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2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
editorial@hematology.org

B-cell Acute Lymphoblastic Leukemia in Patients with Chronic Lymphocytic Leukemia Treated with Lenalidomide

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Moritz Fürstenau (University Hospital Cologne, Germany) Anna Maria Fink (University Hospital of Cologne, Germany) Anke Schilhabel (2nd Department of Internal Medicine, University of Schleswig-Holstein, Campus Kiel, Germany) Jonathan Weiss (University of Cologne, Germany) Sandra Robrecht (University Hospital of Cologne, Germany) Robert Eckert (Onkologische Schwerpunktpraxis Esslingen, Germany) Javier de la Serna (Hospital Universitario 12 de Octubre, Spain) Marta Crespo (Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Spain) Marta Coscia (University of Torino, Italy) Candida Vitale (Univeristy of Torino, Italy) Sebastian Böttcher (8Medical Clinic III, Hematology, Oncology and Palliative Medicine, Rostock University Medical Center, Rostock, Germany) Gesche Weppner (2nd Department of Internal Medicine, University of Schleswig-Holstein, Campus Kiel, Germany) Matthias Ritgen (University Clinic of Schleswig-Holstein, Kiel, Germany) Stephan Stilgenbauer (Ulm University, Germany) Eugen Tausch (Ulm University, Germany) Kirsten Fischer (University Hospital Cologne, Germany) Michael Hallek (University Hospital, Germany) Barbara Eichhorst (University Hospital Cologne, Germany) Monika Brüggemann (Universitätsklinikum Schleswig-Holstein, Germany) Carmen Herling (University of Cologne, Germany)

Abstract:
The placebo controlled CLLM1 trial evaluated the efficacy of lenalidomide maintenance treatment in patients with high-risk chronic lymphocytic leukemia (CLL) in first remission after chemoimmunotherapy (CIT). Upon observation of three cases with acute lymphoblastic leukemia (ALL) in overall 56 lenalidomide treated patients (5.4%), the study treatment was prematurely stopped.

Using next generation sequencing of B cell and T cell receptor (TR) rearrangements, we here report common clonal B cell ancestry between CLL and ALL in one of those three patients, in whom both diseases shared the same VDJ- as well as crosslineage TR rearrangements. Chromosomal/mutation analyses indicated that in this patient the ALL developed from a common B cell precursor which lacks genomic lesions acquired in the CLL subclone, but shares a BIRC3 frameshift deletion (p.L421fs*). In two cases we found independent IGH rearrangements indicating de novo ALL development from a different B cell clone. A retrospective cohort analysis of >1600 CLL patients treated with first-line CIT in previously reported phase 2-3 studies of the German CLL study group, yielded a significantly lower cumulative incidence of ALL at 12.6 cases/100,000 patient years, compared to 1345.5 cases/100,000 patient-years observed in the lenalidomide arm of the CLLM1 study. Given our results and increasing knowledge on the biological effects of lenalidomide in bone marrow precursor cells, we discuss the potential involvement of lenalidomide in the pathogenesis of ALL in CLL patients.

Conflict of interest: COI declared - see note

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B-cell Acute Lymphoblastic Leukemia in Patients with Chronic Lymphocytic Leukemia Treated with Lenalidomide

Short title: B-ALL in CLL Patients Treated with Lenalidomide

Moritz Fürstenau[1], Anna Maria Fink[1], Anke Schilhabel[2], Jonathan Weiss[1], Sandra Robrecht[1], Robert Eckert[3], Javier de la Serna[4], Marta Crespo[5,6], Marta Coscia[7], Candida Vitale[7], Sebastian Böttcher[2,8], Gesche Weppner[2], Matthias Ritgen[2], Stephan Stilgenbauer[9,10], Eugen Tausch[9], Kirsten Fischer[1], Michael Hallek[1,11], Barbara Eichhorst[1], Monika Brüggemann[2*] and Carmen D. Herling[1*]

[1]Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, German CLL Study Group, University Hospital Cologne, University of Cologne, Cologne, Germany

[2]2nd Department of Internal Medicine, University of Schleswig-Holstein, Campus Kiel, Kiel, Germany

[3]Onkologische Schwerpunktpraxis Esslingen, Esslingen, Germany

[4]Servicio de Hematología, Hospital Universitario 12 de Octubre, Madrid, Spain

[5]Experimental Hematology, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona, Hospital Campus, C/ Natzaret, 115-117, Barcelona, Spain

[6]Department of Medicine, Universitat Autònoma de Barcelona, Bellaterra, Spain

[7]University Division of Hematology, A.O.U. Città della Salute e della Scienza di Torino and Department of Molecular Biotechnology and Health Sciences, University of Torino, Italy

[8]Medical Clinic III, Hematology, Oncology and Palliative Medicine, Rostock University Medical Center, Rostock, Germany (current affiliation)

[9]Department of Internal Medicine III, University of Ulm, Ulm, Germany,

[10]Department of Internal Medicine I, Saarland University Medical School, Homburg/Saar, Germany

[11]Cologne Excellence Cluster on Cellular Stress Response in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

*M.B. and C.D.H contributed equally to this study.

Corresponding author

Dr Moritz Fürstenau, moritz.fuerstenau@uk-koeln.de

German CLL Study Group (DCLLSG), Gleueler Straße 176-178, 50935 Köln, Germany

phone: +49-221-478-88220, fax: +49-221-478-86886

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Lenalidomide is an oral thalidomide analog, approved for the treatment of multiple myeloma, 5q-syndrome, mantle cell lymphoma and follicular lymphoma. In the phase III CLLM1 trial, lenalidomide maintenance after first-line chemoimmunotherapy (CIT) was shown to improve progression-free survival (PFS) in patients with chronic lymphocytic leukemia (CLL) compared to placebo[1].

Here, we report clinical and molecular data on three cases with B-cell progenitor acute lymphoblastic leukemia (B-ALL) observed in 56 lenalidomide-exposed patients (5.4%) during follow-up of this trial. Upon safety evaluation of those cases, an independent data safety monitoring board (DSMB) and the GCLLSG decided for early termination of lenalidomide treatment in remaining CLLM1 study patients.

Overall, the CLLM1 study enrolled 89 patients with CLL who had responded to first-line CIT and had a high risk of early disease progression. 60 patients were randomized to receive lenalidomide maintenance, 56 of which were treated with at least one dose. 29 patients were assigned to the placebo arm. After a median observation time of 47.7 (IQR: 39.3 – 60.1) months from first-line treatment, diagnosis of B-ALL was confirmed in three of the 56 lenalidomide-exposed patients (5.4%) 15, 33 and 48 months after the start of maintenance. No case of ALL was observed in the control arm.

Cumulative dosing of lenalidomide was similar between the three patients who developed B-ALL and the remaining 53 lenalidomide-treated patients (**Table S5**). All three patients with B-ALL had a history of high or very high-risk CLL according to CLL-IPI assessment before first-line CIT (**Table S3**). Prior to the start of lenalidomide maintenance, all three patients exhibited detectable MRD ($>10^{-4}$) in peripheral blood with descreasing MRD levels under lenalidomide therapy (**Figure 1A**).

The predicted incidence rate for B-ALL in lenalidomide-exposed patients derived from these observations made in the CLLM1 study was calculated at 1345.5 cases/100,000 patient-years (95%-CI: 277.5-3932.2). We performed a comparative retrospective cohort analysis on the cumulative

incidence of B-ALL observed in CLL patients treated with first-line alkylator-containing chemotherapy (CT) or CIT without lenalidomide across previously reported trials of the GCLLSG. Overall, 1679 patients fulfilled the criteria for inclusion in this analysis (**Table S1**)[2-6]. A significantly lower incidence rate of 12.6 cases/100,000 patient-years (95%-CI: 0.3-70.1) was estimated for this patient population ($p<0.001$) (**Table S6**).

To assess the clonal relationship between CLL and ALL, we sequenced disease-specific immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements[7]. In patient 1 and patient 3, no mutual IG/TR gene rearrangements were identified between the paired CLL and ALL samples (**Figure 2A**). In patient 2, CLL and ALL samples shared the same dominant IGHV1-18(D)/J6 clonotype (**Figure 2B**) strongly suggesting a common B-cell origin of both diseases. The detection of an identical clonal cross-lineage TR beta (TRB) gene rearrangement in both, CLL and ALL further supports this assumption. True cross-lineage TRB rearrangements are frequently found in B-ALL but rather rare in CLL[8,9]. Therefore, we confirmed our finding by TRB clone-specific ddPCR that not only proved high abundance of the clone carrying rearranged TRB genes but also high concordance with levels of the dominant IGHV rearrangement described above suggesting the concurrent presence of rearranged IGHV and TRB genes in the same B-cell clone.

To further assess clonal relationship in patient 2, we applied NGS for selected somatic mutations in 141 cancer-related genes. In the CLL sample before start of lenalidomide therapy a dominant clone harboring a *NOTCH1* (p.P2514fs*) frameshift deletion could be detected as well as a subclone carrying a *BIRC3* (p.L421fs*) frameshift deletion (**Figure 2C**). While the *NOTCH1* mutation could not be found in the ALL sample, CLL and ALL shared presence of an identical subclonal *BIRC3* mutation.

In the ALL sample, additional mutations in *DNMT3A* (p.V716D) and *IKZF1* (p.G89fs*) were detected as well as various variants of unknown significance and recurrent ALL-associated alterations such as dup(21q22) or monosomy 7 (**Figure 1B**). Taken together, the mutational profiles of both samples support a common clonal ancestry of CLL and ALL with persistence of a *BIRC3*-altered subclone and

treatment-related suppression of the *NOTCH1*-mutated clone (**Figure 2D**). *BCR-ABL1(e1-a2)* transcripts were detected at ALL diagnosis with a BCR-ABL1/ABL ratio of 0.1% and *BCR-ABL1* FISH analysis was negative, suggesting a subclonal event. If we consider the presence of initiating (but not necessarily CLL-specific) mutations in hematopoietic progenitor cells in CLL patients[10], the transformation towards B-ALL may have occurred from a *BIRC3*-altered precursor, rather than from mature CLL cells.

Increasing knowledge about the mode of action of lenalidomide supports hypotheses that the drug may facilitate clonal evolution towards acute leukemia. *In vitro* data have shown that lenalidomide induces proteasomal degradation of the transcription factors Ikaros and Aiolos[11]. Genetic deletions or mutations in *IKZF1*, which encodes the Ikaros protein, are frequent driver lesions in ALL and promote leukemogenesis and treatment resistance through various biological pathways[12,13]. It is known that lenalidomide is able to suppress Ikaros protein levels in human CD34+ cells almost equivalent to a genetic loss of *IKZF1*[14]. The drug also protects self-renewal activity resulting in an expansion of the CD34+ progenitor cell pool, while it has anti-proliferative effects on malignant mature B-cells[12,15]. It has been hypothesized that the lenalidomide-associated increase in cycling CD34+ cells and a deregulated metabolic state - due to loss of Ikaros -, may predispose patients to acquire DNA damage including leukemogenic events, particularly under therapeutic selection pressure[16,17].

Interestingly, we found an emerging *IKZF1* frameshift deletion at the time of ALL in patient 2, which may be related to a possible selection benefit for this subclone under lenalidomide maintenance. In patient 2, dup(21q22) and associated *RUNX1* amplification might have also constituted a selection advantage through *RUNX1*-mediated inhibition of *IKZF1* degradation and consequent desensitization towards lenalidomide, as recently described[18].

All patients in the CLLM1 trial were treated with alkylators that have been attributed to increase the risk for second primary malignancies (SPM) in patients with myeloma[19]. Male gender and age have also been identified as independent risk factors for SPM in CLL[20]. Further, profounder remissions induced by the use of anti-CD20 antibody combinations, have been associated with accelerated clonal evolution in CLL[18]. Of note, the three patients reported herein belonged to a subset minority who either achieved undetectable or at least strongly decreasing MRD levels during lenalidomide treatment, suggesting an association between depth of response and B-ALL occurrence.

In the CLLM1 study, all patients were pre-selected for an unfavorable disease course after CIT frontline therapy. As CLL patients have been demonstrated to carry initiating genetic lesions in hematopoietic progenitor cells[21], pre-existing as well as acquired genomic instability induced/selected by CIT might have contributed to genetic hits in lymphatic precursor cells, which finally outgrew as acute leukemia under/after lenalidomide maintenance.

In previous reports, ALL has mostly been described as an unrelated second malignancy in patients with CLL and only recently as a clearly clonally related transformation[8,22-24]. In this analysis we report the first clonally related transformation of a CLL into a *BCR-ABL* positive B-ALL.

The significant cumulative incidence of B-ALL in lenalidomide-exposed CLL patients of the CLLM1 study, when compared across GCLLSG first-line trials, points to a potential relationship between lenalidomide and the risk to develop B-ALL. As hematological SPM in CLL have been rising over past decades according to public registry data[25], meticulous reporting of such events and identification of independent risk factors in meta-analyses would be desirable.

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Authorship Contributions

M.F., A.M.F., J.W., S.R., M.H., B.E., C.D.H. designed the study. **R.E., J.d.l.S., M.Cr., M.Co., S.B., S.S., E.T., M.H., B.E.** enrolled patients and contributed data. **A.S., J.W., C.V., M.R., G.W., M.B.** contributed and interpreted data. **A.S., J.W., G.W., N.A., M.B., C.D.H**. performed and analyzed experiments. **M.F. and S.R.** designed and performed statistical analyses. **M.H., B.E., M.B. and C.D.H.** supervised the project. **M.F., A.M.F., B.E., M.B. and C.D.H.** drafted the first version of the manuscript. All authors revised and approved the final version of the manuscript.

Disclosure of Conflicts of interest

M.F., A.S., J.W., S.R., R.E., J.d.l.S., M.Cr., M.Co., C.V. and G.W. have nothing to disclose.

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Figure Legends

Figure 1

A Absolute lymphocyte counts and minimal residual disease (MRD) of the patients during lenalidomide maintenance. Red lines show the time point of ALL diagnosis. Green lines show the threshold for undetectable MRD (10^{-4}). **Patient 1:** CLL MRD levels in peripheral blood decreased steadily from 8.6x 10-4 to 1.6x 10-4 after 16 months of lenalidomide treatment. *BCR-ABL1* positive common-B-ALL (c-ALL) was diagnosed while no bone marrow infiltration of CLL cells could be detected morphologically (Figure 1C). **Patient 2** received lenalidomide for 15 months before discontinuation due to increased creatinine levels. Undetectable MRD in PB ($<10^{-4}$) was achieved after 6 months and maintained at month 12 of lenalidomide therapy. When *BCR-ABL1* positive c-ALL was diagnosed, there were no signs of CLL cells in the peripheral blood or bone marrow by cytomorphology and immunophenotyping. **Patient 3** received lenalidomide for 45 months before treatment was stopped following the DSMB recommendation. Although his MRD level in PB had dropped sustainably below 10^{-4} after 6 months on lenalidomide, a reciprocal increase of residual CLL cells to $8x10^{-3}$ was observed two months after

discontinuation of lenalidomide. Three months after the end of maintenance treatment, Ph-negative c-ALL was diagnosed following a bone marrow biopsy prompted by persisting pancytopenia. **B** Summary of selected genetic alterations at CLL and ALL time points including: FISH for recurrent aberrations in CLL, *IGHV* status, cancer-related mutations according to NGS, *BCR/ABL* status. Genomic alterations are annotated according to the color panel below the image. Sample type is indicated. We performed *BCR-ABL1* qRT-PCR in earlier PB samples of patient 1 and patient 2. No *BCR-ABL1* fusion transcripts were detected in a blood sample 12 months after initiation of maintenance in patient 2 and 11 months after start of lenalidomide treatment in patient 1. **C** Giemsa stains from bone marrow samples of all three patients at the time point of ALL diagnosis.

Figure 2

A Next-generation sequencing (NGS)-based clonality assessment of immunoglobuline rearrangements in CLL (dark blue) and ALL (yellow) samples of each patient. Samples were analyzed for IGH (IGHV-IGHD-IGHJ and IGHD-IGHJ) and IGK gene rearrangements. Clonal rearrangements with an abundance >5% are shown. Productive rearrangements are depicted in dark blue (CLL) and yellow (ALL). Clonotypes of productive IGH rearrangements are indicated. In patient 2 ALL and CLL share the same productive IGH rearrangement. **B** Patient 2. The nucleotide and amino acid sequence of the CDR3 gene fragment of the abundant clonotype is shown. **C** Patient 2. NGS assessment was performed using the Qiagen Myeloid panel encompassing 141 cancer-related genes at the time of CLL and ALL diagnosis. Alterations in cancer-related genes are shown in blue, variants of unknown significance are shown in yellow. **D** Patient 2. Dynamics of variant allele frequencies of cancer-related genes show persistence of a *BIRC3*-mutated clone while the *NOTCH1* mutation, present in the CLL population, is not detected in the ALL

sample. *IKZF1* and *DNMT3A* mutations occur in the ALL sample and are not present in CLL.

Figure 1

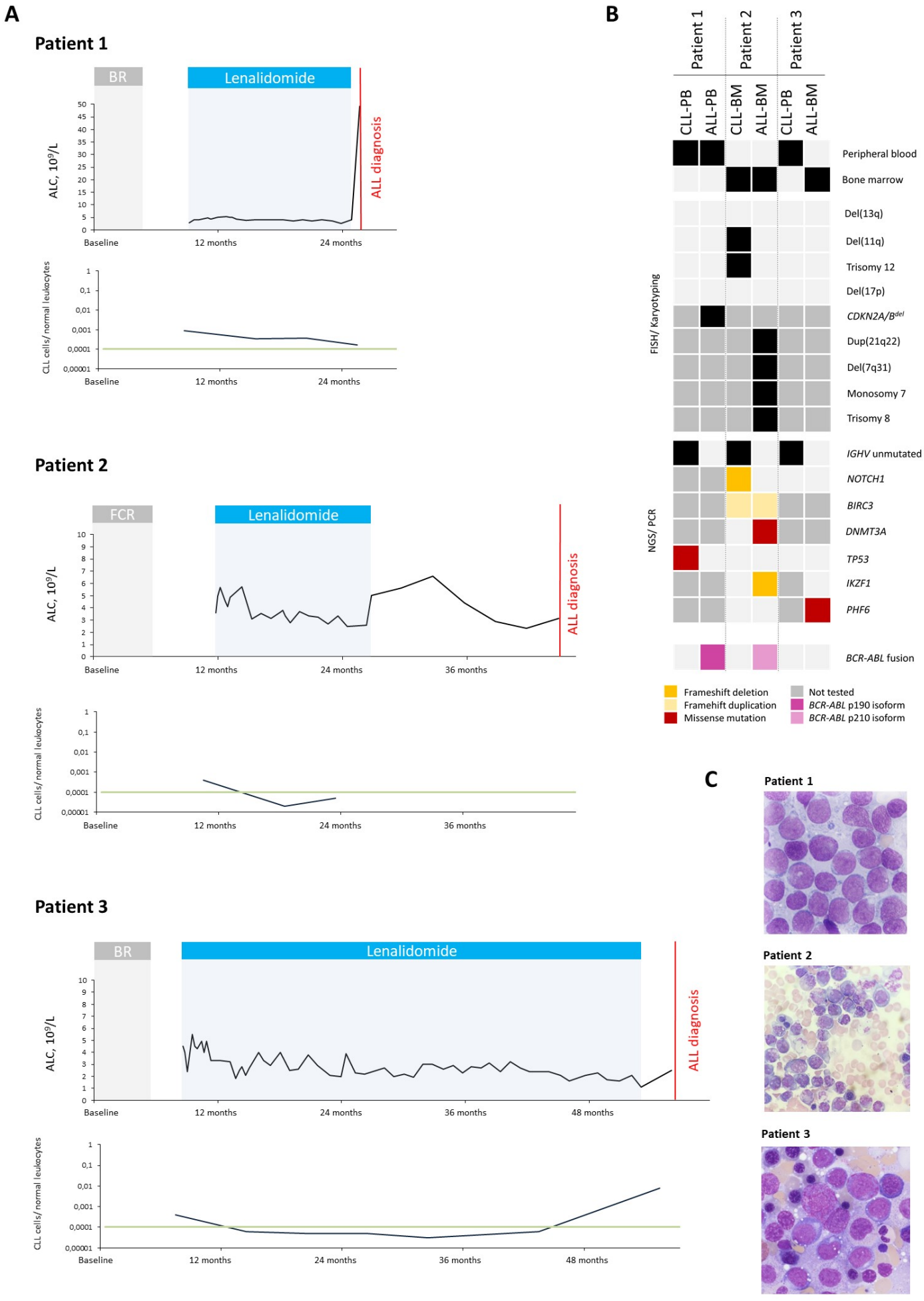
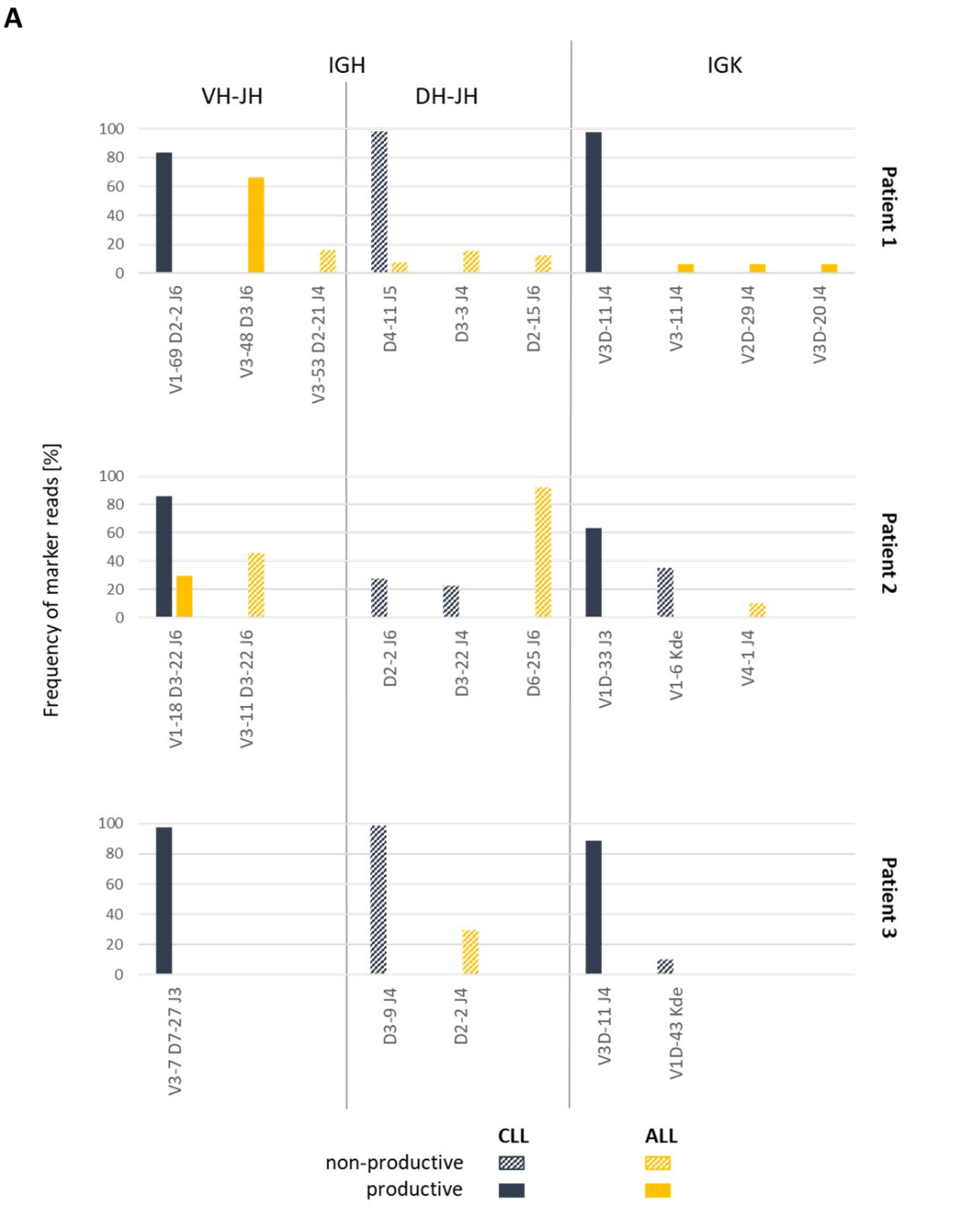


Figure 2



B

Patient 2
Mutual productive IGH rearrangement

Clonotype
VJ:Vh-(Dh)-Jh V1-18 /41/ J6 productive

Junction amino acid sequence
CARDRGLWTFYDSSESFYYYYYGMDVW

Junction nucleotide sequence
TGTGCGAGAGATCGAGGTCTGTGGACCT
TCTATGATAGTAGTGAGTCATTCTATTACTA
CTACTACTACGGTATGGACGTCTGG