

# Immunodeficient mice differentially sensitize to estrogen and exhibit severe estrogen-related adverse effects in orthotopic breast cancer model

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## Introduction

Estrogen-induced growth induction and stimulation of hormone receptor positive breast cancer is commonly acknowledged, and external estrogen stimulus is used in animal models to support and accelerate growth of the tumors. However, estrogen is known to induce adverse effects in female mice, and the experiments may have to be prematurely terminated due to animal welfare issues.

## Aims of the Study

The aims of the study were 1) to compare the tumor growth rate and the estrogen stimulation of tumor growth in nude and NOG mice; 2) to characterize and compare estrogen caused adverse effects in athymic nude and NOG mice in orthotopic breast cancer model.

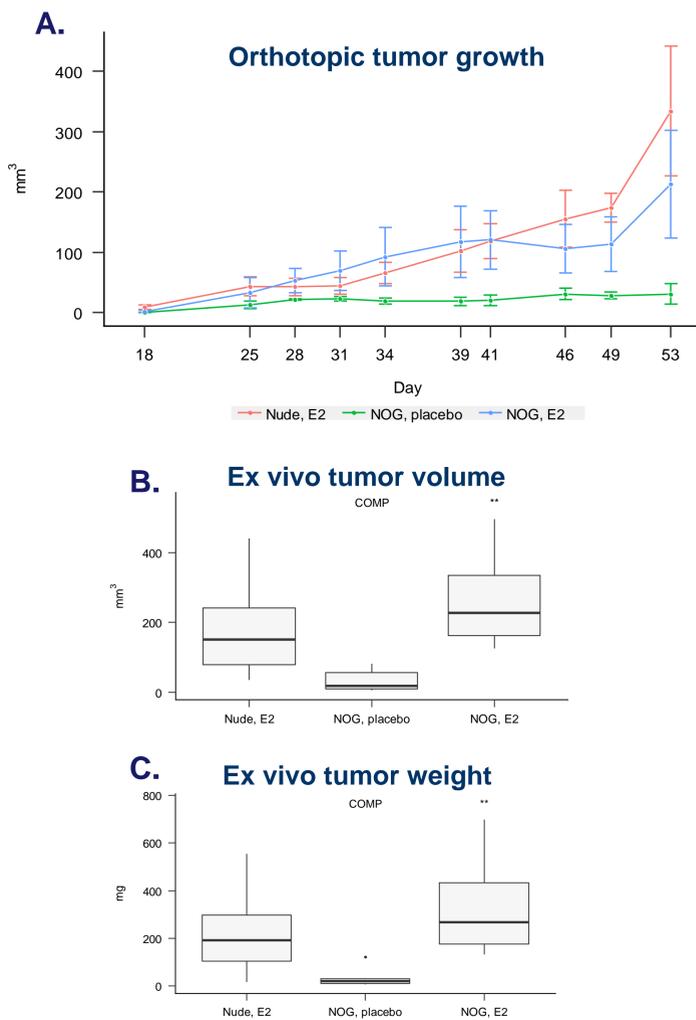
## Materials and Methods

Athymic female nude (Hsd:Athymic Nude-Foxn 1nu; Envigo) and NOG (NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Sug</sup>/JicTac, provided by Taconic Biosciences) mice were implanted with estrogen (17 $\beta$ -estradiol, E2; PreclinApps) releasing hormone rods (5  $\mu$ g/day) prior to cancer cell inoculation. 5x10<sup>6</sup> BT-474 (ER, PR and HER+; ATCC) human breast cancer cells were inoculated to the left inguinal mammary fat pad, and the tumor growth was monitored by measuring the tumor volumes twice a week by caliper. The clinical condition and weight development of the mice was carefully monitored with special attention to E2 caused adverse effects. If the mice met the termination criteria (i.e. significant weight loss or general worsening of the overall health) they were sacrificed individually before the intended end of the study.

## References

- Pearse G, Frith J, Randall KJ, Klinowska T: Urinary Retention and Cystitis Associated with Subcutaneous Estradiol Pellets in Female Nude Mice. 2009. Toxicologic Pathology, 37: 227-234.
- Collins DE, Mulka KR, Hoenerhoff MJ, Taichman RS, Villano JS: Clinical Assessment of Urinary Damage during sustained-release estrogen supplementation in mice. 2017. Comp Med, 1:67(1): 11-21.

## Results



**Figure 1.** A) Tumor growth in nude and NOG mice with and without E2 supplement. The tumor growth was measured twice a week by caliper and the tumor growth (volume, (L\*W\*H)\* $\pi$ /6, mm<sup>3</sup>) by time is presented (mean $\pm$ SD). Tumor volume B) was measured and tumors weighed C) ex vivo (mean with 50% confidence interval). In the figures, COMP=comparison group, \*\*p<0.01.

### A. Rash



### B. Wounds

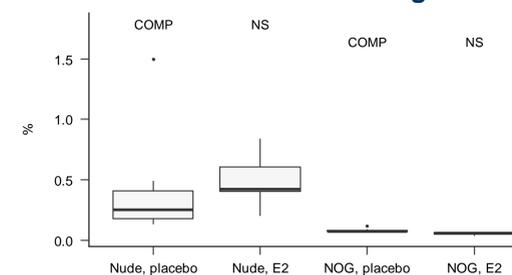


### C. Secretion, irritation and skin retrieval



**Figure 2.** E2 caused adverse effects in nude mice. External adverse effects were mainly A) skin changes which were rash in the upper or lower back of the mice, B) wounds, and the most severe C) secretion in the lower urinary tract and redness, irritation and finally skin retrieval. In most cases, the adverse effects started in the lower urinary tract and extended to cover other parts of the body. At sacrifice, gross necropsy was performed and abnormalities were found in kidney, spleen and bladder, most abundant being bladder stones (see Table 1).

### A. Relative bladder weight



### B.



### C.



**Figure 3.** A) Bladder weight was recorded at sacrifice and presented relative to body weight (%). Illustrative image of B) bladder full of bladder stones and C) opened bladder revealing the bladder stones inside.

**Table 1:** Summary of the adverse effects in the study groups

Study group	Skin changes	Bladder stones	Kidney defects	Other	Early sacrifice
Nude, E2	4/8; 50%	5/8; 62,5%	1/8; 12,5%	3/8; 37,5%	4/8; 50%
NOG, E2	0/6; 0%	0/6; 0%	0/6; 0%	0/6; 0%	0/6; 0%
NOG, placebo	0/8; 0%	0/8; 0%	1/8; 12,5%	0/8; 0%	0/8; 0%

Altogether 62,5% of the nude mice with E2 supplement developed E2 caused adverse effects during the 8 week study. 50% of the mice had to be prematurely sacrificed due to severity of the effects. At earliest, the animals had to be sacrificed at 4 weeks. The most common adverse effects were bladder stones which were found in 62,5% on the mice. Skin changes were observed in 50% of the mice, and in most cases the appearance of skin changes also indicated abnormal findings in lower urinary tract. In 12,5% of the mice appearance of the kidneys was changed and 37,5% of the mice had also other symptoms, including inflammation and change in the appearance of the spleen. No adverse effects were observed in NOG mice with E2 supplementation

## Conclusions

External estrogen stimulation for estrogen-responsive cancer cells was a necessity for orthotopic tumor growth. A clear mouse strain specific impact was observed in the study. Nude mice sensitize to estrogen and exhibit severe estrogen-related adverse effects, while no adverse effects were observed in NOG mice. In breast cancer studies where E2 supplementation is needed for sufficient tumor growth, the choice of mouse strain should be carefully considered. Special attention should be paid to possible adverse effects, keeping in mind animal welfare issues.

## Acknowledgements

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