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ORIGINAL ARTICLE: CLINICAL

Correlation of the microculture-kinetic drug-induced apoptosis assay with patient outcomes in initial treatment of adult acute myelocytic leukemia

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Abstract

Overall survival (OS) with acute myeloid leukemia (AML) remains poor. Determining prognostic factors will help in selecting patients for appropriate treatments. Our aim was to determine whether the level of drug-induced apoptosis (chemosensitivity) demonstrated by the microculture-kinetic drug-induced apoptosis (MiCK) assay significantly predicted outcomes after standard AML induction therapy. A total of 109 patients with untreated AML had blood and/or bone marrow aspirate samples analyzed for anthracycline-induced apoptosis using the MiCK assay. The amount of apoptosis observed over 48 h was determined and expressed as kinetic units of apoptosis (KU). Complete remission (CR) was significantly higher (72%) in patients with high idarubicin-induced apoptosis >3 KU compared to patients with apoptosis ≤3 KU ($p = 0.01$). Multivariate analysis showed the only significant variables to be idarubicin-induced apoptosis and karyotype. Median overall survival of patients with idarubicin-induced apoptosis >3 KU was 16.1 months compared to 4.5 months in patients with apoptosis ≤3 KU ($p = 0.004$). Multivariate analysis showed the only significant variable to be idarubicin-induced apoptosis. Chemotherapy-induced apoptosis measured by the MiCK assay demonstrated significant correlation with outcomes and appears predictive of complete remission and overall survival for patients receiving standard induction chemotherapy.

Keywords: Acute myeloid leukemia, apoptosis, MiCK assay, idarubicin, cytarabine

Introduction

Outcome data regarding standard therapy for acute myeloid leukemia (AML) show only 20–30% long-term disease-free survival [1] and demonstrate the challenge of selecting appropriate treatment strategies for individual patients. Scientific advances in cytogenetics [2,3] and molecular profiling [4,5] have enabled us to stratify patients into appropriate prognostic categories, but as of yet have failed to allow us to replace "7 plus 3" as the standard induction chemotherapeutic regimen [6]. Risk stratification based on clinical and molecular correlates of outcome helps to identify patients who might benefit from enrollment in frontline therapeutic trials and/or those who should be offered hematopoietic stem cell transplant in first complete remission (CR), rather than simply proceeding with standard "7 plus 3" induction and conventional consolidation strategies.

The use of chemotherapy sensitivity and resistance assays to direct therapeutic strategies has been evaluated within multiple tumor types, with mixed results [7–11]. Responses of leukemia cells to drug exposures *in vitro* have correlated with remission but rarely with survival. Such assays have included drug-induced changes in dye exclusion [12–14], dye metabolism [15–20], DNA synthesis [21] and/or cell clonal growth *in vitro* [20,22–25].

Given that standard AML induction chemotherapeutics induce apoptosis [26–29], it is hypothesized that an assay which effectively measures drug-induced apoptosis *in vitro* may allow more appropriate drug selection and more accurate prediction of clinical outcomes. An *in vitro* assay for drug-induced apoptosis, the microculture-kinetic (MiCK) assay, has been applied to AML cells [30–33]. MiCK assay analysis of tumor samples allows reproducible and quantifiable measurement of the increased optical density (OD) due to increasing light scattering associated with cultures of cells undergoing drug-induced apoptosis [32]. There is a good correlation between drug-induced changes in optical density and drug-induced increases in caspase activity.

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This study was conducted to determine the clinical correlations between the MiCK assay results and the clinical outcomes of complete response, relapse-free interval and overall survival in a series of adult patients with AML of all ages treated with 7 days of continuous infusion cytarabine plus 3 days of an anthracycline.

Methods

Patients

Eligibility

Patients with previously untreated adult AML aged 15 or above were eligible. AML was diagnosed by criteria that included: (a) microscopic examinations of blood or bone marrow smears stained with Wright's, Sudan black, periodic acid–Schiff, chloroacetate esterase and α-naphthylacetate esterase stains; (b) cytogenetic analyses; (c) flow cytometry immunophenotypic analyses with a panel of monoclonal antibodies that included antibody pairs for dual staining of CD2/CD4, CD13/CD19, CD20/CD10, CD7/HLA-DR, CD33/CD34 and CD14/CD45. Samples for karyotypic analysis were obtained simultaneously with samples for the MiCK assay. Karyotypes were classified according to accepted standards at the time of study initiation and were considered to have a good prognosis if they were inv(16) or t(8;21), an intermediate prognosis if they were normal, and a poor prognosis if they included other chromosomal abnormalities. Antecedent myelodysplastic syndrome (MDS) was diagnosed by standard morphologic criteria using bone marrow aspiration and biopsy either prior to or at the time of the diagnosis of AML.

Six years elapsed between development of the assay and initiation of this trial and collection of the initial cohort of patients, and the collection of a second cohort of patients. This was due to licensing of the technology to DiaTech Oncology by Vanderbilt University, the patent holder, and moving the laboratory to the McGill University campus in Montreal, Canada. Quality control standards were implemented in Montreal and the laboratory was approved by CAP (College of American Pathologists) and CLIA (Clinical Laboratory Improvement Amendments) prior to initiation of the trial. The initial cohort consisted of 55 patients, and the later cohort consisted of 54 patients.

Laboratory sample submission

After obtaining voluntary informed consent, venous or bone marrow aspirate samples were collected and subsequently evaluated. Initial laboratory assessment was performed at Vanderbilt University. These services were later performed at DiaTech Oncology in Montreal, Canada. Vanderbilt University and the University of Pittsburgh participated in this trial under grant funds provided by DiaTech Oncology to the respective universities.

Therapy

From 1996 through 2010, 109 patients had adequate samples for MiCK assay and were treated with a "7 plus 3" regimen. Since this study covered a period of 14 years, it must be interpreted in the light of gradually improving supportive care

and increasing use of allogeneic transplant. Therefore, multivariate analyses were performed considering the variables of use of allogeneic transplant and karyotypic risk. Correlation of idarubicin-induced apoptosis with response and survival did not vary according to early versus later patient accrual (see Tables III and IV).

Treating physicians were blinded to the laboratory results of the MiCK assay. CR was defined as less than 5% blasts and at least 20% cellularity in a representative bone marrow sample following blood cell count recovery after induction chemotherapy. In addition to the marrow criteria, those patients considered as having achieved CR had more than 1.0×10^9/L absolute neutrophils and more than 1.0×10^{11}/L platelets in the peripheral blood, independent of platelet transfusion support [34].

Drug-induced apoptosis (MiCK) assay

The MiCK assays were performed as described [35] and briefly as follows.

Sample preparation

Heparinized bone marrow aspirates or peripheral blood samples were obtained from patients after obtaining informed consent. Flow cytometry was performed to analyze the antigenic character of the leukemic cells for subsequent purification using appropriate antibodies. Samples were diluted with an equal volume of RPMI-1640 medium without phenol red. The mononuclear cell fraction was then collected by Ficoll–Hypaque gradient. B lymphocytes, T lymphocytes and monocytes were removed from the mononuclear cells by using magnetic beads conjugated with appropriate antibodies to CD19, CD2 and CD14, respectively (Invitrogen Canada Inc., Burlington, ON, Canada). Resultant samples contained an approximately 95% pure population of 95% viable blasts. This was performed to be certain that optical density changes induced by chemotherapy drugs were associated with changes in leukemic cells, not normal cells. Positive selection was not used since the antigenic pattern of the myeloblast was not known prior to sample preparation and many specific antibody bead complexes were not commercially available, there would likely be a large loss of cells in trying to identify a specific antigen that could be used for positive selection, and there was the potential for positive selection to alter the malignant cells in unpredictable ways. If there was aberrant expression of antigens on the blast surface, such as CD19 on myeloblast, an alternative antibody coated bead (e.g. CD20 coated bead) was used.

The MiCK was performed as previously described with minor modifications [30]. Malignant blasts were suspended in RPMI-1640 medium without phenol red and with 5% fetal bovine serum and then placed in 96-well microtiter plates (Corning, Cambridge, MA) in 240 μL aliquots. Aliquots of approximately 1×10^5 cells were used to provide a near uniform covering of the plate well bottoms. With the exception of three patients, all patients had MiCK assay results with serial dilutions of idarubicin (0.1, 0.5, 1, 2.5, 5, 10 and 20 μmol/L) and cytarabine (5, 10, 20, 40, 80, 160, 320 and 640 μmol/L). When adequate numbers of blasts were available, MiCK assays for daunorubicin (1, 5, 10, 20 and

40 μmol/L) and mitoxantrone (0.1, 0.5, 1, 2.5 and 3.3 μmol/L) were also performed. These concentrations were selected based on a starting molar concentration of the clinically used drug dose assuming distribution in total body water, with 1–2-log range of other concentrations above and below the starting molar concentration.

Aliquots of the mixture were then incubated at 37 °C for 30 min in a humidified incubator with 5% CO_2. Sterile mineral oil (30 μL; Sigma, St Louis, MO) was then layered onto the surface without disturbing the cells and the plates were then placed into a 37 °C incubated chamber of a spectrophotometer (BioTek Powerwave X series; Winooski, VT). The OD was then read every 5 min for 48 h. All assays were performed with a concurrent control using JURL-MK2 or HL60 leukemic cell lines as a positive control for apoptosis. The positive and negative sensitivity patterns for each were previously established in this laboratory.

Data processing

Each of the OD readings was then plotted versus time to yield a kinetic plot of the individual drug responses. Apoptosis has been previously shown to be quantified by the apoptotic curve thus generated [31,33]. The steepest slope of this curve is referred to as the maximum kinetic rate (V_{max}) and is generated by proprietary software (DiaTech Oncology, Nashville, TN). The V_{max} has been previously shown to directly correlate with the percent of apoptotic cells in the wells [31]. To control for spontaneous blast apoptosis and for blast proliferation during the assay, the OD readings from the same period of time as those used in generating V_{max} for the control cultures without drug exposure were subtracted from those of the drug treated wells:

$$V_{max} \text{ apoptosis} = (V_{max} \text{ with drugs} - V_{max} \text{ control})$$

Using the resulting values allowed quantification of apoptosis associated with each of the drugs or drug combinations at each of the concentrations. The occurrence of high spontaneous apoptosis was rare (11%), while a low degree of spontaneous apoptosis was sometimes seen (28%), and no spontaneous apoptosis was the most frequent condition (61%). There was no association between idarubicin-induced apoptosis, rate of complete remission or overall survival with degree of spontaneous apoptosis. Because test results are dependent on proprietary preparation methods and proprietary test conditions, the test is not practically performed in a routine clinical laboratory.

Statistical analyses

Apoptotic responses in kinetic units (KU) were calculated for each dose of cytarabine or idarubicin. The maximal KU induced by a drug was considered the "best apoptotic response" to the drug. In order to discriminate between the drug-sensitive and drug-resistant leukemia cell populations, the best responses to cytarabine and idarubicin were compared in a receiver operator characteristic (ROC) analysis as well as logistic regression analysis with attainment of CR after induction therapy (CR vs. no CR) in an initial 23-patient training cohort. Based on the best combination of statistical

sensitivity, specificity and *p*-value, thresholds demarcating drug sensitivity and resistance were established for idarubicin. A 3 KU cut-off point was found optimal for demarcating drug sensitivity and resistance with idarubicin. A 3 KU cut-off might not be appropriate for other drugs whose mechanism of action might be different from idarubicin or other anthracyclines. No cut-off point was optimal for cytarabine. By Fisher's exact test and Pearson χ^2 test, the 3 KU cut-off point for idarubicin provided 100% sensitivity and 67% specificity ($p = 0.0008$). The remaining patients ($n = 86$) formed the test cohort to validate the idarubicin cut-off of 3.0 for complete response.

The Fisher's exact test, χ^2 test, logistic regression and analysis of variance methods were employed to test the correlation between the clinical response results and other clinical variables. For lifetime data analyses, the possible risk factors were compared for survival with Kaplan–Meier estimates and log-rank tests. The Cox proportional hazards model was used for adjusting the tests of significance and estimating the adjusted hazard ratios. Significance was based on two-sided tests. The statistical analyses were completed using S+ 6.1, JMP 8.0 or SAS 8.2 statistical programs.

IRB approval

Prior to study initiation, this study was approved by the Vanderbilt University Medical Center Insitutional Review Board (IRB), and subsequently the Western IRB. The study was registered with the National Cancer Institute as study NCT00286845 at www.clinical.trials.gov.

Results

Patient and assay characteristics

Patient characteristics are shown in Table I. Of the entire patient cohort, 38% were ≥60 years of age, 35% had prior myelodysplastic syndrome, 42% had an unfavorable karyotype and 21% ultimately received a hematopoietic stem cell transplant.

Table I. Patient characteristics.

Characteristic	No. of patients (%)	Median
Age (years)		55
15–59	68 (62%)	
≥60	41 (38%)	
Pretreatment WBC (per μL)		17 600
Gender		
Female	58 (53%)	
Male	51 (47%)	
Specimen source		
Blood	60 (65%)	
Bone marrow	32 (35%)	
Karyotype risk		
Favorable	5 (5%)	
Intermediate	55 (53%)	
Unfavorable	44 (42%)	
Prior MDS		
Yes	38 (35%)	
No	71 (65%)	
Post-induction stem cell transplant		
Yes	23 (21%)	
No	86 (79%)	

WBC, white blood cell count; MDS, myelodysplastic syndrome.

Table II. Drug-induced apoptosis in the MiCK assay.

Drug	Number	Mean KU	Median KU	Minimum KU	Maximum KU
Idarubicin	107	3.88	3.60	0.00	13.10
Daunorubicin	72	2.87	2.74	0.00	12.20
Mitoxantrone	65	2.81	2.55	0.00	10.88
Cytarabine	108	1.32	1.16	0.00	7.77

MiCK, microculture-kinetic; KU, kinetic units of apoptosis in MiCK assay.

The *in vitro* responses of AML cells to drug-induced apoptosis are shown in Table II. Both idarubicin and cytarabine distributions of KU were unimodal. The mode for idarubicin was 3–4 KU, and for cytarabine was 0–1 KU. Comparison of the KU values for anthracyclines revealed that idarubicin produced significantly more apoptosis compared to daunorubicin (difference 0.5 KU, $p = 0.0003$) or mitoxantrone (0.6 KU, $p = 0.003$). No statistical difference was found between the drug-induced apoptosis observed with daunorubicin and mitoxantrone ($p = 0.32$).

Analysis of apoptotic activity within individual patient samples demonstrated at least one standard deviation (1SD; 0.57 KU) greater apoptosis for idarubicin compared to daunorubicin in 43.1% of patients, and in 49.2% for idarubicin compared to mitoxantrone. Improved apoptosis from daunorubicin over idarubicin was seen in 11.1% of samples and mitoxantrone produced more apoptosis than idarubicin in 20.0% of samples. These findings demonstrate the ability of the MiCK assay to differentiate *in vitro* anthracycline sensitivity on an individual patient basis.

Correlations with complete response

CR was achieved in 61.2% ($n = 85$) of patients within the test cohort. Statistically significant correlation existed between achievement of CR and MiCK assay results for idarubicin. Idarubicin apoptosis level ≤3 KU was associated with a CR rate of 45.7%, while an idarubicin apoptosis level >3 KU was associated with a CR rate of 72% ($p = 0.01$). No correlation was identified between CR and MiCK assay results incorporating cytarabine.

Patients with a MiCK assay result for idarubicin of ≤3 KU had a mean apoptotic score of 2.38 KU for those who achieved CR compared to 1.60 KU for those who failed to achieve CR ($p = 0.0012$). Thus, among patients with low apoptototic scores, idarubicin-induced apoptosis predicted CR on a univariate basis.

Multivariate analysis

Individual patient characteristics were compared including MiCK assay results for idarubicin and cytarabine (Table III). The only significant variables correlating with CR were karyotype ($p = 0.0091$) and idarubicin-induced apoptosis ($p = 0.036$). In order to simplify the multivariate model shown in Table III, a backward elimination logistic regression model was run. Idarubicin-induced apoptosis remained significant ($p = 0.0009$) as did karyotype ($p = 0.02$).

Relapse-free interval

All patients attaining CR were consolidated with high-dose cytarabine, and 23 patients ultimately received a hematopoietic stem cell transplant. No correlation was seen between idarubicin-induced apoptosis and relapse-free interval ($p = 0.96$). In patients with intermediate or favorable karyotype, idarubicin-induced apoptosis >3 KU was associated with a median relapse-free survival (RFS) of 37.6 months, compared to 13.5 months in patients with apoptosis ≤3 KU, although this was not statistically significant ($p = 0.22$).

Overall survival

Overall survival in all patients correlated with idarubicin MiCK assay results (Figure 1). The survival of patients with idarubicin-induced apoptosis >3 KU was a median of 16.1 months (95% confidence interval [CI]: 9.7–24.9), significantly longer than the median of 4.5 months (95% CI: 2–9.66) in patients with apoptosis ≤3 KU ($p = 0.004$). Since stem cell transplant can influence overall survival, this analysis was repeated for only patients without stem cell transplant, with a similar correlation (Figure 2). The survival of patients without stem cell transplant who had idarubicin-induced apoptosis >3 KU was a median of 11.9 months, significantly longer than the median of 3.4 months in similar patients with apoptosis ≤3 KU ($p = 0.0024$).

Table III. Multivariate analysis of complete response.

Variable	Comparator	Odds ratio for CR = yes vs. CR = no (95% CI)	p-Value
Idarubicin KU	KU change of 1	1.32 (1.02–1.80)	0.036
Cytarabine KU	KU change of 1	1.06 (0.57–2.11)	0.85
Age	Age change 1 year	0.995 (0.95–1.04)	0.82
Pretreatment WBC	WBC change of 1000	0.996 (0.99–1.004)	0.35
Gender	Female to male	1.95 (0.63–6.11)	0.24
Karyotype	Intermediate to unfavorable	4.22 (1.42–13.8)	0.009
Prior MDS	Yes to no	1.24 (0.38–4.37)	0.73
Post-induction SCT	Yes to no	1.96 (0.48–9.10)	0.35
Cohort	Original to new	1.67 (0.44–6.87)	0.46
Source of specimen	Blood to marrow	1.64 (0.42–6.76)	0.48

KU, kinetic units of apoptosis in the MiCK assay; WBC, white cell blood count; MDS, myelodysplastic syndrome; SCT, stem cell transplant; CR, complete response; CI, confidence interval.
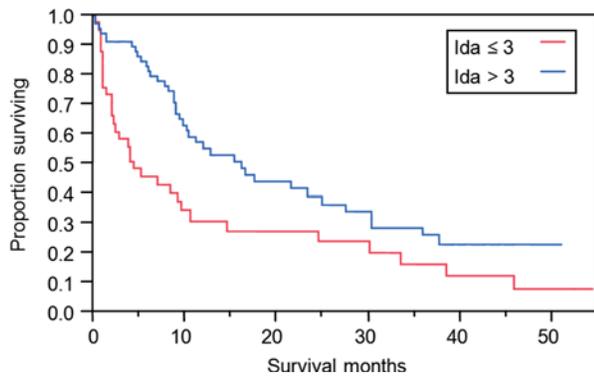
Figure 1. Overall survival of all patients according to idarubicin-induced apoptosis; $p = 0.004$.

Multivariate analysis of variables associated with overall survival of patients indicated statistical significance for only idarubicin-induced apoptosis as measured by the MiCK assay (hazard ratio of 0.52; CI: 0.31–0.88; $p = 0.01$), despite the inclusion of variables generally accepted as being predictive of survival such as a prior history of myelodysplasia, white blood cell count (WBC) at presentation or karyotype (Table IV).

Comparison of idarubicin with daunorubicin induction chemotherapy

During induction chemotherapy, 95% of patients received idarubicin and 5% received daunorubicin. No differences were observed in CR rate or overall survival comparing patients who received either anthracycline for induction. However, in patients who received idarubicin there was a higher rate of CR, 72% in patients with assay idarubicin-induced apoptosis >3 KU versus a CR rate of 40.5% in patients with apoptosis ≤3 KU ($p < 0.01$). In patients who received daunorubicin there was no significant difference in CR rate by idarubicin apoptosis >3 KU vs. ≤3 KU. Similarly, in patients who received idarubicin there was a significantly longer overall survival of 16.1 months in patients with idarubicin apoptosis >3 KU compared to a survival of only 5.3 months if idarubicin apoptosis was ≤3 KU ($p < 0.01$). In patients who received daunorubicin, there was no significant difference in survival by idarubicin apoptosis >3 KU vs. ≤3 KU. This indicates that drug-induced apoptosis in the MiCK assay is predictive of
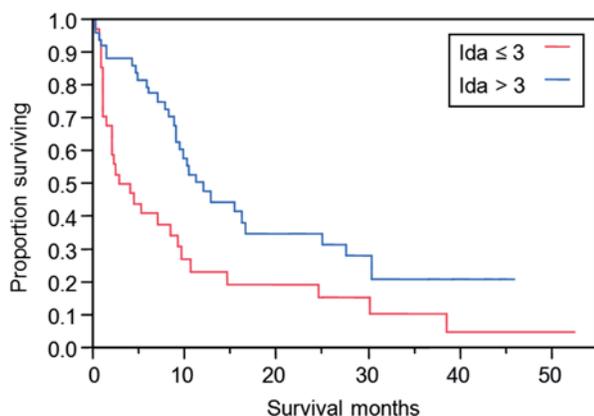

Figure 2. Overall survival of patients who did not have a stem cell transplant according to idarubicin-induced apoptosis; $p = 0.0024$.

response and survival on a drug specific basis, and is not just prognostic regardless of chemotherapy actually used.

Discussion

MiCK assay apoptotic results were predictive of CR and OS in this mixed patient population. Idarubicin-induced apoptosis was significantly correlated with both outcomes for patients with favorable/intermediate karyotype, whereas cytarabine-induced apopotosis was not. While further studies are in progress to better understand the marked differences relative to idarubicin- versus cytarabine-induced apoptosis, it is possible that these findings indicate a greater importance of individual patient sensitivity to anthracyclines.

In patients failing to achieve a CR, the MiCK assay was able to identify another drug or combination that gave higher apoptotic scores by at least 0.57 KU (1SD) compared to idarubicin in 11 of 42 patients (26%). Because of the wide range of concentrations of drugs tested, we assume that the maximally potent concentration of each of the antracyclines has been tested. Differential sensitivities of leukemic cells from individual patients to anthracyclines suggest an ability of the MiCK assay to help clinicians' select particular anthracyclines for individual patients and also suggest a possible role for use of this assay in drug discovery (see below). Prospective validation of this hypothesis is warranted in future controlled studies to determine the ability of the MiCK assay to personalize clinical care and possibly improve outcomes of adult patients with AML.

Correlation of outcomes and idarubicin sensitivity in the MiCK assay indicates the possibility of identifying patients whose therapy should include alternative treatments in addition to or in place of standard induction, consolidation and hematopoietic stem cell transplant. The finding that clinical response and survival are correlated with *in vitro* idarubicin-induced apoptosis is not surprising. The MiCK assay is the first *in vitro* assay to demonstrate such a correlation and represents a new tool for risk assessment and possible drug selection. Lack of correlation of cytarabine-induced apoptosis is likely related to a low fraction of leukemic cells undergoing DNA synthesis during the brief cell culture conditions. Current studies are under way to evaluate the utility of this test with other agents such as etoposide, clofarabine, azacytidine, decitabine and other newer agents that might be appropriate to consider in a patient's regimen to increase CR rates, relapse-free interval and/or overall survival.

The ability to accurately assess apoptosis, a mechanism of cytotoxicity present in all malignancies, underscores the potentially broad applicability of this test to numerous clinical situations. Successful studies utilizing the MiCK assay have been conducted in solid tumors, including blinded, prospective validation studies in breast cancer [36], endometrial cancer [37] and ovarian cancer [38]. A non-blinded, prospective multicenter utility trial demonstrated statistically improved overall survival, relapse-free interval and complete plus partial response rates if the MiCK assay was used to select therapy for patients with solid tumors and leukemia [39].

Table IV. Multivariate analysis of overall survival.

Variable	Comparator	Hazard ratio for death (95% CI)	p-Value
Age	Age change 1 year	1.005 (0.99–1.03)	0.60
Idarubicin KU	Above 3 KU to ≤3	0.52 (0.31–0.88)	0.01
Prior MDS	No to yes	0.64 (0.38–1.11)	0.11
Cohort	Original to new	0.64 (0.37–1.08)	0.09
Post-induction SCT	Yes to no	0.61 (0.29–1.20)	0.15
Gender	Female to male	0.66 (0.38–1.16)	0.15
Karyotype	Favorable + intermediate vs. unfavorable	0.97 (0.57–1.67)	0.92
Pretreatment WBC	WBC change of 1000	1.00 (0.998–1.01)	0.28

KU, kinetic units of apoptosis in MiCK assay; MDS, myelodysplastic syndrome; SCT, stem cell transplant; WBC, white cell blood count; CI, confidence interval.

The assay has been used successfully in over 50 different tumor types including breast, lung, colon, gastric, pancreatic, esophageal, rectal, thyroid, head and neck, skin (squamous cell), renal, prostate, bladder, endometrial and cervical cancers, and melanoma, lymphoma, soft tissue sarcoma, myeloma, myelodysplastic syndrome and glioblastoma. All chemotherapy drugs which produce apoptosis have been used in the assay.

Because of the proprietary nature of certain aspects of the cell purification steps and test culture conditions, it is not feasible to perform the assay consistently with satisfactorily low coefficients of variation in routine clinical laboratories. Since this test was performed efficiently following sample submission via FedEx, this should be feasible to conduct future trials and clinical studies throughout North America and other countries. The assay is thus practical for central reference laboratories to perform to give clinicians data on which chemotherapy produces the best apoptosis, to help in treatment planning. Use of this assay is consistent with the use of such laboratories for other predictive assays (e.g. OncotypeDx).

The MiCK assay has been tested *in vitro* with other measures of apoptosis. The results have indicated good correlation with tritiated thymidine uptake, DNA fragmentation analyses, annexin V binding, flow cytometric analyses of annexin V fluorescein isothiocyanate labeling, morphological analyses of Giemsa stained leukemic cells, and time lapse video microscopy of plasma membrane blebbing [32,33]. While flow cytometric viability dye staining is a commonly used technique, the lack of correlation with clinical outcome has limited its usefulness to the preclinical setting. However, the statistically significant correlation of the MiCK assay with clinical outcome supports its incorporation not only into preclinical drug development but also into clinical treatment planning and the provision of personalized cancer care.

The finding of significant differences in outcomes associated with high optical density changes in the conditions of the assay compared to prior poor correlations of outcomes with other types of assays is important [40]. It is probably due to differences of testing purified leukemic cell populations rather than cell mixtures including normal cells, which can generate confounding signals of cytotoxicity. Also, the MiCK assay is a kinetic assay that takes sequential readings every 5 min for as long as 48 h, it is not destructive, and the same cell population can be monitored over the course of the assay. Virtually all other assays are destructive and not kinetic. They are limited to a single reading taken at a single point in time. Since the length of time necessary for complete apoptosis to occur varies from patient to patient and between drugs and concentrations, the MiCK assay is uniquely suited to measure apoptosis over time (it is not necessary to know in advance when to take the measurement because measurements are taken sequentially and continuously). Additionally, the MiCK assay is automated after the cells are isolated, purified and placed in a spectrophotometric plate with the drugs.

The finding of an association between drug-induced apoptosis in the MiCK assay and overall survival suggests potential for incorporating this assay into drug development. Analysis of novel agents in preclinical settings may allow reliable comparison to conventional agents for better drug selection for future clinical trials, and thereby increase the odds of clinical benefit being demonstrated in these clinical studies. Utilization of this assay as a companion diagnostic may provide the potential to select patients for phase II or III trials who might demonstrate higher response rates or longer survivals when treated with a specific agent. This assay also has the potential to identify synergistic drug combinations *in vitro*, leading to subsequent improvement of patient outcomes.

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Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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