) and then subjected to library preparation with the Ion Plus Fragment Library Kit (Thermo Fisher Scientific). The concentrations of the libraries were measured with an Ion Library TaqMan Quantitation Kit (Thermo Fisher Scientific). The libraries were subjected to amplicon sequencing on Ion PGM according to the standard protocol using the Ion 314 or 318 Chip Kit v2 (Thermo Fisher Scientific). The data were analyzed with the Torrent Suite software program (v5.2.2; Thermo Fisher Scientific). The main variant calling settings were as follows: variant frequency filter, 0.005; base quality Q-value, ≥20; minimum coverage of depth, 1000; and maximum strand bias, 0.95 (SNP), 0.9 (INDEL). The called variants were annotated by wANNOVAR (http://wannovar.wglab.org/index.php), and mutations considered to be single-nucleotide polymorphisms or silent mutations were eliminated.

Validation of candidate somatic mutations by Sanger sequencing

PCR amplification was performed using primers as previously described [8, 12], and PCR products were purified by gel electrophoresis followed by extraction with a QIAExII Gel Extraction kit (QIAGEN) or Agencourt AMPure XP beads (Beckman

Coulter, Brea, CA, USA). The purified PCR products were then sequenced with a BigDye v1.1 Cycle Sequencing kit and an ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific).

Sorting of cell subpopulations

In selected patients, target cell subpopulations were separated using a fluorescence activated cell sorter (FACS). Specifically, $CD2^{+}3^{+}$ T cells, $CD2^{+}3^{-}16^{+}$ NK cells or $CD2^{+}3^{-}56^{+}$ NK cells were separated using antibodies against CD2 (phycoerythrin [PE], clone S5.2; BD Bioscience, San Jose, CA, USA), CD3 (fluorescein isothiocyanate [FITC], clone UCHT1; BD Bioscience), CD16 (peridinin chlorophyll protein complex-cyanine 5.5 [PerCP-Cy5.5], clone 3G8; BD Bioscience) and CD56 (allophycocyanin [APC], clone N901; Beckman Coulter) with the FACSAria cell sorter (BD Bioscience).

Statistical analyses

Comparisons between different groups were carried out using a Fisher's exact test, a two-sided *t*-test, a Mann-Whitney U test, a log rank test or a Kruskal Wallis test, as

appropriate. *P*-values of <0.05 were considered to indicate statistical significance. All of the statistical analyses were performed using the EZR software program [13].

Results

Patient demographics

The demographics and laboratory data of the patients are summarized in Table 1. The complications of CLPD-NK were as follows: neutropenia, n=4; anemia with erythroid hypoplasia, n=3; autoimmune hemolytic anemia (AIHA), n=2; and neurological impairments, n=4.

STAT3 mutations detected by AsPCR

Among the 17 patients, 3 were positive for the *STAT3* D661Y mutation by an AsPCR. A patient with the D661Y mutation was also positive for the *STAT3* Y640F mutation. None of the patients were positive for *STAT5B* hot-spot mutations.

Amplicon sequencing of the STAT3, STAT5B and TNFAIP3 genes

In amplicon sequencing for MNC-derived DNA, the median depth of coverage was 2,947× (range: 783-8,099). Four patients were positive for the *STAT3* Y640F or D661Y mutations, and one was positive for the *STAT3* S614R mutation. In addition, one patient was positive for the *TNFAIP3* A94Efs*9, W448* and C579R mutations. No *STAT5B* mutations were detected.

Amplicon sequencing detected *STAT3* mutations in the sorted $CD2^{+}3^{-}16^{+}$ NK cells but not in the $CD2^{+}3^{+}$ T cells from 3 patients from whom MNCs were available for cell sorting (Fig. 1). One patient (UPN 10) whose MNC-derived DNA was negative for *STAT3* mutations was found be positive when their sorted NK cells were analyzed (Table 2).

All *STAT3* mutations identified in this study were located in the SH2 domain. The *STAT3* D661Y mutations of UPN 3, 6 and 11 were confirmed by Sanger sequencing. The *STAT3* Y640F mutation of UPN 3, *STAT3* S614R mutation of UPN 14 and *TNFAIP3* mutation of UPN 4 were validated by AsPCR or the repeated analysis of amplicon sequencing from another batch of samples. In total, *STAT3* mutations were detected in 5 of 17 (29%) patients. The mutations in the *TNFAIP3* gene detected in UPN 4 were located in the ovarian tumor domain (OTU) and between the zinc finger domains (ZFs) and were validated by the repeated analysis of amplicon sequencing from another batch of samples.

The chronological analysis of STAT3 mutations

Serial blood samples from one patient (UPN 6) were available for amplicon sequencing of sorted NK cell fractions. The patient showed *STAT3* D661Y (variant allele frequency

[VAF] 46.5%) and D661V (VAF 2.0%) mutations at the diagnosis. Five years after the diagnosis, D661Y (VAF 50.2%) was detected, but the VAF of D661V did not reach the cut-off value. However, the D661V mutation was still manually recognized in 5/5487 reads, which suggested that the rate of the D661V mutation had merely significantly decreased.

Clinical characteristics of CLPD-NK patients according to STAT3 mutations

When the clinical characteristics of *STAT3* mutation-positive (*STAT3* mutation[+]) and *STAT3* mutation-negative (*STAT3* mutation[-]) CLPD-NK patients were compared, the *STAT3* mutation(+) patients were more frequently complicated with anemia (5/5; 100% vs. 0/12; 0%, p= 0.00016), had a lower hemoglobin level (median; 6.6 g/dL vs. 13.9 g/dL, *P*= 0.0044), and tended to have lower neutrophil counts (1.22×10^9/L vs. 3.10×10^9/L, *P*= 0.070) than the *STAT3* mutation(-) patients. Platelet counts did not differ markedly between these populations.

When we compared the three subgroups of CLPD-NK depending on complications with cytopenia, neuropathy or without complications (five, four and eight cases, respectively), *STAT3* mutations were exclusively found in the cytopenia-having subgroup. This subgroup also had significantly fewer $CD4^+$ lymphocytes than the

subgroup without complications (median: 0.26 $\times 10^9$/L vs. 1.70 $\times 10^9$/L, P= 0.046) (Table 3).

Discussion

This is the first study to show that CLPD-NK patients can be classified into three groups by *STAT3* mutations and clinical complications. The *STAT3* mutation in CLPD-NK was exclusively detected in patients with anemia (100%; 5/5), and *STAT3*-mutated CLPD-NK cases also tended to have fewer neutrophils than the cases without *STAT3* mutation. In contrast, none of CLPD-NK patients with neurological impairment had *STAT3* mutations. Thus, CLPD-NK comprise three subgroups: CLPD-NK with cytopenia, CLPD-NK with neuropathy, and CLPD-NK without any complications (Table 3).

CLPD-NK and T-LGLL are heterogenous diseases possessing several common features, including indolent clinical courses with proliferation of cytotoxic lymphocytes, frequent *STAT3* mutations and immune-mediated complications, such as cytopenia [4]. LGL leukemia-associated neurological complications have been reported in both T-LGLL and CLPD-NK; however, the frequency of neuropathy in CLPD-NK is much higher than that in T-LGLL [14]. Furthermore, the phosphorylation and activation status of STAT3 may also differ between these two disorders. Kurt et al. recently showed that no STAT3 overexpression was recognized in CLPD-NK [5], in contrast to findings regarding T-LGLL [2, 3, 15]. These results imply that CLPD-NK is a distinct disorder

with specific clinical and pathophysiological features in addition to an NK immunophenotype.

Although previous reports have also suggested that *STAT3*-mutated CLPD-NK patients tended to have cytopenia [5, 16], the details were unclear. Among the five *STAT3*-mutated patients in our study who received therapies for cytopenia, one became refractory to cyclosporine A (CsA), and all three receiving cyclophosphamide (CY) treatments responded to the therapy with persistent remission. We recently reported that *STAT3*-mutated pure red cell aplasia (PRCA) patients were more refractory to CsA treatment than *STAT3*-negative patients [12]. Most of the patients who became refractory to CsA responded well to CY. However, further studies will be needed to clarify the cellular mechanisms underlying how the *STAT3*-mutated NK cells are involved in the cytopenia and activities of immunosuppressants in CLPD-NK.

Our study revealed *STAT3* mutations in 5 of 17 (29%) with the VAFs ranging from 0.012 to 0.371. These frequencies of *STAT3* mutations were similar to those in previous reports [3]. *STAT3* mutations were exclusively detected in the sorted NK cells of the three patients whose MNCs were available for cell sorting. No mutations were found in the $CD2^{+}CD3^{+}$ T cells. Unlike with T-LGLL, it is usually difficult to obtain proof of clonality in CLPD-NK. Evaluating killer-immunoglobin-like receptors might

be useful for determining the clonal natures of NK cells [11]; however, reliable methods, such as assessing TCR gene rearrangements in T cell neoplasms, have yet to be established. A *STAT3* mutational analysis of the NK cell fractions might provide important information for diagnosing CLPD-NK.

We identified a *TNFAIP3* mutation-positive case with CLPD-NK for the first time in the present study. The *TNFAIP3* mutation has been reported not only in patients with B cell lymphoma [17, 18], but also in those with T/NK lymphoma [19, 20]. A previous report showed that 3 of 39 (8%) patients with T-LGLL were *TNFAIP3* mutation-positive [8]. Among the mutations detected in our study, W448* has been reported in marginal zone lymphoma [21] and follicular lymphoma [22], while the other two mutations have not yet been reported. A previous study reported that the monoallelic loss of *TNFAIP3* alone did not affect the prognosis of patients with extranodal NK/T-cell lymphoma, nasal type and suggested the importance of other mechanisms, including haploinsufficiency or the additional inactivation of the other allele [20]. Since only one patient was positive for *TNFAIP3* mutation in our study, significance of *TNFAIP3* in CLPD-NK needs further studies with more cases.

In this study, we were unable to identify any *STAT5B* mutations in CLDP-NK. Rajala et al. reported that only 1 of 38 CLPD-NK patients was positive for an *STAT5B*

mutation [7]. Taken together with our results, *STAT5B* mutations appear to be infrequent in CLPD-NK.

In conclusion, CLPD-NK might comprise three subgroups in terms of the clinical features and *STAT3* mutations: a group with cytopenia and *STAT3* mutations, a group with neurological complications, and "others". The identification of *STAT3* mutations would be useful for determining the subgroup of CLPD-NK and provide meaningful clues to help determine appropriate therapeutic approaches.

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Authorship

Contribution: T.K. designed the study, performed experiments, and analyzed the data. N.S. designed the study and collected clinical data and analyzed the data. J.K., K.M. and T.Y. performed experiments. S. N., Y.S., H.S., Y.H., H.N. and F.I. collected

samples and clinical data. F.I. conceived and designed the study, analyzed the data and supervised the research. T.K., H.N. and F.I. wrote the manuscript.

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FIGURE LEGENDS

Figure 1. Representative examples of scatterplots of sorted lymphocytes and the results of amplicon sequencing in sorted fractions. The $CD2^{+}3^{-}$ fraction of UPN 10 was CD16-positive and CD56-negative, and this sorted fraction was positive for the *STAT3* S614R mutation with a variant allele frequency of 7.6%. The $CD2^{+}3^{+}$ fraction was negative for mutations (A). No mutations were found in the $CD2^{+}3^{+}$ or $CD2^{+}3^{-}56^{+}$ fractions of UPN 9 (B).

Table 1. Characteristics of the 17 patients with CLPD-NK in this study

UPN	sex	Age, years	complication	WBC, $\times 10^9$/L	Lym, $\times 10^9$/L	Neu, $\times 10^9$/L	CD4, $\times 10^9$/L	CD8, $\times 10^9$/L	Hb, g/dL	Hct, %	Plt, $\times 10^9$/L	Ret, $\times 10^9$/L	LDH, IU/L	Cr, mg/dL	erythroid cells in BM, %	therapy	outcome	follow up duration, months
1	F	67	neutropenia	6.32	4.48	1.33	0.76	0.44	14.3	41.0	25.6	51.7	267	0.5	NA	watch	alive	55
2	M	49		7.47	4.71	2.32	1.94	1.20	14.2	38.1	53.9	347.5	184	0.4	NA	watch	alive	233
3	M	71	anemia with erythroid hypoplasia neutropenia	2.50	1.70	0.28	0.23	0.18	6.7	19.5	22.6	23.5	284	0.85	8.8	CY	alive	133
4	M	75		17.22	3.44	2.24	2.07	9.13	13.5	41.3	16.2	54.0	276	0.61	8.6	watch	alive	69
5	F	57	neurological impairment	12.15	8.63	3.16	1.82	1.09	12.6	37.3	28	19.4	137	0.73	7.6	PSL	alive	37
6	F	83	AIHA neutropenia	9.40	7.71	1.22	0.29	2.27	5.8	17.2	15.1	357.0	340	0.53	3.2	PSL, CsA, CY, VP-16	dead	97
7	F	73		9.83	6.19	3.05	0.59	0.59	12.0	38.8	27.4	NA	229	0.65	3.6	watch	alive	108
8	M	69	neurological impairment	11.46	5.84	5.16	1.38	3.32	16.4	47.8	22.2	65.6	256	1.22	17.4	neural block	alive	8
9	M	77		5.20	2.70	1.92	1.40	1.66	11.3	33.5	2.5	71.7	213	0.8	9.6	watch	alive	65
10	M	76	anemia with erythroid hypoplasia	5.49	2.85	NA	1.04	0.38	7.2	21.3	46.4	6.3	171	0.7	NA	CsA	alive	110
*11	F	64	anemia with erythroid hypoplasia neutropenia	1.30	0.31	0.59	0.53	365.30	9.4	27..9	21.5	13.5	NA	NA	NA	PSL, CY	alive	121
12	M	73		15.90	7.63	7.00	5.09	5.88	13.5	41.0	9.2	NA	759	0.58	NA	watch	dead	3
13	M	82	neurological impairment	11.71	7.26	3.86	1.29	6.44	15.9	48.0	28.6	77.3	209	1.16	11.8	watch	alive	24
14	NA	74	AIHA	6.01	2.52	2.64	0.22	0.21	6.5	19.6	25.2	325.1	2158	1	13.2	PSL, CsA	alive	4
15	M	68	neurological impairment	7.40	5.17	1.92	1.44	0.61	12.7	37.5	16.5	NA	166	1.12	20.9	mPSL, PSL	alive	4
16	M	80		12.70	8.61	3.44	1.45	2.93	15.6	45.6	12.1	NA	211	0.7	NA	watch	alive	2
17	M	71		11.36	6.82	3.64	2.50	2.61	16.5	48.1	30.1	NA	177	0.88	NA	watch	alive	5

UPN, unique patient number; WBC, white blood cells; Lym, lymphocytes, Neu, neutrophils; Hb, hemoglobin; Hct, hematocrit; Plt, platelet; Ret, reticulocytes; BM, bone marrow; CY, cyclophosphamide; PSL, predonisolone; CsA, cyclosporine A; AIHA, autoimmune hemolytic anemia; mPSL, methylpredonisolone; NA, not assessed.

*Only samples collected after therapy were available.

Table 2. *STAT3*, *STAT5B* and *TNFAIP3* mutational status in CLPD-NK

UPN	allele-specific PCR		Amplicon (PB or BM MNCs)			Amplicon ($CD2^{+}3^{-}16$ or 56^{+}NK)		
	STAT3	*STAT5B*	*STAT3*	*STAT5B*	*TNFAIP3*	*STAT3*	*STAT5B*	*TNFAIP3*
1	-	-	-	-	-	N.T	N.T	N.T
2	-	-	-	-	-	N.T	N.T	N.T
§‡3	Y640F D661Y	-	Y640F(4.4%) D661Y(31%)	-	-	D661Y(50.1%)	-	-
4	-	-	-	-	A94Efs*9 (19.4%) W448* (3.9%) C579R (1%)	N.T	N.T	N.T
†‡5	-	-	-	-	-	-	-	-
§6	D661Y	-	D661Y(37.1%)	-	-	D661Y(46.5%) D661V(2%)	-	-
7	-	-	-	-	-	N.T	N.T	N.T
8	-	-	-	-	-	N.T	N.T	N.T
†‡9	-	-	-	-	-	-	-	-
§10	-	-	-	-	-	S614R(7.6%)	-	-
11	D661Y	-	D661Y(36.5%)	-	-	N.T	N.T	N.T
12	-	-	-	-	-	N.T	N.T	N.T
13	-	-	-	-	-	N.T	N.T	N.T
14	-	-	S614R (1.2%)	-	-	N.T	N.T	N.T
15	-	-	-	-	-	N.T	N.T	N.T
16	-	-	-	-	-	N.T	N.T	N.T
§17	-	-	-	-	-	-	-	-

-, negative for mutation; N.T, not tested

§ CD2+3-16+ NK cells were sorted, †CD2+3-56+ NK cells were sorted, ‡samples derived from bone marrow

Table 3. Three subgroups of CLPD-NK and their characteristics

	CLPD-NK with cytopenia (n=5)	CLPD with neuropathy (n=4)	CLPD-NK without complications (n= 8)	p-value
Age, median (range), years	74 (64-83)	69 (57-82)	73 (49-80)	0.665
Male, n (%)	3 (60%)	3 (75%)	6 (75%)	1
WBCs, median (range), $\times 10^9$/L	5.75 (2.50-9.40)	11.59 (7.40-12.15)	10.60 (5.20-17.22)	0.089
Neutrophils, median (range), $\times 10^9$/L	1.22 (0.28-2.64)	3.51 (1.92-5.16)	2.68 (1.33-7.00)	0.14
Lymphocytes, median (range) $\times 10^9$/L	2.69 (1.70-7.71)	6.55 (5.17-8.63)	5.45 (2.70-8.61)	0.2
$CD4^+$ lymphocytes, median (range), $\times 10^9$/L	0.26 (0.22-1.04)	1.41 (1.29-1.82)	1.70 (0.59-5.09)	*0.024
$CD8^+$ lymphocytes, median (range), $\times 10^9$/L	0.30 (0.18-2.27)	2.21 (0.61-6.44)	2.14 (0.44-9.13)	0.092
Hemoglobin, median (range), g/dL	6.6 (5.8-7.2)	14.3 (12.6-16.4)	13.9 (11.3-16.5)	*0.014
Reticulocytes, median (range), $\times 10^9$/L	38.7 (6.3-325)	65.6 (19.4-77.3)	62.9 (51.7-347)	0.57
Platelets, median (range), $\times 10^9$/L	239 (151-464)	251 (165-286)	209 (25-539)	0.81
LD, median (range), IU/L	312 (171-2,158)	188 (137-256)	221 (177-759)	0.127
Cr, median (range), mg/dL	0.78 (0.53-1.00)	1.14 (0.73-1.22)	0.63 (0.40-0.88)	§0.034
STAT3 mutation	5 (100%)	0	0	†0.000162

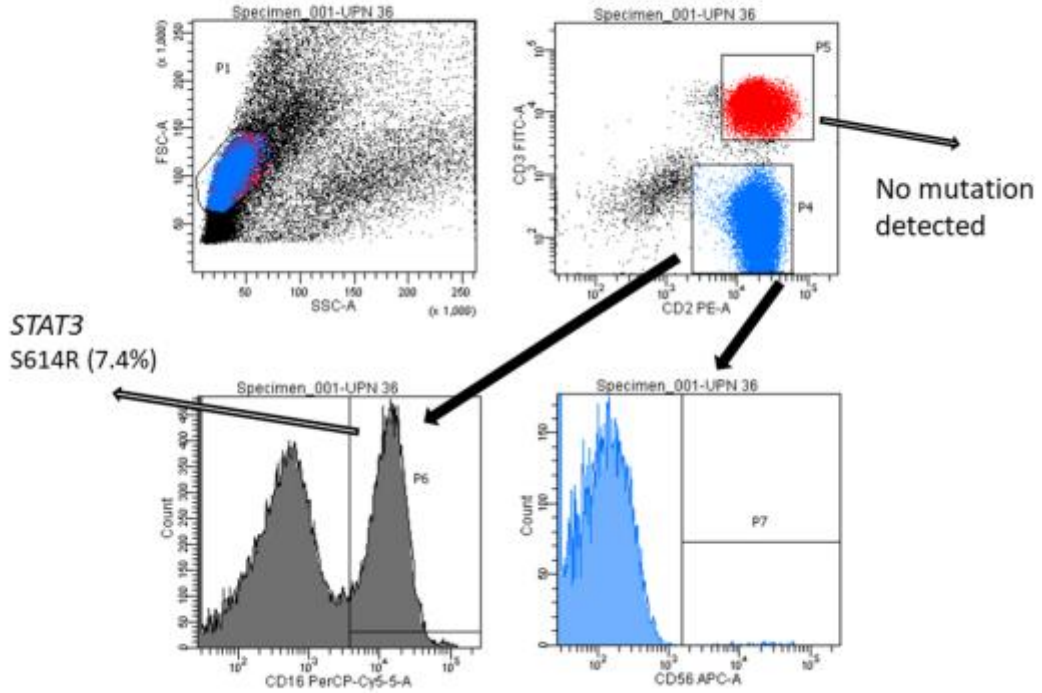
Significant difference between *the group with cytopenia and without complications,

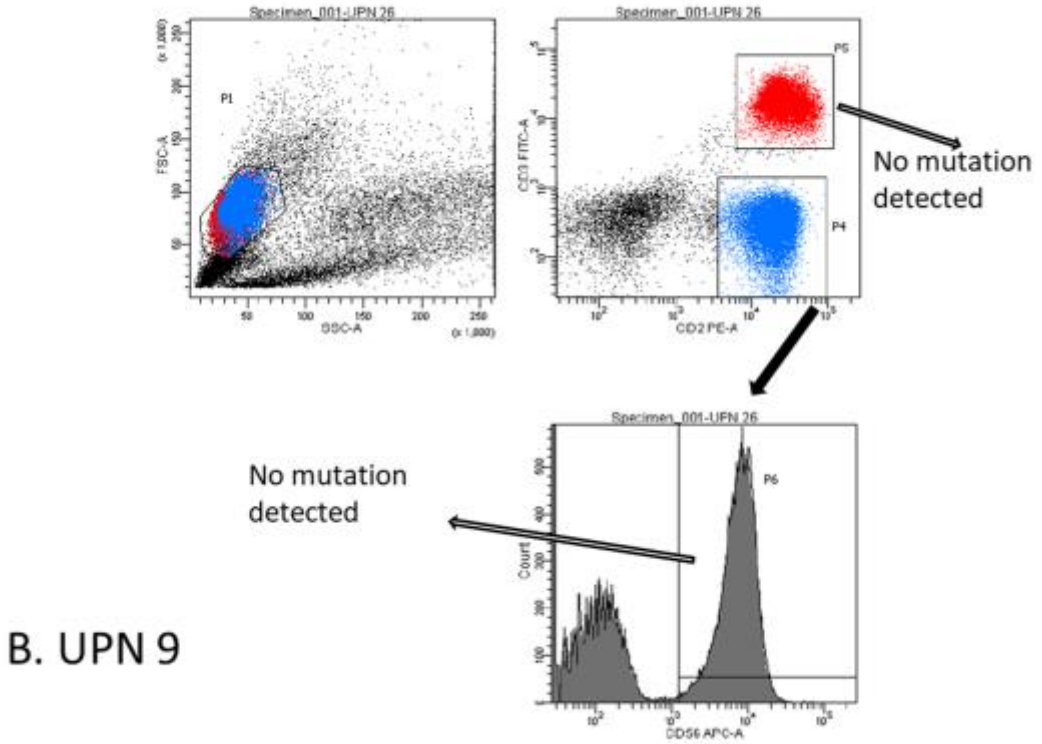
§ the group with neuropathy and without complications

† Significantly higher rate of mutation in CLPD-NK with cytopenia than in the other groups

Cytopenia is defined as follows: anemia, hemoglobin level < 10 g/dL; neutropenia, neutrophil count < 1.5×10^9/L; thrombocytopenia, platelet count < 100×10^9/L

A. UPN 10





B. UPN 9