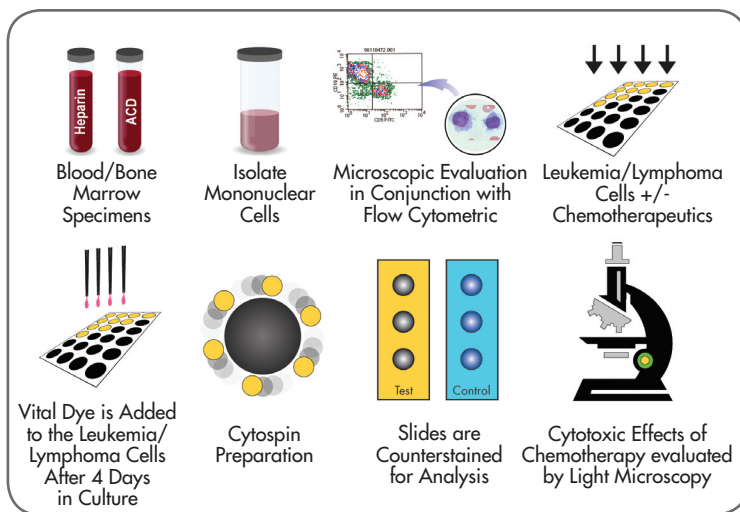


ONCOTECH DiSC[®] ASSAY

For Hematologic Malignancies



DiSC[®] Assay Methodology

The DiSC[®] Assay is well suited for analysis of *in vitro* resistance to chemotherapy. The Assay is based on culturing malignant cells in suspension in the presence of various chemotherapeutic agents.

- Specimens are processed to separate viable mononuclear cells.
- The isolated mononuclear cells are evaluated both microscopically and by flow cytometry to confirm the presence of malignant cells.
- Isolated cells are combined with chemotherapeutic agents.
- After a 4 day period of drug exposure, cells are exposed to a vital stain that interacts preferentially with the membranes of dead/dying cells.
- The vital stain subsequently prevents counterstaining of the dead/dying cells with conventional hematoxylin and eosin.
- After exposure to the vital stain, cells are spun down onto slides, counterstained and the cytotoxic effects of chemotherapy are examined by light microscopy.
- Percent cell kill is determined by comparing treated cell death with untreated controls.

Clinical Correlations

The Differential Staining Cytotoxicity (DiSC[®]) Assay has been studied extensively by numerous independent investigators. The DiSC[®] Assay has been repeatedly found to accurately predict resistance to antineoplastic drugs and has undergone rigorous scrutiny for over 15 years. More than 50 publications report correlation between assay results and patient response or survival. The table below shows that in 510 published clinical correlations the negative predictive accuracy is 92%.¹

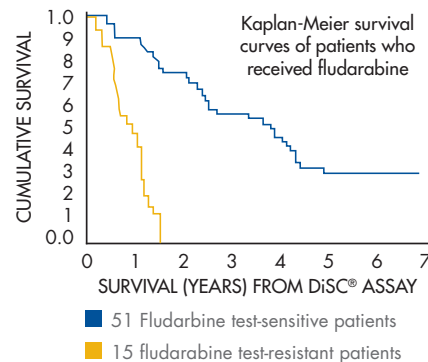
*Correlations of In Vitro Test Results with Patient Response**

Assay Type	Total N	TP	TN	FP	FN	% Negative Predictive Accuracy	% Sensitivity
DiSC [®] Assay	510	247	175	72	16	92	94

*Summary of clinical correlations pooled from individual studies referenced and reported in PPO Updates, 4th Edition, Vol. 7 *In Vitro Determination of Drug Response: A Discussion of Clinical Applications* by J.P. Fruehauf M.D., Ph.D and A.G. Bosanquet, Ph.D.

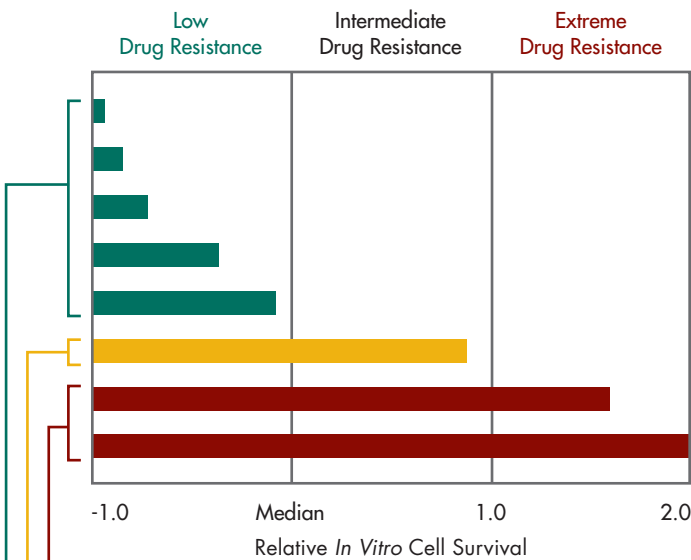
Survival Study

A study was published on the results of the DiSC[®] Assay to determine fludarabine response for CLL patients.² The study demonstrated that fludarabine test-resistant patients, as compared with fludarabine test-sensitive patients, had significantly poorer response rates (7% versus 69%) and poorer survival (median 7.9 months versus 41.7 months; relative risk = 14.8; p < 0.0001). The study demonstrated that DiSC[®] Assay results were a powerful independent prognostic factor, and suggested that treatment of patients who showed *in vitro* resistance to fludarabine had a significantly poorer outcome, particularly because treatment with potentially better alternatives were delayed while they received ineffective therapy with fludarabine.



ONCOTECH DiSC[®] ASSAY REPORT

Drug Resistance Assay



	Assay-Predicted Response Probability	Literature Response Rate
2-Chlorodeoxy-Adenosine	20	10
Dexamethasone	20	10
Cyclophosphamide	37	20
Vincristine	19	10
Etoposide	17	10
Cytarabine	3	10
Doxorubicin	<3	10
Fludarabine	<3	10

Extreme Drug Resistance (EDR): Extreme Drug Resistance (EDR) indicates that tumor cell growth was virtually unaffected by the high chemotherapeutic agent exposure (growth greater than 1 SD above the median growth). Data published in the April 1990 edition of the Journal of the National Cancer Institute (JNCI) and other published data show that patients had very little chance of responding to EDR category agents. Overall, probabilities of clinical response to EDR agents were shown to average less than 1%.

Intermediate Drug Resistance (IDR): Intermediate Drug Resistance (IDR) indicates moderate tumor growth (greater than the median growth but less than 1 SD above the median). In published studies, patients treated with agents in this IDR category had response rates that were about half of the rates reported in the medical literature.

Low Drug Resistance (LDR): Low Drug Resistance (LDR) indicates that tumor cell proliferation was inhibited by the tested agent. Tumor cells demonstrated very little growth (less than the median). Patients treated with agents in the Low Drug Resistance category had response rates that were approximately 1 1/2 to 2-fold greater than the literature reported rates.

Literature Response Rate: Determined from an extensive review of clinical trials in which each drug was administered as a single agent therapy in the tumor type in question.

Assay Predicted Response Probability: Derived from an algorithm involving *in vitro* tumor cell proliferation, literature response rate, patient treatment status, and comparison with a database of over 100,000 previous *in vitro* assays, in accordance with the Bayesian mathematical model.

DiSC[®] ASSAY FEATURES AND BENEFITS

Accurate	<ul style="list-style-type: none"> 92% accuracy for identifying ineffective (resistant) agents Independent of host factors has been documented in published reports
Cost Effective	<ul style="list-style-type: none"> Avoids direct costs of ineffective therapies Avoids costs of managing treatment related morbidity
Humane	<ul style="list-style-type: none"> Spare patients unnecessary toxicity Saves valuable treatment time Avoids the potential of inducing cross resistance to other effective agents
Reliable	<ul style="list-style-type: none"> Over 90% of leukemia specimens submitted yield successful assay results
Fast	<ul style="list-style-type: none"> Test results are available in 7-10 days

References

- Fruehauf, J.P. & Bosanquet, A.G. (1993) *In vitro* determination of drug response: a discussion of clinical applications. *Principles and Practice in Oncology Updates*. 7 (Dec). 1-16.
- Bosanquet, A.G., Johnson, S.A., & Richards, S.M. (1999) Prognosis for fludarabine therapy of chronic lymphocytic leukemia based on ex vivo drug response by DiSC[®] assay. *British Journal of Haematology*. 106. 71-77.