

Male Breast Cancer in Mice

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Biotechnische dagen – Den Bosch

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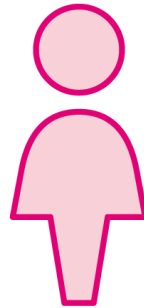
Female breast cancer incidence in NL

Borstkanker in feiten en cijfers

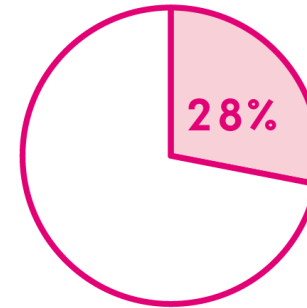
Pink Ribbon 



1 op de 7 vrouwen
krijgt borstkanker, aantal
diagnoses in 2021: **15.746**



Borstkanker is de **meest**
voorkomende kankersoort
bij vrouwen



28 % van alle
kankerdiagnoses
bij vrouwen is borstkanker

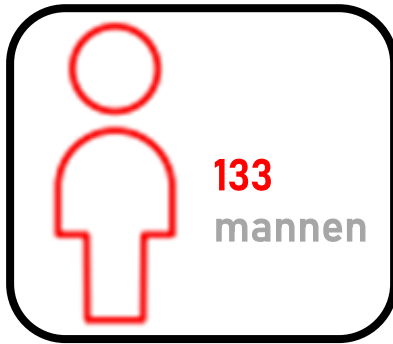


In Nederland zijn er meer dan
230.000 mensen die borstkanker
hebben of hebben gehad.

Male breast cancer ~1% of all breast cancer diagnoses in NL

2021

15.613
vrouwen



Risk factors

- High estrogen levels
- Prior radiation exposure
- Family history of breast cancer

Incidence

- Male breast cancer (MBC) is rare = 1% of all breast cancer cases
- <1% of all male cancers each year

MBC poor prognosis

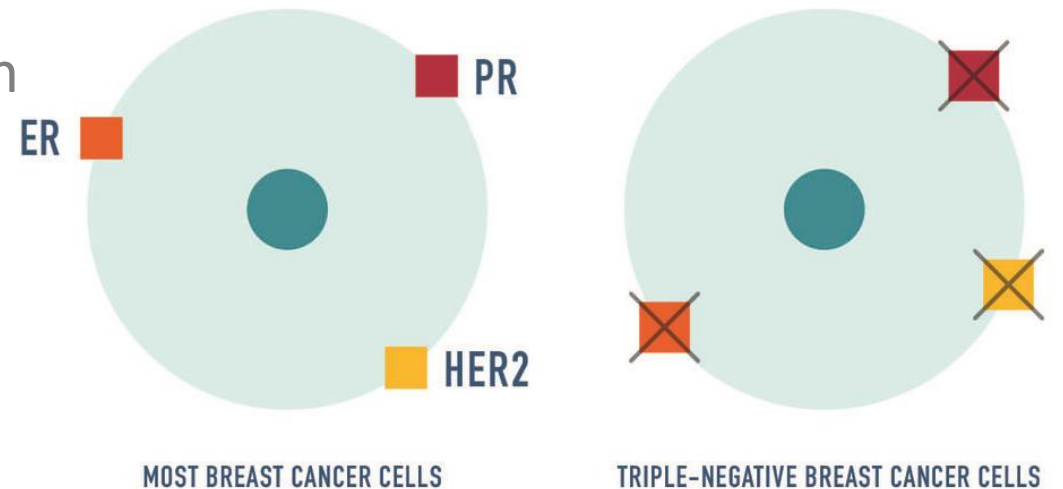
- Lack of awareness & counsel
 - Advanced disease detection
- Sub-optimal clinical management
 - Reliance on female treatment strategies

Triple-negative breast cancer (TNBC)

TNBC is an aggressive breast cancer which is defined by the lack of the estrogen (ER), progesterone (PR), and HER2 receptor expression

TNBC accounts for 12-17% of all female cases and is characterized by poor survival and limited treatment options

<1% of MBCs are identified TNBC



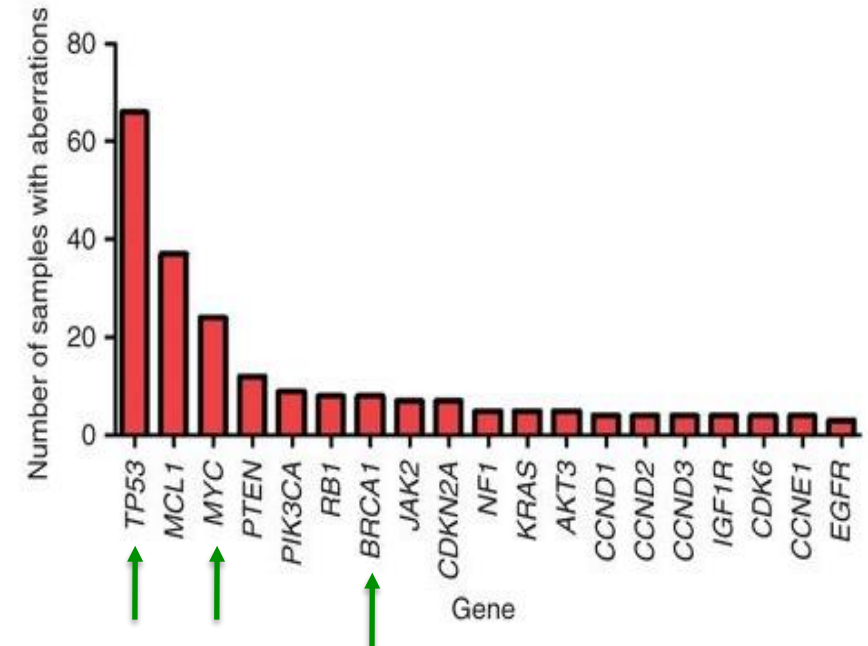
Triple-negative breast cancer (TNBC)

BRCA1 is the main hereditary breast cancer susceptibility gene that is frequently mutated in TNBC

Loss of the tumor suppressor gene **TP53** often occurs in BRCA-associated TNBC

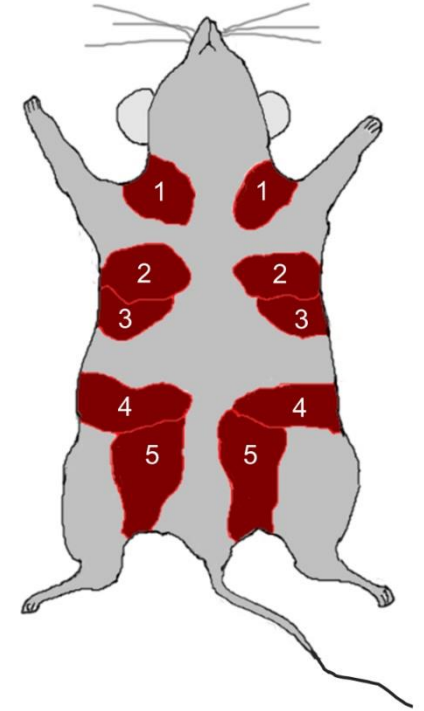
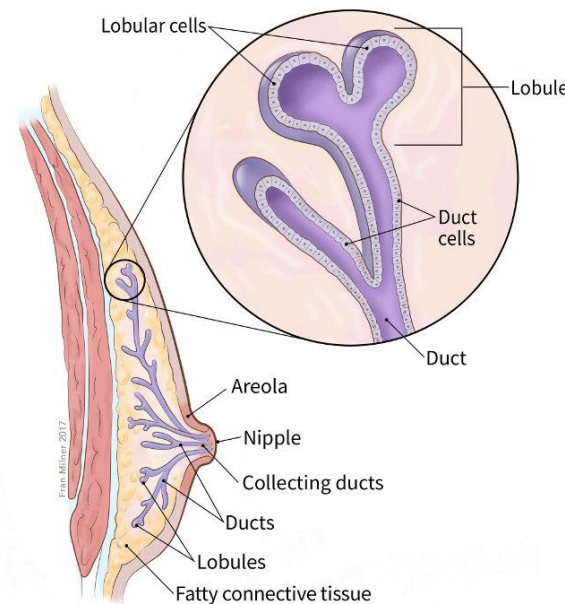
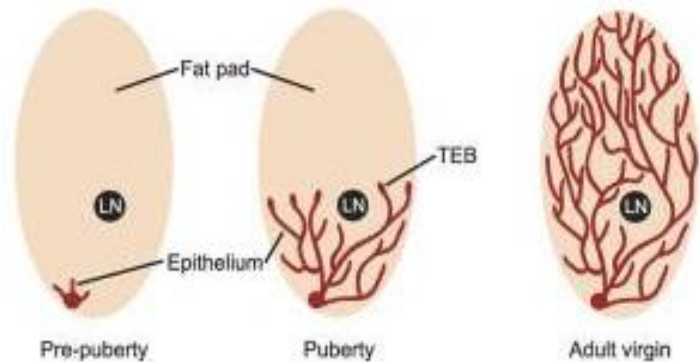
MYC, a proto-oncogene that plays a role in cell cycle regulation and growth, is also commonly overexpressed in BRCA TNBCs

Catalog of mutations in female TNBC

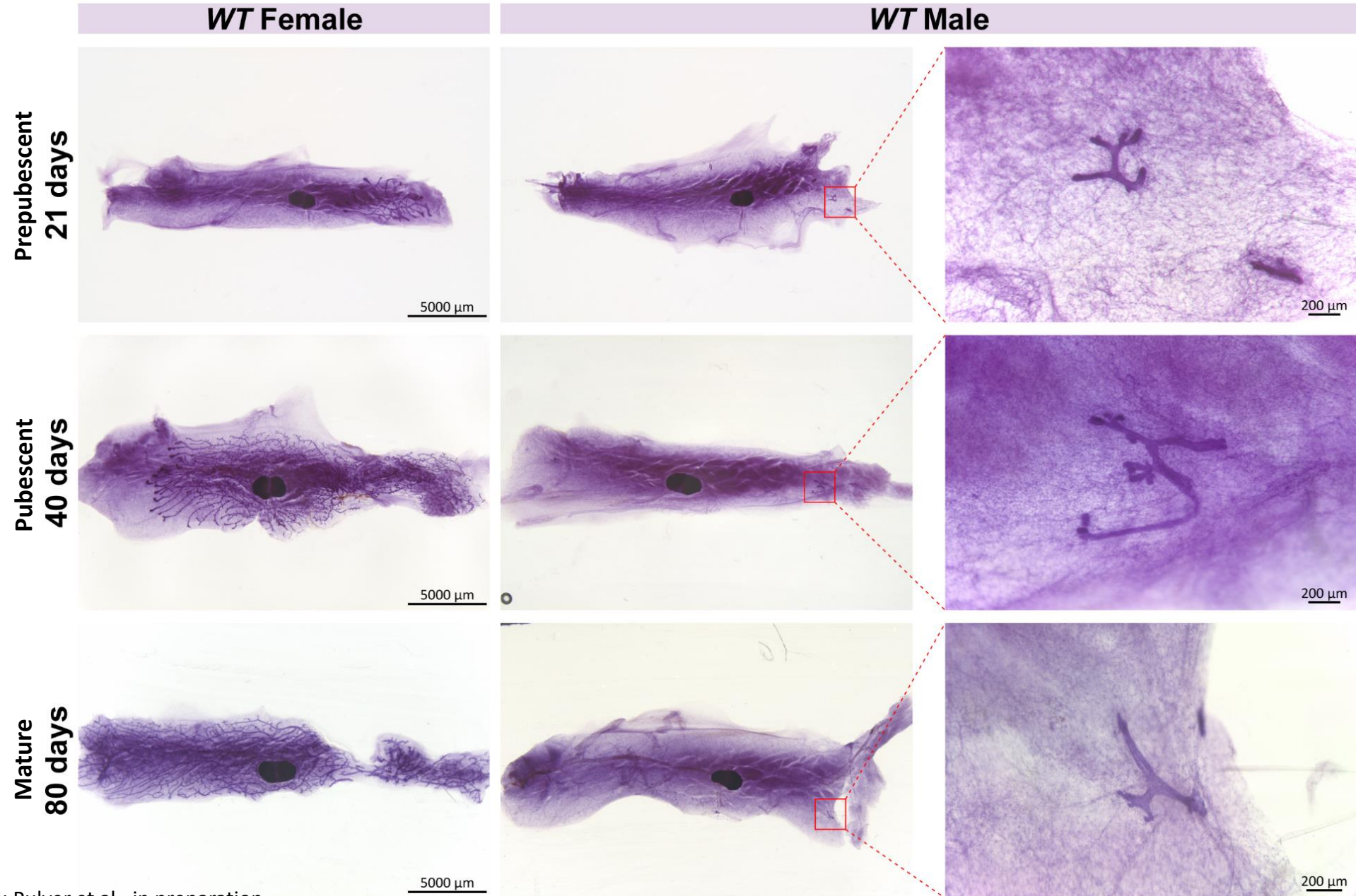


Breast tissue

- Before puberty, all young boys and girls have a small amount of breast tissue
- During puberty, increased levels of **estrogen** drive female breast development
 - Fatty tissue, epithelial ducts, and lobules that produce and secrete milk
- Adult males have lower levels of estrogen so breast tissue development is suppressed and the duct remains rudimentary



FVB/NRj mouse mammary gland development



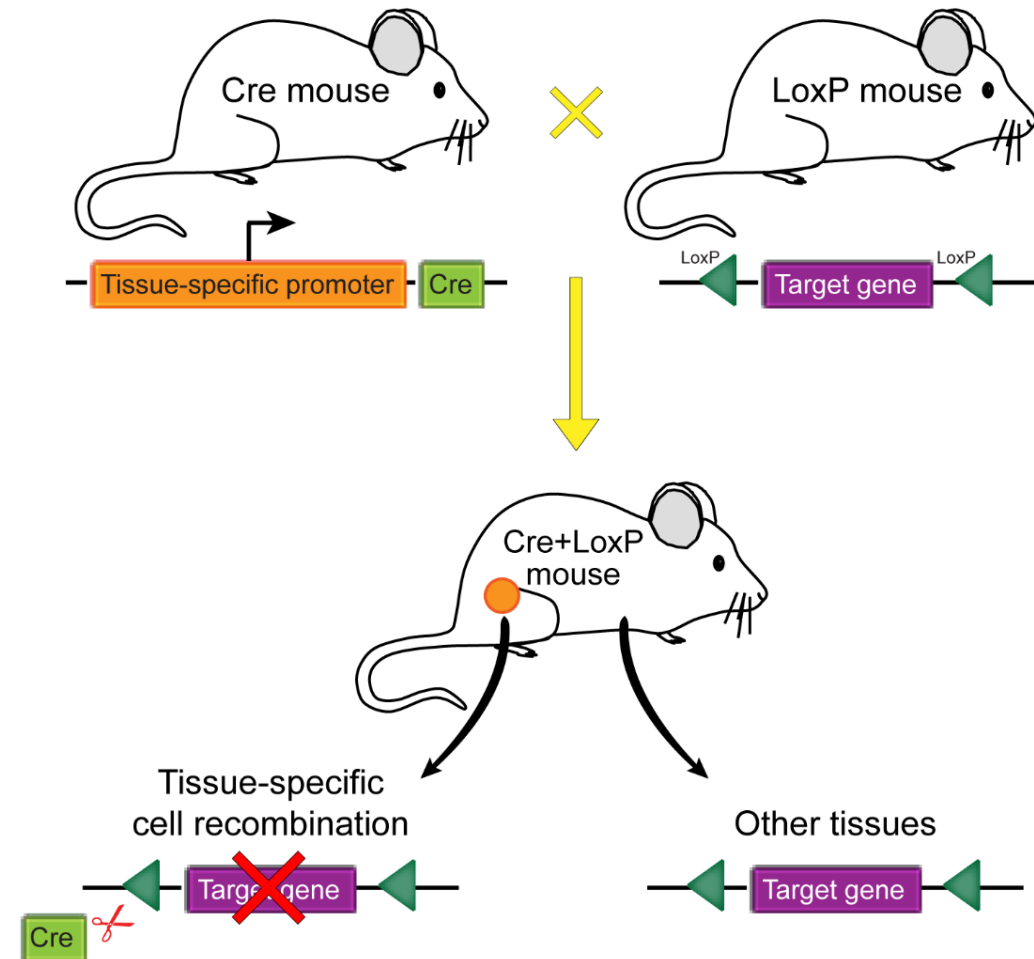
unpublished data: Pulver et al., in preparation

Mouse models of TNBC

Conditional Cre recombination

Cre recombinase → site-specific recombination of DNA flanked by LoxP sites

- Tissue-specific and/or
- Time-specific



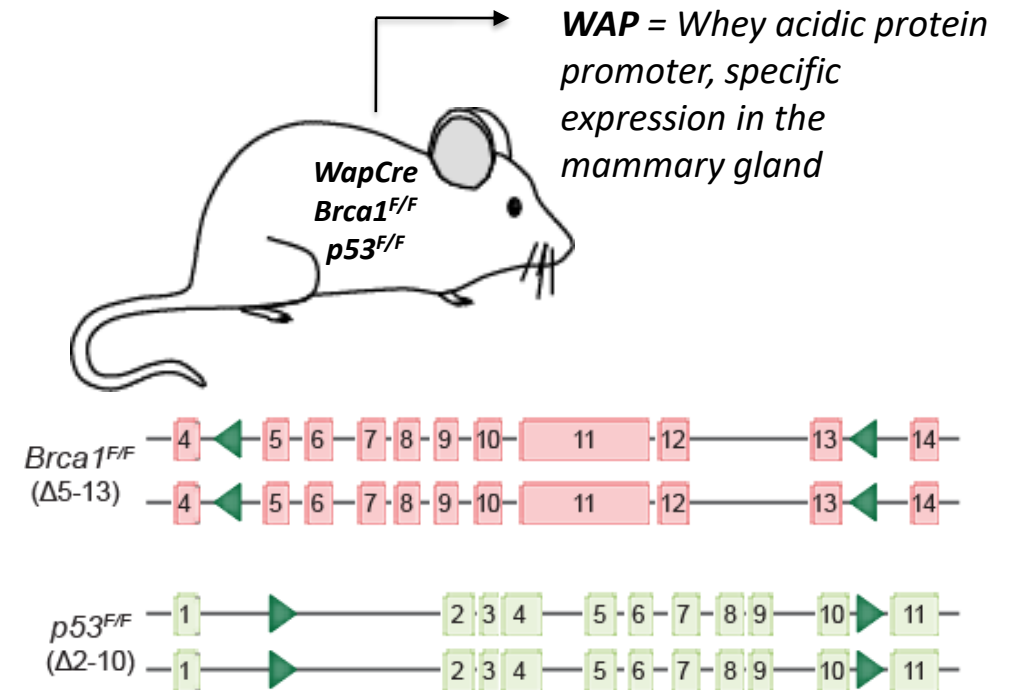
Mouse models of TNBC

Conditional Cre recombination

Mice with conditional inactivation of ***Brca1*** and ***p53*** specifically in the mammary gland epithelium:

WapCre; Brca1^{F/F}; p53^{F/F} → “**WB1P**”

Development of spontaneous mouse mammary tumors are similar to human TNBC



Mouse models of TNBC

Alleles

Generation of additional conditional GEMMs with floxed breast cancer susceptibility genes **Brca2** or **Myc** oncogene overexpression:

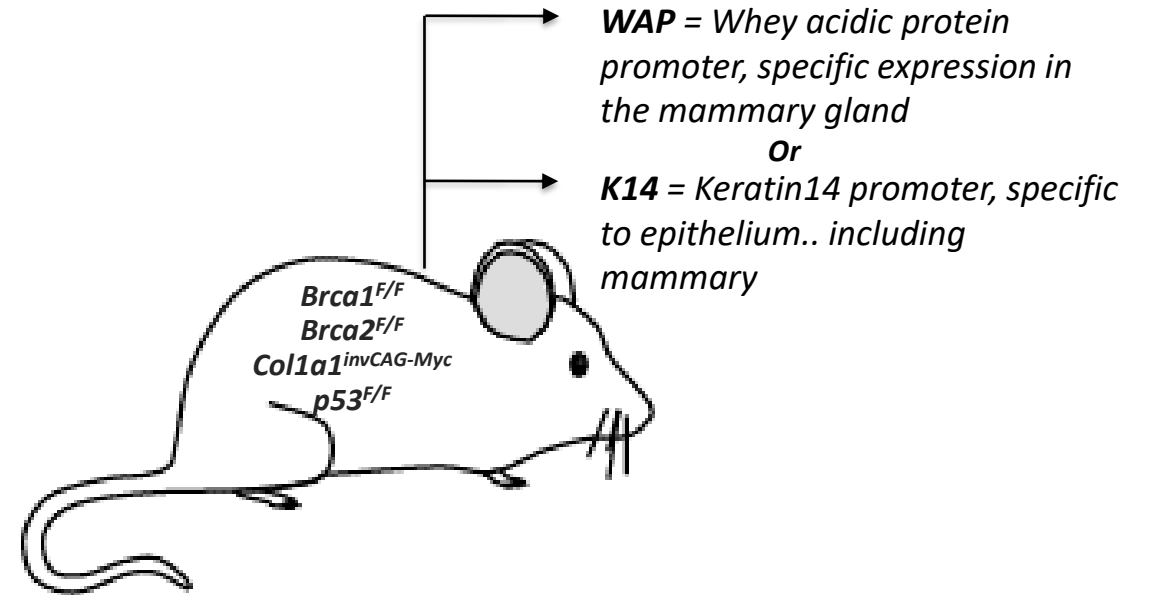
| | | |
|---------------|-----------------|-----------------|
| <i>WP</i> | <i>WB1P</i> | <i>WB2P</i> |
| <i>WP-Myc</i> | <i>WB1P-Myc</i> | <i>WB2P-Myc</i> |

Cre Promoters

Conditional mice under Keratin14 promoter in epithelial cells (mammary gland but also skin):

K14Cre; Brca1^{F/F}; p53^{F/F} → “KB1P”

Etc...



Animal Laboratory NKI



NKI Animal Laboratory Facility

Mouse room
conditions

Temperature
- 21 °C

Humidity
- 55%

Light cycle
- 12 h light /12 h
dark cycle

SPF conditions

Animal Laboratory NKI

Disposable single filtered IVC cage (M-BTM) from PET plastic with Corn Cob, nest sheet and red dome
Aquavive® Mouse Pre-Filled Acidified water



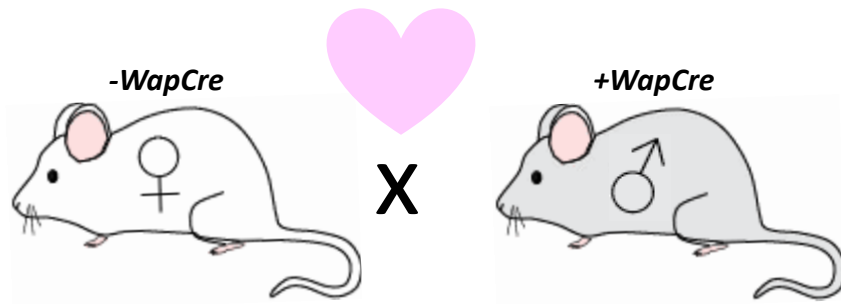
NKI Animal Laboratory Facility



Image: www.innovive.com

Discovered by chance...

Some *WapCre* breeding males got tumors



X Days
Tumor Latency



X Days
Tumor Latency



Tumor watch

Tumor palpation and caliper measurements

Twice/week

Tumor volume formula: $(L \times W^2)/2$

Maximum tumor volume: 1500mm³ for one or multiple tumors

Tissue collection once humane endpoint is reached



WapCre
p53^{F/F}



WapCre
p53^{F/F}
Myc



WapCre
Brca1/2^{F/F}
p53^{F/F}

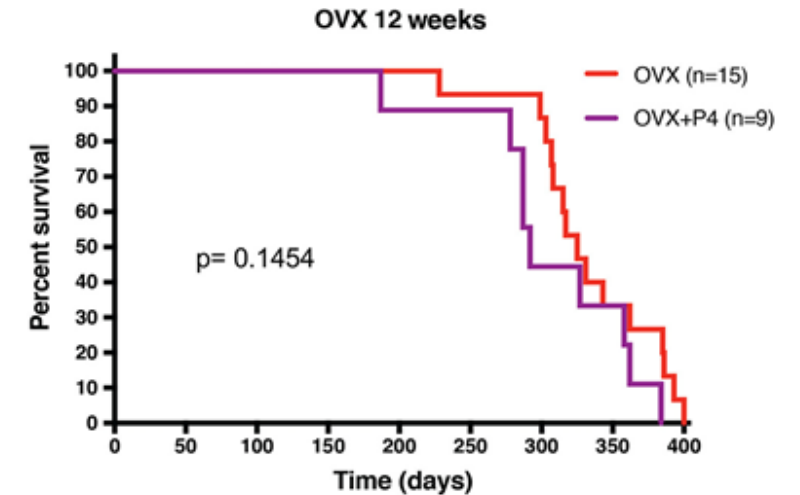
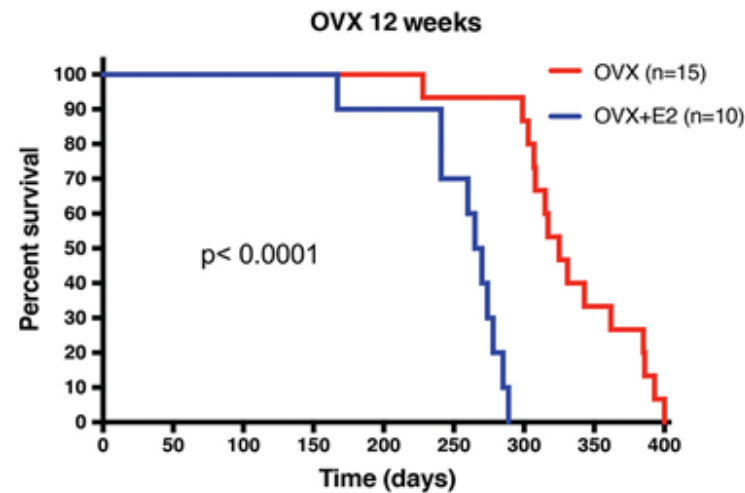
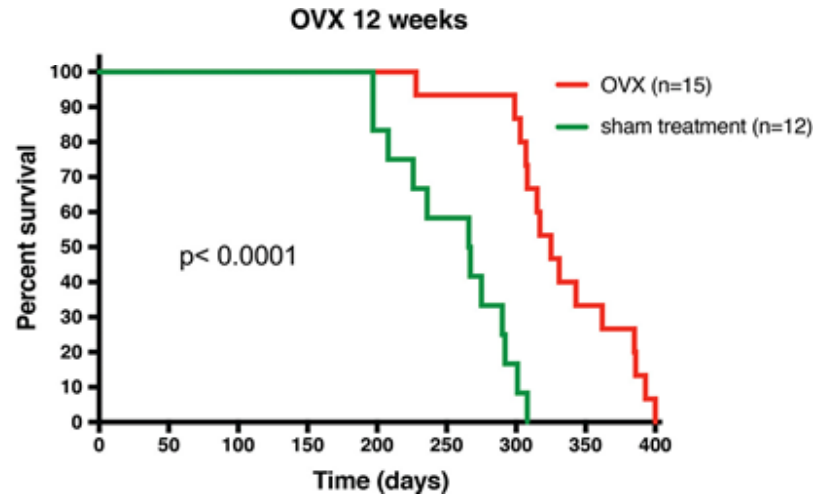


WapCre
Brca1/2^{F/F}
p53^{F/F}
Myc



Influence of hormones on mammary TNBC tumor formation

WB1P female

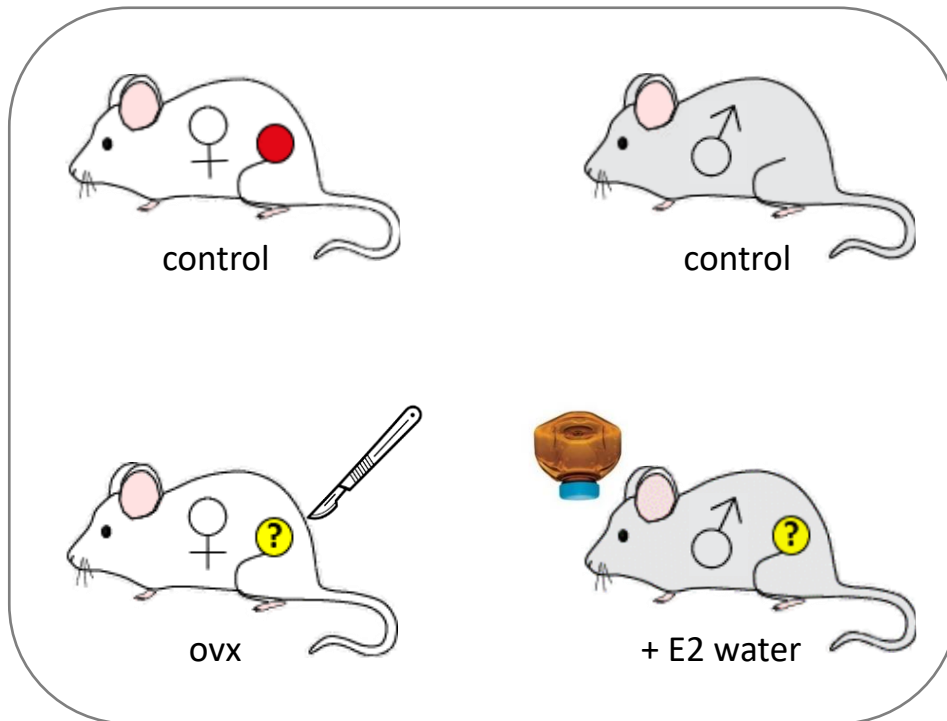


Estrogen drives female mammary tumorigenesis
...but what about *Myc*?

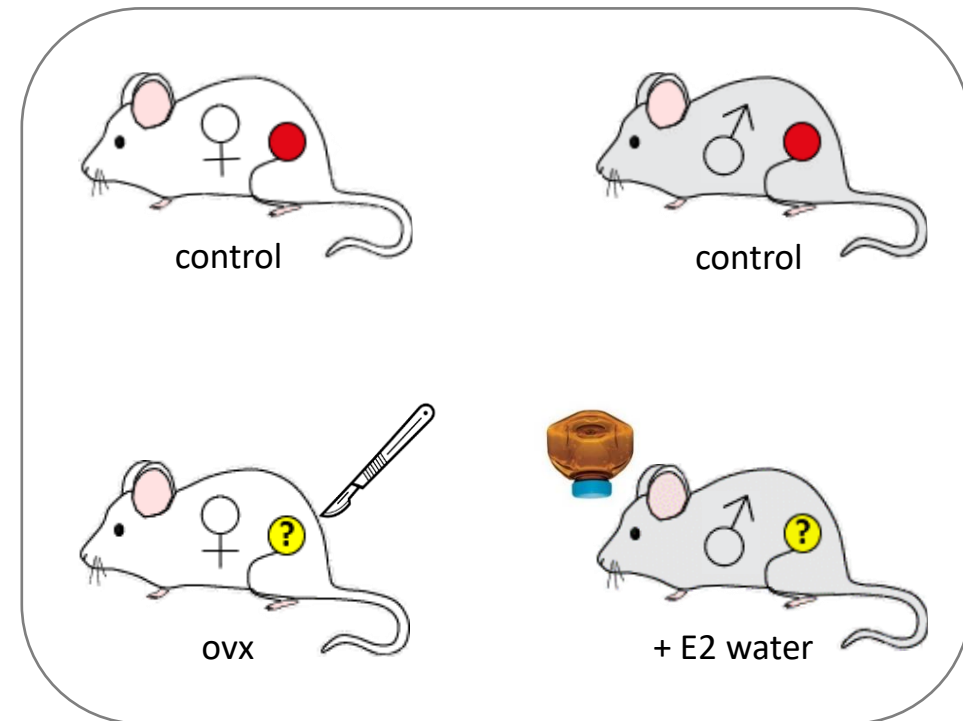
Influence of hormones on mammary TNBC tumor formation

Experiment: ovariectomize (ovx) ♀ or add estrogen (E2) to water ♂ to 6 week old mice

WapCre; p53^{F/F}



WapCre; p53^{F/F}; Myc



Ovariectomy to sterilize females

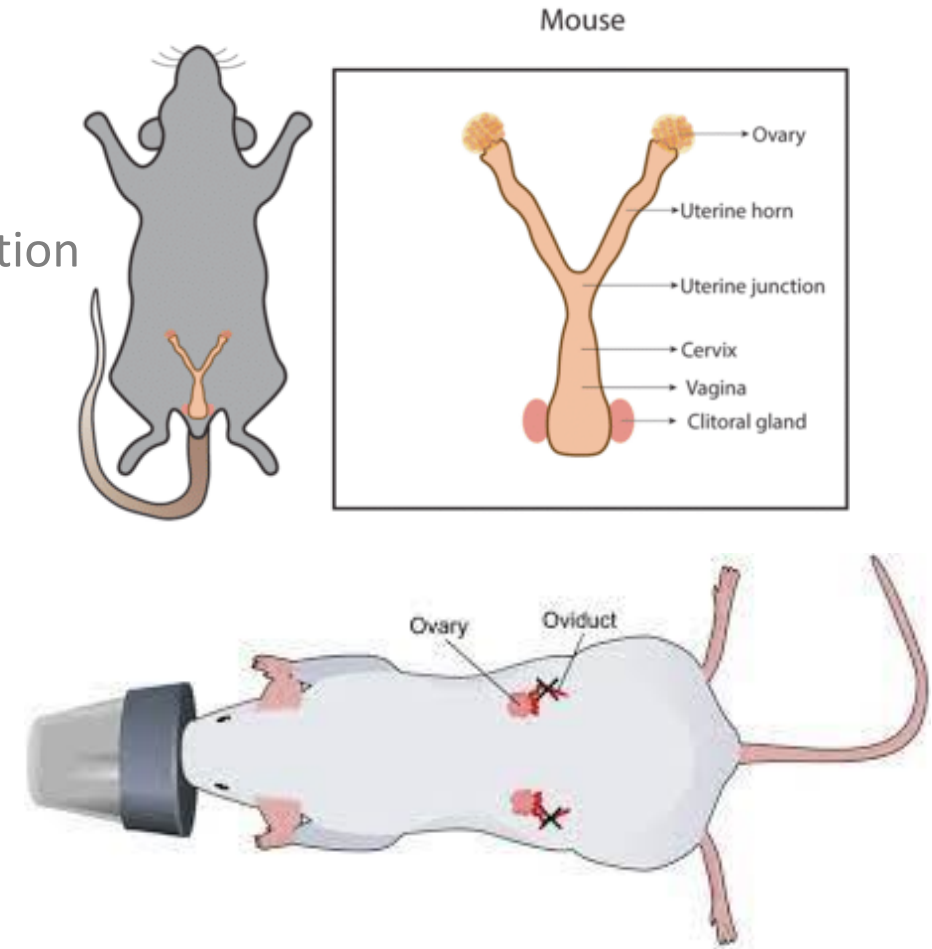
Bilateral ovariectomy removes both ovaries to prevent the production and release of estrogen & progesterone

Pre- & post-surgery:

- Pain relief → 1 day before and 3 days post-surgery
- Rimadyl 0.067 mg/ml (carprofen/NSAID) in drinking water

Surgery:

- Isoflurane anesthesia: induction 3%, maintenance 1-2%
- Heat pad and eye drops on both eyes
- Shave and disinfect the skin with Betadine
- Incision skin and peritoneum



Ovariectomy to sterilize females

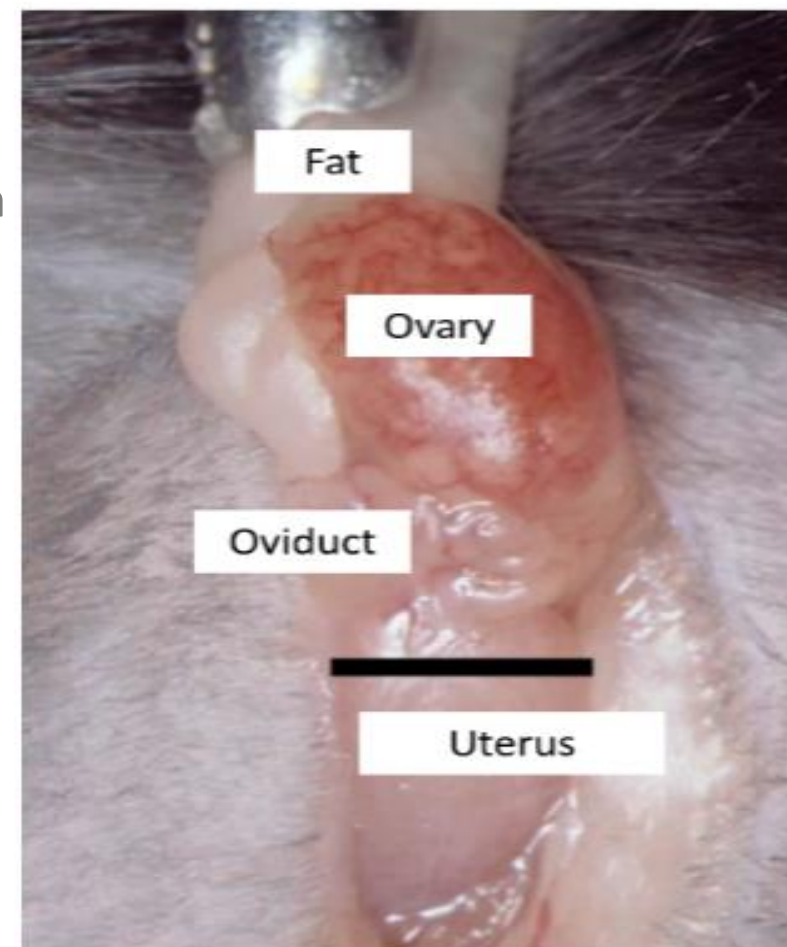
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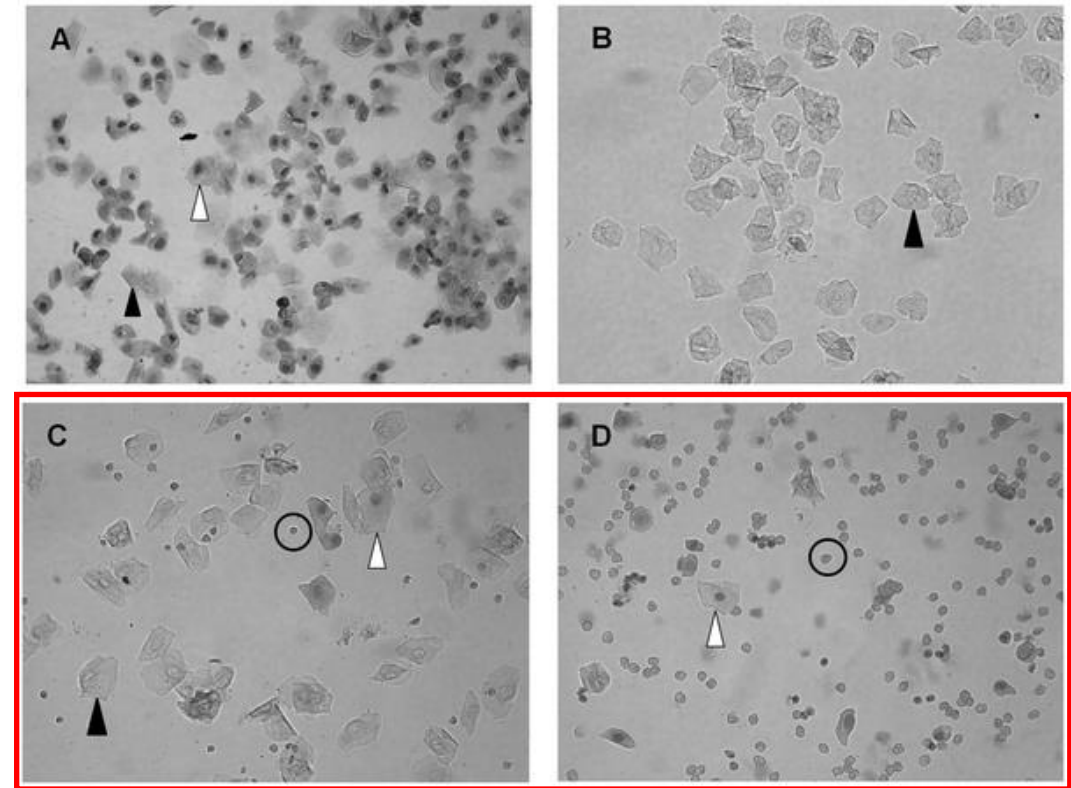
- Isoflurane anesthesia: induction 3%, maintenance 1-2%
- Heat pad and eye drops on both eyes
- Shave and disinfect the skin with Betadine
- Incision skin and peritoneum
- Removal of the ovary. With ligature (Ethicon® Perma-Hand™ zijde) and cauterizer
- Suture peritoneum (Ethicon® Perma-Hand™ zijde) and skin (Ethicon® Vicryl™ Plus)
- Tissue glue (3M Vetbond)



Ovariectomy to sterilize females

Halting of estrous cycle confirmed by vaginal cytology:

- 6 weeks after ovx → 4 consecutive daily vaginal swabs
- PBS flush $\pm 20 \mu\text{l}$ with pipet
- Sample spread on glass slide, dried, fixed, and stained with 0.5% crystal violet for brightfield microscopy

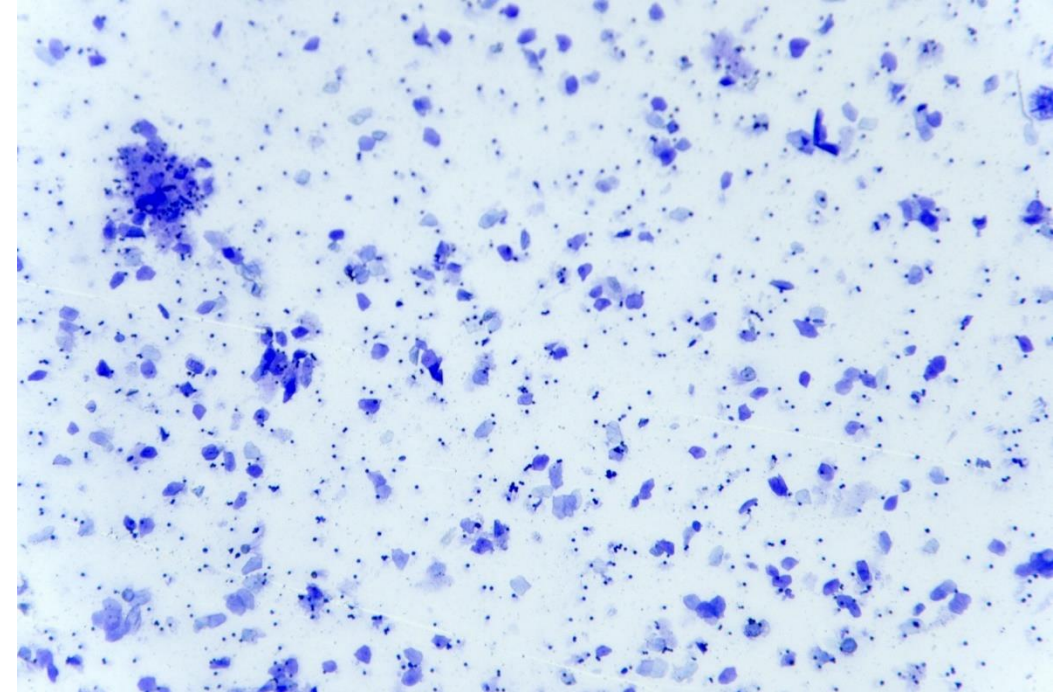


Vaginal swab (A) proestrus, (B) estrus, (C) metestrus and (D) diestrus. Black arrow= cornified epithelial cells, white arrow= nucleated epithelial cells, and black circle= leukocytes.

Ovariectomy to sterilize females

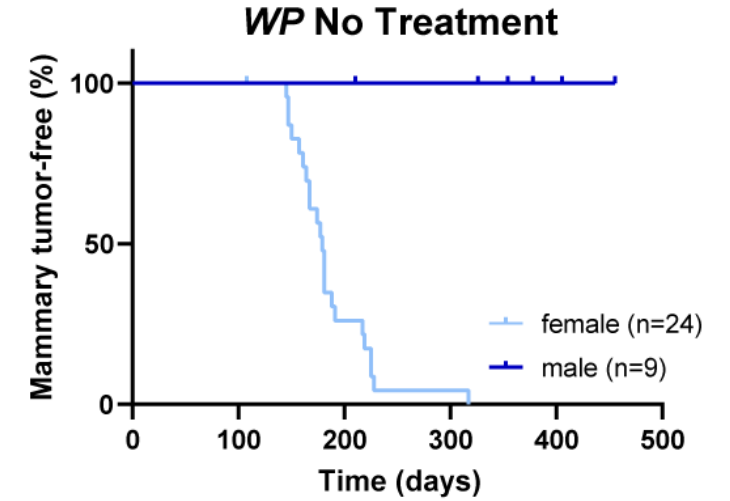
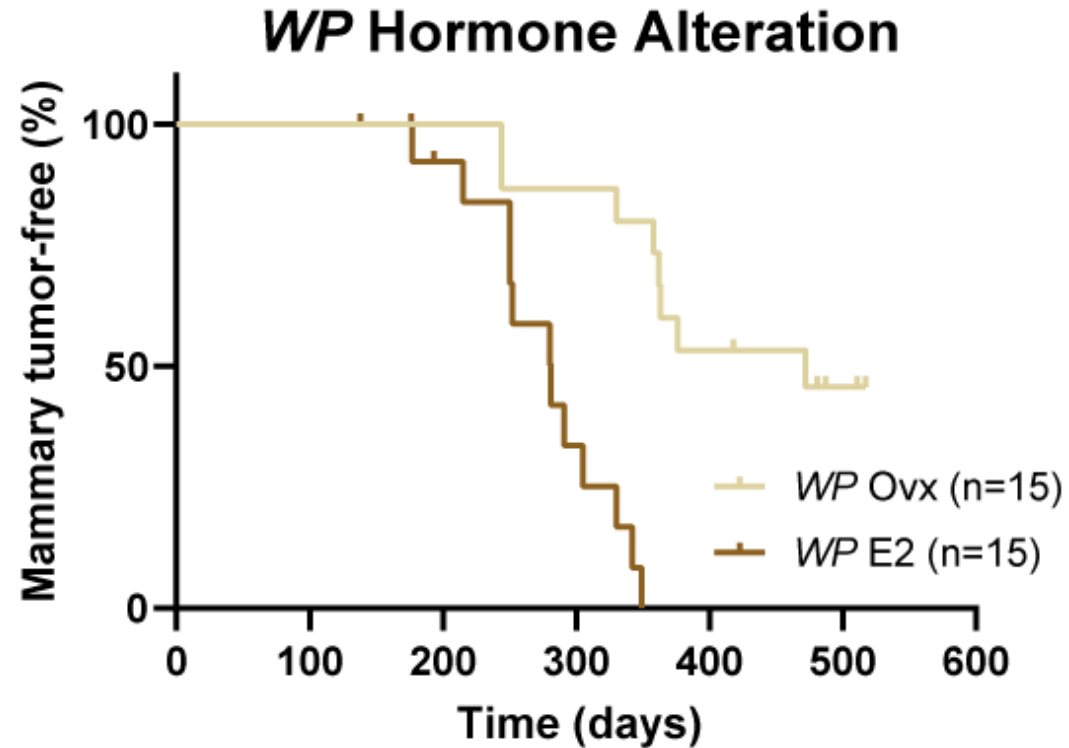
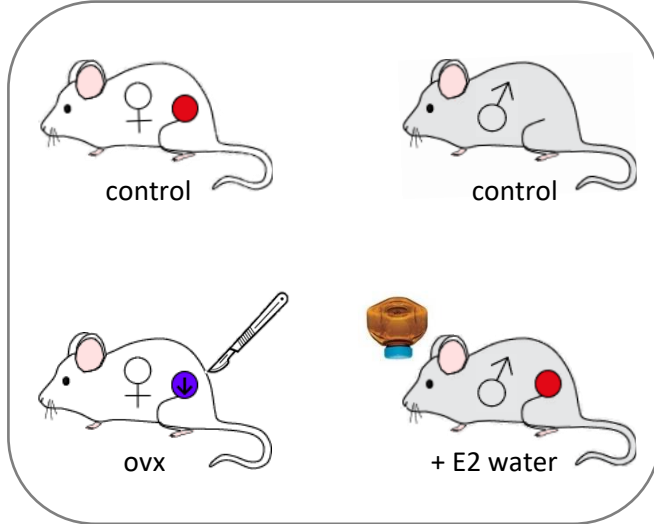
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- PBS flush $\pm 20 \mu\text{l}$ with pipet
- Sample spread on glass slide, dried, fixed, and stained with 0.5% crystal violet for brightfield microscopy
- If ovx is successful, all females should be in metestrus or diestrus

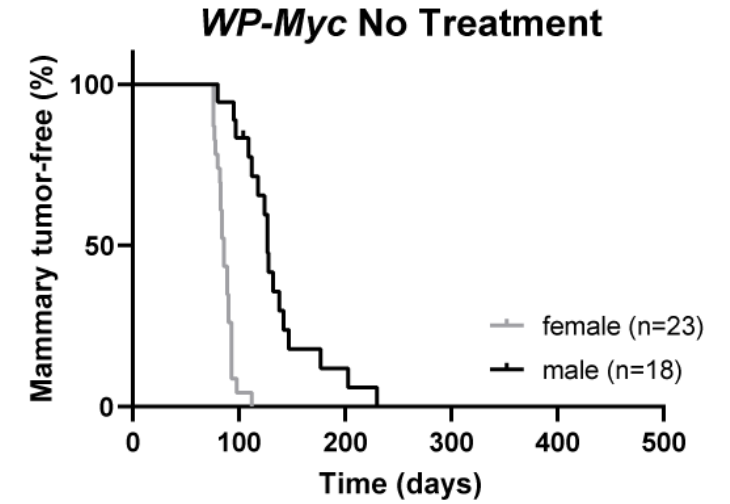
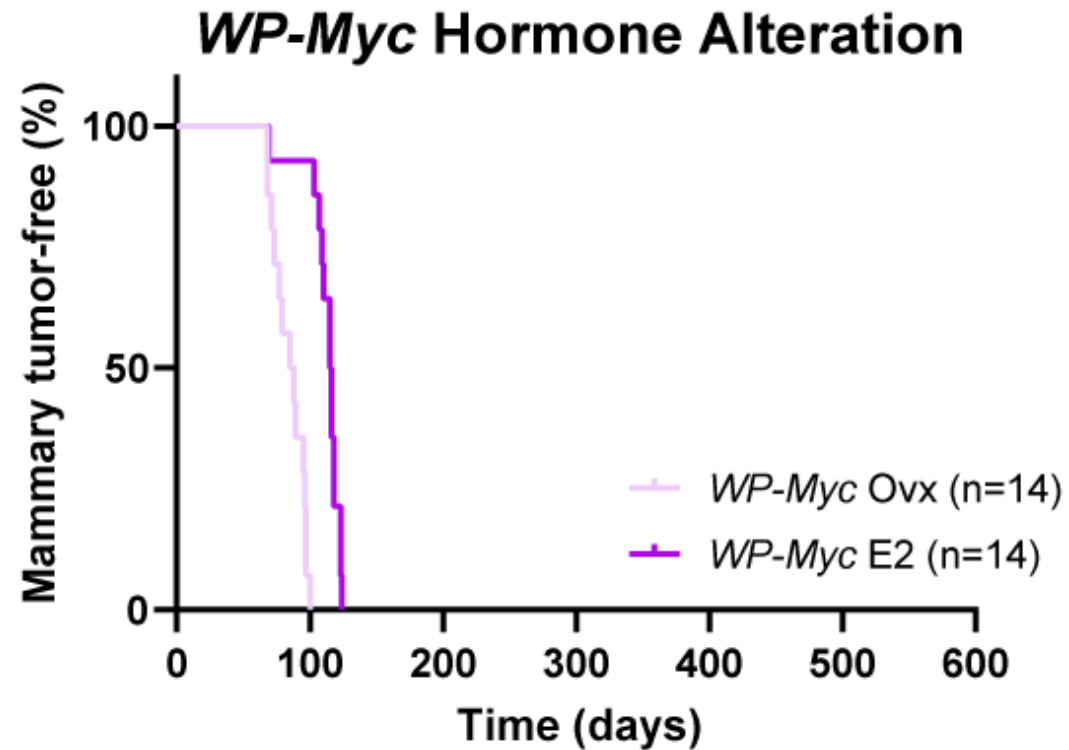
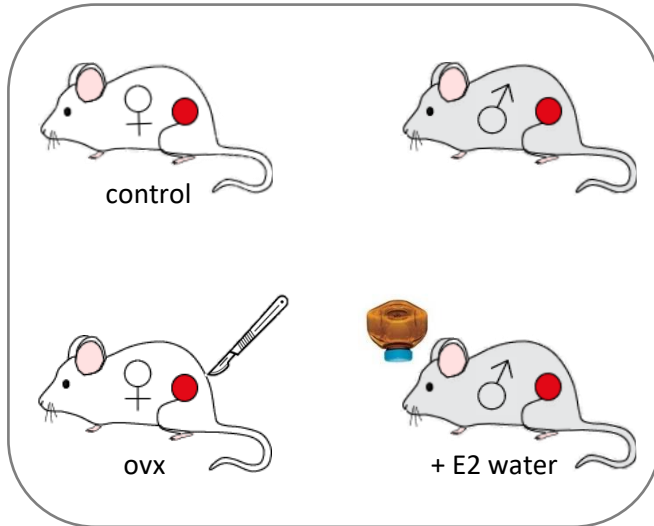


Vaginal swab crystal violet stain from *WP* female day1 post-ovx in diestrus

Results: *WapCre;p53^{F/F}*



Results: *WapCre;p53^{F/F};Myc*



Summary

Biotechnical:

- Advanced mouse model engineering → TNBC breast cancer**male breast cancer!**
- Surgical expertise & animal handling → male vs female husbandry/conditions, maintaining genotypes/breeding, experimental group procedures
- State of the art animal laboratory facility

Cancer biology:

- Male TNBC mice require *Myc* overexpression to prompt mammary tumor formation
- *Myc* overexpression replaces the estrogen-dependence that is required for female TNBC mice

| Sex | Estrogen | Myc | Mammary tumors? |
|-----|----------|-----|-----------------|
| ♀ | ✓ | ✗ | ✓ |
| | ✓ | ✓ | ✓ |
| | ✗ | ✗ | ↓ |
| | ✗ | ✓ | ✓ |
| ♂ | ✗ | ✗ | ✗ |
| | ✗ | ✓ | ✓ |
| | ✓ | ✗ | ✓ |
| | ✓ | ✓ | ✓ |

For more information

<https://www.nki.nl/research/facilities-platforms/>



RESEARCH

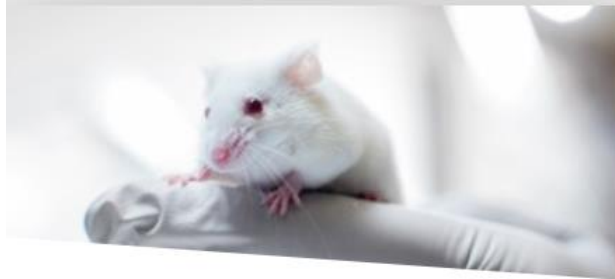
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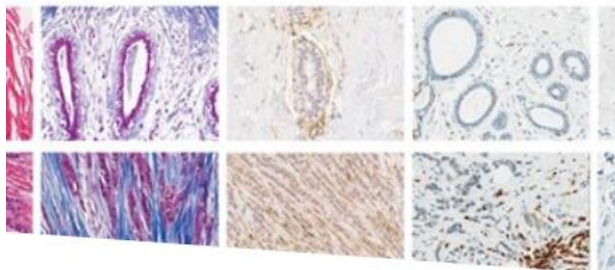
Animal facility - Breeding & experimental units



Animal facility - Mouse cancer clinic



Animal facility - Animal modeling facility



Experimental Animal Pathology



Bioanalytical Laboratory of the Pharmacy



Bioimaging

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Pathology**

Sjoerd Klarenbeek

All members of the

Jonkers Lab

NKI BioImaging Facility

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