Response-adjusted regimen combining ruxolitinib, etoposide and dexamethasone (adRED) in adult patients with acute myeloid leukemia-associated hemophagocytic lymphohistiocytosis: a single-center pilot trial

Secondary malignant hemophagocytic lymphohistiocytosis (mal-HLH) can be diagnosed in up to 10% of patients with acute myeloid leukemia (AML) undergoing intensive chemotherapy (IC).[1,2] The mechanisms implicated in AML-associated HLH are unknown but other HLH subgroups are characterized by a large spectrum of cytokine-driven immune disorders such as interferon-γ (IFN-γ), interleukin (IL)-6, IL-10, IL-1β, and tumor necrosis factor-α (TNF-α).[3] Mal-HLH has the worst prognosis of all HLH subgroups[4] with a median survival of less than 2 months.[5] Current recommendations for mal-HLH advocate the use of corticosteroids as first-line treatment with the addition of dose-adjusted etoposide (ETP) (50-150 mg/m^2) in highly active HLH or in severe cases.[5-7] The heterogeneity of mal-HLH prohibits a "one-size-fits-all protocol".[6] Recently, the use of therapies to attenuate hypercytokinemia has been reported with an encouraging clinical response obtained with other HLH subgroups.[8-10] Ruxolitinib (RXT) has not yet been investigated as frontline therapy for AML-associated HLH, but some studies suggest its effectiveness in a murine model of HLH,[11] in adults with non-malignant secondary HLH[12] and for patients with lymphoma-associated hemophagocytic syndrome (LAHS) in combination with doxorubicin, ETP and dexamethasone (R-DED).[13]

We retrospectively investigated the cytokine profile of AML-associated HLH at diagnosis and during treatment, tested the efficacy and tolerability of a dose-adjusted ruxolitinib, ETP and dexamethasone (DEX) (adRED) regimen, in conjunction with IC, as frontline therapy for patients with newly diagnosed AML-associated HLH and investigated the potential use of RXT as an ETP/DEX-sparing strategy. A per protocol (PP) analysis was realized without deviation from the protocol to avoid underestimating the benefit in patients who did not comply with the protocol. As reported before,[10] only patients who received at least 14 days of regular RXT therapy were considered evaluable for the activity endpoints. The primary objective of this study was to assess whether the use of RXT for a short minimum duration of 2 weeks provides clinical benefit for our patients. The ethics committee of CHUV approved the study (2018-01391). Patients received a response-adjusted regimen combining ETP (50-150 mg/m^2), RXT (10 mg twice a day with dose reduction for toxicity to a minimum of 5 mg a day or augmentation to a maximum of 20 mg twice a day) and DEX (10 mg/day). Administration of ETP was twice a week for the first week, and then repeated based on the degree of cytopenia and the evolution of HLH parameters. ETP was administered at a dose between 50 and 150 mg/m^2, according to cytopenia and/or concomitant use of IC. DEX was administered at a dose of 10 mg a day and then progressively tapered according to the evolution of HLH.

Primary endpoints were the response of AML-associated HLH to treatment and assessment of the cytokine profile at HLH diagnosis. The best overall response was the best response recorded from the start of the treatment until disease progression or recurrence.[10] Secondary endpoint was early mortality analysis at 2 months from the diagnosis of HLH, safety (only grade ≥3 adverse events [AE] have been reported in accordance with the CTCAE V5.0), duration of response, changes in biological and inflammatory biomarkers between the levels at baseline and during treatment, and the possibility of undergoing an allogeneic hematopoietic stem cell transplant (alloHSCT).

Between 2017, and 2021, in a series of 160 patients with AML, 11 patients had a diagnosis of HLH. Five patients were excluded: two patients with <169 Hscore[14] points and <5 HLH-2004 criteria.[15] One patient did not receive treatment for leukemia or HLH because of age and comorbidities. Two patients received less than 2 weeks of RXT due to gastrointestinal volvulus complicated by necrosis followed by death and one due to death from septic shock and multiple strokes. We finally identified six HLH treated with adRED, four males and two females (*Online Supplementary Table S1*). The median age was 52 years (range, 34-72 years). According to the ELN 2017 risk category for AML, five patients were in the adverse risk category, and one was in the intermediate risk category. Complex karyotype was present in two patients. Two patients harbored *TP53* and two had *FLT3-ITD* mutations. HLH diagnosis was concomitant with initial AML diagnosis in two and with AML relapse in four patients. HLH diagnosis was made before initiation of IC in two and after in four patients. HLH diagnosis occurred during the first course of IC in five patients (induction regimen were respectively CLAG-IDA, CLAG, 7+3, FLAG, FLAG-IDA) and the second course of IC in one patient (7+3 followed by FLAG in the absence of a response at D21 to 7+3). All patients had at

least five of the eight HLH-2004 criteria for the diagnosis of HLH[15] and an Hscore[14] >169 (mean 247; range, 206-263). Prior to HLH treatment initiation, all patients had extensive infectious work-up including blood and urine cultures, viremia for Cytomegalovirus and Epstein-Barr virus, blood galactomannan and β-d-glucan assay, and whole body computed tomography scan. Different treatments for each patient are shown in Figure 1A. The overall response rate of HLH was 100% (6/6), with 83.4% (5/6) in CR and 16.6% (1/6) in PR. Among the patients achieving a CR, 60% (3/5) maintained a CR condition for >2 months. Interestingly, the other two patients (2/5) required only one single ETP administration to maintain CR. The 2-month early mortality rate was 33% (2/6). Two patients died of a

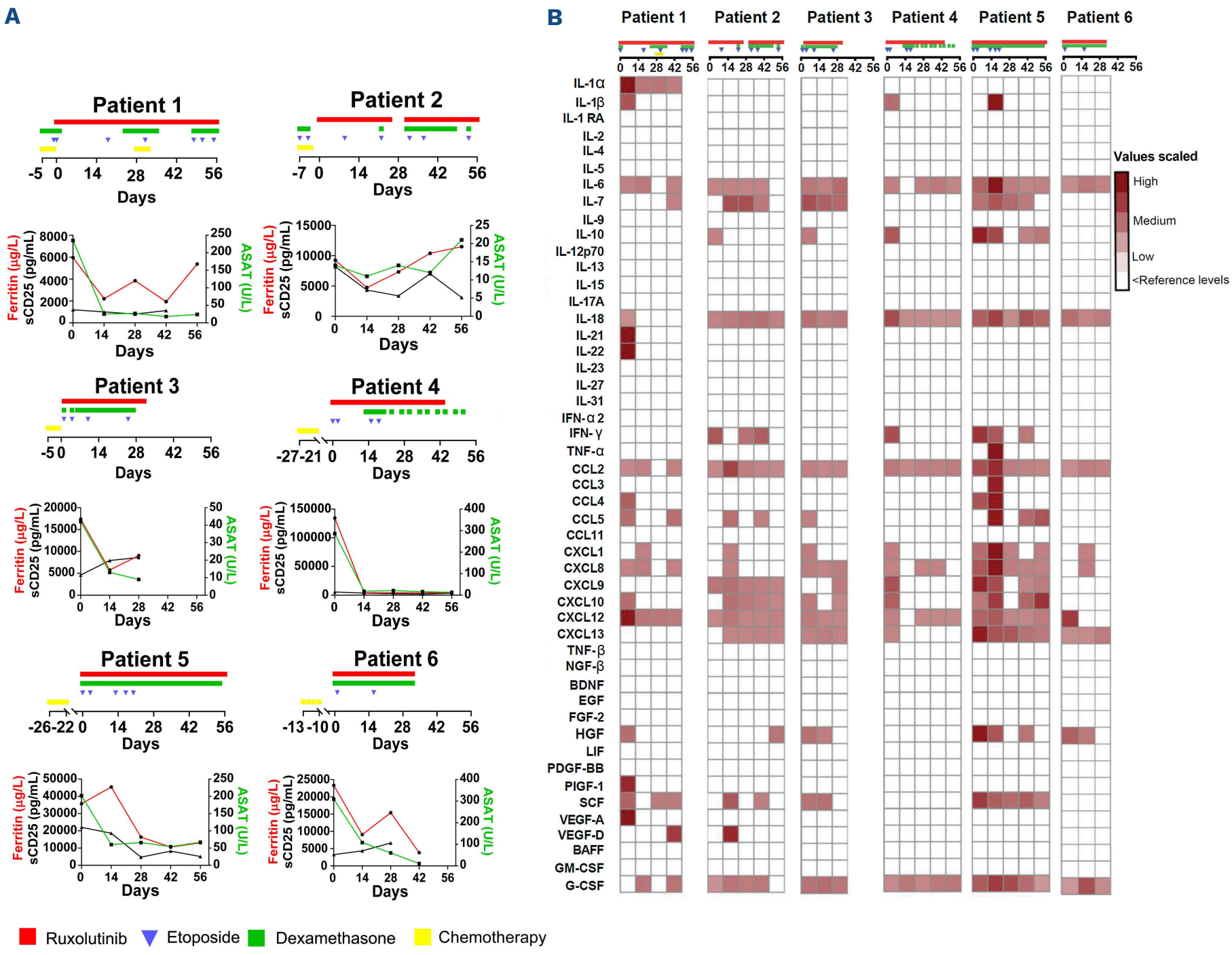

Figure 1. Treatments and studies of the different biological hemophagocytic lymphohistiocytosis parameters in patients treated with adRED. (A) Treatment timelines and changes in selected hemophagocytic lymphohistiocytosis (HLH) parameters from day 0 (HLH diagnosis) and then every 2 weeks for 8 weeks or until death. Treatment with ruxolitinib (RXT) is shown in red, dexamethasone (DEX) is shown in green, intensive chemotherapy is shown in yellow and etoposide (ETP) administration is shown in blue, ferritin (µg/L), ALAT (U/L), sCD25 (pg/mL). (B) Assessment of cytokines from day 0 (HLH diagnosis) and then every 2 weeks for 8 weeks or until death. Heatmap of scaled picogram per microliter values. Columns (i.e., cytokines) are scaled to facilitate the comparison of the detected levels of the cytokines, which are color-coded from white to red (low to high levels of cytokines detected). The treatment timeline is described for each patient. (C) Correlation network analysis of the plasma cytokine and biological parameter concentrations. Color scale (blue-to-red) and thickness of lines connecting variables represent Pearson's correlations. adRED: dose-adjusted ruxolitinib, ETP and DEX.

cause other than their HLH. The time to the best response to HLH was 1 week for all patients (Table 1). Concerning AML, five of six patients could be put into remission and three patients could undergo an alloHSCT.

The evolution of classic biological parameters (ferritin, ASAT, sCD25) and cytokines over time is shown in Figure 1. As all patients received IC, cytopenia parameters were not reliable tools for following patients. The liver function, coagulation (D-dimers and fibrinogen), classic inflammatory parameters (CRP, sCD25 and ferritin) and cytokines were changed favorably quickly within the first days following the adRED regimen.

At diagnosis, the majority of patients exhibited high proinflammatory cytokines levels of IL-6, IL-18, CXCL2, CXCL9, CXCL12, CXCL13, and IFN-γ (Figure 1B). We noticed a cytokine profile characterized by a dominant TH1 cytokine signature with the concomitant exhibition of multiple upstream and downstream HLH-related cytokines with no increase in cytokines or chemokines associated with a TH2 response. In responding patients, there was a decrease in proinflammatory cytokine IL-6-, IL-18-, IFN-γ- and IFN-γ-induced chemokines. When assessing the linear relationship between classic parameters and cytokine levels, we observed a significant positive correlation between ferritin and IL-8 (r=0.79, P=0.000482), CXCL10 (r=0.65, P=0.000798), CXCL13 (r=0.66, P=0.03842), CCL4 (r=0.58, P=0.000976) and sCD25 (r=0.69, P=0.000269). In addition, we observed a significantly lower level of CCL4 in the absence of splenomegaly (P=0.00106) or hepatomegaly (P=0.0107) (*Online Supplementary Figure S2*).

In order to better characterize the network of biological parameters and the inflammatory cytokine/chemokine-receptor network in AML-related HLH, we performed correlation network analysis. This analysis further supported the strong positive correlation between CXCL1, CXCL12, CXCL13, CCL2, CCL3, CCL4, CCL5, CXCL8 (IL-8), CXCL9, IL2R (sCD25), IL-6, IL-10, and IL-18, together with CRP, bilirubin, LDH, ferritin and ALAT. Strong negative correlations were observed between G-CSF and neutrophils and leucocytes and between neutrophils and IL-7 and CCL2. Notably, the cytokines IL-6, IL10, CXCL13, CXCL1, CCL3, and CCL4 are located at the center of an intercorrelated cytokine-inflammatory marker network with CRP, LDH and bilirubin. Our comprehensive correlation network analyses showed that IL-6 was located at the center of a cytokine network with IL-8, CXCL1 and CCL4 (Figure 1C).

We performed a *post hoc* analysis to compare patients treated with RXT with that of a historical control group (n=5) treated with ETP/DEX without ruxolitinib. In the five historical control group patients, induction regimen were respectively CLAG, 7+3, 7+3, FLAG, FLAG. The 2-month early mortality rate was similar at 40% (2/5 patients) for the two groups (Table 1).

In the RXT group, the median cumulative dose of ETP and DEX was significantly lower (287.5 mg/m^2 *vs.* 750 mg/m^2, P=0.0733, and 379 mg *vs.* 725 mg, P=0.0267) than that in the historical control group (Figure 2A). Regarding the improvement of classic parameters, the fold-decrease overtime at days 7 and 14 of liver function tests, lactate dehydrogenase (LDH) (*data not shown*), ferritin, D-dimers (*data not shown*) and triglycerides was quickly favorable in the RXT group compared with the historical control group and not altered by the use of less ETP and DEX (Figure 2B). The differences in the classic parameter between time points of interest at day 0 and day 7 showed similar kinetics over time between the two treatment groups (*Online Supplementary Figure S1*).

Like in the historical control group, the cytopenia and coagulation parameters improved over time (Figures 2B), similarly to inflammatory markers (LDH, ferritin, sCD25 and cytokines) (Figure 1 and *data not shown*). Notably, the data indicate the presence of persistent clinical and biological improvements in the RXT group despite the use of significantly lower cumulative doses of ETP and DEX. As a result, treatment with RXT led to a reduction in both DEX and ETP cumulative doses by 38.3 and 52.2%, respectively, in comparison with the levels in the historical control group (Figure 2A).

Intergroup treatment type data comparisons of low/high doses of ETP/DEX for patients receiving RXT were performed using Fisher's exact test. Notably, 100% of patients receiving high ETP and DEX doses were present in the historical control group *versus* only 33% and 17% in the RXT group, respectively (*data not shown*). The differences over time in the different parameter exhibited similar kinetics between the two low/high doses of ETP/DEX treatment groups (*data not shown*). Taken together, the data suggest that RXT could be an efficient ETP/DEX-sparing therapy.

RXT was well tolerated. The duration of aplasia and infectious episodes are summarized in (Table 1). In the adRED group, the tendency to infectious episodes (grade >3) was less frequent (20 *vs.* 26), and the duration of aplasia war shorter with a mean duration of 33 and 29.5 days between the start of HLH treatment and a thrombocyte count ≥ 50 G/L and a neutrophil count ≥ 0.5 G/L respectively *versus* 48 and 36 days in the historical control group, but the difference was not significant.

This pilot study has a number of limitations, principally the retrospective aspect of this trial, small cohort size and monocentric design, the possible variability in physician judgment for starting ETP and DEX tapering and posology adaptation and some variability in chemotherapy regimen. Diagnostic criteria for HLH (HLH-2004 criteria, HScore) have been developed in cohorts that do not include HLH related to acute myeloid leukemia. Furthermore, the monitoring of cytopenias as parameters of diagnosis and response to treatment does not seem to be optimal in this particular population with bone marrow failure as-

Table 1. Hemophagocytic lymphohistiocytosis-directed treatment and outcomes.

	P1	P2	P3	P4	P5	P6	C1	C2	C3	C4	C5
Ruxolitinib, number of days/cumulative dose in mg	100/4,000	64/765	108/970	31/320	122/840	32/770	-	-	-	-	-
Etoposide, number of administration/cumulative dose in mg/m^2	8/800	9/925	5/250	4/325	4/200	2/150	8/1200	6/750	12/1,200	4/400	4/600
Dexamethasone, number of days/cumulative dose in mg	72/710	25/180	59/500	25/180	52/550	35/258	48/860	63/725	127/1,132	51/580	76/573
Follow-up after HLH diagnosis, days	114	252	40	125	576	46	48	1574	130	51	178
HLH response											
Best response	CR	CR	PR	CR	CR	CR	PR	CR	CR	PR	CR
Time to best response, day	7	7	7	7	7	7	7	28	14	42	28
Best response duration, day	114	14	28	125	14	46	48	1574	130	51	178
Leukemia response											
Leukemia remission	yes	yes	no	yes	yes	yes	no	yes	no	yes	no
Access to alloHSCT afterwards	yes	yes	no	no	yes	no	no	yes	no	no	no
Leukemia remission duration, day	114#	252	-	69	576	46	-	1574	-	51	-
Outcome at end of follow-up (0=Dead, 1=Alive)	0†	1	0‡	0‡	1	0◆	0‡	1	0‡	1	0‡
Aplasia*, day											
Thrombocyte >=50x10^9/L	33						48				
Thrombocyte >=100x10^9/L	43						48				
Neutrophil >=0.5x10^9/L	29.5						36				
Neutrophil >=1x10^9/L	31						36				
Infections	20						26				
Grade 3	16						24				
Grade 4	4						2				
Bacterial	12						16				
Fungal	2						3				
Viral	2						1				
Germ not identified	4						6				

HLH: hemophagocytic lymphohistiocytosis; P: patient; C: control; alloHSCT: allogeneic hematopoietic stem cell transplantation. Complete response (CR): no fever AND no organomegaly + ASAT diminution of at least 50% AND/OR ferritin diminution of at least 50%. Partial response (PR): no fever OR no organomegaly + ASAT diminution at least 25% AND/OR ferritin diminution at least 25%. Progressive disease (PD): fever and organomegaly + ASAT diminution <25% OR ferritin diminution <25%. Stable disease (SD): all other situations.*Days between the start of HLH treatment and an improvement in the blood count. †Transplant-related mortality. ‡ Leukemia progression.◆ Bleeding after transbronchial biopsy. #Death secondary to treatment-related mortality but still in leukemia remission.

sociated to leukemia and/or secondary to treatment. This highlights the need for new diagnostic and monitoring parameters. Accumulating studies suggest that inflammation may play an important role in many aspects of AML such as disease progression, chemoresistance, and myelosuppression.[16] Furthermore, the use of cytokine assessment may be a promising field of exploration in the light of evidences reporting that some cytokines have favorable prognostic factors for survival in AML patients such as low levels of IL-6 and high levels of IL-10.[17] These results suggest that cytokine signatures may be reliable predictive biomarker for clinical evolution in AML patients. Con-

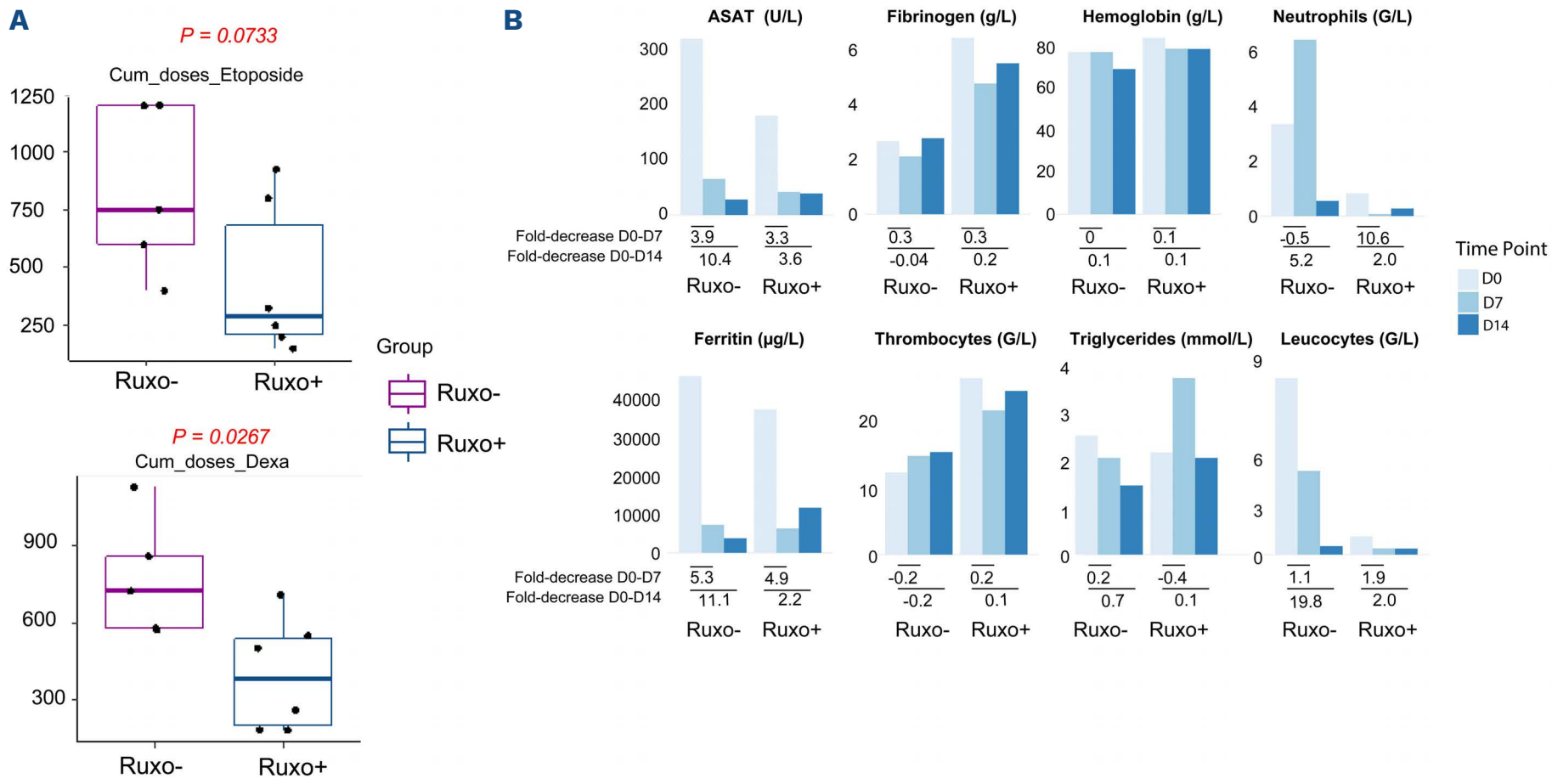

Figure 2. Comparison of cumulative doses of etoposides, dexamethasone and hemophagocytic lymphohistiocytosis parameter evolution between the adRED population and a historical control population. (A) Median cumulative (cum) dose of etoposide (ETP) and dexamethasone (DEX) used in adRED and historical control patients. (B) Fold-decrease in levels of classic parameters at day 7 and day 14 in adRED-treated and historical control patients. adRED: dose-adjusted ruxolitinib, ETP and DEX.

sequently, a longitudinal assessment of the cytokines repertoire in AML patients without HLH need to be explored and identified throughout the treatment and across AML subgroups.

In conclusion, this pilot study shows for the first time that the addition of RXT seems to be safe and an interesting therapeutic option for AML-associated HLH that could reduce the administered doses of DEX and ETP. These preliminary data need to be confirmed prospectively in larger populations with multi-center studies.

Authors

Grégoire Stalder,[1,2,3] Madeleine Suffiotti,[4] Amandine Segot,[1] Alessandra Noto,[4] Giuseppe Pantaleo,[3,4] Olivier Spertini[1,3] and Michel Obeid[3,4]

[1]Service and Central Laboratory of Hematology, Lausanne University Hospital, Lausanne; [2]Service of Hematology and Laboratory of Hematology, Institut Central des Hôpitaux, Hôpital du Valais, Sion and [3]University of Lausanne, Lausanne and [4]Division of Immunology and Allergy, Lausanne University Hospital, Lausanne, Switzerland

Correspondence:
M. OBEID - michel.obeid@chuv.ch

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Disclosures

No conflicts of interest to disclose.

Contributions

MO and OS conceived the study idea. GS, OS and MO had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. GS, MS, AN and MO prepared the figures and tables. GS, AS and MO collected the data. MS and AN did the statistical analyses. GS and MO wrote and prepared the manuscript. All authors participated in discussions and critically read the manuscript.

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Data-sharing statement

Requests for access to research data should be sent to the corresponding author.

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