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In Vitro Treatment of Bone Marrow from Patients with T-Cell Acute Lymphoblastic Leukemia and Non-Hodgkin's Lymphoma Using the Immunotoxin WT1-Ricin A

M. J. Barnett [1], A. Z. S. Rohatiner [1], J. E. Kingston [1], K. E. Adams [1], E. L. Batten [1], R. Bassan [2], P. E. Thorpe [3], M. A. Horton [1], J. S. Malpas [1], and T. A. Lister [1]

A. Introduction

The murine monoclonal antibody WT1 [1] identifies a glycoprotein of molecular weight 40000 present on normal thymocytes and blasts from patients with T-cell acute lymphoblastic leukaemia (T-ALL) [2]. The antibody may be linked by a disulphide bond to the A chain of ricin [3] to form an immunotoxin (WT1-ricin A) which kills cells that express the WT1 antigen but does not have an inhibitory effect on haematopoietic precursors [4].

WT1-ricin A has been used to treat bone marrow from patients in remission of T-ALL and non-Hodgkin's lymphoma (NHL), in an attempt to eradicate occult neoplastic cells. The study was conducted with the intention of then using such cryopreserved marrow to support the treatment of these patients with intensive chemoradiotherapy in second remission.

[1] ICRF Department of Medical Oncology, St. artholomew's Hospital, London EC1A 7BE, UK
[2] Ospedali Ruiniti, Bergamo, Italy
[3] ICRF Drug Targeting Laboratory

B. Materials and Methods

I. Patients

Clinical details are shown in Table 1.

Table 1. Results obtained in patients studied

Patient	Age	Sex	Diagnosis	Pre WT1-ricin A				Post WT1-ricin A		
				BM vol. (ml)	MNC ($\times 10^9$)	WT1 (%)	CFU-GM on day 12 (per 10^5 MNC)	MNC ($\times 10^9$)	WT1 (%)	CFU-GM on day 12 (per 10^5 MNC)
1	33	M	ALL[a]	1420	2.2	5	50	1.7	<1	36
2	41	M	NHL[a]	1437	2.7	5	45	1.7	<1	51
3	49	M	ALL	1376	2.3	8	36	1.8	<1	27
4	13	M	ALL	1239	2.0	10	157[b]	1.1	<1	183[b]
5	26	M	ALL	1736	2.5	13	28	1.6	<1	3
6	9	M	ALL	579	0.7	20	72	0.6	<1	36
7	12	M	ALL	740	1.4	27	49	1.2	<1	147
8	14	F	NHL	578	1.4	22	26	0.6	<1	80

BM, bone marrow.
[a] Second remission, all others first remission.

II. Methods

In Vitro Treatment of Marrow

Bone marrow was harvested during morphological and immunological remission (no detectable WT1+/TdT+ cells), and the mononuclear cell (MNC) fraction was isolated. Half the cells were cryopreserved untreated and half were treated with WT1-ricin A. The cells were transferred to tissue culture flasks at a concentration of 5×10^6 cells/ml in incubation medium (RPM1 1640 + 15% autologous serum + penicillin and streptomycin). Ammonium chloride and WT1-ricin A were then added to a final concentration of 6 *M* ammonium chloride and 0.25 μg ricin A/ml. The flasks were incubated for 20 h at 37 °C in a 5% CO_2-humidified incubator. The cells were then resuspended and washed twice prior to cryopreservation.

C. Results

In all cases, the percentage of WT1+ cells decreased from between 5% and 27% to less than 1% after incubation with the immunotoxin. In four of eight cases, this was accompanied by a decrease in colony-forming unit–granulocyte macrophage (CFU-GM) numbers.

D. Discussion

The method described is practical and, as far as can be deduced from this study, effective. Experience needs to be gained with the reinfusion of such marrow and the monitoring of subsequent haematological and immunological reconstitution. Furthermore, the interest in such in vitro methods of treating the autograft should not eclipse the major issue of disease eradication in the patient.

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