

# Comparison of ex vivo drug sensitivity by TRAC assay and patient response in the UK LRF CLL4 trial

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## Background

Previous results have suggested that ex vivo drug sensitivity test results are independent prognostic or predictive factors for subsequent patient response in chronic lymphocytic leukaemia (CLL).

## Aims

In order to determine its accuracy at predicting response and survival, drug sensitivity is being tested in the UK Leukaemia Research Fund (LRF) CLL4 trial – both at initial entry (closed October 2004) and at second randomisation (still open).

## Methods

At first randomisation, blood specimens were sent to Bath for drug sensitivity testing: initially by DiSC assay; subsequently by its development, the TRAC assay. Ten drugs were tested including chlorambucil, fludarabine and mafosfamide (used in vitro in place of cyclophosphamide). LC90s were calculated. Patients were randomised into Trial arms to receive chlorambucil (Chl, 50% patients), fludarabine (Flu, 25%) or fludarabine+cyclophosphamide (FluCy, 25%). Numbers of patients with any response versus no response were compared. 2P is by Fisher's exact test.

## TRAC (DiSC) assay method

The TRAC (Tumour Response to Anti-neoplastic Compounds) assay is a development of the DiSC (Differential Staining Cytotoxicity) assay. (We no longer differentially stain – hence the new name.)

Mononuclear cells are isolated and incubated with drugs in the Octospot system. At the end of 4 days incubation, this system is used to transfer the contents of the 8 wells of a microtitre strip to one microscope slide.

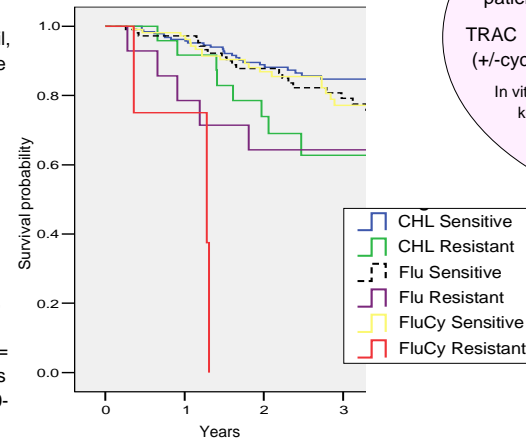
Slides are scored for cell survival – from 0%, if all cells are dead, to 100% if survival is the same as control. LC<sub>90</sub>s are calculated.

## Results

From 777 randomised, LC90 results from 442 patients could be compared with subsequent patient response. Definitions of TRAC-sensitive were LC90s of  $\leq 6.3$  ug/ml for chlorambucil, and  $\leq 10.0$  ug/ml for both fludarabine and mafosfamide. No difference in average drug LC<sub>90</sub>s was found between Trial arms. Results are presented in the Table (below) and Graph of survival (right).

All differences between response rates in the TRAC-sensitive and TRAC-resistant groups were highly statistically significant. For instance, for those treated with Flu or FluCy, 90.7% (95% confidence interval (CI) = 86.8-94.6) of TRAC-sensitive patients responded compared with 22.2% (3.0-41.4) of TRAC-resistant patients.

Survival of patients according to whether they were TRAC-sensitive to the drugs given or resistant



Comparison of Ex vivo drug sensitivity with subsequent patient response (numbers of patients)

Trial arm	No.	TRAC-sensitive		TRAC-resistant		Odds ratio (95% CI)	2P
		Response	No response	Response	No response		
Chl	210	141	45	9	15	5.2 (2.1-13)	0.0004
Flu+FluCy	232	194	20	4	14	34.0 (10-113)	<0.00001
Total	442	335	65	13	29	11.5 (5.7-23)	<0.00001

## Conclusions

At diagnosis of CLL, even within a group of patients with a high clinical response rate, ex vivo drug sensitivity can be used to identify a proportion of patients with a significantly poorer probability of clinical response. TRAC results predict better for patient response to fludarabine (+/-cyclophosphamide) than for response to chlorambucil.

In vitro resistance pre-treatment predicts a poor prognosis, but we do not yet know whether choosing a different treatment out of currently available ones would improve this. The second randomisation of the CLL4 trial is addressing this question with respect to second line treatment.

### DID YOU KNOW?

Bath Cancer Research is now offering a 5-drug CLL test for just £95. Test all your CLL patients before treatment and identify those with a low probability of responding to fludarabine. For more information on drug sensitivity testing for CLL and other leukaemias and lymphomas, see <http://caltri.org>, phone +44 1225 824 124 or email [bcr@caltri.org](mailto:bcr@caltri.org)

### Drugs tested

Fludarabine, Cladribine, Pentostatin, Chlorambucil, Mafosfamide/Cyclophosphamide, Doxorubicin, Mitoxantrone, Prednisolone, Methylprednisolone, Vincristine

The Octospot system can be obtained from TEST Laboratories Ltd, Bath. Tel +44 (0)1225 840593