Interleukin-1α Administered After Autologous Transplantation: A Phase I/II Clinical Trial

By Daniel Weisdorf, Emmanuel Katsanis, Catherine Verfaillie, Norma K.C. Ramsay, Robert Haake, Leslie Garrison, and Bruce R. Blazar

Interleukin-1α (IL-1α) can act as both a hematopoietic growth factor and a stimulant of cellular and humoral immune responses. To promote acceleration of hematologic recovery and induce immune antitumor activity, we initiated a phase I/II dose escalation trial of 6-hour daily infusions of recombinant human IL-1α after autologous transplantation. Forty patients with Hodgkin's disease (n = 9) and non-Hodgkin's lymphoma (n = 31) transplanted with unmobilized autologous peripheral blood stem cells or bone marrow stem cells received daily 6-hour infusions of IL-1α (day 0 to day +13) at daily doses between 0.1 to 10 µg/m²/d; 7 patients received only 7 planned days of IL-1α (day 0 through day 6). Most patients received all 14 days of therapy, although 5 patients discontinued treatment early (after 1 to 6 doses) because of fever and severe chills. Toxicity included IL-1α-related fever that showed extremely diverse effects throughout the immune system, protected against radiation injury, and augmented antimitotic activity against human tumors, and can accelerate hematologic recovery and induce immune antitumor activity. We initiated a phase I/II dose escalation trial of daily infusions of IL-1α at 1.0 µg/m²/d for only 7 days (day 0 through day +6). Three to five evaluable patients were planned to be enrolled at each IL-1α dose level including both PBSC and BMSC recipients, and 14 patients were enrolled at the maximally tolerated dose to assess clinical efficacy. IL-1α dosage was administered at 0.1, 0.3, 1.0, 3.0, and 10 µg/m²/d. Subsequently, to test the value of a more brief IL-1α course, a second cohort of patients was designated to receive IL-1α at 1.0 µg/m²/d for only 7 days (day 0 through day +6). Fourteen immediately preceding, consecutive patients receiving no IL-1α (7 with BMSC and 7 with PBSC) were evaluated as control patients.

Eligible patients were those undergoing autologous transplantation for non-Hodgkin's lymphoma and Hodgkin's disease. Patients with BM in morphologic remission and adequate hematopoietic function were participating in a trial comparing unmobilized BMSC to unmobilized apheresis-collected PBSC. Patients with persisting BM tumor (but transplant eligible) were administered BMSC. The clinical characteristics of patients are shown in Table 1. All patients and controls had been treated with one or more alkylator-based chemotherapy regimens. BMSC collections included bilateral iliac crest aspirate harvests containing at least 2 × 10⁸ nucleated cells/kg. BMSC collections were performed by 4 to 6 apheresis runs (3 to 4 hours each processing 10 to 12 L of blood) using a Fenwal CS3000 stem cell processor running a modification of program 1 to enhance stem cell collections. A minimum of 3.0 and a target of 6.0 × 10⁶ nucleated cells/kg were collected over 8 to 12 days, usually Monday, Wednesday, and Friday of 2 successive weeks.

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Patients were conditioned for transplantation using cyclophosphamide (60 mg/kg × 2 doses) followed by fractionated total body radiation (165 cGy twice a day for 4 days, totaling 1,320 cGy) or cyclophosphamide (1.5 mg/m²), carmustine (300 mg/m²), and etoposide (500 mg/m²) as described. Specific infusion-associated toxicities were prospectively recorded by the physicians and nurses attending each patient during the infusions.

Supportive care practices for BM transplant recipients at our institution have been described and included single rooms on the University of Minnesota Hospital Bone Marrow Transplant Unit (Minneapolis, MN). Protective, reverse isolation techniques were provided using point-of-use high efficiency particulate air filtration, frequent room air exchanges, positive room air pressure, and strict adherence to hand-washing before entry. Prophylactic antibiotics included trimethoprim-sulfamethoxazole, nystatin or fluconazole, and antiviral suppressive therapy (with acyclovir ± intravenous IgG) depending on the patients' serostatus for herpes simplex and cytomegalovirus. Fever greater than 101°F was investigated promptly and treated with broad-spectrum empiric antibiotic therapy (usually vancomycin, pipercillin, and tobramycin). After 72 hours, persistent, culture-negative fever was treated with empiric amphotericin B, especially in patients colonized with yeast or patients with sinus or pulmonary findings.

Hematopoietic progenitor cells in BMSC or PBSC collections were assessed in methylcellulose cultures (colony-forming units [BFU-E]) using standard methods. In addition, long-term BM culture-initiating cells (LTBMC-IC) were assessed in stroma-dependent cultures as previously described.

Cytokine levels (IL-1α, granulocyte-macrophage colony-stimulating factor [GM-CSF], granulocyte-CSF [G-CSF], IL-6, tumor necrosis factor [TNF], and interferon-γ [IFNγ]) were assessed in heparinized plasma and/or serum collected at varying times during infusion of IL-1α and after transplantation. Heparinized plasma and serum samples were collected on ice, separated from red blood cells (RBCs), frozen promptly, and stored at −20°C until assayed. The samples were used to measure IFNγ (Endogen, Boston MA), TNF, IL-6, G-CSF, and GM-CSF (all from R & D Systems, Minneapolis, MN). The serum was also used to measure IL-1α levels with an enzyme-linked immunosorbent assay (ELISA) system. The sensitivities for each assay system are as follows: TNF, 7.5 pg/mL; IFNγ, 5 pg/mL; IL-6, 3.35 pg/mL; G-CSF, 10.9 pg/mL; GM-CSF, 5 pg/mL; IL-1α, 0.5 pg/mL.

For the IL-1α assay a monoclonal antibody (supplied by Immunex) directed against recombinant human IL-1α was diluted to 5 μg/mL in phosphate-buffered saline (PBS). A total of 100 μL of this solution was added to a 96-well Nunc Maxisorp Immunoplate (Nunc, Naperville, IL), covered with an acetate plate sealer, and incubated overnight at 2 to 8°C. The wells were emptied by inversion, and 250 μL of PBS with 5% nonfat dry milk (Carnation, Glendale, CA) was added to each well to block any unbound sites. The plate was covered and incubated at room temperature for 1 hour. The wells were emptied and washed 4 times with PBS with 0.05% Tween-20 (Sigma, St Louis, MO). The plate was then blotted on paper towels until dry. Recombinant human IL-1α was diluted to 100 pg/mL in PBS with 10% goat serum (GIBCO/BRL, Grand Island, NY). The standard was aliquoted in a series of 1:2 dilutions in PBS/10% goat serum, and each dilution was added to the plate at 100 μL/well. The samples were also diluted 1:5 in PBS/10% goat serum and added at 100 μL/well to the plate. The plate was covered and incubated at 37°C for 1 hour. After the incubation step, the contents were washed 4 times and blotted. A polyclonal antibody directed against recombinant human IL-1α was added to the wells at 100 μL/well. The plate was covered and incubated at room temperature for 1 hour. The plate was then washed 4 times and blotted dry. Goat antirabbit horseradish peroxidase (Immunex) was added at 100 μL/well. The plate was covered and incubated at room temperature for 1 hour. The wells were washed 4 times and blotted. A total of 100 μL of single-component tetramethylbenzidine substrate (TMB; Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added to each well. The plate was incubated at room temperature for 20 to 30 minutes to allow full color development. The reaction was stopped with 100 μL of 1 N sulfuric acid, and the plate was read within 15 minutes at 450 nm.

Statistical comparisons of time-to-engraftment and time-to-discharge were compared by the log rank test statistic. Multivariate analysis was performed using a Cox model. Hospital charges were compared by the method of Kaplan and Meier using the log rank test statistic. Multivariate analysis was performed using a Cox model. Comparison of hospital charges was assessed by nonparametric, rank sum statistics, and the association of specific clinical toxicities with IL-1α therapy was tested using χ² analysis. Cytokine levels were compared using the Student’s unpaired t test and nonparametric, rank sum statistics to assess dose-response relationships.

The clinical protocol and patient treatment were initiated only after review and approval by the Food and Drug Administration and the University of Minnesota Committee on the Use of Human Subjects in Research. All patients exercised written informed consent before enrollment.

RESULTS

Clinical toxicity of IL-1α infusion. Infusion of IL-1α was regularly accompanied by fever and chills. Although
patients enrolled at doses between 0.1 to 3.0 μg/m²/d received IL-1α infusions for a median of 14 of 14 planned days (Table 2). 5 patients chose to discontinue therapy early (after 1 to 6 doses) because of uncomfortable fever and chills. As institutional experience with IL-1α therapy continued, anticipation of these fever and chill toxicities led to improved nursing administration of adjunct medications to minimize these toxic symptoms. Medications included acetaminophen to blunt or abort fever and meperidine administered prophylactically or promptly to abate chills; these medications are similar to those used in the adjunctive care accompanying amphotericin therapy. This management expertise allowed completion of therapy for nearly all patients at the higher doses. Fever greater than 101°F associated with the IL-1α infusion was common and developed between 2 and 6 hours into the 6-hour infusion. However, fever was not universal and did not appear to be dose-related. At the three higher doses of IL-1α (0.3, 1.0, and 3.0 μg/m²/d), only 10 days (median) of the 14 daily infusions were accompanied by significant fever.

The first fever during IL-1α therapy was not distinguishable from a first neutropenic fever and, therefore, was often investigated and treated with empiric antibiotic therapy. However, IL-1α fever was characteristic in its pattern and usually occurred between 2 and 6 hours into the infusion with no fever during the other 18 hours of the day. This fever pattern was not interpreted as suggestive of infection (ie, empiric amphotericin was not usually instituted).

Hypotension (systolic blood pressure less than 90 mm Hg) was common and dose-limiting. Significant, though rarely symptomatic, hypotension accompanied all IL-1α courses at 3.0 μg/m²/d, but occurred on only a few days of the 2-week infusion course. Supplemental intravenous saline was often administered to treat this modest hypotension, and only 7 of the 40 patients received low-dose dopamine. Invasive blood pressure and electrocardiographic monitoring was not required. The hypotension nadir usually occurred in the later hours of the 6-hour infusion and usually resolved by 2 hours later. Only 2 patients received 10 μg/m²/d IL-1α, and both had significant hypotension (systolic pressure of 60 and 64 mm Hg) that precluded administration of any additional IL-1α at that dose. One of these patients elected no further IL-1α therapy, and the other successfully completed 13 days of IL-1α therapy at the next lower dose level (3.0 μg/m²/d), which was the maximum tolerated dose. No other serious toxicities associated with IL-1α therapy were observed.

Other toxic effects observed during IL-1α therapy were uncommon. Of 40 patients, 10 developed transient hyperbilirubinemia (≥2.0 mg/dL); 2 developed aspartate aminotransferase levels 3 times greater than normal, and only 5 developed creatinine ≥2.0 mg/dL. One patient died at day +18 of gram-negative sepsis and adult respiratory distress syndrome. No late effects attributable to IL-1α therapy were apparent.

Hematopoietic effects of IL-1α. Infusion of recombinant IL-1α, particularly at the higher doses, was associated with accelerated recovery of neutrophils after transplantation. As shown in Table 3, patients receiving 3.0 μg/m²/d of IL-1α had significantly quicker recovery to 100 or 500 neutrophils/μL than did control patients or those receiving low-dose IL-1α (0.1, 0.3, and 1.0 μg/m²/d; P < .0001). In addition, IL-1α 3.0 μg/m²/d was associated with a stronger trend towards earlier independence from RBC transfusion (P = .06) and from platelet transfusion (P = .09). Although hematopoiesis was accelerated after IL-1α therapy when compared with that of control patients or that of those receiving low-dose IL-1α, no effects of higher dose IL-1α therapy on BM cellularity at day +14 were observed (data not shown).

Although BM cellularity was not enhanced, IL-1α therapy was associated with increased numbers of granulocytic precursors in the BM. In control and low-dose (0.1 to 1.0 μg/m²/d) IL-1α patients (n = 11), BM aspirates at day +14 after transplantation were most often markedly hypocellular and contained only low numbers of lineage-committed hematopoietic progenitors (0 to 10.5 CFU-GM/10⁵ cells [median, 2.5] and 0 to 19/10⁵ BFU-E [median, 0.9]). In contrast, day-14 BM from patients receiving 3.0 μg/m²/d IL-1α (n = 9), though still hypocellular, were markedly enriched for myeloid precursors (CFU-GM, 0.5 to 22.9/10⁵; median, 14.2; P = .03), but not for BFU-E (0.7 to 9.3/10⁵; median, 3.3; P = .44). More primitive pluripotential stem cells capable of sustaining stroma-dependent LTBMIC-IC were infrequent in the day-14 posttransplant BM of both groups (0 to 0.9 [median, 0] for low-dose IL-1α versus 0 to 0.5 [median, 0.1] for high dose IL-1α).

Because other factors (including BMSC v PBSC) may affect hematologic recovery after autotransplantation, we performed a series of Cox model multivariate regressions to assess the independent contribution of IL-1α therapy. In this model, IL-1α dose was confirmed to be an independent factor significantly associated with time to neutrophil recovery (P = .002); and demonstrated a strong independent trend to

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<tr>
<th>Days of treatment</th>
<th>IL-1α Dose (μg/m²/d)</th>
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<tr>
<td></td>
<td>0.1 (n = 7)</td>
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<tr>
<td></td>
<td>0.3 (n = 5)</td>
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<tr>
<td></td>
<td>1.0 (n = 6)</td>
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<td></td>
<td>3.0 (n = 13)</td>
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<tr>
<td></td>
<td>10.0 (n = 2)</td>
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<tr>
<td>Days of fever greater than 101°F</td>
<td>14 (12-14)</td>
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<tr>
<td>Days of hypotension</td>
<td>5 (2-12)</td>
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<tr>
<td>Hypotension &lt;90 mm Hg (%)</td>
<td>3 (43)</td>
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<tr>
<td>Days of hypotension</td>
<td>7 (3-11)</td>
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Data shown are the median (range) of days of IL-1α infusion, days of fever greater than 101°F, and days of IL-1α infusion-related hypotension. Also shown are the no. (%) of patients developing IL-1α-related hypotension.
earlier platelet transfusion independence ($P = .095$) and to earlier RBC transfusion independence ($P = .07$). In additional analyses, neither BM cell dose nor progenitor content was of importance in relation to any of the three clinical recovery end points. However, BM origin of the stem cells as opposed to PBSC was also independently associated with earlier neutrophil recovery ($P = .002$) but not with RBC or platelet transfusion independence.

**Hospital discharge and hospital costs.** For 40 control and low-dose (0.1 to 1.0 $\mu g/m^2/d$) IL-$\alpha$–treated patients, hospital discharge alive occurred at a median of 37 days (range, 19 to 85) after transplantation. IL-$\alpha$ treatment at 3.0 $\mu g/m^2/d$ was associated with a 12-day shortening of the median hospital stay to 25 days (range, 19 to 43 days; $P = .0001$). This shorter hospital stay was accompanied by substantial reduction in hospital charges from a median of $145,000 (mean \pm SE, $177,000 \pm $24,000) in the control and low-dose IL-$\alpha$–treated patients to a median of $107,000 (mean \pm SE, $113,000 \pm $7,000) in the high-dose IL-$\alpha$–treated patients ($P = .01$).

**IL-$\alpha$ and cytokine plasma levels.** Peak plasma concentrations of IL-$\alpha$ in patients receiving 0.1, 0.3, and 1.0 $\mu g/m^2/d$ were unmeasurable by ELISA (level of detection, 0.5 pg/mL). Patients receiving 3.0 $\mu g/m^2/d$ had peak concentrations ranging from 27.4 to 158 pg/mL (median, 54.4 pg/mL; mean, 68.3). There was no demonstrable accumulation of IL-$\alpha$ over the 14 days of administration.

Throughout the course of IL-$\alpha$ infusions, we also assessed the plasma concentration of additional inflammatory and hematopoietic cytokines that might be induced by treatment with IL-$\alpha$. Throughout the 6-hour infusions of IL-$\alpha$ and the 18 hours thereafter, there was no measurable accumulation of either IFN$\gamma$ or TNF$\alpha$ in the plasma of patients receiving IL-$\alpha$ at any dose level (data not shown). A modest, but inconsistent, increase in plasma IL-3 and GM-CSF levels was observed in some patients treated with IL-$\alpha$, but no apparent dose–response effect or correlation with hematologic recovery was observed. Additionally, no change in plasma c-Kit ligand levels was observed (data not shown).

In contrast, IL-$\alpha$ infusion was regularly accompanied by dose-related increases in plasma IL-6 levels. Baseline mean plasma IL-6 levels were 2.5 $\pm$ 1.5 pg/mL. Treatment with low-dose (0.1 to 1.0 $\mu g/m^2/d$) IL-$\alpha$ resulted in IL-6 levels of 106 $\pm$ 27 pg/mL, whereas IL-6 levels increased significantly more in patients treated with 3.0 $\mu g/m^2/d$ (341 $\pm$ 77 pg/mL; $P = .004$).

IL-$\alpha$ therapy was also accompanied by profound and dose-related increases in plasma G-CSF concentrations that peaked at 4 to 6 hours into the 6-hour IL-$\alpha$ infusion. For IL-$\alpha$ doses of 0.1, 0.3, 1.0, and 3.0 $\mu g/m^2/d$, the median peak G-CSF plasma concentrations were 797, 3,377, 12,496, and 15,015 pg/mL, respectively ($P = .0005$).

**DISCUSSION**

In light of its ability to augment proliferation of hematopoietic cells and to enhance immunologic activity, IL-$\alpha$ could favorably influence therapeutic effects of BM transplantation. However, its powerful capacity to induce fever, chills, headache, nausea, vomiting, and dose-limiting hypotension required that its use in BM transplantation patients be evaluated cautiously. In this trial, in comparison with contemporary controls, we have recognized favorable effects of IL-$\alpha$ in induction of more rapid trilineage engraftment leading to shorter and less expensive hospitalization for transplantation. The clinical toxicity of IL-$\alpha$ infusions was modest and tolerable when dosages of $\leq$3.0 $\mu g/m^2/d$ were used; however, clearly demonstrable dose-limiting hypotension was identified at 10 $\mu g/m^2/d$.

Earlier clinical trials of IL-1 therapy have shown moderation of myelosuppression and induction of neutrophilia in conjunction with fever, rigors, and headache. IL-$\alpha$ and IL-1$\beta$ have both shown modest ability to augment platelet counts or to blunt chemotherapy-induced thrombocytopenia accompanying modestly myelosuppressive drug therapy. It is uncertain whether this thrombopoietic effect is direct or whether it is indirectly mediated through IL-6. We observed dose-related increases in IL-6 secretion in response to IL-$\alpha$ therapy as well as a stimulatory effect of IL-$\alpha$ infusion on platelet recovery. Modifications of this IL-$\alpha$ schedule or, perhaps, extended therapy after autotransplantation might enhance this latent potential for stimulating thrombopoiesis and ameliorate prolonged posttransplant thrombocytopenia. This might be especially valuable in patients with extensive pretransplant chemotherapy and compromised BM reserve.

Although showing myeloprotective and thrombopoietic effects, earlier clinical reports of IL-1$\beta$ or IL-$\alpha$ therapy

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<tr>
<th>Days to ANC 100</th>
<th>Controls + IL-$\alpha$ 0.1, 0.3 $\mu g/m^2/d$ (n = 19)</th>
<th>IL-$\alpha$ Dose (mg/m$^2$/d)</th>
<th>7 days of 1.0 (n = 7)</th>
<th>14 days of 1.0 (n = 6)</th>
<th>14 days of 3.0 (n = 13)</th>
<th>P Value</th>
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<tr>
<td>Days to ANC 500</td>
<td>24 (16-34)</td>
<td>27 (19-63)</td>
<td>22 (9-58)</td>
<td>12 (11-27)</td>
<td>.0001</td>
<td></td>
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<tr>
<td>Days to platelet independence</td>
<td>39 (12-182)</td>
<td>41 (26-66)</td>
<td>63 (19-157)</td>
<td>29 (15-92)</td>
<td>.09</td>
<td></td>
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<tr>
<td>Days to ANC independence</td>
<td>39 (12-182)</td>
<td>41 (26-66)</td>
<td>63 (19-157)</td>
<td>29 (15-92)</td>
<td>.09</td>
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Data shown are the median (range) of days to ANC $\geq$100/μL and $\geq$500/μL. The day of transfusion independence was defined as the day posttransplant when no platelet transfusions were required for $\geq$15 days or no RBC transfusions were required for $\geq$30 days. P values represent log rank comparisons between control and lower dose IL-$\alpha$ (0.1 to 1.0 pg/m$^2$/d) treated patients compared with those receiving high-dose IL-$\alpha$ (3.0 pg/m$^2$/d) for 14 days.

Abbreviation: ANC, absolute neutrophil count.
have identified either no increases or mild increases in BM cellularity.\textsuperscript{2,14} We observed that IL-1\(\alpha\) enhanced peripheral leukocyte recovery without increases in BM cellularity. However, a marked augmentation of clonogenic progenitors was observed in BM obtained at the end of the IL-1\(\alpha\) infusions. Therefore, the observed hematopoietic effects of IL-1\(\alpha\) may be the result of both direct enhancement of BM progenitor proliferation and expansion as well as differentiation resulting in accelerated hematologic recovery.

Secondary cytokine release may contribute to both the toxic and therapeutic effects of IL-1\(\alpha\).\textsuperscript{2,22}\textsuperscript{24} However, the observed fever, chills, and hypotension were not accompanied by measurable plasma levels of TNF\(\alpha\), IFN\(\gamma\), or IL-2, suggesting that these cytokines may not mediate these toxic effects. Of course, analysis of plasma levels cannot definitively exclude the possibility that these bioactive molecules are elaborated in response to IL-1\(\alpha\), because membrane-bound or locoregional secretion may manifest their activity. The observed secretion of G-CSF and IL-6, but not GM-CSF or IL-3, suggests that stromal cells (fibroblasts and endothelial cells) rather than hematopoietic cells are the prime sources of this IL-1\(\alpha\)-stimulated cytokine release.\textsuperscript{25,26}

We have also evaluated the immunologic activation induced by IL-1\(\alpha\) therapy.\textsuperscript{27} After high dose IL-1\(\alpha\) therapy, we recognized significantly increased proportions of circulating CD3\(^+\) T cells on day +14 and +28, but slight decreases in CD16\(^+\) or CD56\(^+\) natural killer cells compared with patients treated with lower doses of IL-1\(\alpha\). Additionally, on day +14, cells from patients receiving high-dose IL-1\(\alpha\) had significantly increased killing of natural killer sensitive and resistant lymphoma targets compared with those treated with lower dose IL-1\(\alpha\).

These observations indicate that recombinant IL-1\(\alpha\) treatment may be useful in reducing the myelosuppressive effects of autotransplantation and might be promising in facilitating more effective anitumor therapy. Although in this study IL-1\(\alpha\) could be delivered safely in doses sufficient to enhance hematologic recovery, its administration is neither simple nor easily tolerated by patients. Additional study of IL-1\(\alpha\) scheduling and of techniques to ameliorate its clinical toxicities may be helpful in unraveling its therapeutic potential.

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**REFERENCES**


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