

# **HAELAN RESEARCH FOUNDATION**

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## **Research Summary:**

The effects of Haelan 951 as monotherapy and as a combined treatment with Doxorubicin (Adriamycin) chemotherapy on invasive ductile carcinoma breast cancer cell line BT 474.

## **Background:**

The study was performed at Gemeinschaftspraxis für Laboratoriumsmedizin in Recklinghausen, Germany. Tests on the cancer cell killing effect of both Haelan 951 and Doxorubicin were studied in addition to gene expression. (See Attached Study for complete details)

1) **GAPDH:** Cancer Cell killing was measured by the GAPDH gene expression. Only live cells have GAPDH gene expression. The GAPDH chart in the study measures cancer cell kill and survival by the agent being tested.

Results: Cancer Cell Survival

<b>Individual Treatments:</b>	<b>Cells</b>		<b>Cells</b>
<b><u>Doxorubicin</u></b>	<b><u>Surviving (%)</u></b>	<b><u>Haelan 951</u></b>	<b><u>Surviving (%)</u></b>
.5 % Strength	82 %	.5 % Strength	58 %
1.25 % Strength	56 %		
<b>2.5 % Strength</b>	<b>14 %</b>	<b>1.5 % Strength</b>	<b>20 %</b>
3.75 % Strength	2.3 %		
5 % Strength	4.6 %	<b>3 % Strength</b>	<b>4.8 %</b>

## **Combined Treatment:**

**.5 % Doxorubicin + 3% Haelan 951** **14 %**

(Note: Low Dose Chemotherapy (1/2% by vol. plus 3% by vol. Haelan 951) produces the same cancer cell killing as 5 times greater Doxorubicin strength without the severe toxic side effects and adverse gene expressions caused by stronger doses of the chemotherapy.

2. **Bcl2 Gene Expression:** Bcl2 is an anti-apoptotic gene. Higher gene expression is not desirable because it increases the resistance for the cell to die normally by apoptosis (suicide is the normal cell death). Therefore, the lower the Bcl2 gene expression (down regulation) - the better the conditions are for cancer cells to die.

Results:	<u>Doxorubicin</u>	<u>Bcl 2 Expression</u>	<u>Haelan 951</u>	<u>Bcl2 Expression</u>
	.5 % Strength	.4575	.5 % Strength	1.2468
	1.25 % Strength	.2222		
	2.5 % Strength	.1163	1.5 % Strength	.4675
	3.75 % Strength	.0693		
	5 % Strength	.051	3 % Strength	.026

3.) **Bax Gene Expression:** Bax is an pro-apoptotic gene. Higher gene expression is desirable because it reduces the resistance for the cell to die normally by apoptosis (suicide is the normal cell death). Therefore, the higher the Bax gene expression (up regulation) - the better the conditions are for cancer cells to die.

Results:	<u>Doxorubicin</u>	<u>Bax Expression</u>	<u>Haelan 951</u>	<u>Bax Expression</u>
	.5 % Strength	1.05	.5 % Strength	2.55
	1.25 % Strength	1.09		
	2.5 % Strength	1.91	1.5 % Strength	2.94
	3.75 % Strength	.98		
	5 % Strength	.71	3 % Strength	3.97

Combined Treatment:

**.5 % Doxorubicin + 3% Haelan 951** **2.15**  
 (Note: Low Dose Chemotherapy (1/2% by vol. plus 3% by vol. Haelan 951) produces the same cancer cell killing as 5 times greater Doxorubicin strength and increases the Bax gene expression, which is desirable.).

4.) **Topoisomerase IIa :** Studies have shown that Topoisomerase IIa gene expression levels are increased in breast cancer non-responders to chemotherapy treatments. Likewise, breast cancer treated responders to chemotherapy show decreased gene expression levels of topoisomerase II. Higher gene expression (up regulation) is not desirable because it increases the resistance for the cell to die normally by apoptosis (suicide is the normal cell death). Therefore, the lower the topoisomerase IIa gene expression (down regulation) the better the conditions are for successful cancer treatment survival

Results:	<u>Doxorubicin</u>	<u>Topo IIa Expression</u>	<u>Haelan 951</u>	<u>Topo IIa Expression</u>
	.5 % Strength	1.8908	.5 % Strength	.9195
	1.25 % Strength	1.3793		
	2.5 % Strength	1.0575	1.5 % Strength	.2299
	3.75 % Strength	.5747		
	5 % Strength	.3909	3 % Strength	.2816

Combined Treatment:

**.5 % Doxorubicin + 3% Haelan 951** **.2691**  
 (Note: Low Dose Chemotherapy (1/2% by vol. plus 3% by vol. Haelan 951) produces the same cancer cell killing as 5 times greater Doxorubicin strength and

it gives a lower Topo IIa gene expression, which is desirable.)

5) **MDR1** : The MDR1 gene acts as an efflux pump and gives a cell multi-drug resistance by enhancing the transport of toxic cancer killing compounds out of the cell. Increasing chemotherapy concentrations raises the expression of MDR1. Higher MDR1 gene expression levels raise drug resistance and decrease the effectiveness of chemotherapy drugs on cancer cells. Higher gene expression (up regulation) of MDR1 is not desirable because it lowers chemotherapy concentrations in the cell resulting in increased cancer cell survival. Therefore, a lower MDR1 gene expression (down regulation) produces greater cancer cell kill rates and lower multi-drug resistance to chemotherapeutic agents.

Results:	<u>Doxorubicin</u>	<u>MDR1</u> <u>Expression</u>	<u>Haelan 951</u>	<u>MDR1</u> <u>Expression</u>
	.5 % Strength	1.31	.5 % Strength	.6538
	1.25 % Strength	2.452		
	2.5 % Strength	10.32	1.5 % Strength	.5962
	5 % Strength	17.08	3 % Strength	4.808

Combined Treatment:

**.5 % Doxorubicin + 3% Haelan 951** **3.8245**

(Note: Low Dose Chemotherapy (1/2% by vol. plus 3% by vol. Haelan 951) produces the same cancer cell killing as 5 times greater Doxorubicin strength and it gives a lower MDR1 gene expression, which is desirable.)

In summary, an evaluation of the cytotoxic effects of Haelan 951 shows dose dependent cancer cell kill as well as desirable gene expression when compared to Doxorubicin (Adriamycin) chemotherapy treatment at toxic levels. The toxic level increases with higher concentrations of Doxorubicin and adverse gene expression is experienced resulting in multi-drug resistance and resistance to apoptosis by the cancer cells being targeted by the treatment. The addition of Haelan 951 to a low dose Doxorubicin (Adriamycin) chemotherapy treatment of .5 % (1/2 %) produced the same cancer cell kill as monotherapy treatment with 2.5% Doxorubicin (5 times stronger) and in addition produced favorable gene expressions involved with these breast tumors.

A review of the attached study and the pathology showing anti-angiogenesis gives strong indications that the combination of Haelan 951 and Doxorubicin (Adriamycin) offers a better treatment option than Doxorubicin as a monotherapy. Estrogen metabolism and other mechanisms of action also favor the addition of Haelan 951 to the treatment of breast cancers with Doxorubicin by itself.

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