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Erythropoietin Induced Tumour Mass Reduction in Murine Lymphoproliferative Models

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Recombinant erythropoietin (rHuEPO) is widely used in clinical practice in the treatment of several types of anaemia [1–4]. We observed that patients with end-stage multiple myeloma (MM) treated with EPO live longer than expected, despite their original poor prognostic features [5, 6]. BALB/c mice in which MM was induced by transplantation of mineral oil-induced plasmacytoma cells (MOPC-315) showed complete T cell-mediated tumour regression in 30–60% of the animals after treatment with EPO [7], suggesting that EPO may also act as an antitumour immunotherapeutic agent.

Here, we raised a question regarding the effect of EPO on tumour load, rather than on the ultimate survival, by studying two lymphoproliferative murine models, MOPC-315 MM [8] and B cell leukaemia/lymphoma (BCL1) [9]. BCL1 is a B-cell leukaemia/lymphoma that developed spontaneously in a 2-year-old female BALB/c mouse [10]; its advantage as a model is due to its analogies to human chronic lymphocytic leukaemia/lymphoma. In both models, we focused on tumour-bearing mice that did not achieve complete tumour regression after EPO treatment ('progressors').

Female inbred BALB/c mice, aged 6–8 weeks, were obtained from the Tel-Aviv University Breeding Center. BCL1 tumour cells (10^4 cells), derived from spleens of tumour-bearing BALB/c mice, were injected intra-peritoneally into syngeneic mice. Typically, peripheral blood leukocyte (PBL) counts begin to increase a few weeks following injection, exceeding 20×10^6 cells/ml. Mice were injected (subcutaneously) with 10^4 MOPC-315 tumour cells in the abdomen area as described [7]. The rHuEPO (epoetin alfa, Eprex®; Janssen-Cilag, Baar, Switzerland) treatment (rHuEPO 30 U) was administered daily for 10 consecutive days, followed by three times per week for an additional 2–3 weeks to the MOPC-315-bearing mice as described [7]. The linear mixed effect (repeated measures) model was used to determine the effect of EPO on tumour size. Variance among mice was taken into account in the analysis by considering the mice to be randomly selected from a larger population.

Starting 9 days after tumour cell injection, 28 MOPC-315-bearing mice were injected with rHuEPO or albumin (control). The survival curve, shown in figure 1d, demonstrates approximately 50 and 15% survival of the EPO-treated and control mice, respectively, which is in line with our previously documented observations [7]. Tumour growth kinetics in each of the EPO-treated and albumin-treated mice are presented in figures 1a and b, respectively. Statistical analysis of the tumour size of 7 EPO-treated and 11 control progressor mice, which displayed only one localized tumour, using the linear mixed effect (repeated measures) model, yielded an R value of 0.875 (fig. 1c). The predicted mean rate of tumour growth was 0.539 and 1.238 mm/day for EPO and albumin-treat-

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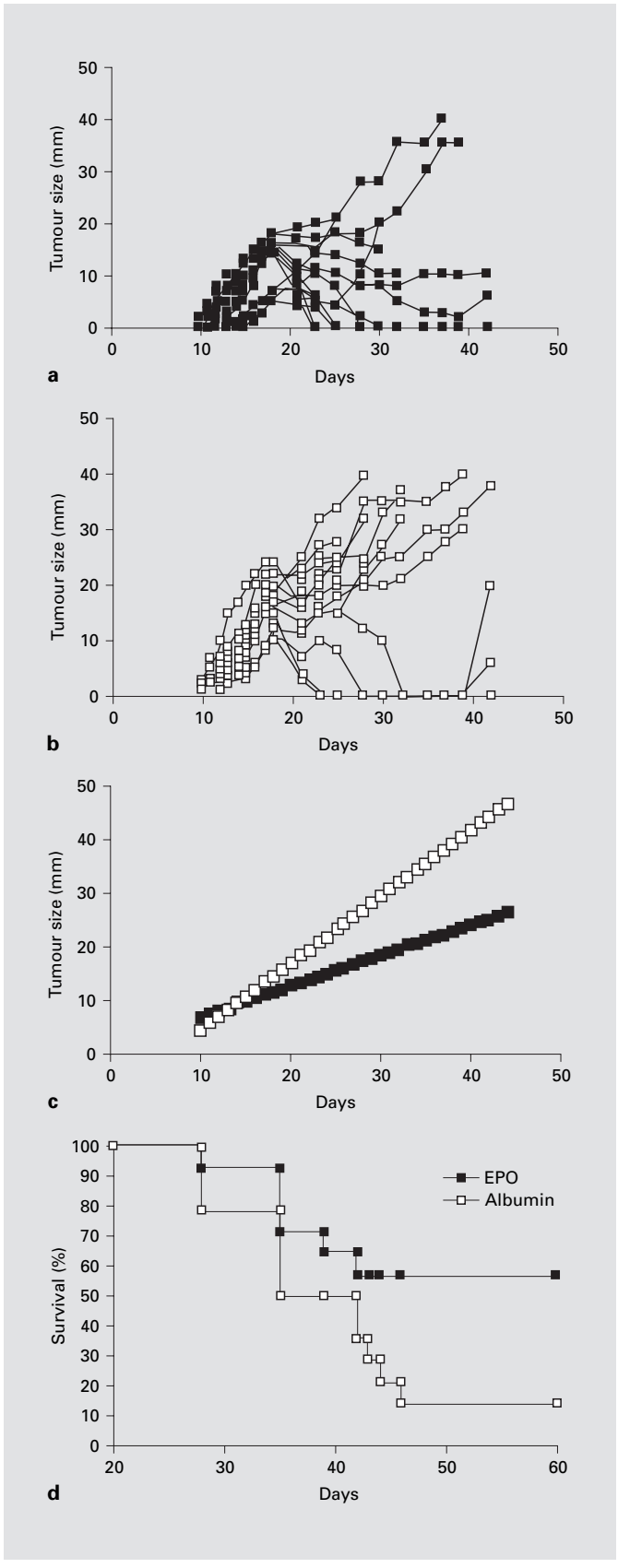

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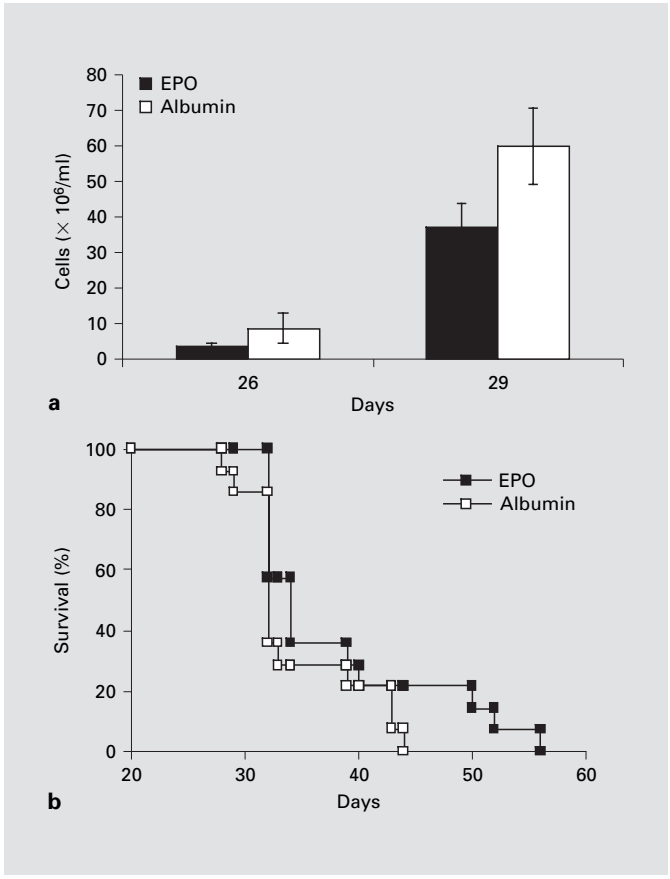

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Fig. 1. Kinetics of MOPC-315 tumour growth. Twenty-eight mice (14 in each group) were subcutaneously injected (day 0) with 10^4 MOPC-315 cells. **a**, **b** Tumour size in each of the EPO- and albumin-treated (control) mice. Tumour measurement started 10 days after MOPC-315 challenge. The graphs depict tumour growth in all mice from both groups. **c** Statistical analysis of MOPC-315 tumour size in progressor mice using the linear mixed effect model. The mean daily rate of tumour growth, based on the data obtained in **a** and **b** for EPO-treated (■) and albumin-treated (□) progressors is 0.569 and 1.238 mm, respectively ($p < 0.0001$). **d** Survival over a 60-day period. This figure represents one of three experiments displaying similar results.

Fig. 2. Leukocyte count and survival of BCL1-bearing mice. Twenty-eight mice (14 in each group) were injected intra-peritoneally with 10^4 BCL1 cells (day 0). **a** PBL count at days 26 and 29 ($p = 0.019$ and $p = 0.041$, respectively). It should be noted the blood counts were still normal on day 21. The course of this disease is extremely fast and aggressive, and mice began to die shortly after day 29. **b** Survival over a 56-day period. This figure represents one of three experiments displaying similar results.

ed progressors, respectively ($p < 0.0001$). The remaining mice either achieved complete regression or developed more than one localized tumour. Thus, even in those EPO-treated mice that did not achieve complete tumour regression, the treatment led to a significant reduction in tumour mass and slower tumour progression.

In order to assess the effect of EPO on the B cell lymphoma/leukemia murine model, BCL1-bearing mice were injected with rHuEPO (5 injections per week, 30 U each, till death) at different times following BCL1 challenge (data not shown). Mice that received the first rHuEPO injection on day 19 after tumour cell injection demonstrated a smaller tumour mass. Hence, PBL counts at days 26 and 29 indicated a lower tumour mass ($p = 0.019$ and $p = 0.041$, respectively) in EPO-treated mice in comparison with controls (fig. 2a). This was further supported by a tendency for prolonged survival in the EPO-treated mice (fig. 2b). Indeed, the effect of EPO on the BCL1 model is not as robust as that seen in the MOPC-315 model. This difference probably results from the aggressive nature of the disease.

Based on our clinical observations [6], as well as the data provided here and in our previous study [7], clinical trials are now underway to test this new, hitherto unrecognized EPO-induced anti-neoplastic effect. The role of EPO in the biology and prognosis of certain neoplasms has not been fully elucidated. Despite reports that raise concerns [11], others favour the use of EPO not only for anaemia but also to improve prognosis [12, 13]. The data presented here further support an anti-neoplastic effect of EPO at least in certain malignancies. The implications for the therapeutic approach, as well as for the prognosis of various lymphoproliferative diseases, could be most significant. Potentially, cancer patients may benefit from a slower tumour progression even without the effect on ultimate survival. Such slower tumour progression may eventually translate into better prognosis and an improved quality of life.

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