# International Chinese Medicine Cancer Research Center of Israel Recent Research Report

## Introduction

ACNO is anti-neoplastic drug developed from Chinese medicine. It has been used for 30 years in china and 20 years in Israel. Thousands of cancer patients were treated with ACNO. The efficacy of ACNO is 69.7% and ACNO combined with electrochemotherapy group is 84.3%. the clinic results should that ACNO can improve immune system, inhibit cancer cells ,improve blood circulation to suppress the growth and metastasis tumor ,increase the sensitivity to other therapies , diminish toxic and adverse effect of other therapies and drugs , improve sleep, increase appetite, alleviate pain and stress, improve liver and kidney function, and prevent leukemia cause by radiotherapy and chemotherapy. It also elevates the body's anti-tumor capacity to reduce the relapse, metastasis and improve the patients' quality of life. To further prove the medicine, a series of experiments were made in Tel-Aviv University and The Chaim Sheba Medical Center Tel-Hashomer Hospital, affiliated to the Tel-Aviv University Sackler School of Medicine, Ministry of Health, The State of Israel .

## 1.Clinic results

In the research center clinic of Israel, more than 15000 cancer patients are treated by Chinese medicine ACNO or ACNO combination with conventional treatments. Patients come from Israel, America, England, China, Canada, Germany, France, Switzerland, Russia, Turkey, Malaysia, Singapore etc. with problems including different kinds of cancer : breast cancer ,ovary cancer , lung cancer ,brain tumor , stomach cancer , kidney cancer , colon cancer , bladder cancer ,pancreas cancer , liver cancer , gall bladder cancer, prostate cancer , uterus cancer , oral cancer , esophagus cancer, nasopharyngeal carcinoma , thyroid cancer, throat cancer etc., lymphoma , sarcoma , leukemia ,melanoma , multiply myeloma etc. Efficacy is 70 and 88% combination with chemotherapy and radiotherapy. The therapeutic was graded as CR(complete remission), PR(partial remission) , NR(no remission , stable), PD (progressive disease) according to the standard put forth by WHO in 1987.

## 2.Killing Jurkat Tumor Cells by three Kinds of ACNO Formula

**1.Human Cells Culture** Jurkat histocytic lymphoma cells were cultured in RPMI-1640 medium, supplemented with 4 m ML-glutamine, 30Mm Hepes(pH 7.3), 100 u of penicillin per ml, 100ug of streptomycin per ml, 10% fatal calf serum(FCS), at 37°C and 5% CO2 condition.

**2.Killing of tumor cells by ACNO-1, ACNO-2, and ACNO-3.**

$5X10^5$ Jurkat tumor cells were transferred into well of 24-well plate with 1ml medium. 20ul ACNO-1 ,ACNO-2 ,ACNO-3 and Chinese medicine 'Shen Qu'(the concentration of the medicine is 0.5mg/ml) were added into wells, and incubated for overnight at 37°C under5% $CO_2$ Condition. Cells was transferred into glass tube, and wash with 2ml $PBS^{++}$ containing 10% heat inactivated FCS, and centrifuged at 1,000 rpm at 4°C for 8 min. 50ul 0.2 % Trypan blue in saline was added into pellet of tumor cells, and mixed well on ice. Dead and alive tumor cells were counted under microscope.

Experiments are triplicated

**3. Results: Percent killing of Jurkat tumor cells**

| Formula | percentage of killing the tumor(%) |
|---|---|
| ACNO-1 | 81.6-81.7 |
| ACNO-2 | 80.7-85.7 |
| ACNO-3 | 93.4-95.1 |
| Shen Qu | 1.8-2.7 |

ACNO formula is concentrated by xiyuan hospital of China.

The results showed that the ACNO -3 is better than 1 -2. The rate of killing the tumor cells in vitro is very high.

## 3.Killing and Inhibited Growth of U937 and JY-5 Tumor Cells by Chinese Medicine ACNO and Dong Chong Xia Cao .

1. **Human cell lines culture**

U937 histocytic lymphoma cells and JY-5 cells (Epstein-barr virus-transformed human B lymphoblastoid cells) were culture in RPMI-1640 medium, supplemented with 4m M HEPES(Ph 7.3), 100U of penicillin per ml, 100 ug of streptomycin per ml, 10% fetal calf serum(FCS), at 37°C and 5% $CO_2$ condition.

1. **inhibition of growth and killing cell by ACNO and Dong Chong Xia CAO.** $5X10^5$ U937 and JY-5 cells was transferred into wells of 24-well plate with 1 ml medium. 20ul ACNO , Dong Chong Xia

CAO( mixture , DCXC-M) and Dong Chong Xia Cao (pure, DCXC-P) (0.5mg/ml concentration ) were added into wells, and incubated for overnight. Cells was transferred into glass tube , and wash with 2ml $PBS^{++}$ containing 10% heat inactivated FCS, and centrifuged at 1,000 rpm at 4°C for 8 min. 50ul 0.2% Trypan blue in saline was added into pellet of cells , and mixed well on the ice. Cells were counted under microscope.

2. **Results:** Percentage of killing and inhibition of cell growth

| | **U937 (inhibition)** | **JY**-5(killing) |
|---|---|---|
| ACNO | 75.3-78.5 | 74.6-82.5% |
| | Cell death: 7.1-10.6% | |
| DCXC-M | 66.1-73.9 | 15.2-17.8% |
| | Cell death:4.3-.5.9% | |
| DCXC-P | 64.3-65.4% | 5.2-7.1% |
| | Cell death:0.3-1.8% | |

The results showed that ACNO is significant in killing the cancer cells more than the DCXC, but inhibit the cell growth almost in the same amount.

## 4.Inhibited Growth of U937and JY-5 Cells by ACNO and DongChong Xia Cao.

**1.Human cell lines culture**

U937 histocytic lymphoma cells and JY-5 cells (Epstein-barr virus-transformed human B lymphoblastoid cells) were culture in RPMI-1640 medium, supplemented with 4m M HEPES(Ph 7.3), 100U of penicillin per ml, 100 ug of streptomycin per ml, 10% fetal calf serum(FCS), at 37°C and 5% $CO_2$ condition.

**2. inhibition of growth and killing cell by ACNO and Dong Chong Xia CAO.** $5X10^5$ U937 and JY-5 cells was transferred into wells of 24-well plate with 1 ml medium. 20ul ACNO , Dong Chong Xia CAO( mixture , DCXC-M) and Dong Chong Xia Cao (pure, DCXC-P) (0.5mg/ml concentration ) were added into wells, and incubated for overnight. Cells was transferred into glass tube , and wash with 2ml $PBS^{++}$ containing 10% heat inactivated FCS, and centrifuged at 1,000 rpm at 4°C for 8 min. 50ul 0.2% Trypan blue in saline was added into pellet of cells , and mixed well on the ice. Cells were counted under microscope.

**3. Results: percentage of inhibition of cell growth**

| | U937 | JY-5 |
|---|---|---|
| **ACNO** | **78.52%(2.6X10$^5$/1.21X10$^{6)}$** | **91.8%(4.1X10$^5$/5X10$^6$)** |
| | cell death: 1.8% | cell death:9.2% |
| Dong Chong Xia Cao | 85.95%(1.7x10$^5$/1.21x10$^6$) cell death: 3.5% | 81.2%(9.4x10$^5$/5x10$^{6)}$ cell death:4.8% |
| Control : medium | cell: 1.21 x10$^6$ Cell death 0.85% | cell : 5x10$^6$ cell death: 2.1% |

The results showed that ACNO inhibit the tumor cells better than Dong Chong Xia Cao. ACNO and Dong Chong Xia Cao are significantly different compared with the control group.

## 5. Detecting of the killing of tumor cells via Necrosis by ACNO with propidium iodide(PI) Staining under fluorescent microscope

1. Cuture Jurkat and k562 tumor cells in RPMI medium containing 10%FCS.
2. Take 0.5ml cells into each well of 24-well plate, add 0.5ml fresh medium and 20ul**ACNO,** ShenQu, and ddw, incubate for overnight at 37$^o$C.
3. Transfer into glass tube, and centrifuge for 8 min at 1,000 rpm, 4$^o$C.
4. Add 1ml PBS into pellet of each tube.
5. Add 5ul propidium iodide (PI, stock 0.5mg/ml), and keep for 5 min at room temperature. PI would specifically stain the necrotic cells with red color, but stain the normal and apoptotic cells.
6. Centrifuge for 6 min at 1,000rpm, and remove most of supernatant , letting about 20 ul solution with pellet.
7. Mix the pellet well.
8. Wash under fluorescent microscope and count the cells.
9. Results:

| | Jurkat | K562 |
|---|---|---|
| *ACNO red staining cells(mean) | 265 | 114 |
| Total cells (mean) | 300 | 298 |
| Ncrosis % | 88.33% | 38.25% |
| **Shen Qu red staining cells (mean) | 15 | 9 |
| Total cells (mean) | 260 | 290 |
| Necrosis % | 5.76% | 3.1% |
| ***ddw red staining cells(mean) | 11 | 8 |
| Total cells (mean) | 250 | 278 |
| Necrosis % | 4.4% | 2.78% |

*ACNO is one kind Chinese medicine to killing the tumor
**Shenqu is negative control group of Chinese medicine
***ddw is saline control group.

The results showed that ACNO kills the tumor cells by the way of Necrosis. It is significantly different compared with the control group. The results equal to the clinic experiments.

## 6. Killing Jurkat Tumor cells and K562 by chinese medicine 'KLN"

**1. Human Cell Lines Culture** . Culture Jukat and K562 tumor cells in RPMI-1640 medium ,supplemented with 4Mml-glutamin, 30mM Hepes(PH 7.3), 100 U of penciling per ml, 100ug of streptomycin per ml, 10% fetal calf serum (FCS), at 37°c and 5% CO2 condition.

**2.killing of tumor cells by Chinese medicine 'KLN'**

$5 \times 10^5$ Jukat tumor cells and K 562 cells were transfer into wells of 24-well plate with 1 ml medium . 20ul KLN ,UFT ( one conventional chemotherapy medicine) and medium were added into wells, and incubated for overnight at 37°C under 5% $CO_2$ condition. Cells were transferred into glass tubes, and wash with 2 ml $PBS^{++}$ containing 10% heat inactivated FCS, and centrifuged at 1,000 rpm at 4°C for 8 min. 50ul 0.2 % Trypan blue in saline was added into pellet of tumor cells , and mixed well on ice. Dead and alive tumor cell were counted under microscope .

**3. Results : percentage killing the tumor cells**

| Agents (20ul) | Jurkat | K56 |
|---|---|---|
| * UFT | 99.4% | 99.2% |
| ** KLN | 99.1% | 99% |
| ***MEDIUM | 1.3% | 1.5% |

*UFT: one kind chemotherapy positive control group.
** KLN one kind Chinese medicine from China
*** medium as blank

The results showed that the Chinese medicine kills the tumor cells equal to the chemotherapy in vitro; also another research showed that Chinese medicine can change the cancer cells to normal .Later on the DNA level will be repeated in the experiment. KLN is used by many thousands of patents in the clinic, which give same results as those in chemotherapy but without side effects. Chinese medicine will open the new road for the treatment of cancer patients.

## 7.DNA extraction from ACNO-Treated Jurkat and K562 Tumor Cells

1. Treat Jurkat and K562 cells with 20ul ACNO for overnight at $37^0$C under 6% $CO_2$ condition.
2. Add 10ml 70% ethanol for 24h at -20°C.
3. Centrifuge for 5 min at 800g, 4°C.
4. Add 40ul phosphate-citrate butter (192:8) into pellet, mix well and keep for 40ul at room temperature.
5. Centrifuge for 5 min at 1000g at room temperature.
6. Keep supernatant in a new Eppendorf and concentrate by vacuum dry for 20 min at room temperature.
7. Add 3ul 0.25% NP-40 in double distilled water, then 3ul Rnase (1mg/ml), and incubate for 30 min at 37°C..
8. Add ---- proteinase K (1mg/ml) and incubate for 30min at 37°C.
9. Prepare 50ml 0.8% agarose gel containing 3ul EB for DNA electrophosis.
10. Take 3ul of each DNA sample into 5ul 3x loading buffer with 7ul ddW.
11. Run DNA gel under 2V/cm condition for 16hr, with DNA ladder standard.
12. Visualize DNA in the gel under UV.
13. Results:
    All DNA samples without fragmentation suggest that the tumor cells treated with ACNO are not under apoptosis, which indicates that ACNO kills tumor cells likely by necrosis.

## 8. Killing of T Lymphocytes By UFT/ACNO/ShenQU

1. Take 10ml blood, dilute in 10ml RPMI.
2. Add into 50ml conical tube containing of 18 ml Ficoll-pague(pharmacia) gently on the top(do not mix)
3. Centrifuge at 2,000 rpm, 30min at room temperature.
4. Pool cells from the interphase area with Ficoll(cloudy area), dispense these cells in new 50ml tube.
5. Centrifuge at 2,000 rpm, 15min, 4°C. Cells are not packed tightly. Discard supernatant with pipette.
6. Resuspend in RPMI. Centrifuge at 2,000, 10min, 4°C.
7. Wash twice with 20ml RPMI, Centrifuge at 2,000 rpm, 15min, 4°C.
8. Resuspend in 10ml of complete medium with 10% FCS(RPMI + FCS+ Penicillin/strep+Glu) for growing cells.

9. Counting cells and dilute to a count of 4 x $10^6$ T cells/ml in Complete Medium.
10. Take 1ml cells into each well of 24-well plate, add 20ulUFT, or ACNO, or ShenQu, transfer into $CO_2$ incubator overnight at 37°C.
11. Counting the killing of cells.
12. Results: T cells death%

| Volume | UFT* | ACNO** | ShenQU*** | Medium**** |
|---|---|---|---|---|
| 20ul | 95.8-96.7% | 11.3-14.7% | 3.7-5.6% | 99.5-100% |

*UFT one kind chemotherapy.
** ACNO Chinese medicine for anticancer
*** ShenQu negative Chinese herb
**** Medium positive control group.

T lymphocytes belong to the human immune system and play first line against cancer. However when the patients take chemotherapy ,the cells are also killed . The results showed that chemotherapy and medium control group almost kill all normal T-lymphcytes. However the Chinese medicine ACNO and ShenQu have a very low influence on the T cells. That means that the Chinese medicine kills the cancer cells and does not damage the normal cells.

## 9. Killing of Tumor Cells by ACNO

1.Culture tumor cells Jurkat( T lymphoma) and K562 ( erythrolukemia) in suspention.
2. take 0.5ml cell solution( about 5x$10^5$ cells ) into each well of 24-well plate, with 20ul ACNO( anti-cancer number one , 0.5mg/ml) , ShenQu ( Chinese medicine , negative control group, 0.5mg/ml), UFT ( Conventional medicine, positive control group, 0.5mg/ml), and double distilled water for overnight in $CO_2$ incubator at 37°C. Duplicate the experiments.
3. Transfer cells into glass tubes, and wash with 2 ml $PBS^{++}$ containing 1% FCS.
4. Centrifuge at 1,000 rpm at 4°C for 8 min.
5. Add 50ul 0.2% TB into pellet, mix well and keep on ice.
6. Counting percentage of killing under microscope.
7. Results

| | ACNO* | Shaqu** | UFT*** | ddW**** |
|---|---|---|---|---|
| Jurkat | 76.3-85.6% | 1.5-2.3% | 98-100% | 1.2-2.6% |
| K562 | 32.9-37.8% | 2.1-2.4% | 93-96.8% | 1.8-2.1%. |

* ACNO Chinese medicine for anticancer

** ShenQu negative Chinese herb
***UFT one kind chemotherapy.
**** ddw negative control group.

The results showed that ACNO can kill the Jurkat cells much better than it can kill k562 cells and is significantly different compared with two control groups. However the UFT kills the Jurkat and K562 on the same level.

## 10. Killing of Tumor Cells by ACNO (anti-cancer number one)

1. Culture tumor cell lines: K562 (erythroleukemia) and Jurkat (T lymphoma) in suspension. Medium: 500ml RPMI, 5ml glutauine, 10ml sodium pyruvate, 0.9ml combined antibiotics, 50ml FCS.
2. Take one plate of each cell line (6ml, about $12x10^6$ cells), add 6ml fresh medium, mix well.
3. Take 0.5ml tumor cell solution (about $5x10^5$ cells) into each well of 24-well plate, add ACNO (0, 5, 10, 20 ul/well, from sterile stock: 0.5mg ACNO in 1ml double distilled water). Duplicate the experiment.
4. Incubate in $CO_2$ incubator at 37°C overnight.
5. Transfer cells from each well into 5ml glass tubes, and wash each well with 2ml $PBS^{++}$ (50ml PBS, 0.5ml 100mM $CaCl_2$, 0.5ml $MgCl_2$) containing 1% FCS, transfer into glass tubes.
6. Centrifuge for 10 min at 2,000 rpm, 4°C, then remove supernatant.
7. Add 50ul o.2% TB into pellet, mix well.
8. Counting percentage of killing under microscope.
9. Result:

| *ACNO(ul) | 0 | 5 | 10 | 20 |
|---|---|---|---|---|
| **Water(ul) | 20 | 15 | 10 | 0 |
| Jurkat | 2.7-3.1% | 8.5-9.7% | 27-33.8% | 73.5-82.5% |
| K562 | 0.8-1.4% | 3.7-4.5% | 13.4-16.7% | 32.8-33.3% |

*ACNO Anticancer Number One, one kind Chinese medicine for anticancer. 0,5,10,20 different concentration of ACNO.
** Water is diluted the medicine for the balance.

The results showed that the high dose group kills the tumor much better than the low dose and is significantly different compared with the low dose.

## 11. Killing of Tumor Cells By ACNO

1. Culture tumor cells Jurkat (T Lymphoma) in suspension.
2. Take 0.5ml cells solution ( about $5x10^5$ cells) into each well of 24-well plate, with 20ul ACNO(anti-cancer number one,0.5mg/ml) , ShenQu( Chinese medicine, negative control,0.5mg/ml, UFT( Western medicine , positive control, 0.5mg/ml) and double distilled water for overnight in $CO_2$ incubator at 37°C. Duplicate the experiments.
3. Transfor cells into glass tube and wash with 2ml $PBS^{++}$ containing 1% FCS.
4. Centrifuge at 1,000 rpm at 4°C for 8 min.
5. Add 50ul 0.2% TB into pellet, mix well and keep on ice .
6. Counting percentage of killing under miceroscope.
7. results

| *ACNO | ** ShenQu | ***UFT | ****ddw |
|---|---|---|---|
| 74.3-85.6 | 1.5-2.3 | 98-100% | 1.2-2.6% |

* ACNO Chinese medicine for anticancer
** ShenQu negative Chinese herb
***UFT one kind chemotherapy.
**** ddw negative control group.

The results showed that the ACNO and UFT kill the tumor in high percentage and are significantly different compared with the control group.