# #4053 A unique nutrient mixture suppresses ovarian cancer growth of A-2780 by inhibiting invasion and MMP-9 secretion

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## Introduction

Ovarian cancer is the deadliest gynecological malignancy in women, and fifth leading cause of death. The American Cancer Society estimated that it would claim 14,250 lives in 2013. Despite the advances made in chemotherapy and surgery, the average time of clinical remission is approximately 2 years and the 5-year survival rate is 45%. Thus, there is an urgent need for the development of a novel therapeutic approach to ovarian cancer treatment.

## Objective

We investigated the effect of a unique nutrient mixture (EPQ) containing ascorbic acid, lysine, proline, green tea extract and quercetin on human ovarian cancer cell A-2780 *in vivo* and *in vitro*.

## Materials and Methods

*In vivo*

1. Athymic female nude mice (n=12) were inoculated by I.P. with $2x10^6$ cells in 0.1ml PBS and randomly divided into two groups. Group A (n=6) was fed a regular diet and group B (n=6) a regular diet supplemented with 0.5% EPQ
2. Four weeks later, the mice were sacrificed and tumors that developed in the ovary were excised, weighed and processed for histology.

*In vitro*

1. A-2780 cells were cultured in Dulbecco modified Eagle medium supplemented with 10% FBS and antibiotics. At near confluence, cells were treated with EPQ in triplicate at concentrations between 0-1000 µg/ml.
2. Cell proliferation was measured by MTT assay, MMP-9 secretion by gelatinase zymography, invasion through Matrigel and morphology by H&E staining.

### Composition of Nutrient Mixture (NM)

| | |
|---|---|
| Vitamin C (as ascorbic acid and as Mg, Ca and palmitate ascorbate) | 700 mg |
| L-Lysine | 1000 mg |
| L-Proline | 750 mg |
| L-Arginine | 500 mg |
| N-Acetyl Cysteine | 200 mg |
| Standardized Green Tea Extract (80% polyphenol) | 1000 mg |
| Selenium | 30 µg |
| Copper | 2 mg |
| Manganese | 1 mg |
| Quercetin | 50 mg |

## Results

1. All control mice developed large ovarian tumors, whereas the EPQ group developed no tumors in 5 of 6 mice and a small tumor in the 6th mouse. EPQ inhibited mean tumor weight by 87% ($p<0.0001$), as shown in Figure 1A. Control group mice showed metastasis to lungs in 6 out of 6 mice, while there was no lung metastasis in the EPQ group (Figure 1C).

*Figure 1A - Effect of EPQ on mean tumor weight*

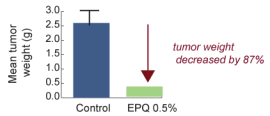
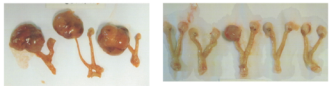



*Figure 1B - Control and EPQ group ovaries*

Control group | EPQ group



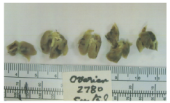
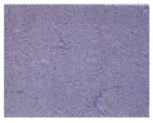


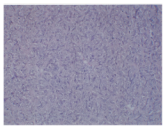
*Figure 1C - Control group lungs*

2. Control group histopathology
In examination of two sections of large, round tumors in the Control group the tumor appeared to have destroyed the ovary or ovaries as elements of an ovary are present in one section. The tumor is composed of neoplastic cells, similar to those in the one tumor found in the EPQ group. Necrosis varied from 35% in one section to 65% in the second section. Mitotic figures ranged from 0-1 per high-powered field.

*Figure 2- Histopathology of representative tumor sections of Control group*



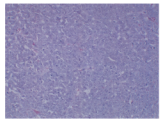
Control #1A - 200x
tumor morphology



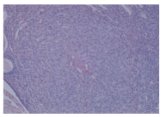
Control #2 - 200x
tumor morphology

3. EPQ group histopathology
Examination of sections of uterus, cervix, vagina and ovaries of EPQ group of mice, showed mild to moderate multiple small glandular cysts in the uterus, One ovary was totally replaced by a large tumor composed of irregularly-round cells with indistinct cell borders and irregularly round nuclei, possibly of luteal or granulosa cell origin. Mitotic figures range from 4-5 per high powered field. Multiple foci of necrosis involved about 40% of the tumor mass (Figure 3).

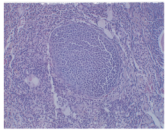
*Figure 3 - Histology of representative sections from ovaries in EPQ group*



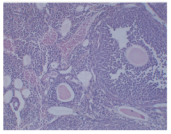
EPQ #1A - 200x
ovarian tumor



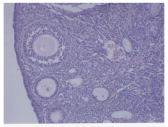
EPQ #1A - 200x
lesion in second ovary



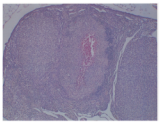
EPQ #1A - 200x
ovary follicular or cell proliferation



EPQ #1A - 200x
multiple developing follicles



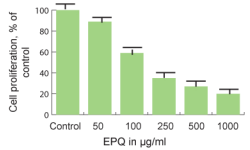
EPQ #1B - 200x
multiple follicles



EPQ #1B - 200x
multiple small nodular lesions

4. *In vitro*, EPQ exhibited dose dependent inhibition of cell proliferation ($p=0.0001$), with 65% toxicity over the control at 250 µg/ml, 73% at 500 µg/ml concentrations, and 80% at 1000 µg/ml, as shown in Figure 4.

*Figure 4 - Efect of EPQ on A-2780 cell proliferation: MTT assay*




5. Zymography demonstrated only MMP-9 secretion by A-2780 cells which was inhibited by EPQ in a dose dependent fashion, with virtual total inhibition at 250 µg/ml, as shown in Figure 5.

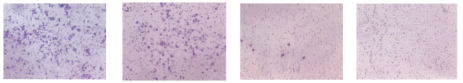
*Figure 5 - Effect of EPQ on A-2789 cell MMP-9 secretion*


MMP-9

Legend: 1 - Markers, 2-Control, 3-7 -EPQ 50, 100, 250, 500, 1000 µg/ml.

6. Invasion of A-2780 cells through Matrigel was inhibited by EPQ in a dose dependent manner, with total block of invasion at 250 µg/ml, as shown in Figure 6.

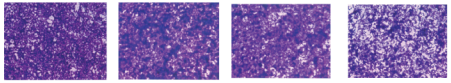
*Figure 6 - Effect of EPQ on A-2780 invasion through Matrigel*



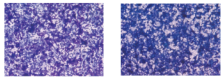
Control | EPQ 50 µg/ml | EPQ 100 µg/ml | EPQ 250 µg/ml

7. H&E staining showed no morphological changes below 500 µg/ml EPQ, as shown in Figure 7

*Figure 7 - Effect of EPQ on A-2780 imorphology: H&E*



Control | EPQ 50 µg/ml | EPQ 100 µg/ml | EPQ 250 µg/ml



EPQ 500 µg/ml | EPQ 1000 µg/ml

## Conclusions

**These results suggest that EPQ has therapeutic potential in treatment of ovarian cancer by significantly suppressing tumor growth and by inhibiting MMP-9 secretion and invasion of ovarian cancer A-2780 cells.**