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## Electro Enhanced Gene Tumour Therapy with IL-18 and IFNgamma transfected tumour cell vaccine

In memory of Bengt Widegren d. 2014

Engström, Per; Persson, Bertil R

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PO Box 117  
221 00 Lund  
+46 46-222 00 00



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Corresponding author:  
Per Engström  
Bertil R.R. Persson,  
Lund University, Dept. of medical radiation physics,  
Barngatan 2, S-22185 Lund Sweden  
E-mail: bertil\_r.persson@med.lu.se

**Research article:**

## **Electro Enhanced Gene Tumour therapy with IL-18 and IFN $\gamma$ transfected tumour cell vaccine**

**Per Engström, Bengt Widegren <sup>\*</sup>), and Bertil R.R. Persson**  
**<sup>\*</sup>)In memory of Bengt Widegren † 2014**

Lund University, Dept. of medical radiation physics, S-22185 Lund Sweden  
E-mail: bertil\_r.persson@med.lu.se

### **Abstract.**

In an attempt to achieve immunoreactions against implanted brain tumours, rats with N29 glioma tumours were treated with electric pulses followed by injections of IL-18 and IFN- $\gamma$  secreting syngeneic tumour cells {Engström, 2018 #17241}.

Tumours were inoculated subcutaneously on both thighs of female Fischer-344 syngeneic rats. The left tumour was treated once with 16 pulses of 1400 V/cm, 1.0 ms duration (time constant). No anticancer drugs were given at any time. The following day and then once weekly for three weeks, the animals were given intraperitoneal injections of irradiated, modified N29 tumour cells, secreting either interleukin-18 (IL-18) or interferon- $\gamma$  (IFN- $\gamma$ ).

The results were evaluated by measuring the growth of the *untreated contralateral* tumours. There was no difference in contralateral tumour growth between animals given no treatment, electric pulses only, IFN- $\gamma$  secreting cells only

or IL-18 secreting cells only. A significantly inhibited growth rate was observed, in animals given electric pulse treatment followed by intraperitoneal injections of IFN- $\gamma$  or IL-18 secreting cells secreting cells. This treatment resulted in a prolonged survival (the time for the contralateral tumour to reach the predetermined limit volume), by 50%.

These results show that a systemic response of the host's immune system can be achieved against the tumour, using *syngeneic* tumour cells. This may be an important step towards development of electro immunotherapy to an effective tumour treatment modality.

**Keywords:** IL-18, IFN- $\gamma$ , Electro Enhanced, Gene, Tumour, vaccine, therapy

## 1. Introduction

In an attempt to achieve immunoreactions against implanted brain tumours, rats with N29 glioma tumours were delivered with electric pulses followed by injections of IL-18 and IFN- $\gamma$  secreting cells {Engström, 2018 #17241}.

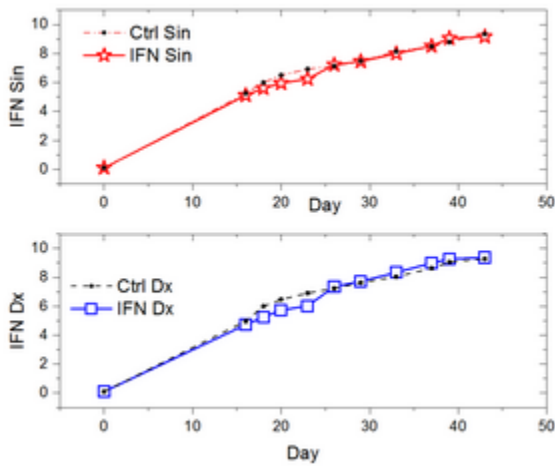
## 2. Material and methods

Tumours were inoculated subcutaneously on both thighs of female Fischer-344 syngeneic rats. The **left tumour** was treated once with 16 exponentially decaying pulses of initial amplitude of 1400 V/cm, 1.0 ms time constant at a frequency of 1 Hz. No anticancer drugs were given at any time. The following day and then once weekly for three weeks, the animals were given intraperitoneal injections of irradiated, modified N29 tumour cells, secreting either interleukin-18 (IL-18) or interferon- $\gamma$  (IFN- $\gamma$ ).

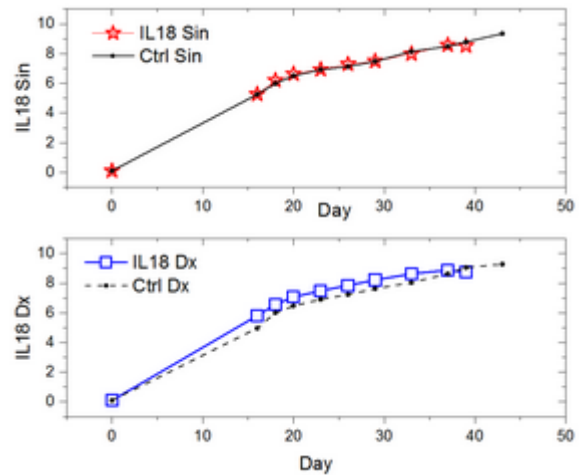
## 3. Results and discussion

The results were evaluated by daily measuring the size of the tumours beginning at 16 days after inoculation, The results are displayed in figures 1-5 for the controls

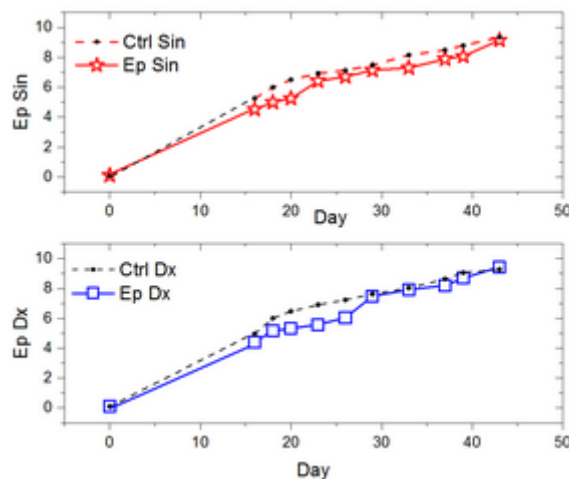
and treated tumours. There was no difference in contralateral tumour growth between animals given no treatment, electric pulses only, IFN- $\gamma$  secreting cells only or IL-18 secreting cells only. An inhibited growth was observed, in animals given electric pulse treatment followed by intraperitoneal injections of IFN- $\gamma$  or IL-18 secreting cells secreting cells. This treatment resulted in a prolonged survival (the time for the contralateral tumour to reach the predetermined limit volume), by 50%.



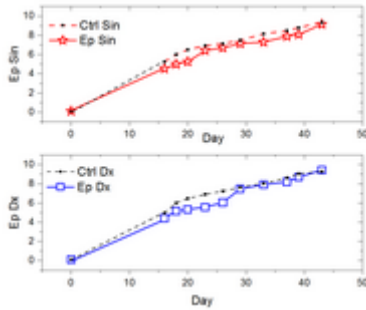
**Figure 1**  
Average growth curves of ln tumour volume in 3 controls and 5 rats treated with IFN $\gamma$  secreting tumour vaccine



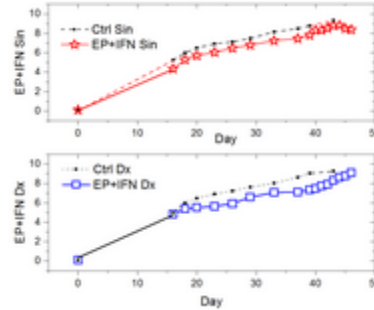
**Figure 2**  
Average growth curves of ln tumour volume in 3 controls and 5 rats treated with IL18 secreting tumour vaccine



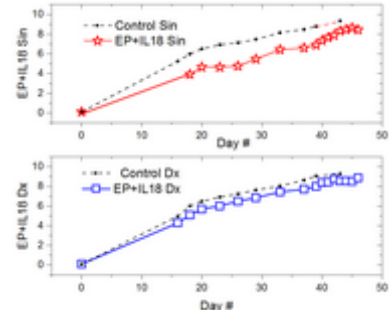
**Figure 3**  
Average growth curves of ln tumour volume in 3 controls and 5 rats treated with EP treatment of left tumour at day 16.



**Figure 4**  
Average growth curves of ln tumour volume in 3 controls and 5 rats treated with EP treatment of left tumour at day 16.



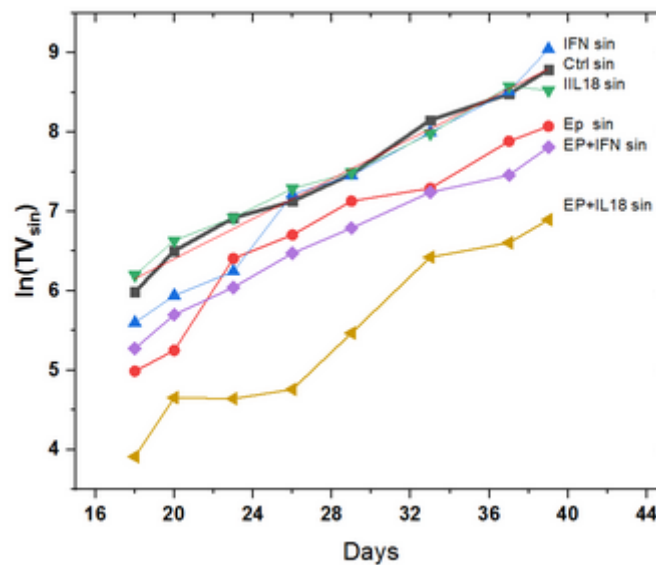
**Figure 5**  
Average growth curves of ln tumour volume in 3 controls and 5 rats treated with IFN $\gamma$  secreting tumour vaccine and EP treatment of left tumour at day 16.



**Figure 6**  
Growth curves of ln tumour volume in rats treated with IL18 secreting tumour vaccine and EP treatment of left tumour at day 16.

In order to evaluate effect of each treatment modality by the response of the left treated tumour the specific therapeutic effect was defined as:

$$STE = 100 * (Treated_{sin} - control_{sin}) / control_{sin}$$



**Figure 7**

Tumour growth of the left tumour displayed as  $\ln(\text{tumour volume})$  after the time the EP treatment occurred in the EP treated cases

**Table 1**

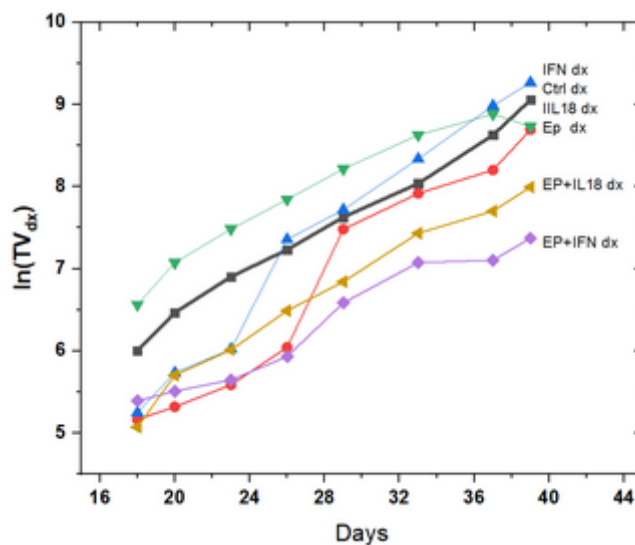
Results of linear fitting of the curves of the left treated tumour in Figure 7

| Treatment              | Intercept | SD  | Slope | Sd   | STE Intc. | STE Slope | STE AVE | p vs ctrl |
|------------------------|-----------|-----|-------|------|-----------|-----------|---------|-----------|
| Ctrl <sub>sin</sub>    | 3,9       | 0,2 | 0,13  | 0,01 |           |           |         |           |
| Ep <sub>sin</sub>      | 2,8       | 0,4 | 0,14  | 0,02 | 29        | -12       | 9       | 0,70      |
| IFN <sub>sin</sub>     | 2,8       | 0,2 | 0,16  | 0,01 | 29        | -27       | 1       | 0,98      |
| IL18 <sub>sin</sub>    | 4,3       | 0,2 | 0,11  | 0,01 | -12       | 12        | 0       | 0,99      |
| EP+IFN <sub>sin</sub>  | 3,4       | 0,2 | 0,11  | 0,01 | 13        | 10        | 11      | 0,70      |
| EP+IL18 <sub>sin</sub> | 1,5       | 0,4 | 0,14  | 0,01 | 61        | -10       | 25      | 0,01      |

The evaluation of the specific therapeutic response of the left show that Electro-pulse treatment combined with IL18 secreting tumour vaccine resulted in the best therapeutic response of 25%. While the single treatments with vaccine only resulted in almost no therapeutic response.

In order to evaluate the specific Abscopal effect of each treatment modality the specific abscopal effect was evaluate by the response of the contralateral right tumour:

$$SAE = 100 * (\text{Treated}_{Dx} - \text{control}_{Dx}) / \text{control}_{Dx}$$



**Figure 8**

Tumour growth of the right tumour displayed as  $\ln(\text{tumour volume})$  after the time the EP treatment occurred in the left EP treated tumours

**Table 2**

Results of linear fitting of the curves of the right tumour in Figure 8

| Treatment              | Intercept | sd  | Slope | sd   | SAE Intc. | SAE slope | SAE | p vs ctrl |
|------------------------|-----------|-----|-------|------|-----------|-----------|-----|-----------|
| Ctrl <sub>dx</sub>     | 3,7       | 0,1 | 0,14  | 0,00 | 0         | 0         |     |           |
| Ep <sub>dx</sub>       | 1,8       | 0,5 | 0,18  | 0,02 | 52        | -33       | 10  | 0,70      |
| IFN <sub>dx</sub>      | 1,9       | 0,4 | 0,19  | 0,01 | 49        | -44       | 3   | 0,98      |
| IL18 <sub>dx</sub>     | 5,0       | 0,3 | 0,10  | 0,01 | -35       | 22        | -6  | 0,98      |
| EP+IFN <sub>dx</sub>   | 3,5       | 0,2 | 0,10  | 0,01 | 6         | 25        | 15  | 0,34      |
| EP+ IL18 <sub>dx</sub> | 3,0       | 0,2 | 0,13  | 0,01 | 20        | 3         | 11  | 0,70      |

The evaluation of the specific abscopal response of the right contralateral tumour show that Electro-pulse treatment of the left tumour combined with IFN $\gamma$  or IL18 secreting tumour vaccine resulted in the best abscopal responses. While the single treatments with vaccine resulted in no abscopal response

**4. Discussion and Conclusion**

These results show that an increase therapeutic and systemic response of the host's immune system can be achieved against the tumour, by treatment with cytokine excreting *syngeneic* tumour cells in combination with pulsed electric fields. This may be an important step towards development of electro-pulse enhanced immunotherapy “EpEIT” to an effective tumour treatment modality.