

# Can we increase response rate (RR) and overall survival (OS) by individualizing chemotherapy in ovarian cancer (OC) – the role of new chemotherapy (CT) induced apoptosis assay

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## ABSTRACT

**Background:** In order to develop an assay to improve OS of OC pts, we tested pt tumor cells (TC) using a novel CT-induced in vitro apoptotic assay (the Microculture Kinetic [MiCK] assay). This was a prospective blinded trial in which physicians selected CT without knowledge of MiCK assay results.

**Methods:** TC obtained at surgery were prepared and cultured with drugs as previously described (Lab Invest 74: 557, 1996). TC apoptosis from single drugs or combination regimens was measured over 48 hours. Assay results were compared to OS and RR following CT. The assay results were measured as kinetic units of apoptosis (KU) with > or =1.7 KU defined highly active vs less active <1.7 KU. The best CT was defined as apoptosis >0.57 KU (>1 measurement s.d.) higher than other drugs.

**Results:** A total 128 pts were evaluable for MiCK results. Combination paclitaxel (Pac) + carboplatin (C) gave significantly more apoptosis 2.6 KU vs single agent Pac 1.8 KU or C 1.4 KU (p<0.0001). However, single drug Pac or C was more effective than Pac+C in 28% and was equal to Pac+C in 9% of pts. In 46% of pts, another drug or combination produced more apoptosis compared to Pac+C. When comparing CT for all stages, OS was significantly better at 24 months in 92% pts who received best CT vs only 76% in pts who received a non-best assay predicted CT (p=0.01). There was a significantly higher overall RR 82% in pts who received the best CT in the assay vs 54% in pts who received a non-best assay predicted CT (p=0.04). In stage 3 or 4 (54/90) OC pts treated with highly active CT by assay had a significantly increased OS (94% alive at 24 mo) compared those with less active CT (77% alive; p=0.02). The hazard ratio for death in pts receiving highly active CT was 0.17 (95% CI 0.03-0.92). The CR, PR, SD, and nonrelapse rate was 85% for pts with highly active CT, compared to 57% for those pts receiving less active CT (p=0.03).

**Conclusions:** The drug induced apoptosis MiCK assay is useful in predicting which CT will result in increased OS and increased RR in this study. This assay can help oncologists to select the best CT for individual pts. This assay may help develop new CT regimens and select responsive pts.

## BACKGROUND

- Survival of advanced ovarian cancer is suboptimal and is due to chemotherapy failure
- Personalized chemotherapy strategies are not effective, and prior chemo-resistance assays have not been adopted
- A novel chemotherapy-induced apoptosis assay (the MiCK assay) has been able to predict survival in acute myelocytic leukemia
- A clinical trial of the MiCK assay in solid tumors, specifically ovarian cancer, is warranted

## CLINICAL TRIAL GOALS

- To determine if the MiCK assay can predict overall survival of patients with ovarian cancer treated with assay-determined effective chemotherapy
- To determine if the MiCK assay can predict response of patients with ovarian cancer treated with assay-determined effective chemotherapy
- To determine if the MiCK assay can compare effectiveness of single agents versus combination chemotherapy
- To determine if the MiCK assay can compare the effectiveness of generic chemotherapy drugs versus proprietary drugs

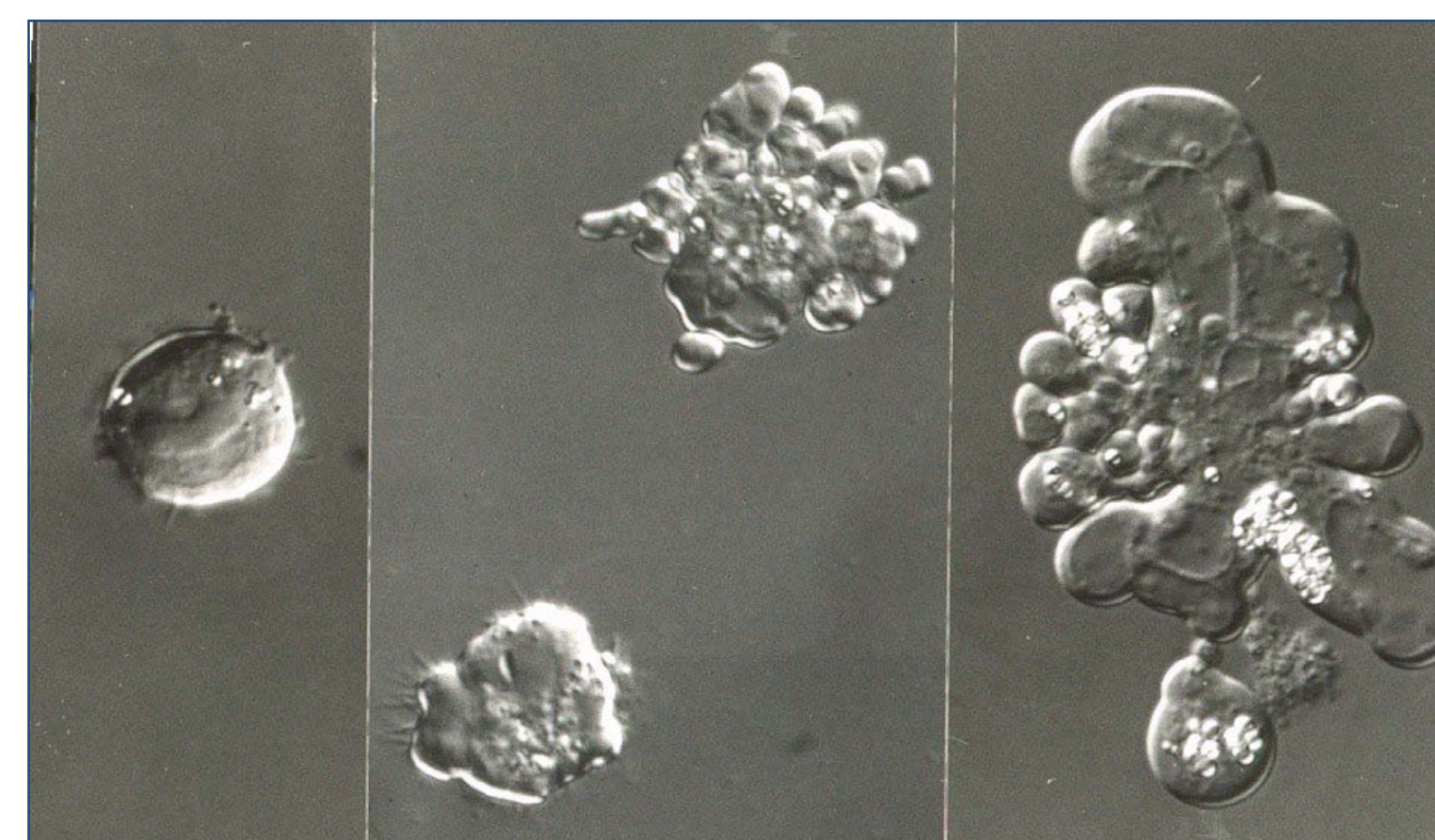
## METHODS

- This clinical trial was a multicenter prospective, nonrandomized, investigator-blinded, IRB-approved trial
- Gyn oncology surgeons enrolled all successive patients giving informed consent
- Specimens obtained at surgery, biopsy, or by paracentesis were sent to diaTech oncology (Montreal) by FedEx and processed with results obtained by 48 hours
- Physicians and patients were not informed of the results at any time, and patients were treated with physician choice of drugs
- MiCK assay results were correlated with clinical outcomes

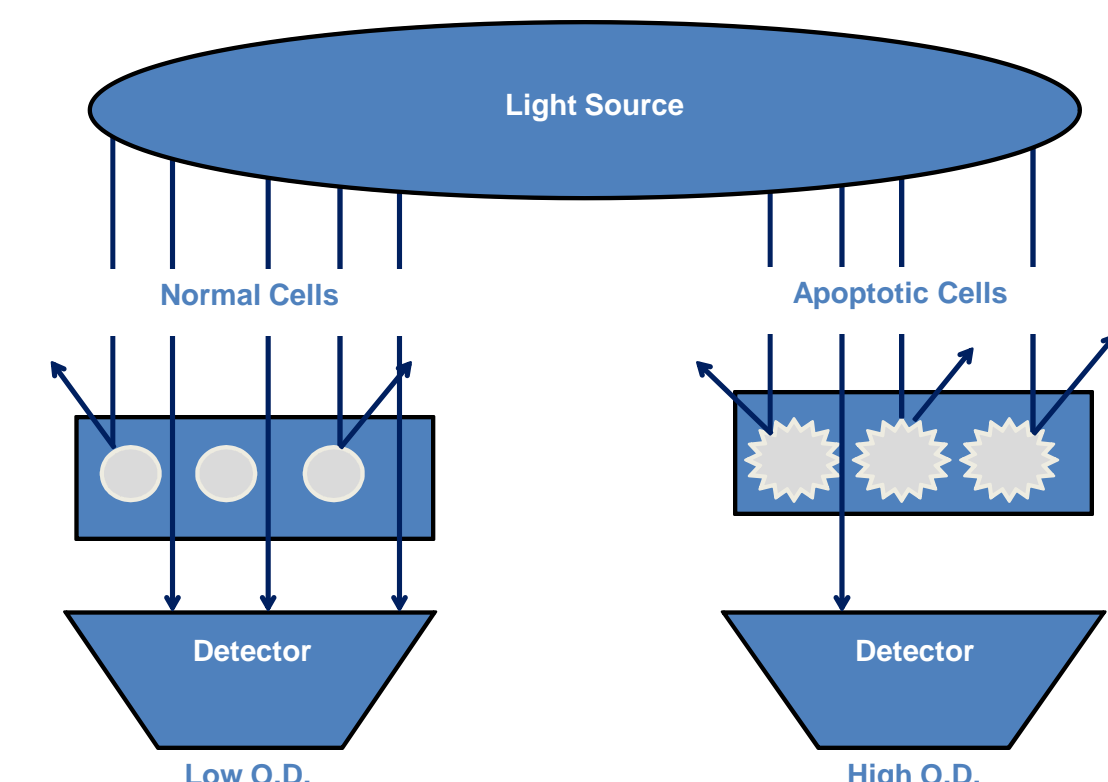
## MiCK ASSAY DESCRIPTION

- Tumors were disrupted mechanically and with collagenase and trypsin
- A homogeneous tumor cell population was obtained by differential centrifugation and affinity adsorption
- Tumor cell viability and homogeneity were assessed by a pathologist using dye exclusion, immunohistochemistry, and flow cytometry before assay
- Tumor cells were cultured for 48 hours with media alone, or media with 3 concentrations of chemotherapy drugs alone or in combination
- Optical density was measured automatically every 5 minutes over 48 hours
- Results were given in kinetic units of apoptosis:
- <1 KU was inactive
- >= 1 KU was active in producing apoptosis in vitro
- >= 1.7 KU was activity higher than the median ku in all patients

## Morphological Changes of Apoptosis

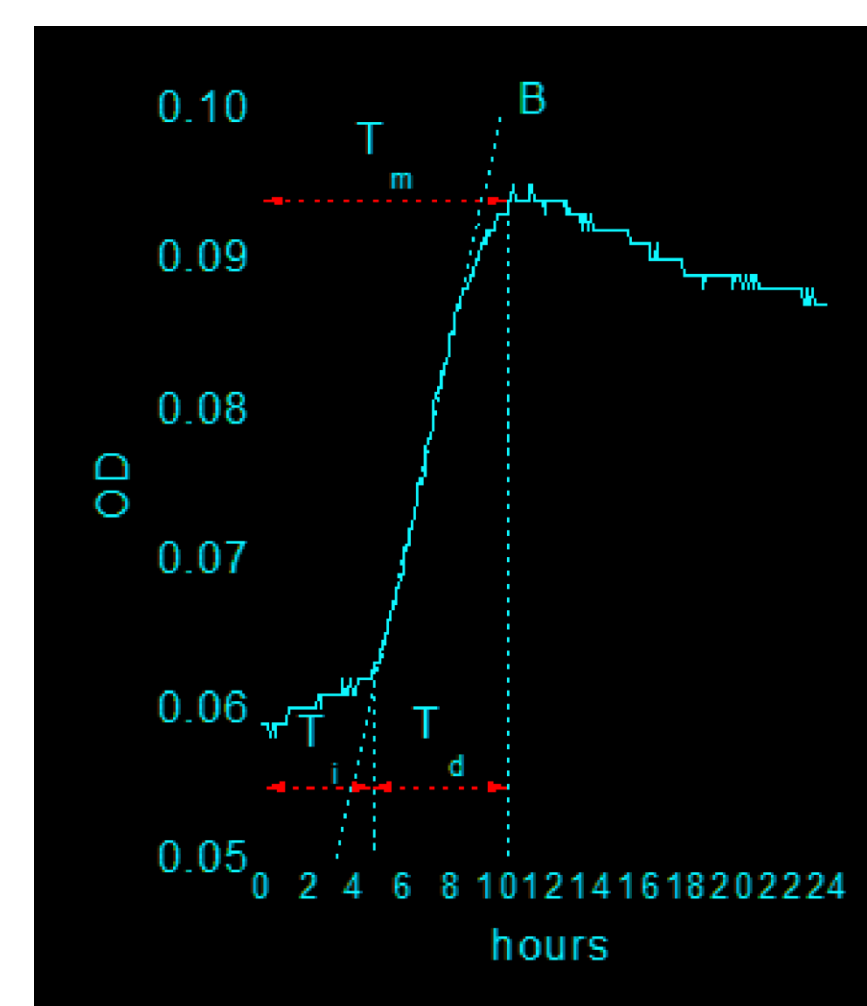


## Scattering of the Light by Normal and Apoptotic Cells



## Quantitation And Timing Of Apoptosis In The MiCK Assay

Wintrobe Clinical Hematology  
Wintrobe's 11th edition, Volume 2, p.2117.



Extent of Apoptosis is expressed in Kinetic Units (KU)

$$KU = B \times k$$

B - slope of the curve  
k - a cell density coefficient

## OVARIAN CANCER STUDY: PATIENT CHARACTERISTICS

Age	Mean = 59.2 Median = 60.5 Range 20 to 88												
Stage	<table border="1"> <tr><th>Stage</th><th>Count</th></tr> <tr><td>1</td><td>8</td></tr> <tr><td>2</td><td>5</td></tr> <tr><td>3</td><td>76</td></tr> <tr><td>4</td><td>14</td></tr> <tr><td>Unknown</td><td>25</td></tr> </table>	Stage	Count	1	8	2	5	3	76	4	14	Unknown	25
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3	76												
4	14												
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Prior Chemo	<table border="1"> <tr><th>Prior Chemo</th><th>Count</th></tr> <tr><td>Yes</td><td>18</td></tr> <tr><td>No</td><td>93</td></tr> <tr><td>Unknown</td><td>17</td></tr> </table>	Prior Chemo	Count	Yes	18	No	93	Unknown	17				
Prior Chemo	Count												
Yes	18												
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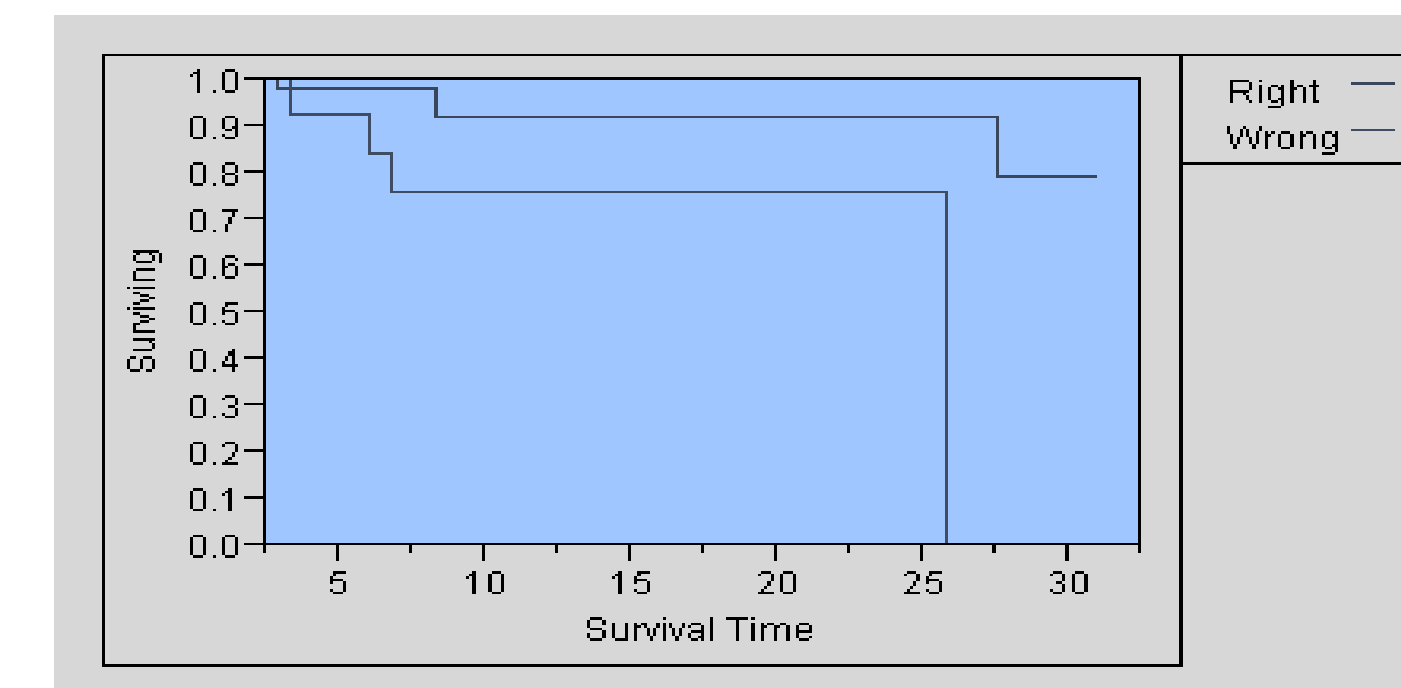
## OVARIAN CANCER STUDY = MiCK RESULTS

Drug	N	Median	Min	Max	#Inactive
Carbo	89	1.4	0.0	12.4	21
Taxol	93	1.8	0.0	9.7	30
Tax	15	2.4	0.0	5.1	1
Cisplatin	53	2.2	0.0	11.3	7
Gemz	47	0.8	0.0	3.4	28
Epi	13	2.7	0.4	7.7	2
Doxil	78	0.9	0.0	5.3	40
Topotecan	80	1.3	0.0	6.8	33
Abiraterone	8	1.0	0.0	1.4	4
HC	25	2.4	0.0	15.4	9
Mitox	2	1.7	1.7	1.7	0
Gleev	3	1.2	1.2	4.3	0
Velcade	3	1.6	0.8	2.9	1
Doxo	8	2.4	0.4	6.9	1
VP16	19	0.7	0.0	2.0	10
Carbo+Taxol	125	2.6	0.0	11.4	5
Cisplatin+Gemz	103	2.1	0.0	12.2	17
Cis+Epi	4	7.4	5.2	10.9	0
HR	4	4.4	0.0	5.0	1

## HAZARD RATIO FOR DEATH IF PATIENT RECEIVED CHEMOTHERAPY WITH APOPTOSIS < 1.7 KU VERSUS CHEMOTHERAPY WITH APOPTOSIS >= 1.7 KU

- All patients with ovarian cancer hazard ratio is 7.2 (p=0.02)  
If a patient received a chemotherapy regimen with apoptosis by MiCK <1.7, her chance of death was 7.2 fold greater than if she received a regimen with apoptosis >=1.7
- Patients with stage III, IV ovarian cancer hazard ratio is 6.0 (p=0.03)  
If a patient received a chemotherapy regimen with apoptosis by MiCK <1.7, her chance of death was 6.0 fold greater than if she received a regimen with apoptosis >=1.7

## OVERALL SURVIVAL OF PATIENTS GIVEN THE BEST CHEMOTHERAPY BY MiCK ASSAY ("RIGHT") VERSUS PATIENTS GIVEN NON-BEST CHEMOTHERAPY ("WRONG")



P=0.01 Best=42 Non Best=13

## CLINICAL RESPONSE OF STAGE III, IV PATIENTS ACCORDING TO USE OF HIGH APOPTOSIS OR LOW APOPTOSIS REGIMEN

APOPTOSIS GROUP	CR, NO POSTOP RELAPSE, PR	CR, NO POSTOP RELAPSE, PR OR SD
MD USED CHEMO WITH APOPTOSIS >= 1.7 KU	73.5%	85.3%
MD USED CHEMO WITH APOPTOSIS <1.7 KU	42.9% P=0.04	57.1% P=0.03

Single agents compared to combination therapy in individual patients with ovarian cancer

- Carboplatin or paclitaxel versus combination
  - Single drugs more apoptosis 25/90 (28%)
  - Single drugs equal to combination 8/90 (9%)
- Cisplatin or gemcitabine versus combination
  - Single drugs more apoptosis 14/44 (32%)
  - Single drugs equal to combination 8/44 (18%)

Generic agents compared to proprietary drugs in individual patients with ovarian cancer

- Best generic drug apoptosis was equal to or better than the best proprietary drug apoptosis in 75%
- Best proprietary drug apoptosis was better than generic drug apoptosis in 25%
- A proprietary drug was better than carboplatin plus paclitaxel in 38%

## CONCLUSIONS

- The MiCK assay can predict which chemotherapy will produce superior survival and superior response rates in patients with ovarian cancer
- The MiCK assay is feasible for solid tumor analysis in multiple centers
- The MiCK assay may impact physician decisions for chemotherapy, single versus multiple drug therapy, and generic versus proprietary drug choice
- Multivariate analyses are being performed

## CHALLENGES IN CONDUCTING A PROSPECTIVE RANDOMIZED TRIAL

- Full information to patients will result in
  - High drop-in rates to MiCK assay directed cohorts
  - High drop-out rates from control cohorts
  - 20% drop-out rates from MiCK assay cohorts due to insurance non-coverage of the best chemotherapy, patient comorbidities, patient copays, and patient preference
- An IRB member has given a written opinion that a prospective randomized trial would be unethical after full knowledge of ovarian cancer and leukemia results
- Prospective nonrandomized trials of impact of the MiCK assay results on physician decision-making are being performed