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Clinical Studies on the Effect of Imuran and Vincristine in the Treatment of Leukaemia

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It is always a difficult task to report the results of a clinical trial with antimitotic drugs in acute leukaemia. The irregular course of untreated cases, the inability to forecast the likelihood or extent of natural or induced remissions, and the difficulty of devising a standard of reference make it almost impossible to draw firm conclusions as to the efficacy of any form of treatment and to state whether in a given case one form of therapy is preferable to another.

The scarcity however of new therapeutic agents for the treatment of leukaemia may perhaps justify our attempt to describe very briefly some of the impressions derived from the use of two drugs, Vincristine and Imuran, in the different forms of acute leukaemia.

In view of the nature of the Symposium, no details will be given of the criteria with which a clinical and haematological remission was assessed, nor will we expand on the difficulties of classifying each case of leukaemia, since all of you are well aware how controversial this differentiation may sometimes prove. However, for a precise evaluation of the efficacy of an antimitotic agent, an exact cytological allocation is most desirable and to this end considerable help may be obtained from the application of cytochemical staining methods. In our case material these were carried out in the most equivocal cases.

Vincristine, of which the history, the chemical structure and the pharmacological effects are well known, is obtained from one of the alcaloids extracted from the Vinca rosea Linn, and is structurally closely related to Vinblastine, from which however it differs in its effectiveness against human malignancies. While Vinblastine appears to be more active in the treatment of Hodgkin's disease (Storti *et al.*, 1963), Vincristine exerts its most favourable therapeutic effects in acute leukaemia (Bohannon *et al.*, 1962; Carbone and Brindley, 1962; Costa *et al.*, 1962).

Although some uncertainty still exists concerning the full extent of its mechanism of action, especially with regard to the submicroscopic sites and the metabolic effects of its antitumour activity, it has now been repeatedly shown that Vincristine, like Vinblastine, produces a characteristic mitotic block at the metaphase stage (Cardinali *et al.*, 1961; Cardinali *et al.*, 1963).

In Tab. 1 are summarized the findings obtained in 27 cases of acute leukaemia treated with Vincristine.

12 of our 27 patients experienced a complete clinical and haematological remission. These cases had not received any previous treatment. In 8 patients the remissions were partial and of shorter duration but still satisfactory. 7 of these had previously been treated with 6-mercaptopurine or had received steroid therapy. In the remaining 7 patients no benefit was derived from the drug.

Tab. 1. Therapeutic response in 27 cases of acute leukaemia treated with Vincristine

Good remissions	N. of cases: 12 Cytological diagnosis: all lymphoblastic leukaemias Average duration of remission: 2 months
Partial remissions	N. of cases: 8 Cytological diagnosis: all lymphoblastic leukaemias Average duration of remission: 2-3 weeks
Minimal or no response	N. of cases: 7 Cytological diagnosis: 2 Myeloblastic leukaemias; 5 Lymphoblastic leukaemias Average duration of remission (if any): no remission

The results of this clinical trial entail the following comments. Firstly a few words regarding dosage and the therapeutic scheme adopted. The initial dose varied from 0.03 to 0.15 mg/Kg body weight and was given by intravenous injection or infusion. After a variable period of time, usually one week, the drug was again given intravenously either at the same concentration or increased by 0.05 mg/Kg body weight. This form of intermittent therapy was continued until the white cell count had dropped to 3000/mm^3 white blood cells or less.

An illustrative example of a case of acute lymphoblastic leukaemia treated with Vincristine (Oncovin) is given in Fig. 1.

The patient, a girl aged 10, was first seen in September 1963. On admission to Hospital, she was febrile and was found to have generalized lymph-node enlargement and moderate hepato-splenomegaly. A blood count showed Hb 10.4 g %, platelets 85 000/mm^3 and leucocyte count 160 000/mm^3, mainly lymphoblasts. She was treated with Vincristine at a dosage of 0.05 mg/Kg. Six days after the first injection the leucocyte count had dropped to 10 000/mm^3, with a decrease in the percentage of lymphoblasts (28%). At the same time both lymph nodes and spleen were considerably reduced in size. Vincristine, at the same dosage, was again given two weeks after the first injection, when the white cell count was rising, and once again the drug caused a fall in the leucocyte count, a decrease in the number of primitive cells and temporary clinical improvement. Owing to the presence of toxic effects, mainly hair loss and constipation, the subsequent injections of Vincristine were given at suitable intervals, usually 2-3 weeks and this therapeutic scheme was continued until April 1964, when the patient eventually died.

The percentage of remissions in our series seems to be around 50% and is similar to that reported by Karon *et al.* (1962) and Johnson *et al.* (1963).

It is apparent from Tab. 1 that the most significant responses were observed in cases of acute lymphoblastic leukaemia and that the two cases of myeloblastic leukaemia included in the trial fell into the group which did not derive any beneficial effect from the use of Vincristine. These findings would suggest that the drug is more effect-

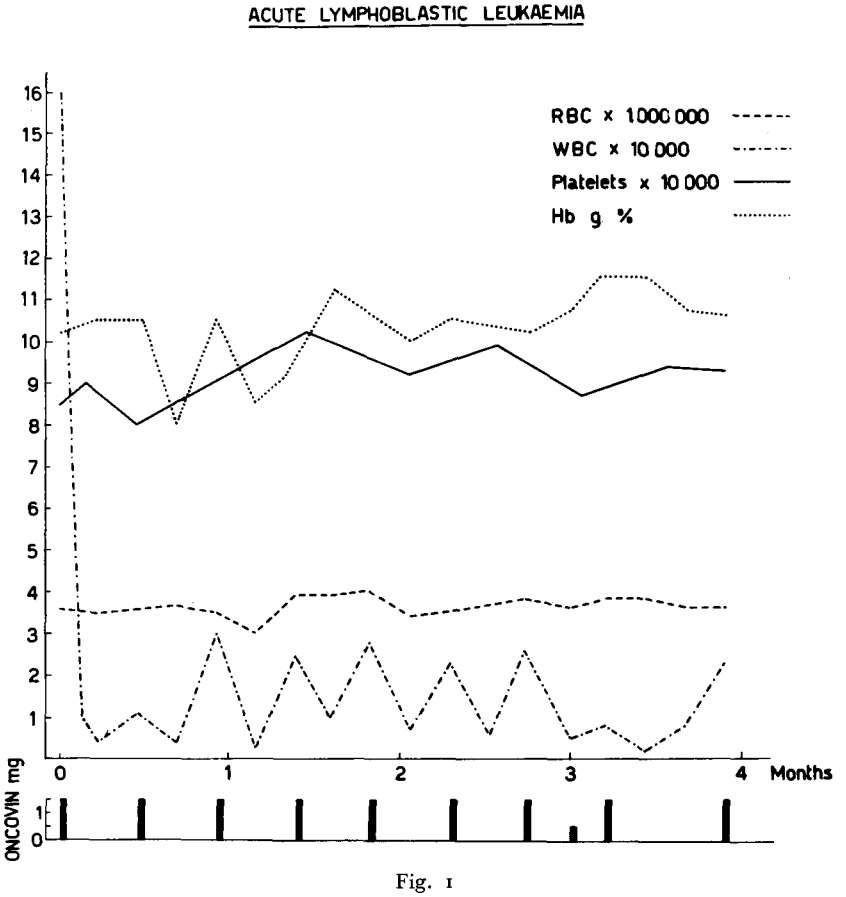

Fig. 1

ive in the former type of leukaemia, in which it is possible to obtain remissions lasting from one to six months (exceptionally), with an average duration of two months.

Therefore in lymphoblastic leukaemia the effects of Vincristine are not dissimilar from those obtained with 6-mercaptopurine or amethopterin, with the difference however that Vincristine suffers from the disadvantage of inducing remissions of shorter duration than those achieved with either 6-mercaptopurine or amethopterin,

and also of causing more toxic side-effects such as hair loss, constipation and neurological complications, such as paraesthesiae, sensory loss, diminished or absent tendon reflexes.

One of the advantages of Vincristine therapy is the absence of cross-resistance with other antimitotics, since it appears to be effective also in those cases which have received prior treatment with 6-mercaptopurine or amethopterin, although in agreement with Johnson *et al.* (1963), remissions are more promptly induced in untreated cases.

In conclusion, although Vincristine may be usefully employed in some cases of lymphoblastic leukaemia either in the initial untreated phase or when the disease has become resistant to steroids or other antimetabolites, the drug does not seem to offer any particular advantage over the other available compounds since it is more toxic, especially in adults, its remissions are of shorter duration and its therapeutic action is substantially confined to the lymphoblastic variety of acute leukaemia.

Imuran, 6-((I-methyl-4-nitro-5-imidazolyl) thio)-purine, is a " masked " derivative of 6-mercaptopurine, in which a substituted imidazole ring replaces the hydrogen of the sulfhydryl group. This compound undergoes *in vivo* cleavage and releases the basic compound 6-mercaptopurine. Over this latter compound, Imuran would present the advantage of producing more gradual and persistent therapeutic effects on account of the progressive release of 6-mercaptopurine (Elion *et al.*, 1961). The activity of the compound differs very little from that of 6-mercaptopurine, inasmuch as the antitumour effects of the two compounds in animals is similar (Elion *et al.*, 1961; Hitchings and Elion, 1959) and both inhibit antibody formation (Schwartz *et al.*, 1958; Zukoski and Callaway, 1962).

Clinical experience with Imuran is still rather limited. The report of Rundles and associates (1961) suggests that the drug is active in chronic granulocytic leukaemia, gives promising results in auto-immune diseases and is not inferior to 6-mercaptopurine in the treatment of acute leukaemia. Rundles and his collaborators point out that the drug shows a selective action on the leucopoietic tissue, with minimal effects on the erythropoietic and megakaryocytic tissues. These characteristics indicate that the drug may be a suitable additional tool in the treatment of acute leukaemias and we therefore set out to assess its efficacy in patients with this disease.

In Tab. 2 are shown the number of cases treated, the average remissions obtained and the dosage employed.

With regard to the latter, it is immediately apparent from the table that the dosage has a considerable importance in assessing the therapeutic value of Imuran. With doses comparable to those of 6-mercaptopurine, i. e. 2-3 mg/Kg body weight daily, the drug induces a fairly low percentage of satisfactory or lasting remissions. With much higher levels, i. e. 7-8 mg/Kg body weight daily, the percentage of remissions is much higher. The policy of employing such large doses of Imuran was adopted following the results obtained in a case of childhood leukaemia which had become resistant to chemotherapy and in which gradually increasing doses of Imuran, far

from causing irreversible toxic effects, eventually succeeded in producing a lasting remission. This unexpected observation induced us to follow the practice of giving for short periods, usually 6-10 days, high doses of Imuran, rather than the reverse as reported by Rundles *et al.* (1961). Treatment is interrupted when the white cell count shows a significant fall. A second course may be given at the same dosage, when and if there is evidence of recurrence of disease in blood and bone-marrow. Whether it is possible to obtain remissions of greater duration by continuing treatment at a maintenance dose until drug resistance finally develops is a question which warrants further investigation. The drug has not been used in the present series of cases in combination with other antimitotic agents and only occasionally with steroids; our impression is that steroids do not enhance the therapeutic effects of Imuran.

In the 19 cases which experienced good remissions, Imuran, used at the appropriate high doses, caused a sharp drop in the white cell count, a reduction in the number of immature cells in the circulation, a decrease in the size of the spleen, liver and lymphnodes and the disappearance of most of the initial symptoms, including fever.

The results of this preliminary trial as well as the observations of Rundles *et al.* indicate that in none of the cases treated with Imuran the high doses of the drug caused a deterioration in the bleeding manifestations, so that therapy could be undertaken in patients who either spontaneously or on account of previous treatment showed marked degrees of thrombocytopenia. This fact is clearly illustrated in Fig. 2.

This was a case of acute myeloblastic leukaemia in a man aged 31, who had previously had a satisfactory remission. On admission (February, 1965) the patient was febrile and showed clinical and haematological signs of an impending relapse. His platelets were few (25 000/mm^3), his white cell count was steadily rising and increased in a few days from 20 000 to 85 000 per mm^3 with numerous myeloblasts and a few promyelocytes. His spleen was enlarged, numerous petechiae were present, with associated bleeding from mucosae and microscopic haematuria. Despite the presence of this bleeding tendency, therapy with Imuran was started at a dose of 600 mg daily and continued for 7 days. During the first days of treatment the haemorrhagic manifestations became more marked and haematuria became massive. However 8 days after therapy had been started a drop in the white cell count to 20 000/mm^3 heralded the first signs of a haematological remission. In parallel with the fall in the white cell count, haematuria became less intense and 15 days after therapy had been discontinued all haemorrhagic manifestations had disappeared. At the same time the white cell count had dropped further to 2 000/mm^3 and neutrophil granulocytes were fairly numerous (25%). A remission lasting two months finally ensued.

Another case (Fig. 3) shows how the massive doses of Imuran did not cause any severe or irreversible toxic effects, excepting a temporary marrow depressant action. The patient was a woman aged 32 with acute lymphoblastic leukaemia. On admission to hospital she was found to have bilateral inguinal lymph node enlargement,

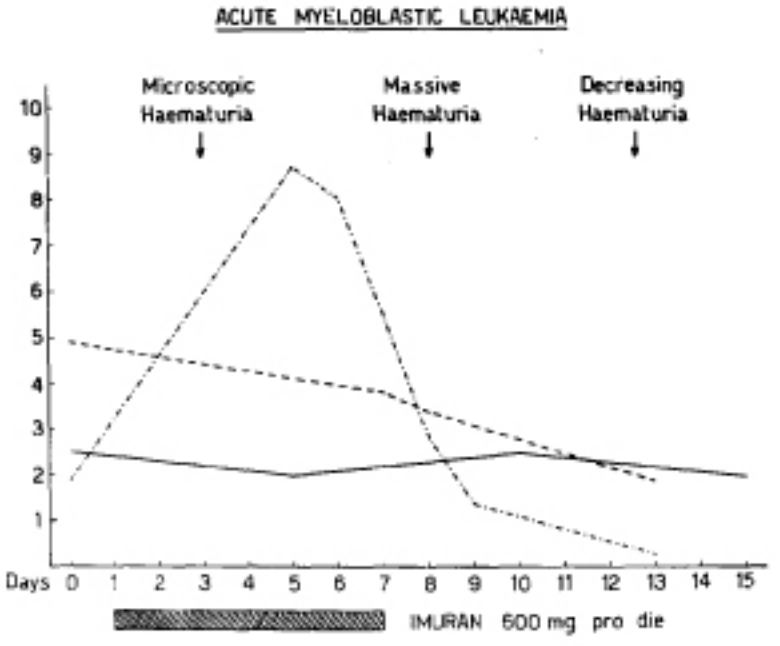

Fig. 2

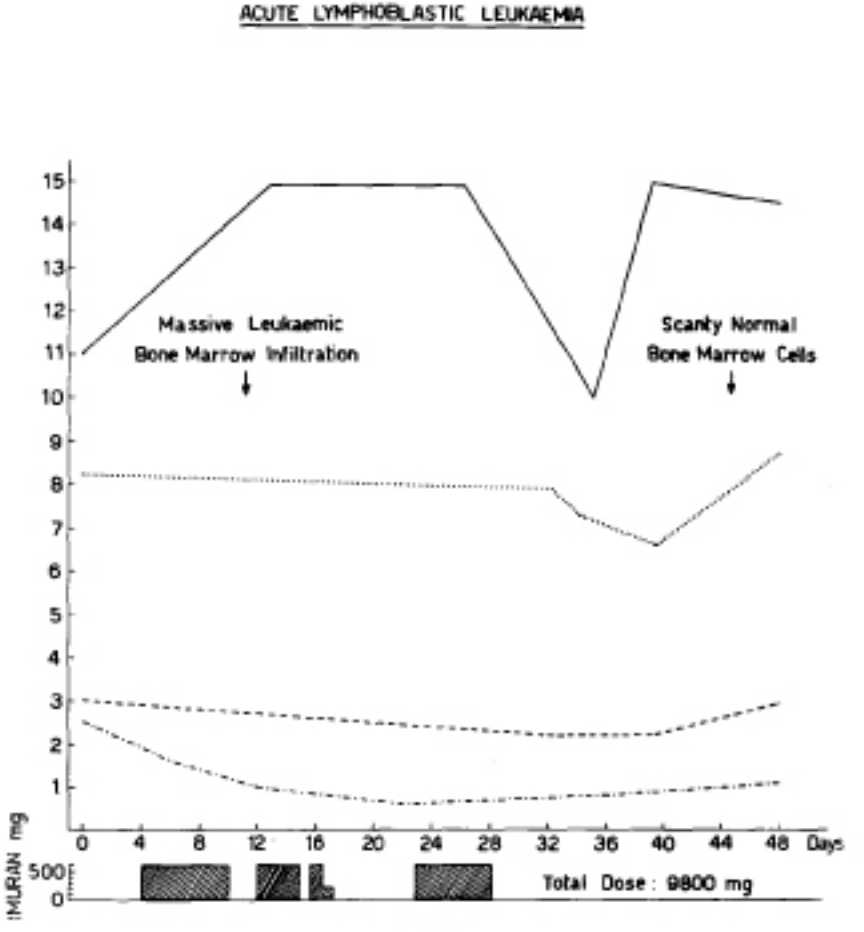

Fig. 3

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anaemia (Hb 8.3 g %), moderate thrombocytopenia (110 000/mm³) and a leucocyte count of 26 000/mm³ with few circulating primitive cells. The bone-marrow was totally replaced by immature nucleolated blast cells. Notwithstanding the relatively low peripheral blood leucocyte count, Imuran was given at a dose of 600 mg daily in three successive courses, until a total dose of 9 800 mg had been reached. Two weeks later a bone-marrow aspirate showed the presence of small numbers of cells of the erythro and granulopoietic tissues with an occasional megakaryocyte.

At the time of writing the patient is in good clinical and haematological remission. Her periods are normal, she has no haemorrhagic manifestations and has been able to resume her normal activity as a house-wife.

Figs. 4 to 7 show the remarkable effects of Imuran in a case of acute lymphoblastic leukaemia. The patient was a five-year old child, who had been previously treated with Vincristine and had remained in good clinical and haematological remission for the last three months. Despite these satisfactory conditions, the pa-

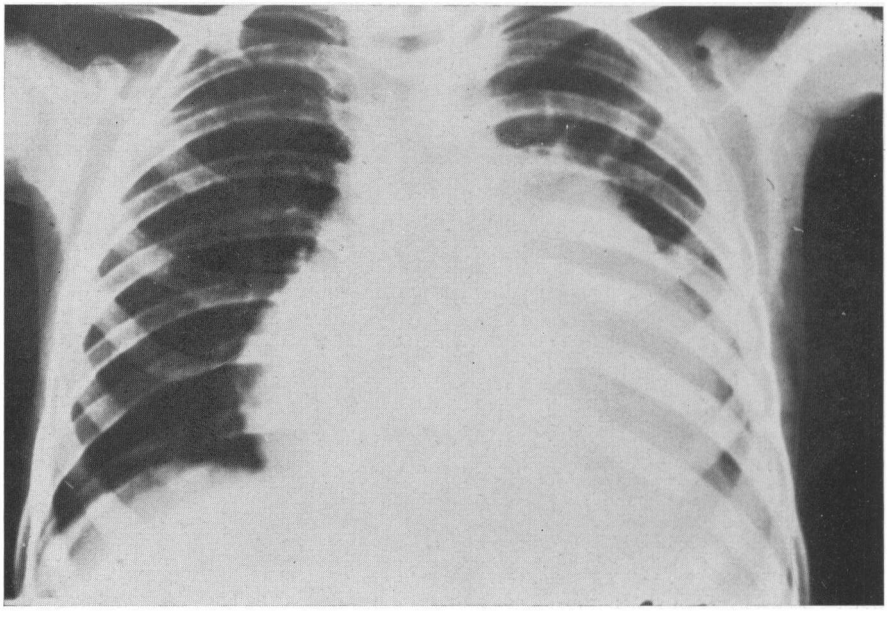

Fig. 4

tient suddenly developed cough and temperature and a chest-ray showed a dense fairly well circumscribed pulmonary opacity, producing mediastinal displacement and atelectatic changes (Fig. 4).

Treatment with Vincristine was immediately resumed, but the pulmonary opacity continued to increase in size (Fig. 5). After 15 days Vincristine was therefore discontinued and Imuran started at a daily dose of 150 mg. After 5 days there

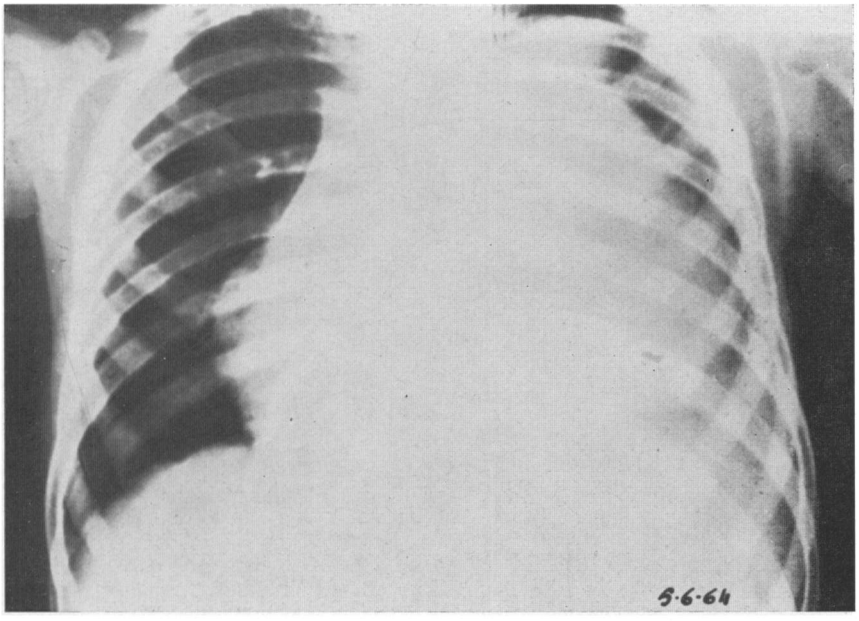

Fig. 5

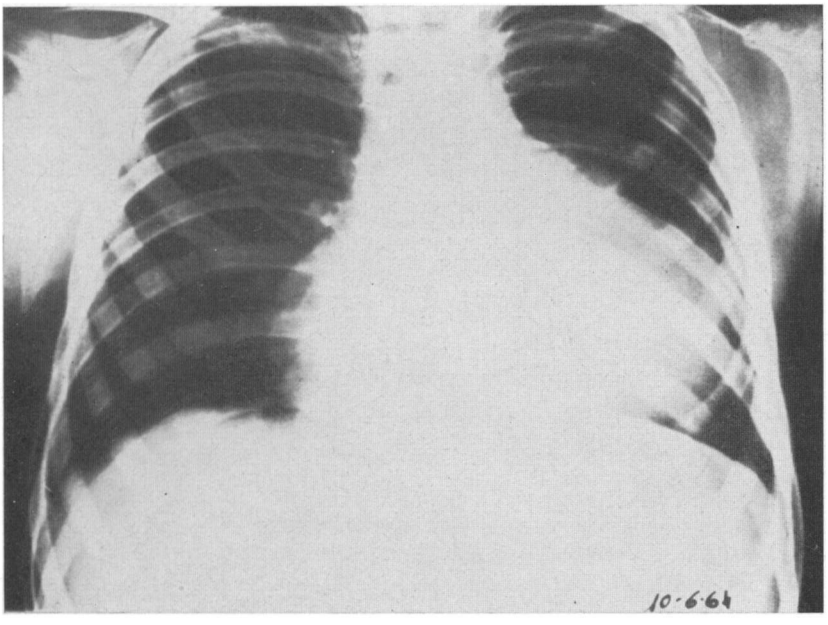

Fig. 6

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was already a noticeable reduction in the size of the pulmonary mass (Fig. 6) and after 11 days the opacity had almost completely disappeared, the mediastinum was no longer displaced and the atelectatic changes were considerably reduced.

During treatment with Imuran the white cell count never fell below 3 800, the differential count remained unchanged and platelets varied between 160 000 and 200 000.

At the time of writing the patient is still in remission.

A few final considerations: as shown in Tab. 2, the duration of the remissions in the group which responded well to treatment ranged from a minimum of two months to an occasional maximum of 8-9 months, the average duration being 4-5

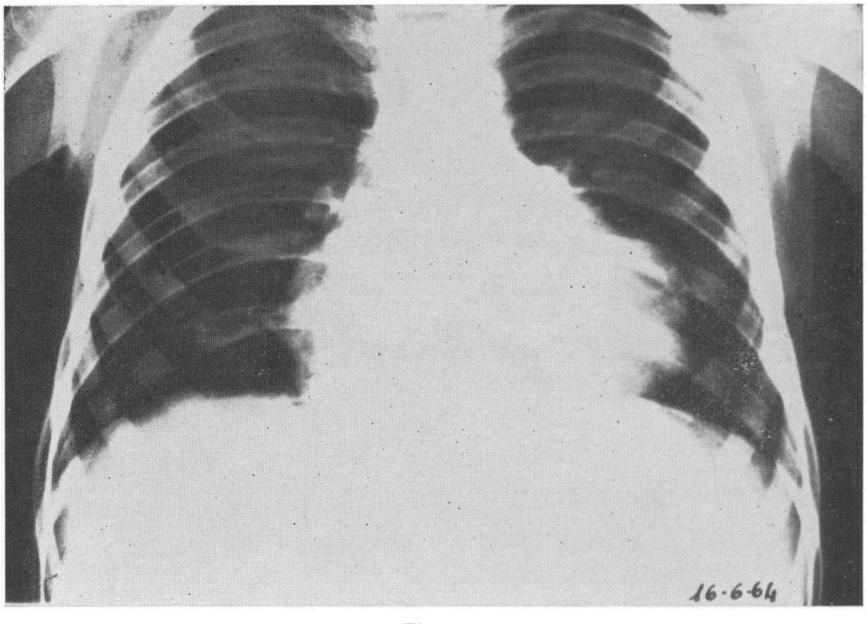

Fig. 7

months. It is noteworthy that in contrast to the results obtained with Vincristine, good remissions were also obtained in myeloblastic leukaemia and in particular in 2 cases of chronic granulocytic leukaemia in blastic crisis which are notoriously difficult to manage and respond only, if at all, to 6-mercaptopurine.

With the doses employed and in the cases so far examined, Imuran has not produced so far any undesirable side-effects, either in the form of mouth ulcerations, drug fever, gastro-intestinal symptoms or toxic hepatitis.

Compared with 6-mercaptopurine, Imuran appears therefore to be better tolerated by patients. Whether instead the latter is able to produce more lasting and

Tab. 2. Therapeutic response in 31 cases of acute leukaemia treated with Imuran

Good remissions	N. of cases: 19 Cytological diagnosis: 14 lymphoblastic leukaemias; 5 myeloblastic leukaemias, of which 2 cases of C.M.L. in blastic crisis Average duration of remission: 4-5 months Maximum dosage employed: 10 mg/Kg pro die
Partial remissions	N. of cases: 3 Cytological diagnosis: all myeloblastic leukaemias Average duration of remission: 4-6 weeks Maximum dosage employed: 10 mg/Kg pro die
Minimal or no response	N. of cases: 9 Cytological diagnosis: 6 lymphoblastic leukaemias; 3 myeloblastic leukaemias Average duration of remission (if any): no remission Maximum dosage employed: 2-3 mg/Kg pro die

satisfactory remissions and may eventually replace 6-mercaptopurine in the treatment of acute leukaemia is a matter which can only be settled by undertaking more extensive clinical trials.

Summary

The Authors describe the therapeutic effects obtained with Vincristine and Imuran in patients with acute leukaemia.

The results obtained with Vincristine indicate that the drug may be usefully employed in some cases of lymphoblastic leukaemia, both in the untreated phase or when the disease has become resistant to other antimetabolites. The remissions are of shorter duration compared with those obtained with other available compounds and are substantially confined to the lymphoblastic variety of acute leukaemia.

The preliminary clinical trial with Imuran has shown that the drug is better tolerated than 6-mercaptopurine and is able to produce good and satisfactory remissions in lymphoblastic and myeloblastic leukaemia, as well as in the blastic crisis of chronic granulocytic leukaemia, provided the dosage employed reaches 7-8 mg/Kg body weight daily.

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RIASSUNTO

Gli AA. descrivono gli effetti terapeutici ottenuti con Vincristina e Imuran in pazienti affetti da leucemia acuta. I risultati ottenuti con la Vincristina indicano che il medicamento può essere usato con successo in alcuni casi di leucemia linfoblastica, sia nella fase non trattata che quando la malattia diventa resistente ad altri antimetaboliti. Le remissioni sono di minore durata rispetto a quelle ottenute mediante le altre sostanze disponibili e sono sostanzialmente limitate al tipo linfoblastico della leucemia acuta. Gli esperimenti clinici preliminari con Imuran hanno indicato che il medicamento viene tollerato meglio della 6-Mercaptopurina, che è in grado di produrre remissioni buone e soddisfacenti nella leucemia linfoblastica e mieloblastica, come pure nella leucemia granulocitaria cronica, purché la dose impiegata raggiunga i 7-8 mg/kg di peso corporeo al giorno.

RÉSUMÉ

Les Auteurs décrivent les effets thérapeutiques obtenus par Vincristine et Imuran chez des patients atteints de leucémie aiguë.

Les résultats obtenus par Vincristine indiquent que ce médicament peut être employé utilement en quelques cas de leucémie lymphoblastique, soit dans la phase non-traitée que lorsque la maladie est devenue résistente à d'autres antimétabolites. Les remissions sont de durée plus brève de celles obtenues par d'autres substances et sont pratiquement limitées à la variété lymphoblastique de la leucémie aiguë.

Les expériments chimiques préliminaires avec Imuran ont démontré que ce médicament est mieux toléré de la 6-Mercaptopurine et est à même de produire des remissions bonnes et satisfaisantes dans la leucémie lymphoblastique et myéloblastique, ainsi que dans les crises blastiques de la leucémie granulocytaire chronique, si les doses employées atteignent 7-8 mg/kg/24 h.

ZUSAMMENFASSUNG

Verf. beschreiben die therapeutische Wirkung von Vincristine und Imuran bei Fällen von akuter Leukämie. Die mit Vincristine erreichten Resultate zeigen, dass man das Medikament erfolgreich bei einigen Fällen von Lymphoblastenleukämie anwenden kann und zwar sowohl, wenn diese noch nicht behandelt wurde als auch, wenn sie bereits gegen andere Antimetaboliten resistent geworden ist. Im Vergleich zu anderen verfügbaren Mitteln halten die Besserungen kürzere Zeit an und sind hauptsächlich auf den lymphoblastischen Typ der akuten Leukämie beschränkt.

Die vorläufigen klinischen Experimente mit Imuran zeigen, dass das Medikament besser als 6-Merkaptopurina vertragen wird, dass man damit sowohl bei Lymphoblasten- und Myeloblastenleukämie als auch bei chronischer Granulozytenleukämie gute und zufriedenstellende Besserungen erzielen kann, vorausgesetzt, dass man Dosen bis zu 7-8/mg/kg Körpergewicht pro Tag anwendet.