Title: T cell clonal expansion and *STAT3* mutations: A characteristic feature of acquired chronic T-cell mediated pure red cell aplasia

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Abstract

Acquired chronic pure red cell aplasia (PRCA) develops idiopathically or in association with other medical conditions, including T cell large granular lymphocytic leukemia (T-LGLL) and thymoma. T cell dysregulation is considered a cardinal pathogenesis of PRCA, but genetic-phenotypic associations in T cell abnormalities are largely unclear. We evaluated an extended cohort of 90 patients with acquired PRCA, including 26 with idiopathic, 36 with T-LGLL-associated and 15 with thymoma-associated PRCA, for their T cell immunophenotypes, clonalities and *STAT3* mutations. TCR repertoire skewing of $CD8^+$ T cells was detected in 37.5% of idiopathic, 66.7% of T-LGLL-associated and 25% of thymoma-associated PRCA patients, and restriction to Vβ1 was most prominent (41%). Clonalities of TCRβ or γ chain and *STAT3* mutational status were statistically associated (P=0.0398), and they were detected in all three subtypes. The overall response rate to cyclosporin A was 73.9%, without significant difference by subtypes nor *STAT3* mutational status. The T cell dysregulations, such as TCR repertoire skewing with predominant Vβ1 usage, clonality and *STAT3* mutations, were frequently found across the subtypes, and the close associations between them suggest that these T cell derangements reflect a common pathophysiological mechanism among these PRCA subtypes.

key words

pure red cell aplasia, *STAT3*, T cell receptor, thymoma, large granular lymphocytic leukemia,

Introduction

Acquired chronic pure red cell aplasia (PRCA) is a syndrome defined by anemia with the

marked reduction or absence of erythroid production and normal hematopoiesis of other lineages, developing predominantly via T-cell-mediated or autoantibody-dependent immune mechanisms [1, 2]. PRCA is classified into idiopathic and secondary types, including T cell-type large granular lymphocytic leukemia (T-LGLL)-associated and thymoma-associated PRCA, accounting for the three most frequent subtypes, with significant responses to immunosuppressants expected [3-5].

T-cell dysfunction and aberrant proliferation have been observed in some patients with PRCA, implying their association with the inhibition of erythropoiesis. For example, erythroid progenitor colony formation was suppressed *in vitro* with T cells in PRCA [6, 7]. Clonal T cells have been frequently recognized in not only T-LGLL-associated PRCA but also in idiopathic and thymoma-associated PRCA [8, 9]. Recently, we and others showed that somatic mutations of *signal transducer activator transcription 3(STAT3)* were frequently detected in $CD8^+$ T cells of PRCA [10] as well as in other cases of bone marrow failure syndrome, such as aplastic anemia and hypoplastic myelodysplastic syndrome [11, 12]. These data indicate that dysregulation of T cells may play a central role in the pathogenesis of PRCA and may be a cardinal feature shared with idiopathic, T-LGLL-associated and thymoma-associated PRCA. However, the details of immunophenotypes and clonalities of T cells in PRCA have yet to be clarified, and the

relationship between *STAT3* mutations and those T-cell abnormalities needs be elucidated through analyses of a PRCA cohort large enough to demonstrate statistical significance.

The present study examined $CD8^+$ T cells in an extended cohort of PRCA patients, and searched for genetic and immunophenotypic features of the T cells, and their associations, among idiopathic, T-LGLL-associated and thymoma-associated PRCA.

Materials and Methods

Patients

Since our previous study on acquired PRCA with a cohort of 42 patients [10], we continued to recruit additional patients for the present study, as an extended cohort. The diagnostic criteria of PRCA and LGLL were in accordance with the previous reports (Supplemental Table S1). Patients with idiopathic aplastic anemia and healthy individuals were also included as controls. The clinical parameters, including PRCA subtypes, and laboratory data were collected from medical charts. The demographic backgrounds of the 29 patients in cohort 2 (Table 1) were not available because of the anonymity of the database to which they belonged. Some of the clinical information of regarding 42 of the 61 patients in cohort 1 has been published previously [10, 13, 14].

This study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review boards of each participating center. Written informed consent was obtained from the patients and healthy controls.

Flow cytometry of the T-cell receptor (TCR) Vβ repertoire and immunophenotyping of T cells

The TCR Vβ repertoire of TCRαβ^{+}CD8^{+} T cells was analyzed using a panel of TCR Vβ antibodies (TCR Vβ Repertoire Kit; Beckman Coulter, Brea, CA, USA). The samples were analyzed using a FACSCantoII and the FACSDiva software program (BD Biosciences, Franklin Lakes, NJ, USA). The Vβ family was considered to be aberrantly expanded when it exceeded 20%. The immunophenotypes of the T cells were characterized using the antibodies listed in supplemental Table S2.

TCRβ and TCRγ gene rearrangement analyses

To analyze TCR gene rearrangements, we performed multiplex polymerase chain reaction (PCR) with the BIOMED-2 protocol [15] and analyzed the data from capillary electrophoresis with a 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) (Supplemental Table S3).

Allele-specific PCR (AsPCR) for STAT3 *mutations and amplicon sequencing of the* STAT3*,* STAT5B *and* TNFAIP3 *genes*

The DNA extracted from the patients' samples was subjected to AsPCR and amplicon sequencing to detect mutations in the *STAT3*, *STAT5B* and *TNFAIP3* genes, as previously described [10, 13]. In brief, mononuclear cells (MNCs) were isolated from the peripheral blood using a Ficoll gradient separation (GE Healthcare, Little Chalfont, UK) and were stored at -80 °C until DNA extraction. With the DNA obtained with a QIAamp DNA blood mini-kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions, we then performed AsPCR to detect *STAT3* Y640F and D661Y using the primers listed in Supplemental Table S4.

Amplicon sequencing was performed with Ion AmpliSeq technology. The libraries were established with an Ion AmpliSeq kit for the Chef DL8 or Ion AmpliSeq™ Library Kit Plus according to the protocol for preparing Ion Ampliseq libraries (Thermo Fisher Scientific). After measuring the concentrations with an Ion Library TaqMan Quantitation kit (Thermo Fisher Scientific), the libraries were subjected to amplicon sequencing on the Ion PGM according to the standard protocol using an Ion 314 or 318 Chip kit (v2; Thermo Fisher Scientific). The data were analyzed with the Torrent Suite software program

(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 (single-nucleotide polymorphism [SNP]), 0.9 (insertion/deletion [INDEL]). The called variants were annotated by wANNOVAR (http://wannovar.wglab. org/index.php), and the mutations considered to be SNPs or silent were eliminated. The median depth of coverage was 6,432 (range: 953-12,017) times.

Statistical analyses

Fisher's exact test or a two-sided *t*-test was used for comparative analyses of categorical variables, while the Mann-Whitney U test was used for continuous variables. The logrank test and logistic regression model were employed for univariate and multivariate analyses. *P*-values <0.05 were considered statistically significant. The statistical analyses were performed using the EZR software program [16].

Results

Demographics of patients with acquired chronic PRCA

Additional 48 patients with acquired PRCA were recruited, and a total of ninety patients

were enrolled in this study (the extended cohort), and their demographics are shown in supplemental Table S2. Subtypes of PRCA were idiopathic (N=26), T-LGLL-associated (N=36), thymoma-associated (N=15), Chronic Lymphoproliferative Disorder (CLPD)-NK-associated (N=4), autoimmune diseases-related PRCA (N=8) and PRCA with other causes (N=5). Subtype classification was the only information available in cohort 2. The median age was 65 years old among the 61 patients with their demographic data available (cohort 1). Idiopathic, T-LGLL-associated and thymoma-associated PRCA were the three major subtypes. While the patients' age, gender, hemoglobin value and platelet count were not significantly different among the three subtypes, the number of lymphocytes was significantly higher in T-LGLL-associated PRCA patients than in others (p=0.002) (Table 2).

TCR repertoire and T-cell immunophenotypes of PRCA

A flow cytometric immunophenotypic analysis was performed with 36 patients in whom a sample was available (Figure 1). T-cell repertoire skewing was recognized in 17 of the 36 patients (47%), including 3 of the 8 patients with idiopathic (37.5%), 12 of the 18 with T-LGLL-associated (66.7%) and 2 of the 8 with thymoma-associated PRCA (25%) (Figure 2a). T cell skewing was not found in 2 patients with autoimmune-associated and

CLPD-associated PRCA. Of note, the most frequent expanded Vβ family was the Vβ1 (n=7, 41% of the cases), followed by Vβ3 (n=2, 12%), Vβ7.1 (n=2, 12%) and Vβ14 (n=2, 12%) (supplemental Figure SF1, supplemental Table S5). T cell skewing in Vβ1 was not found in normal controls (data not shown, N=13).

Clonal T cells in PRCA

We previously applied the BIOMED-2 protocol to a T cell line, showing that its sensitivity for detecting TCRβ gene rearrangement was 5% (supplemental Figure SF2). TCR clonality in either the β or γ chain was observed in 62 of the 90 patients (68.9%) with acquired PRCA, including 73% of those with idiopathic, 96% of those with T-LGLL-associated and 33% of those with thymoma-associated PRCA (Figure 3a-1, supplemental Table S6). In idiopathic aplastic anemia or heathy subjects, by contrast, such rearrangements were only minimally detected. These results suggest that a significant proportion of PRCA patients harbored clonal T cell expansions in the peripheral blood, although the rearrangements were less frequent in thymoma-associated PRCA than in other subtypes.

STAT3, STAT5B *and* TNFAIP3 *gene mutations of T cells and their relationships with T*

cell clonalities

An overview of the mutational landscape in our extended cohort of PRCA patients is shown in Figure 2b. Amplicon sequencing and/or AsPCRs for the *STAT3* gene, with a median variant allele frequency (VAF) of 4.4% (range: 1.1%-58.9%), showed that 41 of 90 PRCA patients (45.6%) were positive for *STAT3* mutations (Figure 3b). Despite an overrepresentation of T-LGL-associated PRCA among *STAT3* mutated individuals, the mutation was also found in a substantial number of those with other subtypes of PRCA. The mutations were also frequently recognized within the SH-2 domains in the extended cohort (Figure 2b). *STAT3* mutations were found specifically in 38% of idiopathic, 61% of T-LGLL and 27% of thymoma-associated PRCA (Figure 3a-2). A χ^2 test showed that an association of T cell clonality and the mutations was statistically significant (P=0.0398). More specifically, of those with clonal T cell expansions, mutations were identified in 47.3% of idiopathic, 64.7% of T-LGLL and 40% of thymoma-associated PRCA (Figure 4). Among the PRCA patients with *STAT3* mutations, T cell clonality was found in 80.5% (33/41), including 90% of idiopathic (9/10), 100% of T-LGLL-associated (22/22) and 50% of thymoma-associated PRCA (2/4), revealing the overrepresentation of T cell clonality among those with *STAT3* mutations (supplemental Figure SF3). A total of 60% of the patients without *STAT3* mutations were still positive for T cell rearrangements

(Figure 2a), reconfirming that T cell clonal expansions are frequently recognized in PRCA, regardless of the *STAT3* mutational status.

A further analysis of the TCR repertoire immunophenotypes revealed that TCRVβ skewing was highly associated with TCR clonalities (*P*=0.0198) and *STAT3* mutations (*P*=0.018) (supplemental Table S7). These data, showing close associations among the genetic mutations, TCR clonalities and TCR repertoire skewing in CD8 T-cells of PRCA in our extended cohort, implied that *STAT3* mutations have developed among clonal T cells. None of the patients analyzed harbored *STAT5B* mutations. *TNFAIP3* mutations were also detected in three individuals: two with TCRαβ–type LGLL-associated PRCA and one with TCRγδ–type LGLL-associated PRCA, the latter of which was described in greater detail previously [14].

Treatments and outcome of PRCA

A total of 67 immunosuppressive regimens were applied in 51 patients, including cyclosporin A (CsA) as a first- (N=37) or a second-or-later-line therapy (N=9), or cyclophosphamide (CY) as a front-line (N=4) or a second-or-later-line therapy (N=17) (supplemental Table S8). Thirty-four patients with PRCA (73.9%) had complete or partial response to CsA: the overall response rate was not markedly different among the PRCA

subtypes (p=0.223). Of note, all 8 patients with thymoma-associated PRCA had undergone thymectomy prior to immunosuppressive therapy and showed a 100% response rate to the subsequent CsA treatment. Thirteen of the 21 patients (62.0%) with CY therapy showed a clinical response, and no significant differences in the response rate were observed among the subtypes. Patients with *STAT3* mutations, however, tended to have a more favorable response rate to CY than those without mutations (P = 0.018) (supplemental Table S9). In contrast, the overall response rate to CsA did not differ by the *STAT3* mutational status (p=0.321). No significant differences were observed in the overall response rate to immunosuppressive treatments between the presence and the absence of TCR clonalities, either. The superiority of *STAT3* mutations in CY treatment was reiterated in a multivariate analysis (p=0.0226) (supplemental Table S10). The estimated 10-year overall survival rate was 82.4% (supplemental Figure SF4-a). There was no significant difference in the overall survival by the *STAT3* mutational status, T cell clonalities or subtypes of PRCA (supplemental Figure SF4-b).

Longitudinal immunogenetic alterations in thymoma-associated PRCA during the clinical course

We longitudinally studied the immunophenotypic and mutational evolutions to determine

their etiological relevance in thymoma-associated PRCA. While PRCA and thymoma were diagnosed almost concurrently in 7 of the 10 studied patients, a thymoma had first developed more than 15 years prior to the PRCA in 2 of the patients, who were positive for *STAT3* mutations (supplemental Table S11). Results of a longitudinal genetic-phenotypic analysis were available in 1 of the 2 patients (UPN690), in whom PRCA had developed after a 19-year history of refractory thymoma and its associated diseases, including myasthenia gravis and graft-versus-host disease-like skin eruption. At 9 months before the development of anemia, the patient had harbored a 6.4% of Vβ1+ cell populations in the peripheral blood, which subsequently expanded to 34.9% by the time of the PRCA diagnosis. Furthermore, the results of amplicon sequencing of the sorted expanded Vβ1+ subpopulation exhibited an *STAT3* H410R mutation at VAF 3.6%, although the mutation had not been detected before PRCA development (Figure 5). The patient's rapidly deteriorating clinical condition had hindered any aggressive treatments with a curative intent, and she was red blood cell transfusion-dependent until she died of pneumonia three months after the PRCA diagnosis.

Discussion

In this study with a large cohort of PRCA patients, clonal T cell expansions and *STAT3*

mutations were frequently observed, regardless of the subtype (idiopathic, T-LGLL-associated or thymoma-associated PRCA). We also demonstrated that clonal T cell expansions were strongly associated with *STAT3* mutations.

It is widely accepted that PRCA is one of the most frequent complications of T-LGLL [14, 17]. The revised diagnostic criteria for T-LGLL included the minimum required LGL count being lowered to 0.5×10^9/L [18], resulting in a less-delineated diagnostic contour between T-LGLL-associated PRCA and idiopathic PRCA. The obscured diagnostic boundary between the two subtypes may reflect their shared pathogenesis and the relevance of clonal cytotoxic T cell expansion and *STAT3* mutations. Likewise, remarkably similar characteristics in clonal T cell expansions with *STAT3* mutations were replicated in 20% of patients with thymoma-associated PRCA in our study.

Frequent skewing of the TCR repertoire that we demonstrated across the three subtypes may also be sufficient for the T-cell derangements to be considered a common pathophysiology in PRCA. Among the skewed Vβ family, a frequent usage of Vβ1 was commonly observed in the three subtypes, occupying 41% of the total skewed repertoire. The post-thymectomy persistence of oligoclonal T cells with Vβ1 in thymoma-associated PRCA, described in a previous study [19], may also support the pathophysiological relevance of Vβ1 in PRCA. A systematic review of 533 patients with T-LGLL, including

80% of CD8+LGLL and 8.4% of PRCA, shows that the most frequent expanded Vβ family was Vβ13.1 (12.5%), while 5.7% had a dominant Vβ1 expansion [20]. Although a simple comparison across different cohorts is difficult, however, the predominance of Vβ1 in our extended cohort may be valid as an immunophenotypic characteristics of PRCA. The immunogenetic role of Vβ1, however, remains poorly understood. While a restricted immunodominant Vβ expansion pattern with different specificities has been observed in patients with T-LGLL, Vβ1 has not always been overrepresented [21-23]. However, Vβ1 was utilized in only roughly 5% of $CD8^+$ T cells in the peripheral blood of healthy subjects, while clonal expansion of $V\beta1^+$ T cells was observed in other pathologic conditions [24].

Given both the variant allele frequencies of *STAT3* mutations and the reasonably high sensitivity of the T cell clonality assay, the association of the mutations and the clonalities demonstrated in the present study may not be a random immunogenetic finding. We therefore speculate that *STAT3* mutations may have occurred in the clonally expanded T cells. However, we are not sure whether or not such immunogenetic sequential events may have occurred in a single T cell, and if they did, we also do not know how they occurred and in what order. A single-cell gene expression analysis might help us to clarify these questions, which are beyond the scope of the present study; for example, *STAT3*-

mutated $CD8^+$ T cells in an AA patient have been shown to express aberrant phenotypes with cytotoxic features [11].

However, a limited number of patients exhibited *STAT3* mutations and clonal T cell expansions in thymoma-associated PRCA, suggesting that T cell abnormalities might be less pertinent in this subtype than in the idiopathic and T-LGLL-associated PRCA subtypes. In our longitudinal analysis among patients with thymoma-associated PRCA, however, a patient (UPN690) demonstrated an informative clinical course, showing the concurrent expansion of *STAT3*-mutated Vβ1+ cells with PRCA development. Such a sequential manifestation may support the hypothesis that the clonal expansions and *STAT3* mutations are closely associated with suppressed erythropoiesis in PRCA, although this case does not fully prove a pathophysiological significance of the sequence. However, evidence concerning the pathogenesis of PRCA without *STAT3* mutations or clonal T cells has been scant; we speculate that the identical T cells with an undetected clone size may be associated. It also remains unclear as to what triggers the genetic alterations, such as *STAT3* mutations, in T or NK cells in the first place and how relevant these genetic events are in the pathogenesis of PRCA.

PRCA has a strong historical association with thymoma; anemia may precede the finding of a thymoma or may occur after thymectomy [1]. A favorable response in

thymoma-associated PRCA treated with immunosuppressors was also confirmed in the present study. Underlying T-cell mediated immunological dysregulations, as such, are relevant to the pathophysiology as well as the treatment of thymoma-associated PRCA. While the mechanisms underlying T-cell derangements in thymoma and thymoma-associated PRCA remain unclear, one hypothesis proposes the failure of the positive/negative selection of T cells and autoimmunity in an autoimmune regulator-poor environment in the thymus [25]. Further studies on T cell dysregulations in thymoma-associated PRCA are expected to help us understand the pathogenesis of thymoma-associated PRCA as well as other thymus-associated pathologies [26]. Our results may provide further evidence that TCR skewing, clonal T cell expansions and *STAT3* mutations are relevant T-cell dysregulations in thymoma-associated immune dysfunction.

Although our results clearly illustrated the unique immunological nature of T-cell-mediated acquired PRCA, several limitations associated with our study should be mentioned. Mainly, the association of T cell clonalities and *STAT3* mutations, especially in Vβ1-positive T-cell populations, was proven with indirect evidence, and a single-cell analysis with target cells may be required to confirm these findings. In addition, the T cell derangement was illustrated mostly with data from the three major subtypes of PRCA, so we might have underestimated the findings for PRCA subtypes with lower prevalence,

such as autoimmune-disease-associated PRCA. Clonal T cell expansions and somatic mutations in nonleukemic T cells are common in other immune mediated bone marrow failure syndrome, such as aplastic anemia [11]. The present study may help understand the close associations between genetic and immunophenotypic T cell derangements in the pathogenesis of PRCA, and we believe that it may not only contribute for a further understanding of pathophysiology of erythroid suppression in PRCA but also it might contribute for a development of treatment strategy in the future.

In conclusion, clonal T cell expansions in close association with *STAT3* mutations were frequently recognized in an extended cohort of PRCA, irrespective of the subtype. Despite difference in frequency, T cell derangement may reflect a common pathophysiological mechanism of impaired erythroid hematopoiesis across these subtypes of PRCA.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Author contributions.

FK designed the study, collected samples, performed experiments, analyzed data, and wrote the manuscript. TK collected samples, performed experiments, and analyzed data. TY, MM and JK performed experiments. SN collected samples and reviewed the manuscript. HS, MH and SN collected samples. YH analyzed data. HN collected samples, analyzed data, and wrote the manuscript. FI designed the study, collected samples analyzed data, wrote the manuscript, and managed the project. HN and FI equally contributed as correspondent authors.

Figure legends

Figure 1. The etiological breakdowns of different T cell dysregulations in PRCA. The pie chart demonstrates the results of a flow cytometric immunophenotypic analysis in 36 patients with PRCA, showing T cell repertoire skewing in 17 patients (47%), including 7 (19.4%) with a restriction in Vβ1. The PRCA subtypes with or without skewed TCRs were idiopathic, T-LGLL-associated and thymoma-associated PRCA, with proportions depicted in the blue bars (c, d). The *STAT3* mutational status with or without TCR skewing are depicted in the brown bars (b, e). A further subtype classification of PRCA in each subgroup with *STAT3* mutations are shown in blue bars (a, f). The number of patients are described as N in each portion, and the parenthesized numbers are their frequencies in percentage.

Figure 2. (a) A summary of the T cell dysregulations in an extended cohort of 90 subjects with PRCA. Each column represents T cell derangement in a patient with PRCA: TCR clonality, mutational status and immunophenotype. TCR clonality was defined as positive when rearrangements were found in either the T cell receptor β gene or γ gene with BIOMED2-porotocol-based PCR methods. (b) A lollipop plot showing the mutational landscape in the *STAT3* gene of 41 cases with PRCA. The mutations were confirmed with

amplicon sequencing method and/or allele-specific PCR. Of note, mutations were most frequently found in the SH-2 domain. idiopathic: idiopathic PRCA, LGLL-associated: large granular lymphocytic leukemia-associated PRCA, thymoma-associated: thymoma-associated PRCA, CLPD-NK-associated: chronic lymphoproliferative disorders NK type-associated PRCA, others: PRCA with other underlying diseases, including autoimmune disease-related PRCA.

Figure 3. (a) Proportions of TCR clonality and *STAT3* mutations in subtypes of PRCA. The bar graphs show the proportions of T-cell dysregulations. (a-1) The dark blue bar indicates the proportion of positive TCR clonality in either the β or γ chain in each subtype of PRCA. The percentage of positive TCR clonality was higher in those with idiopathic and T-LGLL-associated PRCA than in those with thymoma-associated PRCA ($p<0.001$). (a-2) The dark green bar indicates the proportion of patients with positive *STAT3* mutations. The percentages were significantly different among the subtypes ($p<0.05$). (b) Proportions of the subtypes of PRCA in *STAT3* mutated and unmutated patients. Despite an overrepresentation of T-LGL-associated PRCA among *STAT3* mutated individuals, the mutation is also found in a substantial number of those with other subtypes of PRCA.

Figure 4. T cell derangement in each subtype of PRCA. The horizontal dark blue bar indicates the proportion of the patients with TCR clonality, while the gray bar indicates the proportion without the clonality. The pie charts below each bar indicate the percentages of the patients with a *STAT3* mutation (the dark green pie) or those without such a mutation (the orange pie). Idiopathic: idiopathic PRCA, T-LGLL-associate: T cell large granular lymphocytic leukemia-associated PRCA, thymoma-associated: thymoma-associated PRCA, CLPD-NK-associated: CLPD-NK-associated PRCA, others: PRCA with other backgrounds, including autoimmune disease-related PRCA.

Figure 5. Clinical course of a patient with thymoma-associated PRCA. In this patient (UPN690), PRCA developed after a 19-year history of refractory thymoma (the white arrowhead in the CT scan in the upper left window) and its associated diseases, including myasthenia gravis and graft-versus-host-disease-like skin eruption. The red line indicates the hemoglobin level, while the blue arrowheads indicate thymectomy, black arrowhead radiation therapy and black arrows the time of relapse of thymoma. Before the development of PRCA at 230 sick months, the patient had harbored a 6.4% of Vβ1+ cell population in the peripheral blood, which subsequently expanded to 34.9% by the time of

full-blown PRCA. Furthermore, the results of amplicon sequencing revealed that the expanded subpopulation of Vβ1+ cells harbored a *STAT3* H410R mutation at VAF 3.6%, although this mutation had not been detected before the development of PRCA.

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