TAKE LIFE ON

REFIXIA® ONCE-WEEKLY

prophylaxis[1] giving your patients the confidence to live beyond haemophilia B[2–5]

27%

REFIXIA® ALLOWS FOR MORE AMBITIOUS PROPHYLAXIS, IN LINE WITH THE WFH GUIDELINES:[6]

mean trough FIX levels in adolescents and adults[4]

100%

REFIXIA® PROPHYLAXIS ALLOWS FOR AN ALMOST BLEED-FREE LIFE[4,7–10]

target joint declassification[3] with 0 median annualised spontaneous bleeds[3–5]

50%

REAL-WORLD EVIDENCE DEMONSTRATES

reduction in the mean-intra patient ABR after patients switched from SHL rFIX to Refixia®*,**[11,12]

Leopoldo, 61 years old, is an IT engineer and loves spending time sailing. Leopoldo lives with haemophilia B.

*The CBDR collects real-world data for patients with haemophilia.[11,12] Based on a retrospective study on CBDR patients with haemophilia B receiving prophylactic Refixia® for at least 6 months after switching from either rFIXFc or rFIX. Data set contains 5 patients under the age of 18[12]
**Significant reduction using a binomial regression model[12]

ABR, annualised bleed rate; CBDR, Canadian Bleeding Disorders Registry; FIX, factor IX; rFIX, recombinant factor IX; rFIXFc, recombinant factor IX-Fc fusion protein; SHL, standard half-life; WFH, World Federation of Hemophilia

1. Refixia® Summary of Product Characteristics, 2022. 2. Tiede A et al. *Haemophilia* 2017; 23:547–555. 3. Negrier C et al. *Haemophilia* 2016; 22:507–513. 4. Collins PW et al. *Blood* 2014; 124:3880–3886. 5. Young G et al. *Thromb Res* 2016; 141:69–76. 6. Srivastava A et al. WFH Guidelines for the Management of Hemophilia, 3rd edition. *Haemophilia* 2020; 26(Suppl 6):1–158. 7. Collins PW. *Haemophilia* 2012; 18:131–135. 8. Oldenburg J. *Blood* 2015; 125:2038–2044. 9. van Keep M et al. *J Comp Eff Res* 2018; 7:39–48. 10. Jiménez-Yuste V et al. *Blood Transfus* 2014; 12:314–319. 11. Lee A et al. *Blood* 2020; 136:37–38. 12. Matino D et al. *Res Pract Thromb Haemost* 2022; 6:e12661.

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Prescribing Information

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A refined approach to detect and measure minimal residual disease in childhood acute myeloid leukemia by flow cytometry

Event-free survival for children with acute myeloid leukemia (AML) ranges between 40% and 60% [1,2]. Risk stratification of patients with AML mainly relies on cytogenetic/genetic classification and morphological response to induction therapy. However, assessment of response to chemotherapy by conventional morphology is subjective and lacks sensitivity. Thus, high-resolution methods such as real-time quantitative polymerase chain reaction (RQ-PCR) and flow cytometry (FCM) have been recently proposed to detect minimal residual disease (MRD) [3]. Notwithstanding its high sensitivity, RQ-PCR can be used only in a fraction of patients and cannot precisely calculate MRD because the amount of PCR product does not accurately reflect cell number [3]. MRD quantification by FCM has a significant prognostic value in predicting both risk of relapse and survival [4]. Recently, FCM has been successfully used for the final risk assignment determining the timing and the intensity of therapy in childhood AML [2]. The main limitation of MRD monitoring by FCM is the requirement of highly specialized skills and experience to distinguish MRD among the different populations arising in the bone marrow in the reconstitution phase following chemotherapy or transplantation [3,5,6]. Limited information is available on FCM characterization of myeloid developmental stages during normal hematopoiesis [5,6]. This knowledge is indispensable to discriminate MRD by FCM in AML [3,6].

Here, we describe a simple and most effective approach to detect MRD in AML, based on the characterization and distinction of normal precursors from leukemia cells.

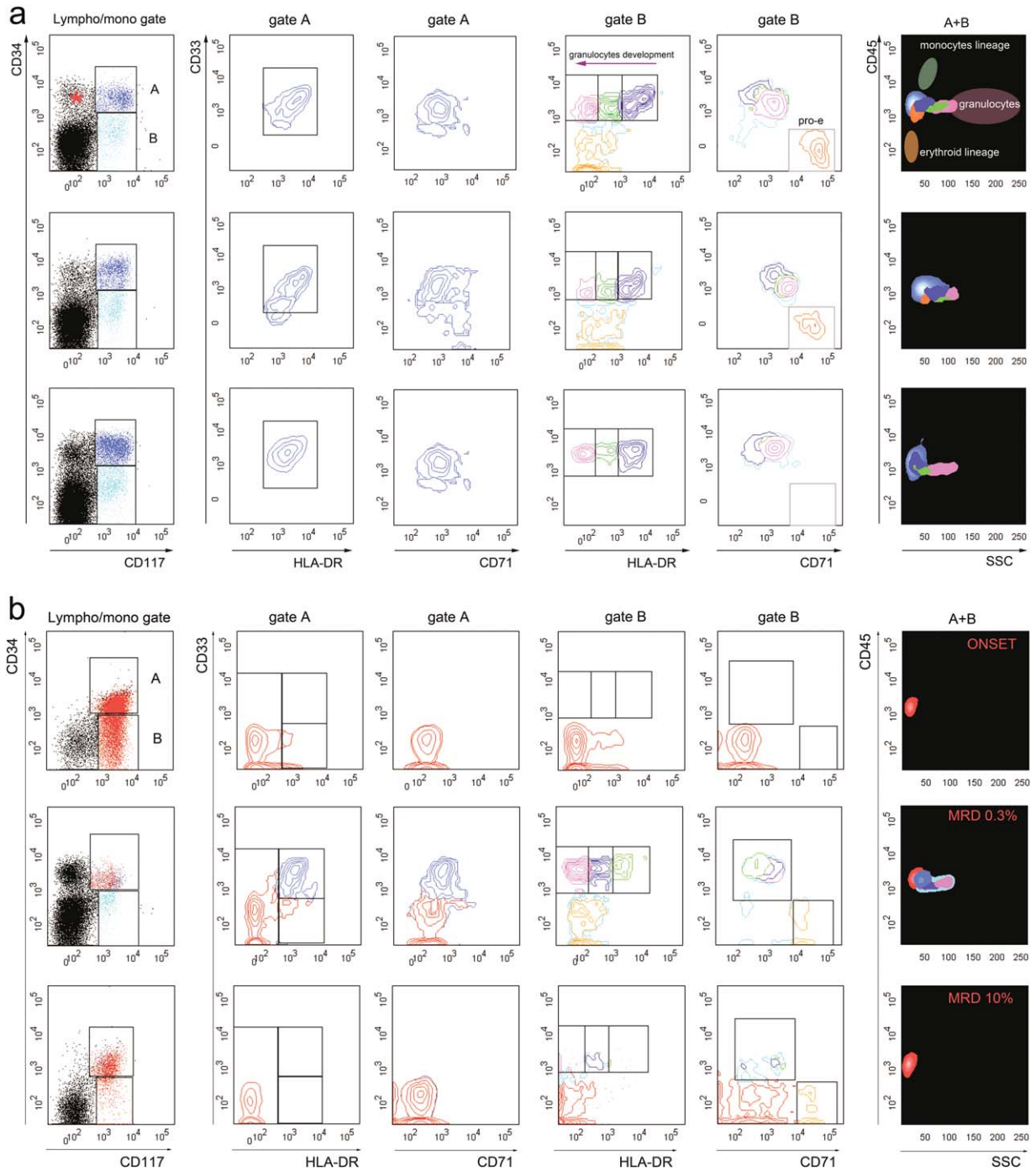

Figure 1. (a) Phenotypical characterization of myeloid precursors in bone marrows from healthy child (upper panels), steroid-treated child (middle panels) and infant with anemia (lower panels). (b) Onset phenotype (upper panels) and example of MRD detection in AML patient (middle and lower panels).

Figure 1 illustrates a prototypical characterization of the phenotype of myeloid precursors using a 6-color staining with antibodies anti-CD33, CD34, CD117, CD71, CD45, and HLA-DR. In Fig. 1a, we show a healthy child bone marrow (upper panels), a steroid-treated child with rheumatic disease (middle panels) and a 1 month-old infant with anemia associated to absence of bone marrow erythroid progenitors (lower panels). Monocyte-myeloid precursors reside in the $CD34^{pos}CD117^{pos}$ population (blue cells, gate A) whereas committed precursors of the granulocytes lineage and erythroid lineage are identified as $CD34^{dull}CD117^{pos}$ cells (light blue cells, gate B). The $CD34^{pos}CD117^{neg}$ population (asterisk) corresponds to $CD19^{pos}$ pro-B cells (data not shown). Using our combination of markers, cells included in gate B can be dissected into three $CD33^{pos}$ myeloid precursor stages, based on the progressively down-regulation of the HLA-DR component (dark blue, green and pink cells). The location of these three stages within the CD45-side scatter plot (right columns, dark blue, green, and pink areas), showing the increasing intracellular complexity, confirms their maturation toward granulocytes. The pro-erythroblast (pro-e) are $CD33^{neg}HLA\text{-}DR^{neg}$ and can be easily identified by their high levels of CD71 (gate B, orange cells).

Figure 1b shows an example of childhood AML followed from onset until relapse, first detected 7 months after bone marrow transplantation (MRD 0.3% of live cells gate) and confirmed 2 months later by relapse (10%). The immunophenotype of blast cells (red cells) at onset is showed in the upper panels. Leukemia cells closely resembled normal precursors based on the expression of CD34 and CD117 (first plot in the left column) but lacked CD33, HLA-DR, and CD71 (upper plots panel). Cells with this phenotype do not exist during normal development (compare with examples shown in Fig. 1a). In the middle panels, blast cells (red cells) appear among the normal precursors in the reconstituting bone marrow 7 months after transplantation (MRD 0.3%). Leukemic blasts increased to 10% in bone marrow taken two months later, when normal precursors had become undetectable. Thus, our approach allows identification and precise quantification of rare leukemia cells based on their distinction from normal precursors. This method also allows to detect and track leukemia cells when information on leukemia-associated phenotype (LAP) at onset is unavailable and when leukemia cells at relapse are not identical to the previously defined LAP. Further studies are necessary to evaluate whether our method to detecting and measuring MRD in AML can be used to improve patient stratification and assign them to personalized treatment protocols.

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References

1. Pession A, Masetti R, Rizzari C, et al. Results of the AIEOP AML 2002/01 multicenter prospective trial for the treatment of children with acute myeloid leukemia. Blood 2013;122:170–178.
2. Rubnitz JE, Inaba H, Dahl G, et al. Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: Results of the AML02 multicentre trial. Lancet Oncol 2010;11:543–552.
3. Coustan-Smith E, Campana D. Should evaluation of minimal residual disease be routine in acute myeloid leukemia? Curr Opin Hematol 2013;20:86–92.
4. Coustan-Smith E, Ribeiro RC, Rubnitz JE, et al. Clinical significance of residual disease during treatment in childhood acute myeloid leukemia. Br J Haematol 2003;123:243–252.
5. van Lochem EG, van der Velden VHJ, Wind HK, et al. Immunophenotypic differentiation patterns of normal hematopoiesis in human bone marrow: Reference patterns for age-related changes and disease-induced shifts. Cytometry Part B 2004;60:1–13.
6. van Dongen JJM, Lhermitte L, Böttcher S, et al. EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes. Leukemia 2012;26:1908–1975.

Restoration of response to ruxolitinib upon brief withdrawal in two patients with myelofibrosis

In 2005, an activating mutation in the *Janus Kinase 2* gene (*JAK2* V617F) was found in the majority of patients with primary myelofibrosis, which led to the development of specific JAK inhibitors [1]. One of these, ruxolitinib, a selective inhibitor of JAK1 and 2, has recently been shown to improve splenomegaly, disease symptoms, and occasionally disease burden in myelofibrosis [2–4]. *JAK2* mutant clones were found to persist in the presence of chronic JAK2 kinase inhibition in mouse models [5]. This persistence was shown to occur via the *trans* activation of JAK2 by other JAK kinases rather than the emergence of a compensating *JAK2* mutation or an activating mutation in a pathway component downstream. Importantly, this meant that the observed persistence was reversible upon temporary withdrawal of the JAK2 inhibitor, at least in vitro [5]. This reversibility suggests that patients who develop resistance to JAK2 inhibitors may also become resensitized upon temporary withdrawal of treatment [6].

Here, we describe two case studies that address this hypothesis. The first patient was a 59-year-old female, diagnosed with primary myelofibrosis. Ruxolitinib treatment was initiated with an oral 20 mg dose bid (Fig. 1A). At study entry, the patient had palpable splenomegaly of 19 cm below the costal margin (cm b.c.m, Fig. 1B). After 3 weeks of treatment, an excellent response was observed in terms of a reduction in spleen volume to 8.5 cm b.c.m. (Fig. 1B), and the loss of all constitutional symptoms as well as a 2 kg weight gain. After 6 weeks, the dose was reduced to 10 mg bid due to a decrease in platelet levels to 81×10^9/l and an increase in transfusion frequency caused by the presence of transfusion-dependent anemia which led to an increase of the spleen size (Fig. 1A–C). After continuous treatment of 15 mg bid for around 15 months, the spleen had again increased in size to 20 cm b.c.m, (Fig. 1A,B), and constitutional symptoms started to reappear. To counteract this, the dose was raised to 20 mg bid (Fig. 1A). However the subsequent response was marginal and after monitoring over a further 16 months of treatment, the patient was eventually classified as a non-responder.

To prepare for an alternative therapeutic strategy, ruxolitinib treatment was gradually withdrawn over a period of two weeks (Fig. 1A, Supporting Information Appendix Fig. 2). However, this resulted in a dramatic reappearance of more severe constitutional symptoms, as well as an increase in leukocyte and platelet counts, lactate dehydrogenase (LDH) levels, and a spleen size of 25 cm b.c.m (Fig. 1B,C, Supporting Information Appendix Fig. 2). Therefore, after 2 days without treatment, and due to the unavailability of the alternative therapy, ruxolitinib treatment was resumed at a reduced dose of 5 mg bid (Fig. 1A–D, Supporting Information Appendix Fig. 2). Unexpectedly, even with this low dose there was an impressive response, particularly of spleen size and LDH levels. Within 11 days, spleen size was reduced to 14 cm b.c.m. There was also an increase in haemoglobin levels and a decrease in transfusion dependency (Fig. 1B,D, Supporting Information Appendix Fig. 2). These effects lasted around 8 weeks.

We also observed similar albeit less pronounced results with a second patient. This patient, a 73-year-old female diagnosed with post-polycythemia vera myelofibrosis, had palpable splenomegaly (15 cm b.c.m) and excessive constitutional symptoms. The patient was treated with a 15 mg bid dose of ruxolitinib and after two months the spleen had reduced to 6 cm b.c.m, and constitutional symptoms had improved. Two months later, hemoglobin levels had decreased to 8.6 g dl^{-1} and the spleen increased to 11 cm b.c.m. Upon a further increase in spleen size to 16.5 cm b.c.m, the patient was classified as a non-responder and ruxolitinib treatment was withdrawn. Again, upon reintroduction of ruxolitinib, the patient regained response, albeit to a lesser extent than previously seen. Spleen size decreased from 16.5 to 13.0 cm b.c.m, and hemoglobin levels increased from 9.2 to 12.8 g/dl.

In conclusion, two patients who initially responded to ruxolitinib but developed resistance regained response upon temporary ruxolitinib withdrawal. This supports previous results in vitro, and demonstrates that intermittent ruxolitinib treatment, at least for some patients, may lead to an enhanced therapeutic response.

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