# *Mouse models of breast and prostate cancer*


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# Need of mouse models of human cancer

- Cancer : complex diseases.

→ **Challenge :** improve diagnosis and treatments.

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→ **Challenge :** improve diagnosis and treatments.

**In vitro studies / in vivo studies**

**Cell lines, Xenografts, PDX, organoids**

# Need of mouse models of human cancer

- **Use of certain mouse strains :**

→spontaneous cancer or increased sensitivity to "environmental" exposure (radiation, chemicals, viruses…)

**Useful for:**

- identification of **oncogenes** and **tumor suppressor genes**, mapping of modifier genes…

- assessment of **carcinogenic** or **chemopreventive** effects of compounds.

- **Limitations :**
  - restricted subset of **tumor types** and **grades**
  - incomplete **penetrance**
  - Variable **latency**

# Need of mouse models of human cancer

→Development of **new technologies**

to provide **mouse models** that :

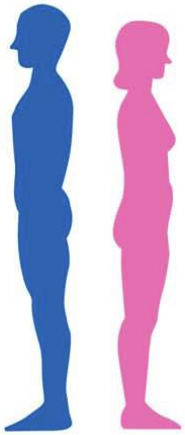
- accurately **reflect the common forms** of human cancer
- allow systematic investigation of **tumor genetics and gene-environment interactions**

**Genetically engineered mouse models**
**(GEMMs)**

# Hormone-dependent cancers

**Estimated New Cases**

| Males | | | Females | | |
|---|---|---|---|---|---|
| Prostate | 161,360 | 19% | Breast | 252,710 | 30% |
| Lung & bronchus | 116,990 | 14% | Lung & bronchus | 105,510 | 12% |
| Colon & rectum | 71,420 | 9% | Colon & rectum | 64,010 | 8% |
| Urinary bladder | 60,490 | 7% | Uterine corpus | 61,380 | 7% |
| Melanoma of the skin | 52,170 | 6% | Thyroid | 42,470 | 5% |
| Kidney & renal pelvis | 40,610 | 5% | Melanoma of the skin | 34,940 | 4% |
| Non-Hodgkin lymphoma | 40,080 | 5% | Non-Hodgkin lymphoma | 32,160 | 4% |
| Leukemia | 36,290 | 4% | Leukemia | 25,840 | 3% |
| Oral cavity & pharynx | 35,720 | 4% | Pancreas | 25,700 | 3% |
| Liver & intrahepatic bile duct | 29,200 | 3% | Kidney & renal pelvis | 23,380 | 3% |
| **All Sites** | **836,150** | **100%** | **All Sites** | **852,630** | **100%** |



**Excludes :**

- basal and squamous cell skin cancers
- in situ carcinoma except urinary bladder.

*Siegel et al., 2017*

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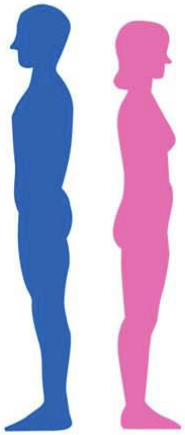
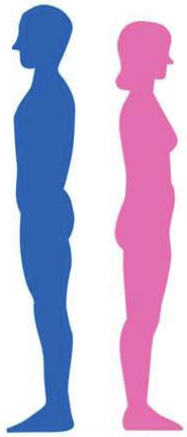


**Excludes :**
- basal and squamous cell skin cancers
- in situ carcinoma except urinary bladder.

**Estimated Deaths**

| Males | | | Females | | |
|---|---|---|---|---|---|
| Lung & bronchus | 84,590 | 27% | Lung & bronchus | 71,280 | 25% |
| Colon & rectum | 27,150 | 9% | Breast | 40,610 | 14% |
| Prostate | 26,730 | 8% | Colon & rectum | 23,110 | 8% |
| Pancreas | 22,300 | 7% | Pancreas | 20,790 | 7% |
| Liver & intrahepatic bile duct | 19,610 | 6% | Ovary | 14,080 | 5% |
| Leukemia | 14,300 | 4% | Uterine corpus | 10,920 | 4% |
| Esophagus | 12,720 | 4% | Leukemia | 10,200 | 4% |
| Urinary bladder | 12,240 | 4% | Liver & intrahepatic bile duct | 9,310 | 3% |
| Non-Hodgkin lymphoma | 11,450 | 4% | Non-Hodgkin lymphoma | 8,690 | 3% |
| Brain & other nervous system | 9,620 | 3% | Brain & other nervous system | 7,080 | 3% |
| **All Sites** | **318,420** | **100%** | **All Sites** | **282,500** | **100%** |



**→ high socio-economical impact.**

*Siegel et al., 2017*

# Breast cancer

**Females**

| | | |
|---|---|---|
| Lung & bronchus | 71,280 | 25% |
| Breast | 40,610 | 14% |
| Colon & rectum | 23,110 | 8% |
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Global gene expression analyses have classified **breast cancer** into at least **five biologically distinct intrinsic subtypes** :

- luminal A,
- luminal B,
- human epidermal growth factor receptor 2 (HER2)- enriched,
- basal-like
- normal-like.

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- basal-like
- normal-like.

**Luminal A and B subtypes** : estrogen receptor α (**ERα**) positive

→ **~ 70% of breast cancers**

→ **Estrogen-dependent growth → Tamoxifen treatment/resistance**

→ **major clinical interest.**

# Hormone-dependent cancers

**Estimated deaths**

**Females**

| | | |
|---|---|---|
| Lung & bronchus | 72,160 | 26% |
| Breast | 40,450 | 14% |
| Colon & rectum | 23,170 | 8% |
| Pancreas | 20,330 | 7% |
| Ovary | 14,240 | 5% |
| Uterine corpus | 10,470 | 4% |
| Leukemia | 10,270 | 4% |
| Liver & intrahepatic bile duct | 8,890 | 3% |
| Non-Hodgkin lymphoma | 8,630 | 3% |
| Brain & other nervous system | 6,610 | 2% |
| **All Sites** | **281,400** | **100%** |

**Genetically modified mouse models** (GEMMs) **of breast cancer**
(overexpression of Myc, ErbB2/Neu, polyoma middle T antigen (PyMT), SV 40 T antigen, wnt-1, TGF-α, c-myc, ras...)

→ mammary carcinomas
- **ER⟨ negative** and hormone independent

→ **do not mimic luminal subtypes**.

# Hormono-dependent cancers

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→ mammary carcinomas

- **prevalently ERα negative** and hormone independent

→ **do not mimic luminal subtypes**.

**Ras :** the **most frequently mutated** dominant acting **oncogene** in human cancer

**MMTV-Ki-Ras(G12V) transgenic mice :**

- develop **mammary adenocarcinoma** with a short tumor latency,
- **high tumor incidence**

**→ Limitations :**

- tumor formation in **various tissues** (e.g. salivary and harderian glands),
- poor **characterization** of mammary carcinomas.

*Omer et al., 2000*

# Mouse model of estrogen receptor α (ERα)-positive mammary adenocarcinoma


*Collaboration with Pr. Ando, University of Calabria*

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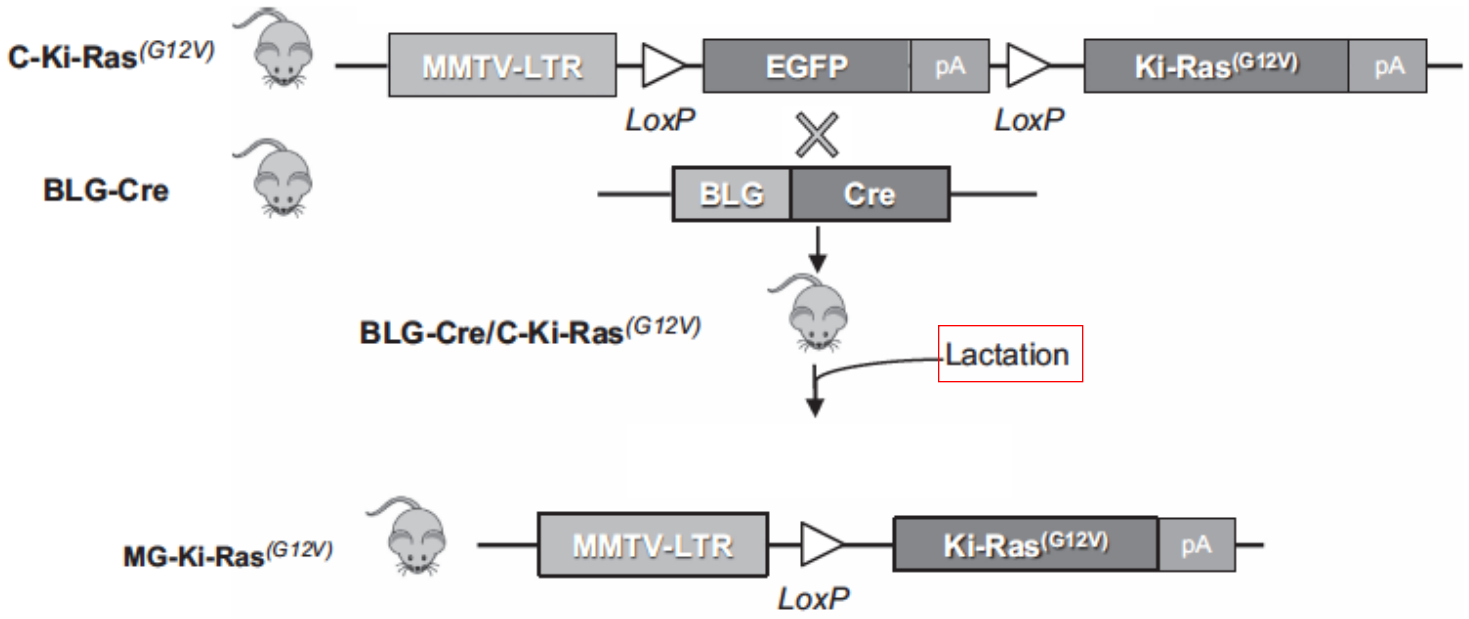


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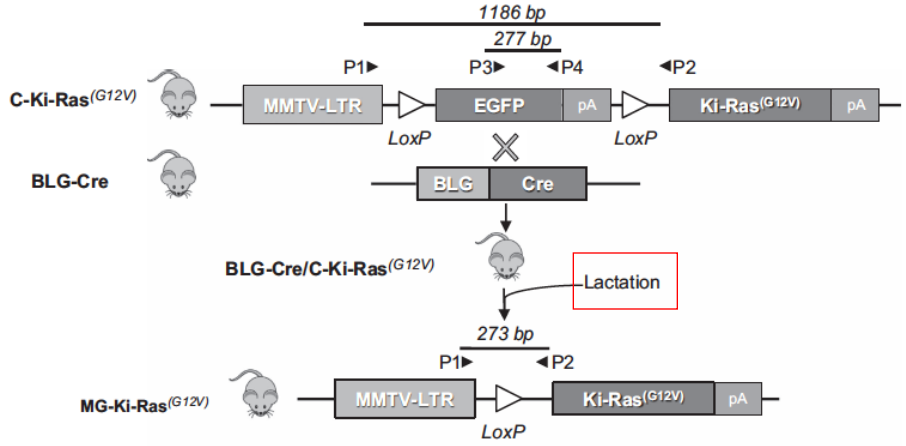


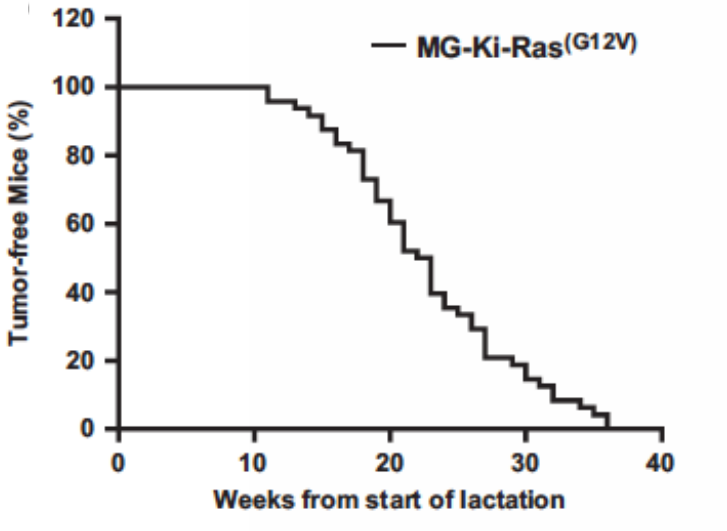


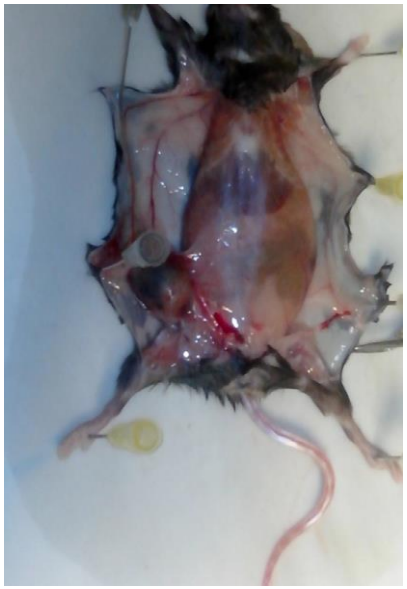

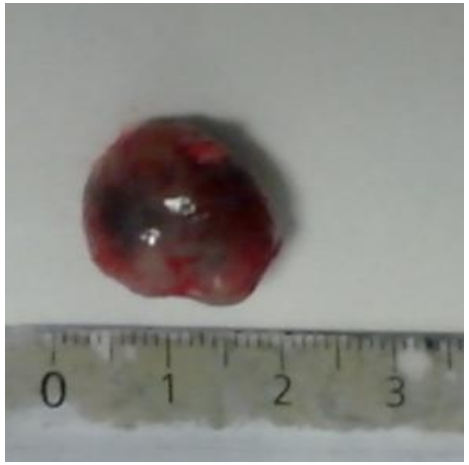


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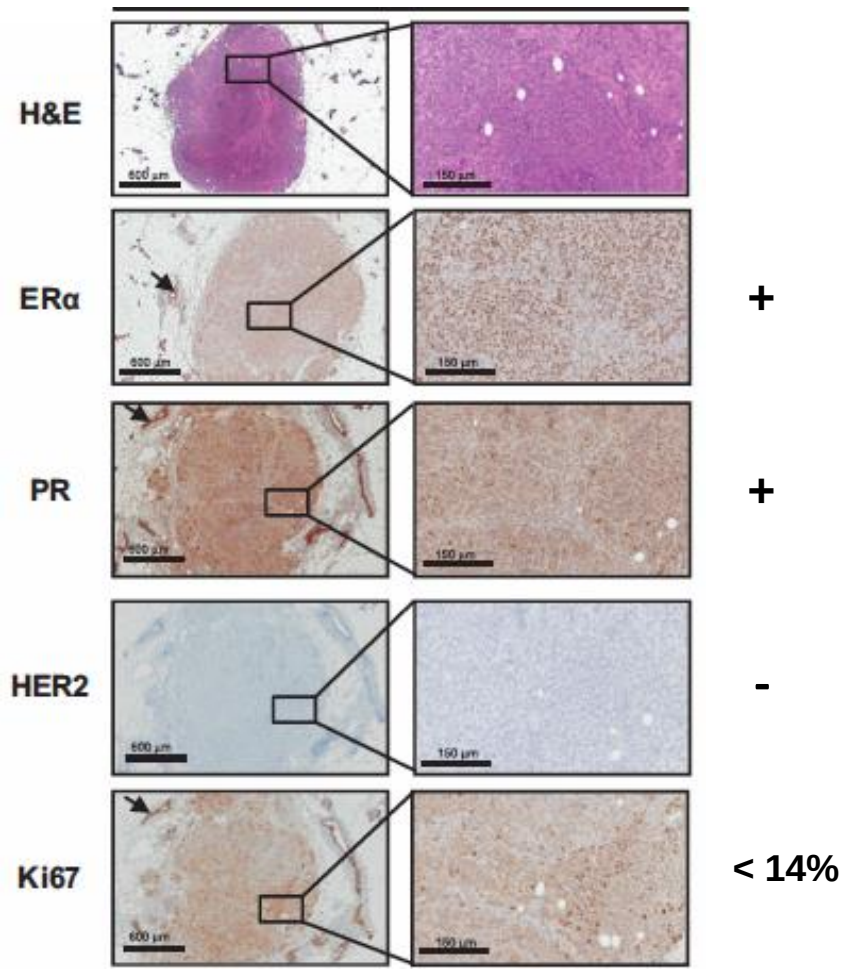


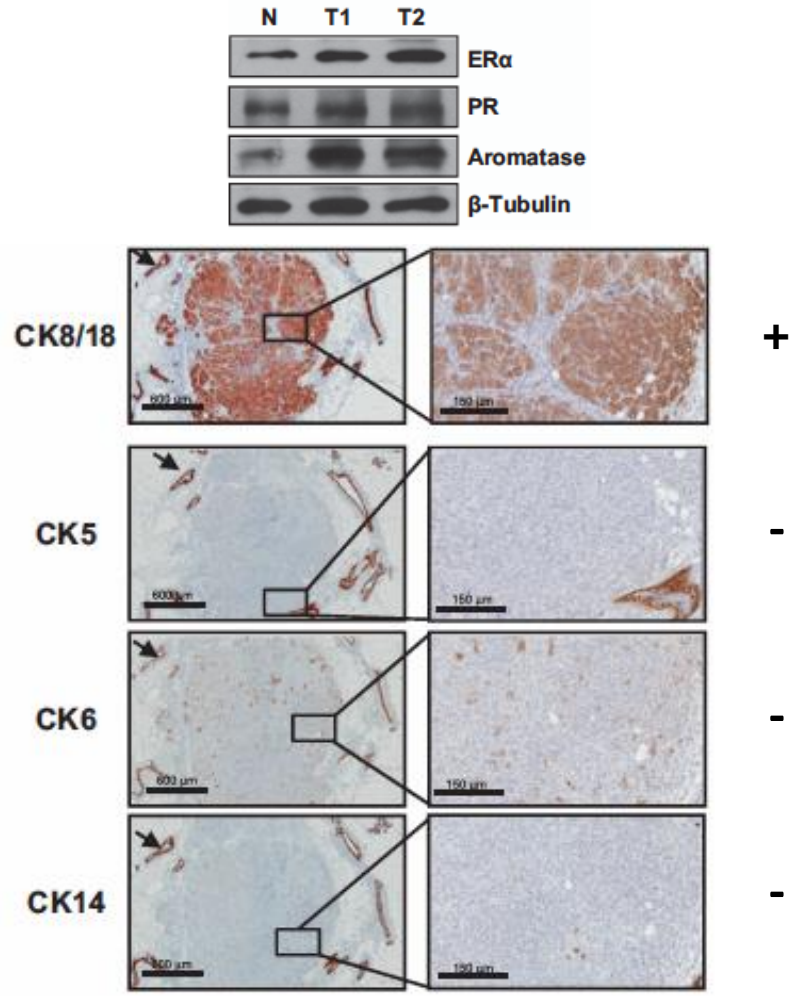


**MG-Ki-Ras(G12V) mice develop mammary tumors that recapitulate then most common human breast cancer subtype : ductal ER-positive invasive adenocarcinoma.**

# Prostate cancer

**Males** — **Estimated deaths**

| | | |
|---|---|---|
| Lung & bronchus | 84,590 | 27% |
| Colon & rectum | 27,150 | 9% |
| Prostate | 26,730 | 8% |
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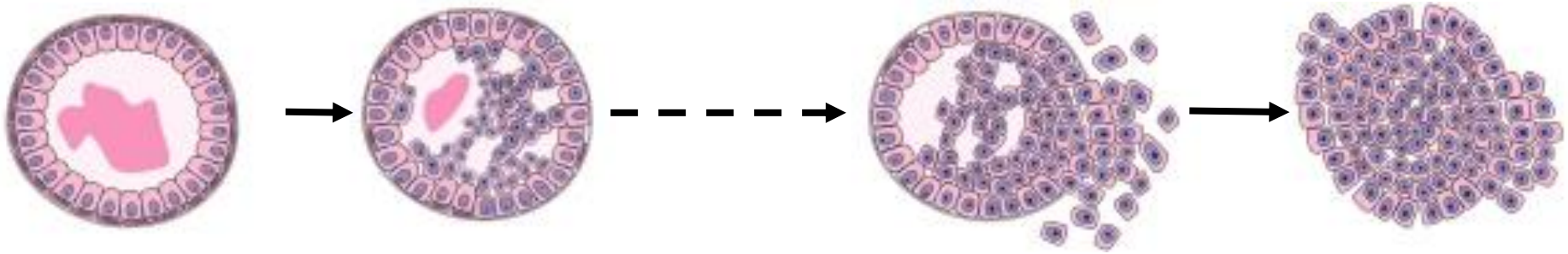
| **All Sites** | **318,420** | **100%** |

Normal epithelium

Prostatic Intraepithelial Neoplasia (PIN)

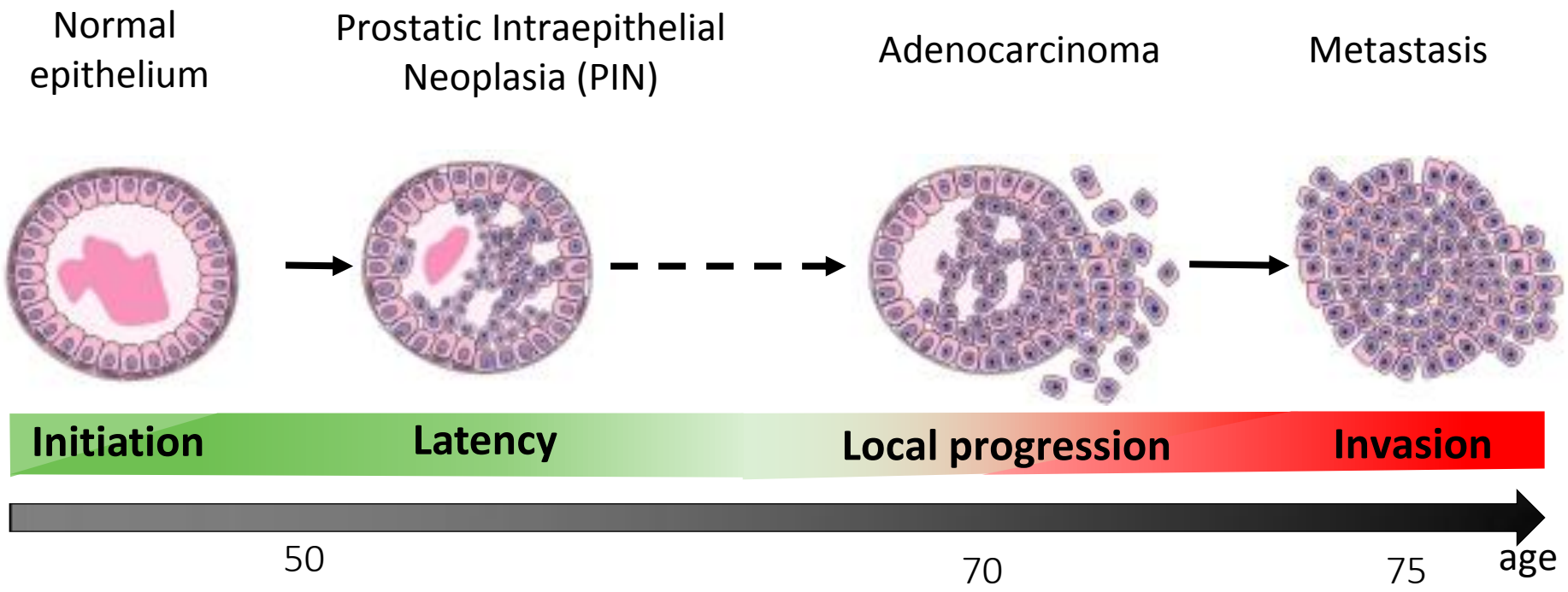
Adenocarcinoma

Metastasis



**Initiation** **Latency** **Local progression** **Invasion**

# Prostate cancer




➢ **Prostate cancer development takes decades**

**Diagnosis** : - Serum PSA (prostate specific antigen)
- rectal digital examination
- histological analysis of biopsies

} **Over diagnosis / over treatment**

# Prostate cancer

Normal epithelium | Prostatic Intraepithelial Neoplasia (PIN) | Adenocarcinoma | Metastasis | Castration resistant adenocarcinoma

Initiation | Latency | Local progression | Invasion | Resistance

50 — 70 — 75 — age

**Treatments:**

**Prostatectomie**
**Radiotherapy**

↓

**Side effects**
- Incontinance
- impotance

**Hormono-therapy**
**(anti-androgens)**

↓

**Resistance**

**Chimio-therapy**
(docetaxel)

↓

**Limited efficacy**

**→ Need to improve diagnosis and treatments**

# TRAMP mice

(transgenic adenocarcinoma mouse model)

**Transgene :**


**-426 - +28 bp**
**rat probasin promoter**

(Greenberg et al., 1995)

## Use :

- **pre-clinical testing of chemoprevention strategies**
- **Identify pathways involved in prostate cancer initiation and progression**

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## Use :

- **pre-clinical testing of chemoprevention strategies**
- **Identify pathways involved in prostate cancer initiation and progression**

## Limitations :

- **Prostate tumour formation driven by viral proteins that are not involved in the generation of human prostate cancers**
- **Develop neuroendocrine carcinoma that rarely occur in human prostate cancer**
- **T antigen is expressed in the prostate at the first week(s) of life**
- **The activity of rPB promoter is regulated by androgens at adulthood**
  **→ effects observed in hormone ablation experiments might result from decreased transgene expression.**

# Prostate cancer

**PTEN (phosphatase and tensin homolog deleted on chromosome 10) :**

→ Negative regulator of PI3K/AKT pathway

→ frequently deleted in various advanced human cancers

→ PTEN mutations in 10 – 15 % of prostate tumors and in 60 % of advanced cancers

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## Consequences of PTEN deficiency in prostate cells ?

**Mouse model : PB-Cre/PTEN$^{L2/L2}$**

**→ Prostatic tumors**

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**Mouse model : PB-Cre/PTEN$^{L2/L2}$**

**→ Prostatic tumors**

A novel type of cellular senescence that can be enhanced in mouse models and human tumor xenografts to suppress prostate tumorigenesis

Andrea Alimonti,[1,2] Caterina Nardella,[1,2] Zhenbang Chen,[1,2] John G. Clohessy,[1,2] Arkaitz Carracedo,[1,2] Lloyd C. Trotman,[2] Ke Cheng,[1,2] Shohreh Varmeh,[1,2] Sara C. Kozma,[3] George Thomas,[3] Erika Rosivatz,[4] Rudiger Woscholski,[4] Francesco Cognetti,[5] Howard I. Scher,[6] and Pier Paolo Pandolfi[1,2]

- **No DNA-damage response**
- **No hyper-proliferation phase**
- **No replicative stress !**

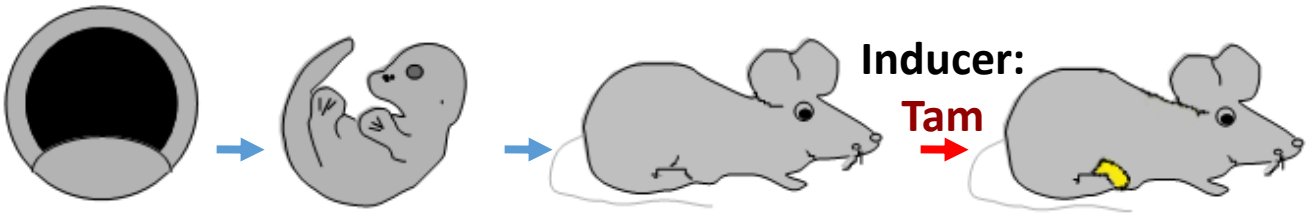
} **≠ Oncogene induced-senescence**

**PTEN-loss induced cell senescence (PICS) represents a new type of premature senescence**

**Relevant model? (gene ablation before puberty !)**

# Site-directed cell-specific temporally-controlled targeted somatic mutagenesis in the mouse.

Transgenic expression of the chimeric Cre-ER$^{T2}$ recombinase


Metzger et al., PNAS, 1995

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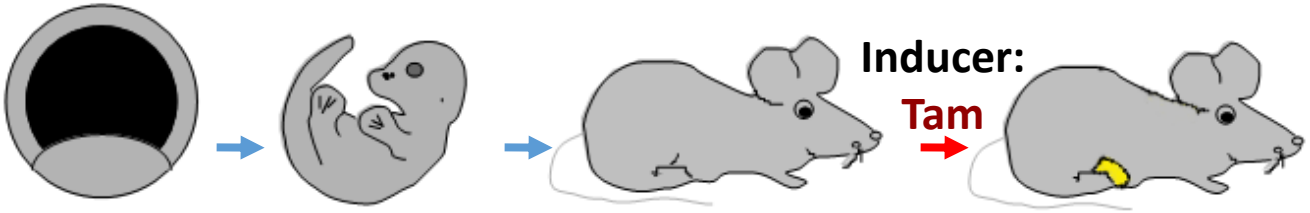


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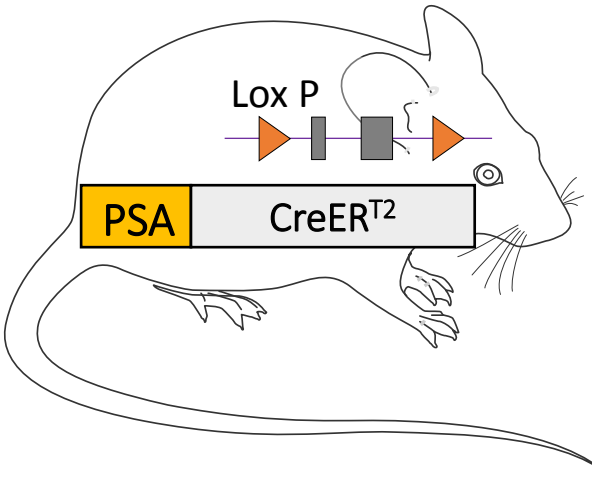
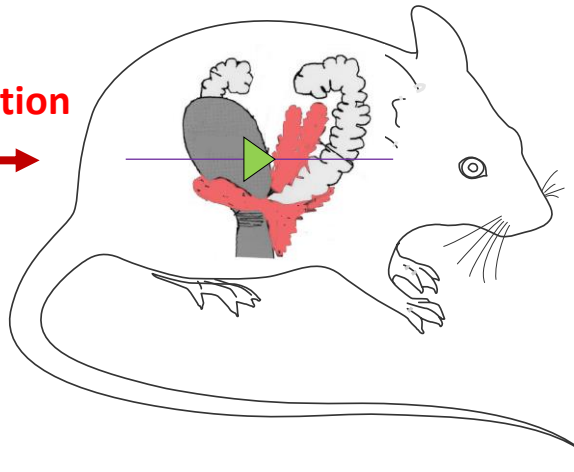



Puberty (8 weeks)

**Tamoxifen administration**



Ratnacaram et al., PNAS 2008

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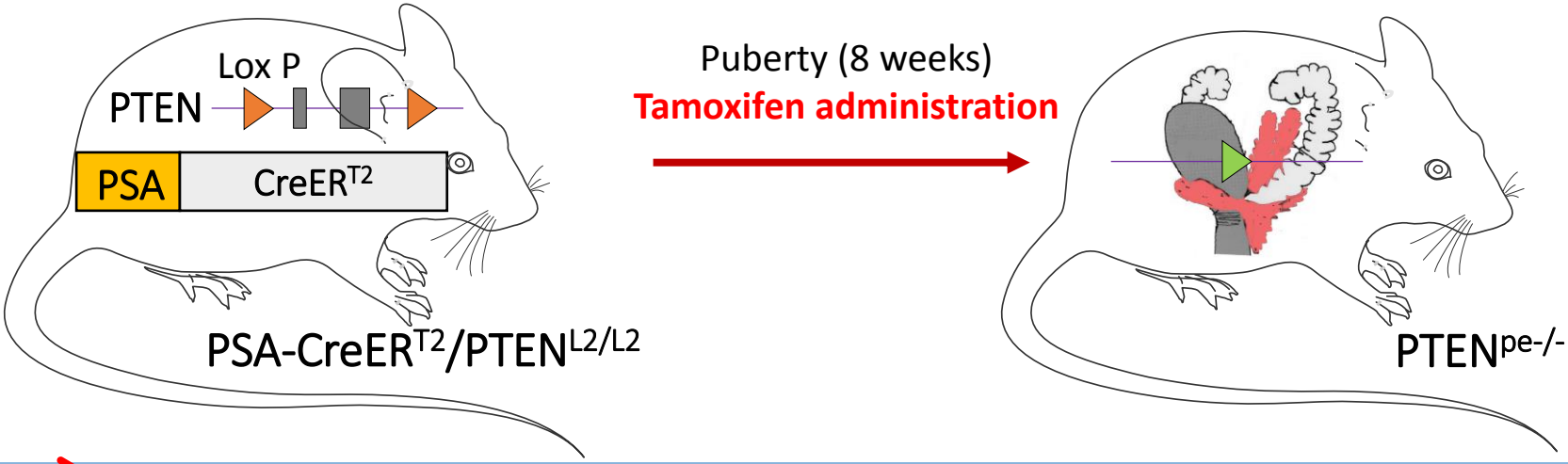
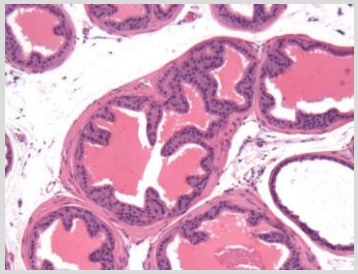


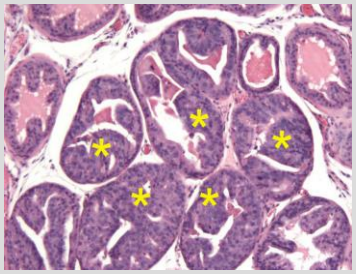

**Generation of PTEN$^{pe-/-}$ mice**

**(**in which PTEN is selectively ablated in prostatic epithelial cells at adulthood; Tam-treated PSA-CreER$^{T2}$/PTEN$^{L2/L2}$ mice)

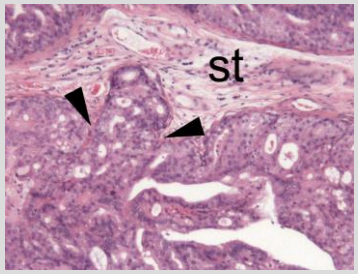
PTEN$^{pe-/-}$ mice



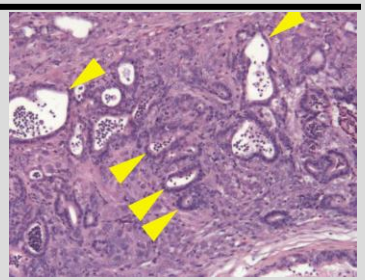
**Normal prostate**



**Prostatic Intraepithelial Neoplasia (PIN)**
(2 - 9 months)




**micro-invasive cancinoma**
(9 - 15 months)



**Local invasive Adenocarcinoma**
(15 - 20 months)

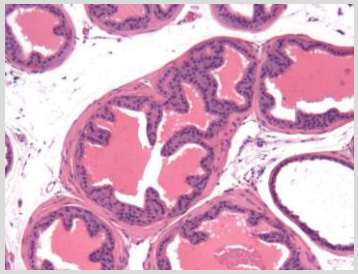
**Mouse model of prostate cancer development**

Ratnacaram et al., PNAS 2008

# Prostate cancer

## Characterisation of PTEN$^{pe-/-}$ mice

PTEN$^{pe-/-}$ mice



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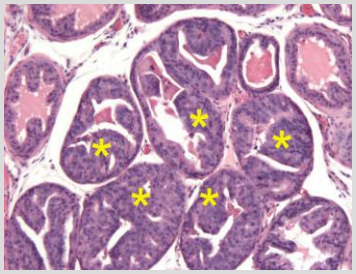

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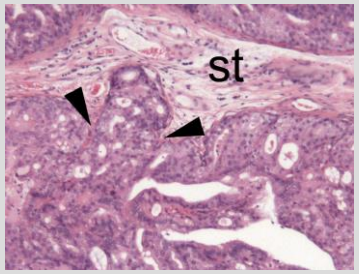


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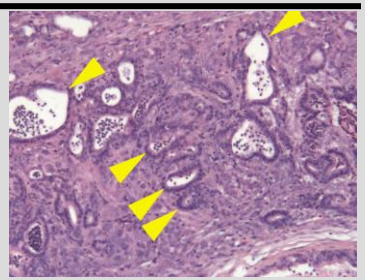

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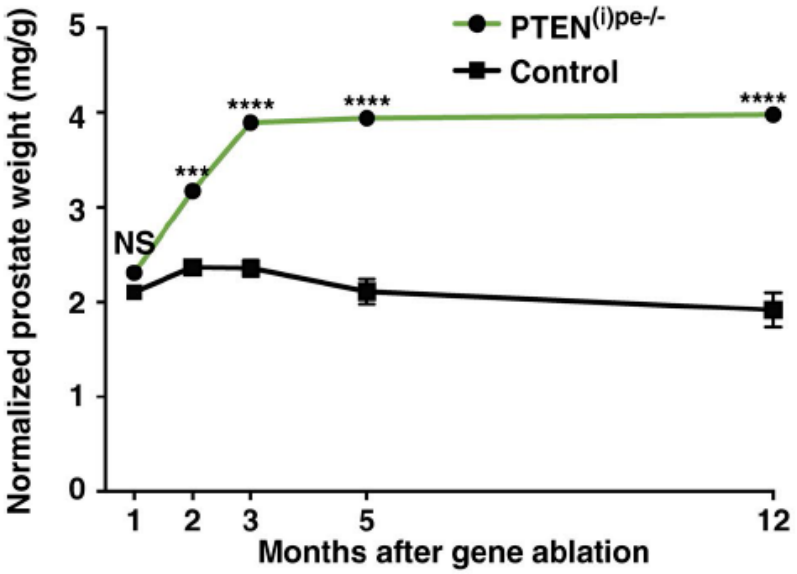


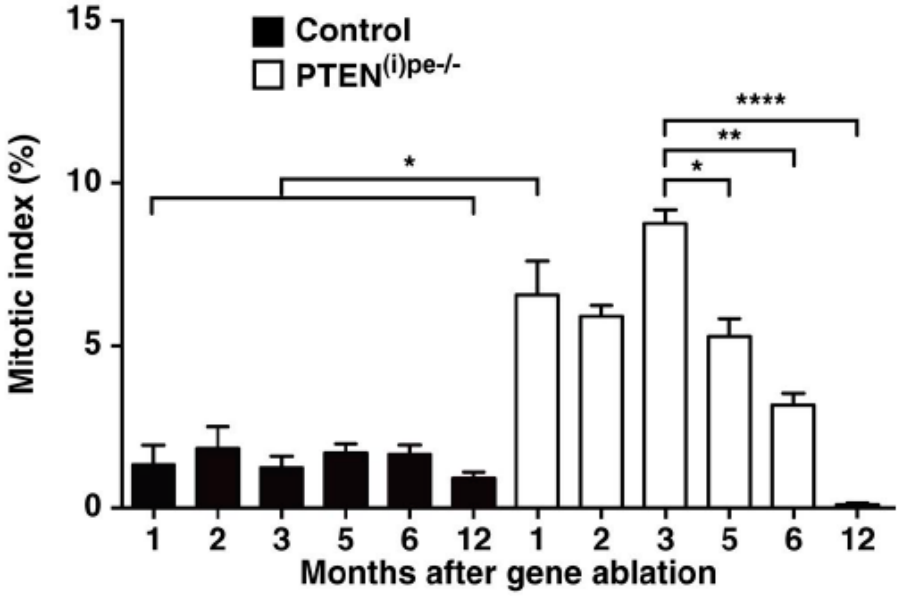

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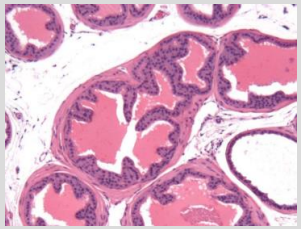
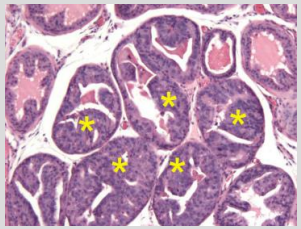
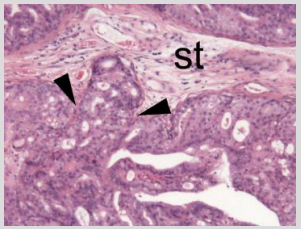


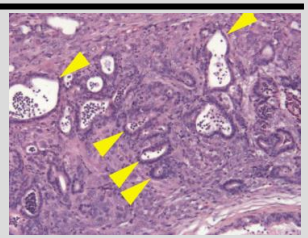
**Normal prostate**



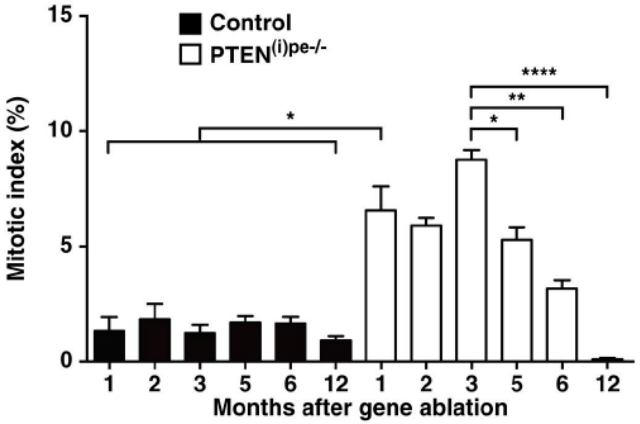
**Prostatic Intraepithelial Neoplasia (PIN)**
(2 - 9 months)

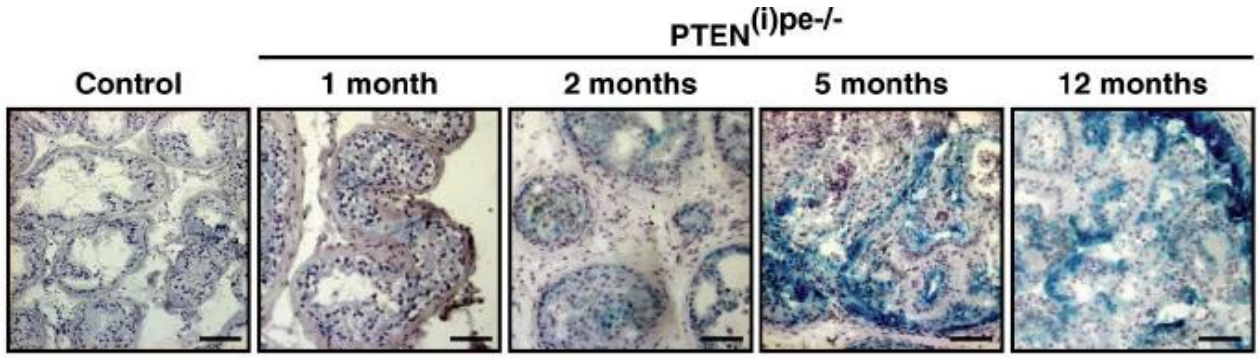



**micro-invasive cancinoma**
(9 - 15 months)



**Local invasive Adenocarcinoma**
(15 - 20 months)




**Senescence-associated β-galactosidase staining**

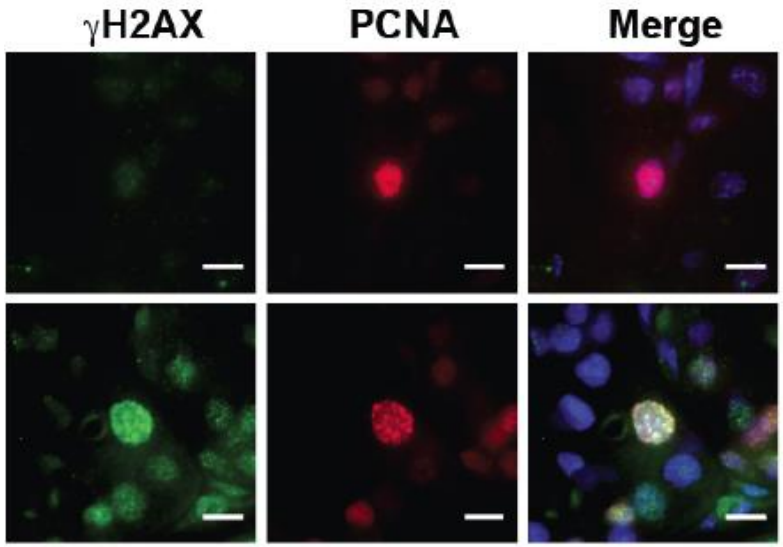
- **pHP1γ positive prostatic epithelial cells**
- **Senescence-associated secretory phenotype**
  (SASP; IL-1α, IL-1β, M-CSF, TNFα…)

**Proliferation → senescence**

Parisotto et al., J. Exp. Med. 2018

# Prostate cancer

## Characterisation of PTEN$^{pe-/-}$ mice




PCNA : proliferation marker

**γH2AX foci colocalize with PCNA foci.**

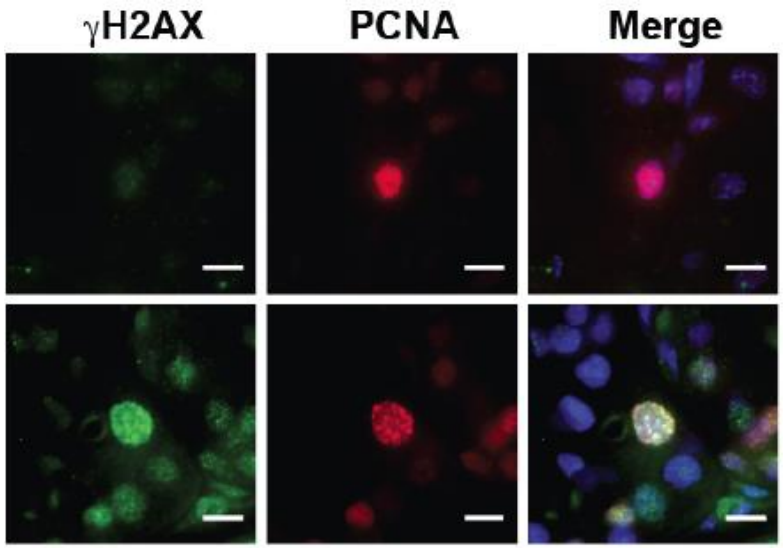
- **γH2AX foci localized at the site of DNA replication**
- **Nuclear foci of RPA32** (coats stretches of ssDNA during replicative stress)
  **ATR** (Ataxia telangiectasia and Rad3 related) and **53BP1**

**PTEN$^{pe-/-}$ prostatic epithelial cells → Replication stress, DNA damage response → Senescence**

Parisotto et al., J. Exp. Med. 2018

# Prostate cancer

## Characterisation of PTEN$^{pe-/-}$ mice

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**PTEN$^{pe-/-}$ prostatic epithelial cells → Replication stress, DNA damage response → Senescence**

**PTEN-loss induced cell senescence (PICS): similar to oncogene-induced senescence !**

**Approaches for cancer prevention and therapy based on PICS induction :**
**→ high risk** [replication stress → accumulation of mutations (e.g. p53)]

Parisotto et al., J. Exp. Med. 2018

# Prostate cancer

**Characterisation of cell populations in senescent PINs of PTEN$^{pe-/-}$ mice**

**→ Single cell sequencing**


**Prostate dissection & dissociation**

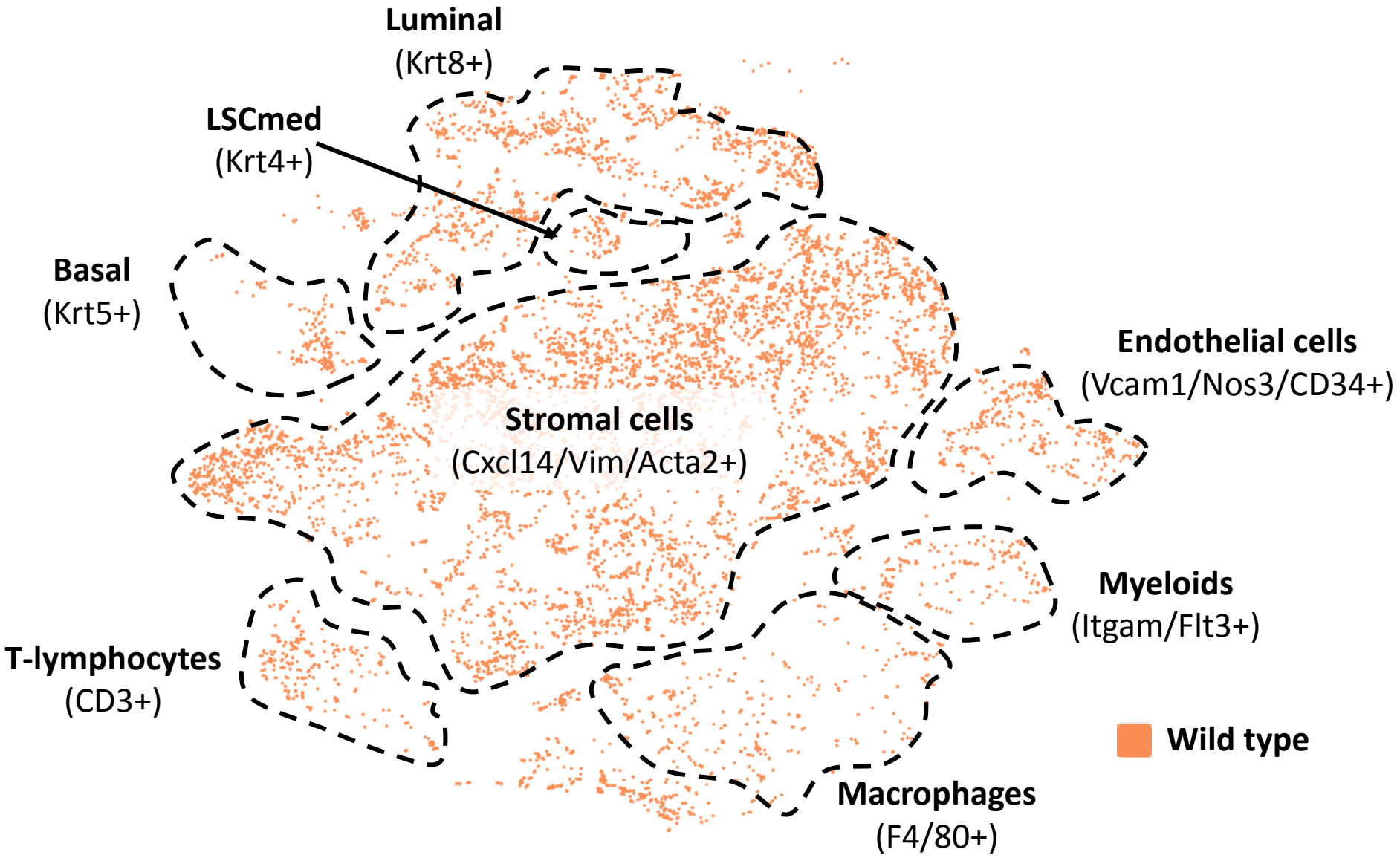
**FACS-sort single/ living cells (viability ≈ 90%)**

**10,000 cells**


**High-throughput sequencing with Illumina HiSeq**

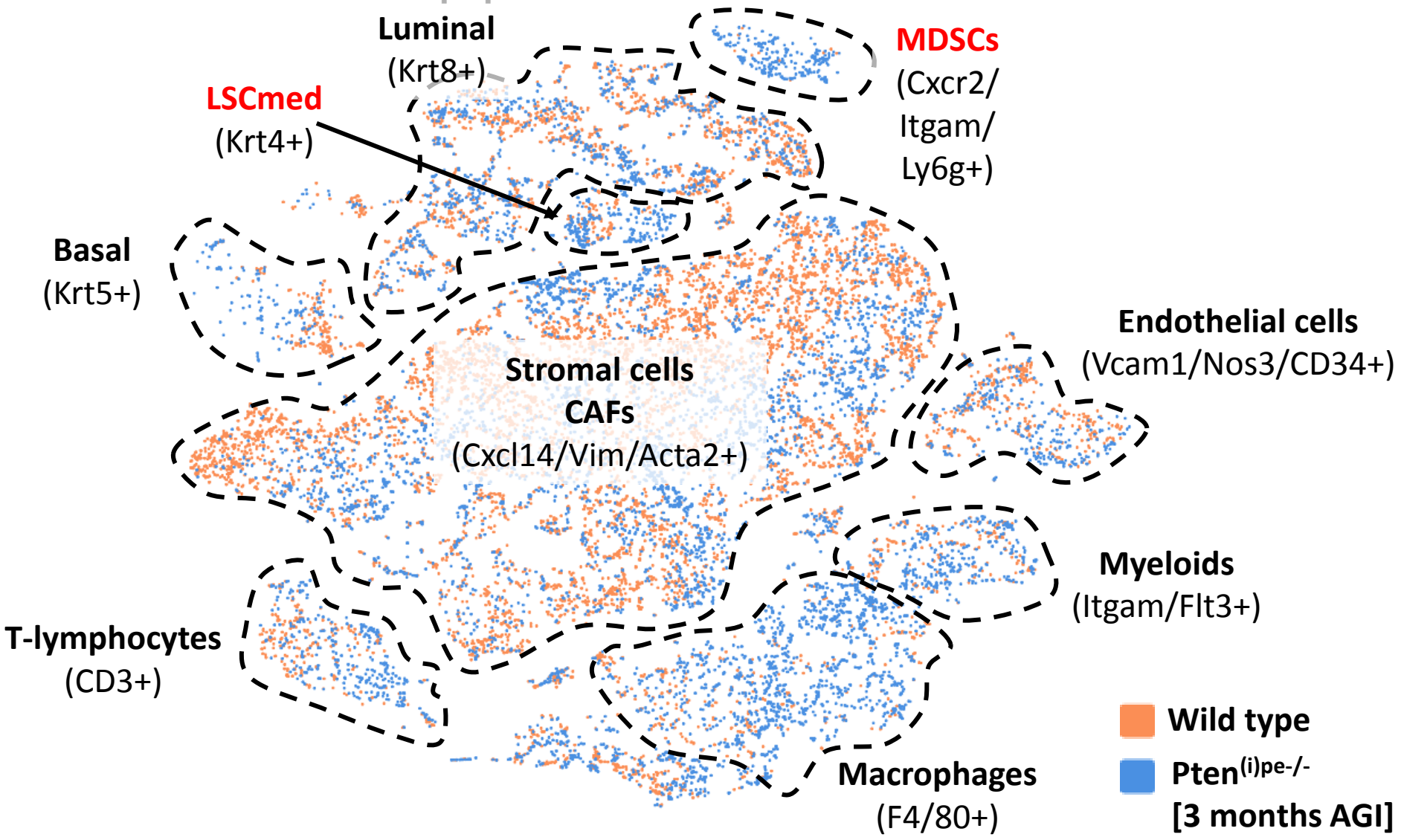
# Prostate cancer

## Characterisation of cell populations in mouse prostate



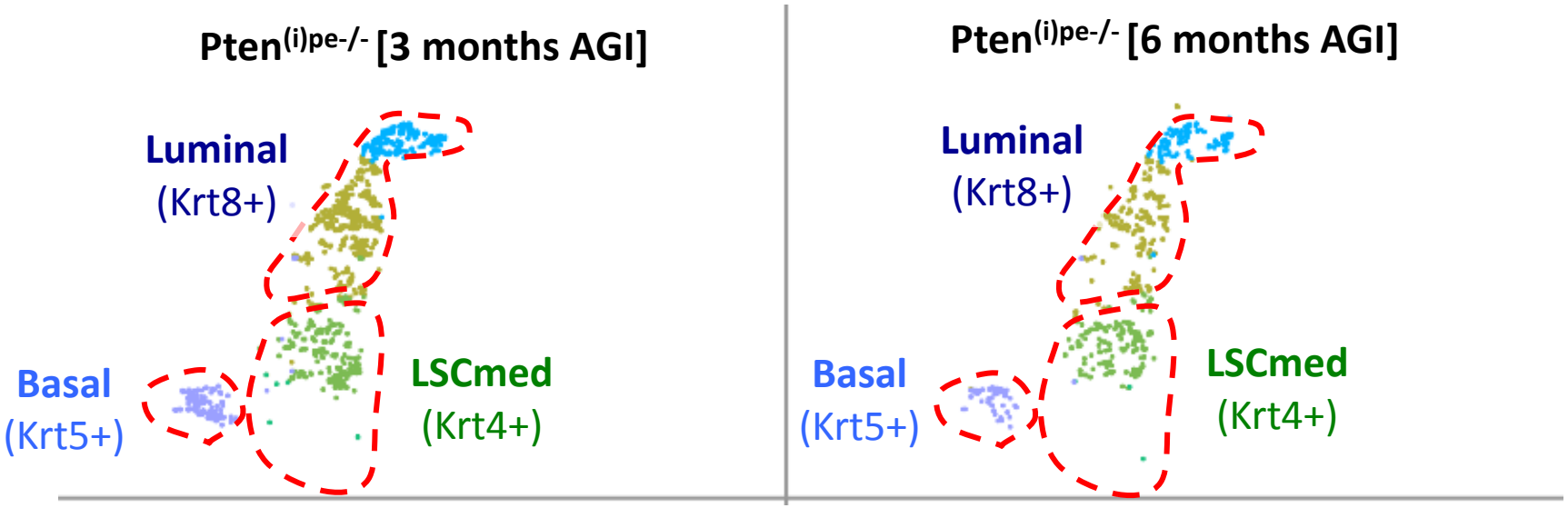
# Prostate cancer

## Characterisation of cell populations in senescent PINs of PTEN$^{pe-/-}$ mice



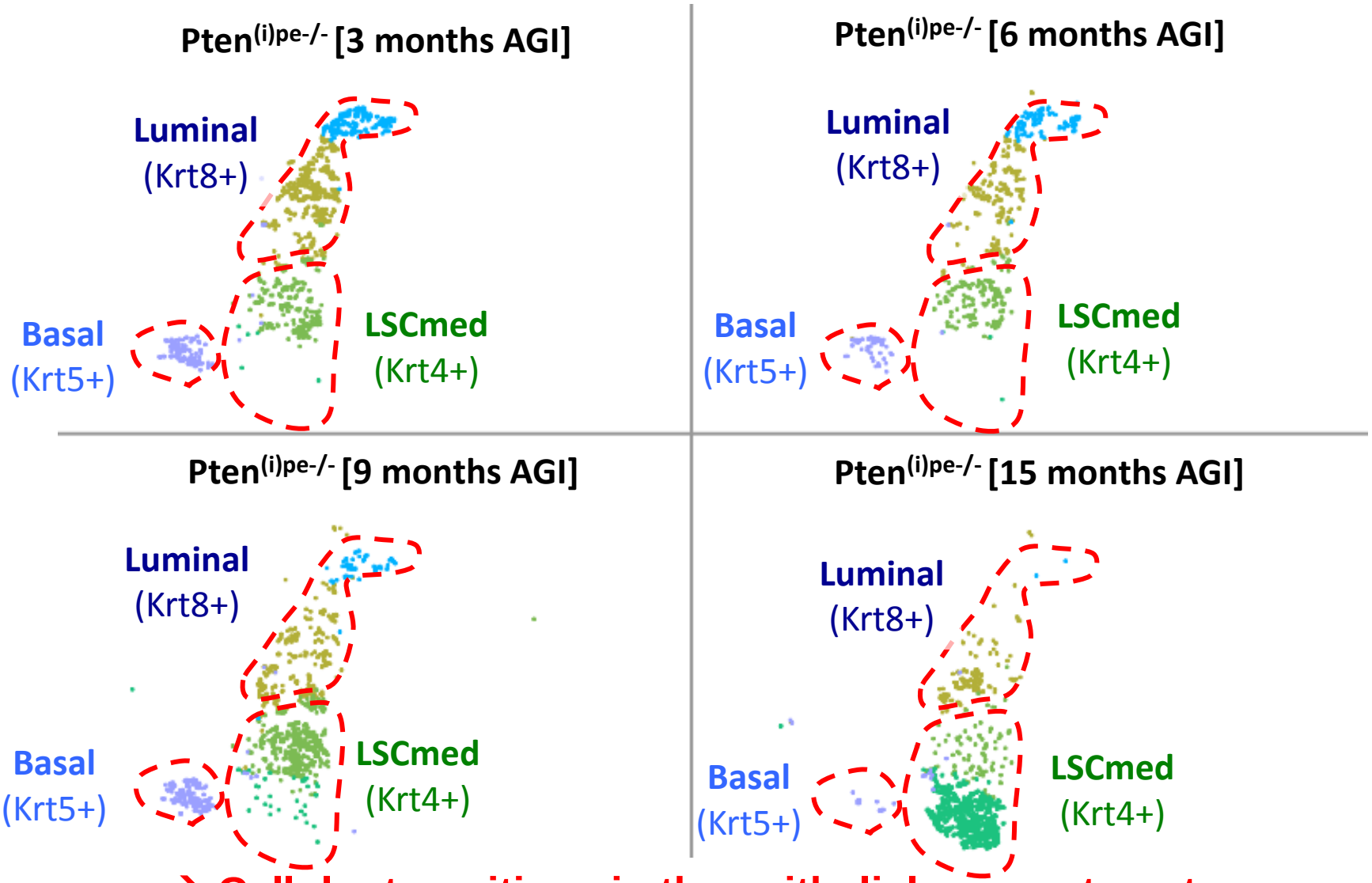
# Prostate cancer

## Characterisation of cell populations in senescent PINs of PTEN$^{pe-/-}$ mice



# Prostate cancer

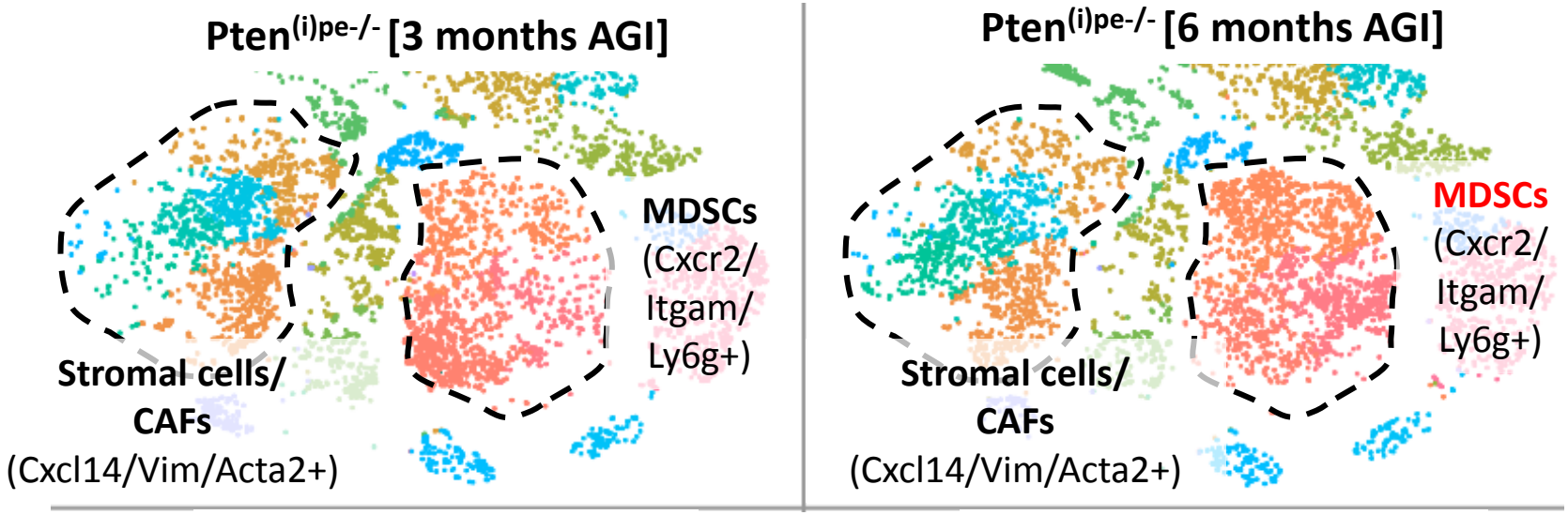
## Characterisation of cell populations in senescent PINs of PTEN$^{pe-/-}$ mice




→ Cellular transitions in the epithelial compartment

# Prostate cancer

## Characterisation of cell populations in senescent PINs of PTEN$^{pe-/-}$ mice



# Prostate cancer

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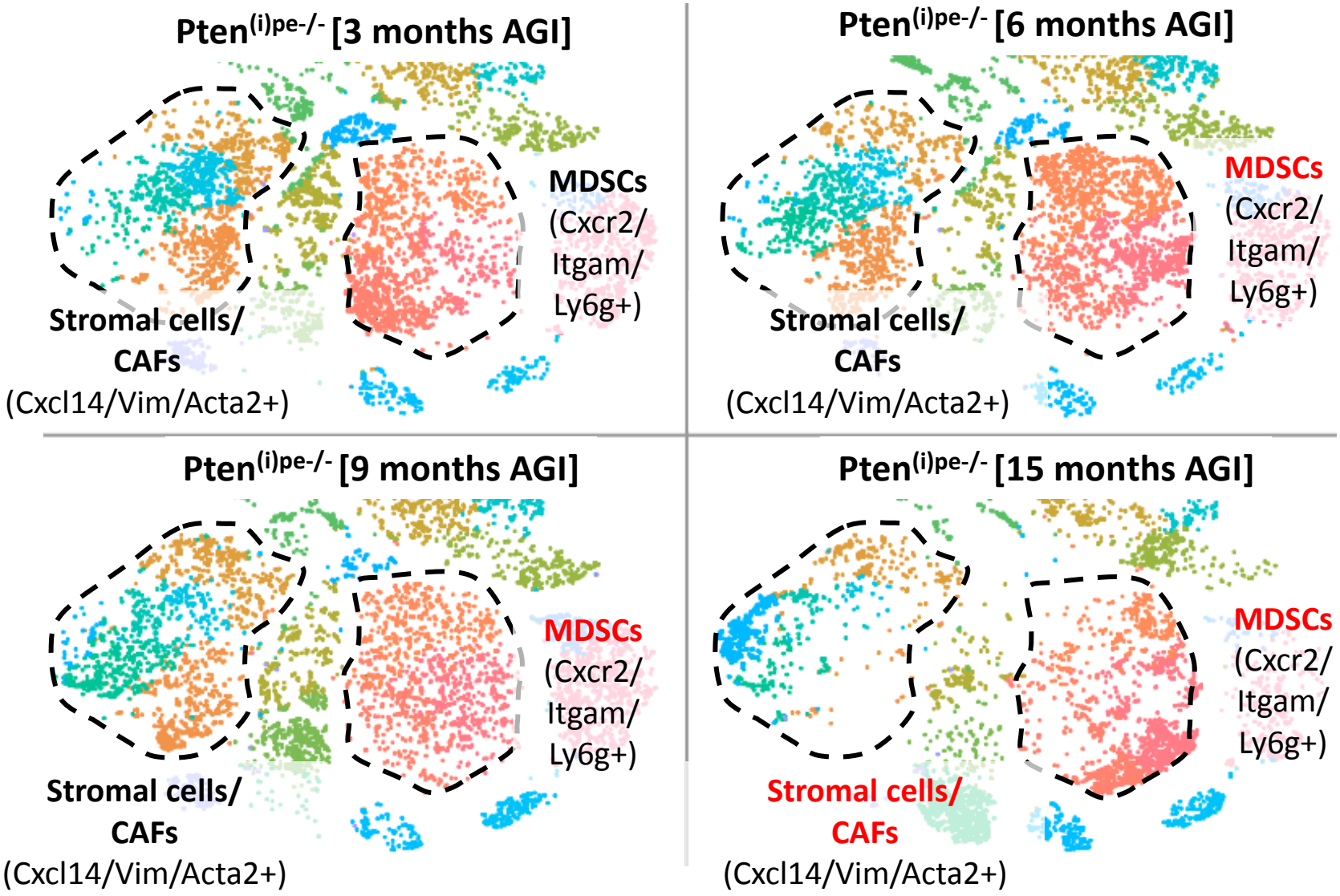
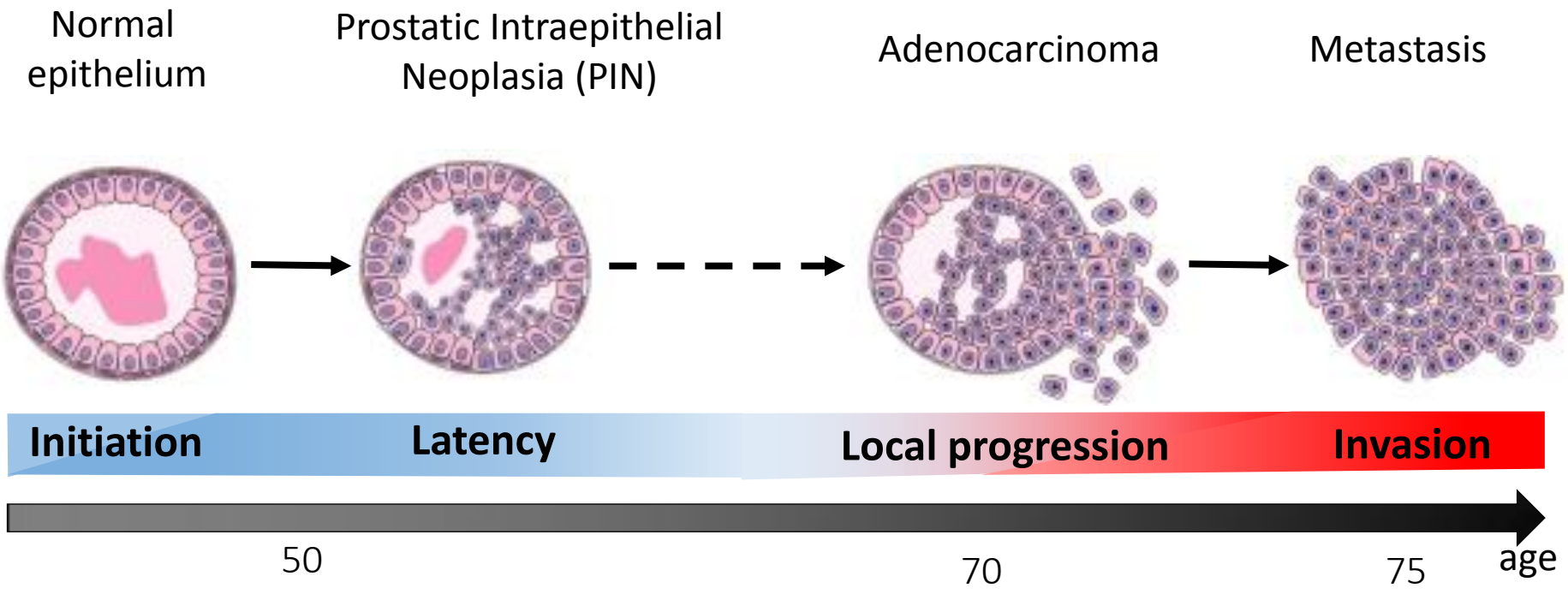



→ **Alterations in the microenvironment during disease progression**

# Prostate cancer and vitamin D




## Epidemiology

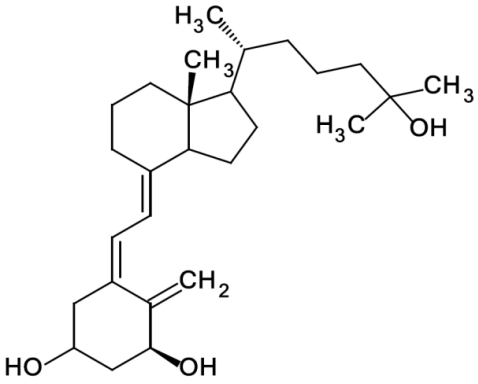
- **correlation between prostate cancer severity and**
  - low circulating levels of **Vitamin D**
  - **low vitamin D receptor** expression

## Preclinical

- **anti-proliferative and anti-inflammatory potency in**
  - prostatic cancer cell lines
  - mouse model of prostate cancer

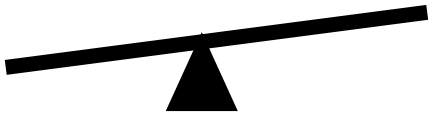
# Role of vitamin D in senescent PIN

$1,25(OH)_2D_3$




Anti-proliferative
Anti-inflammatory

Calcemia



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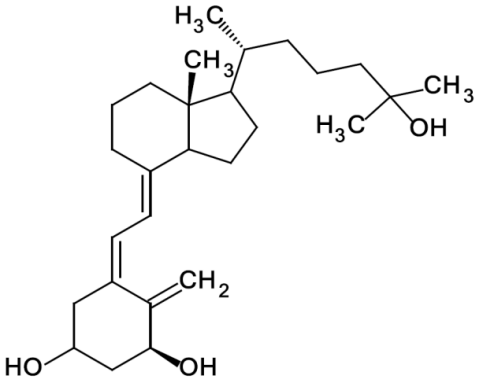
## $1,25(OH)_2D_3$

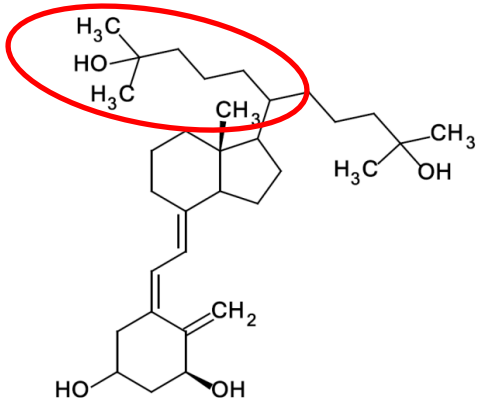



**Gemini-72, a vitamin D analog**

**Calcemia**

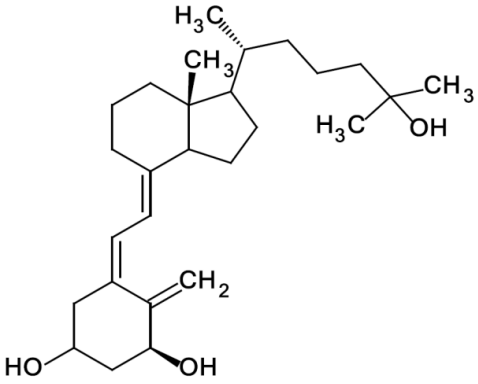
**Anti-proliferative**
**Anti-inflammatory**

## Gemini



# Role of vitamin D in senescent PIN

$1,25(OH)_2D_3$

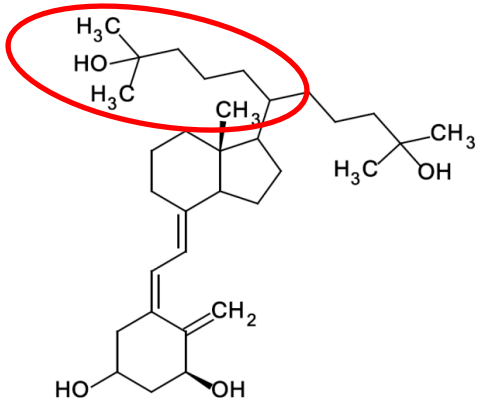



## Gemini-72, a vitamin D analog

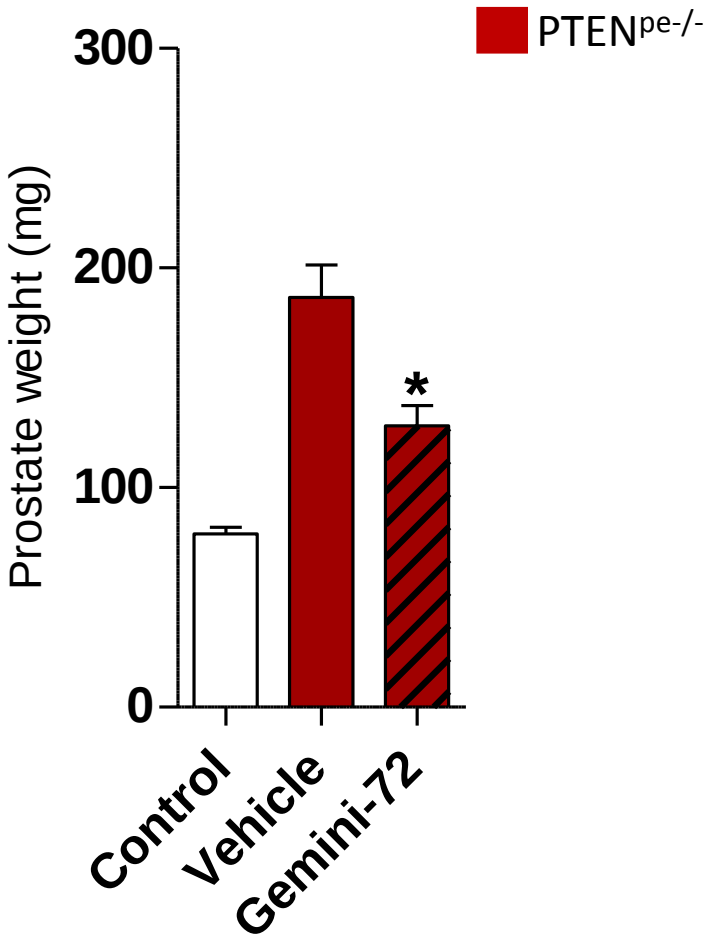
Calcemia

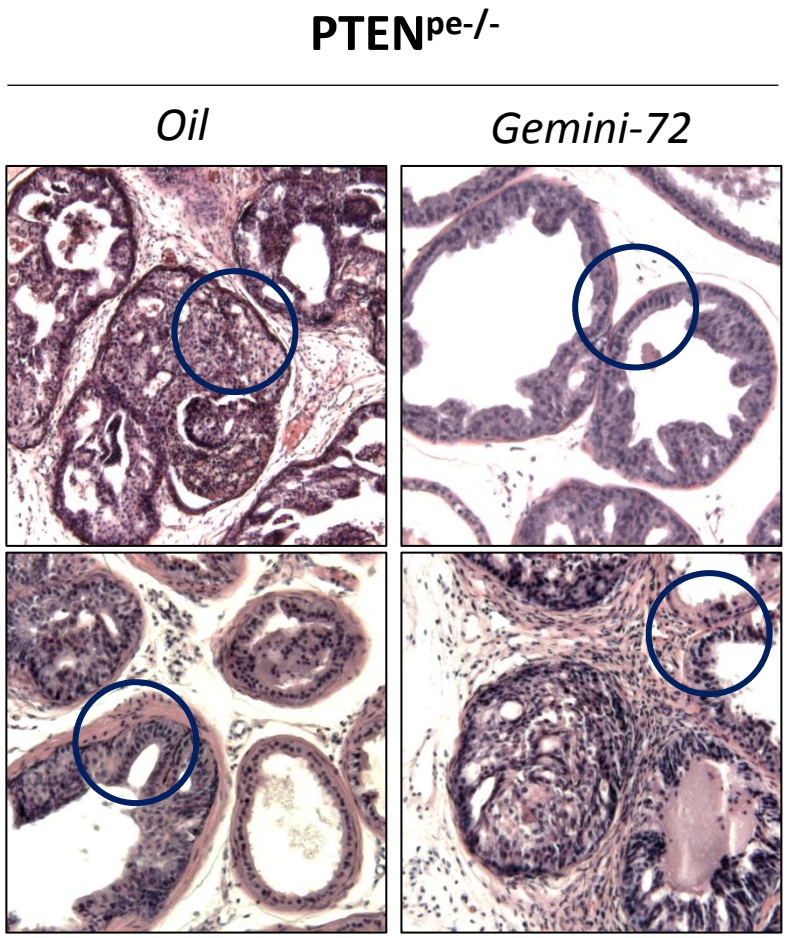
Anti-proliferative
Anti-inflammatory

Gemini



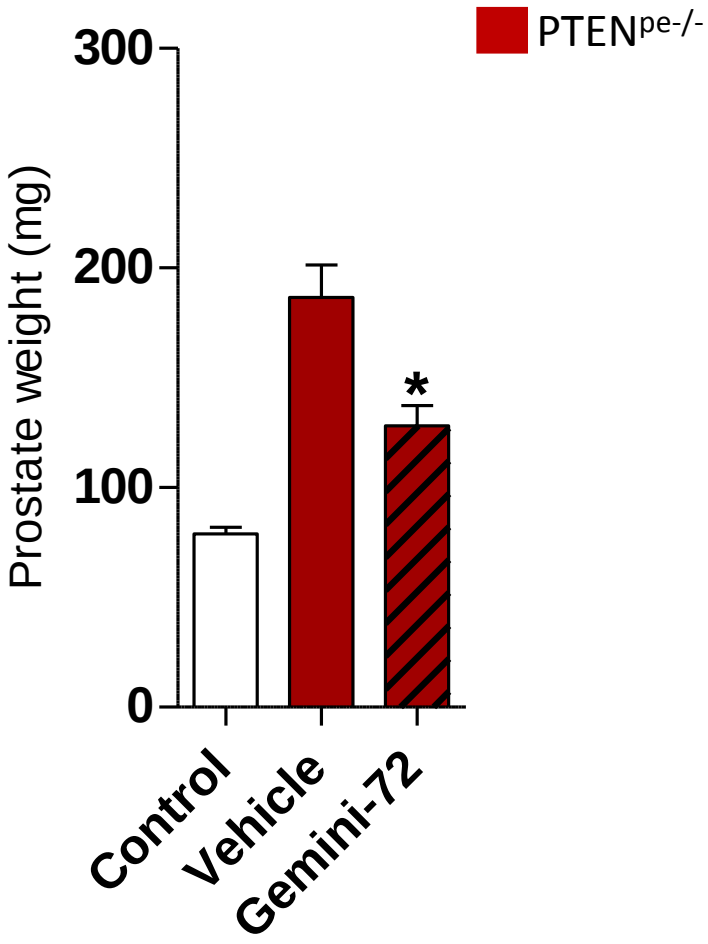

# Therapeutic potency of Gemini-72 for prostate cancer

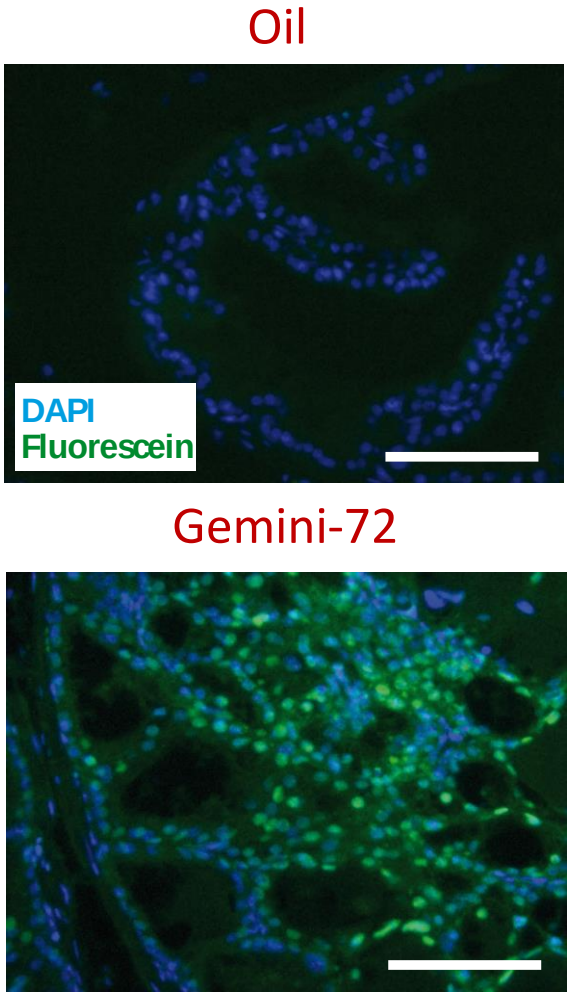



**Gemini-72 decreases the severity of pre-cancerous lesions**

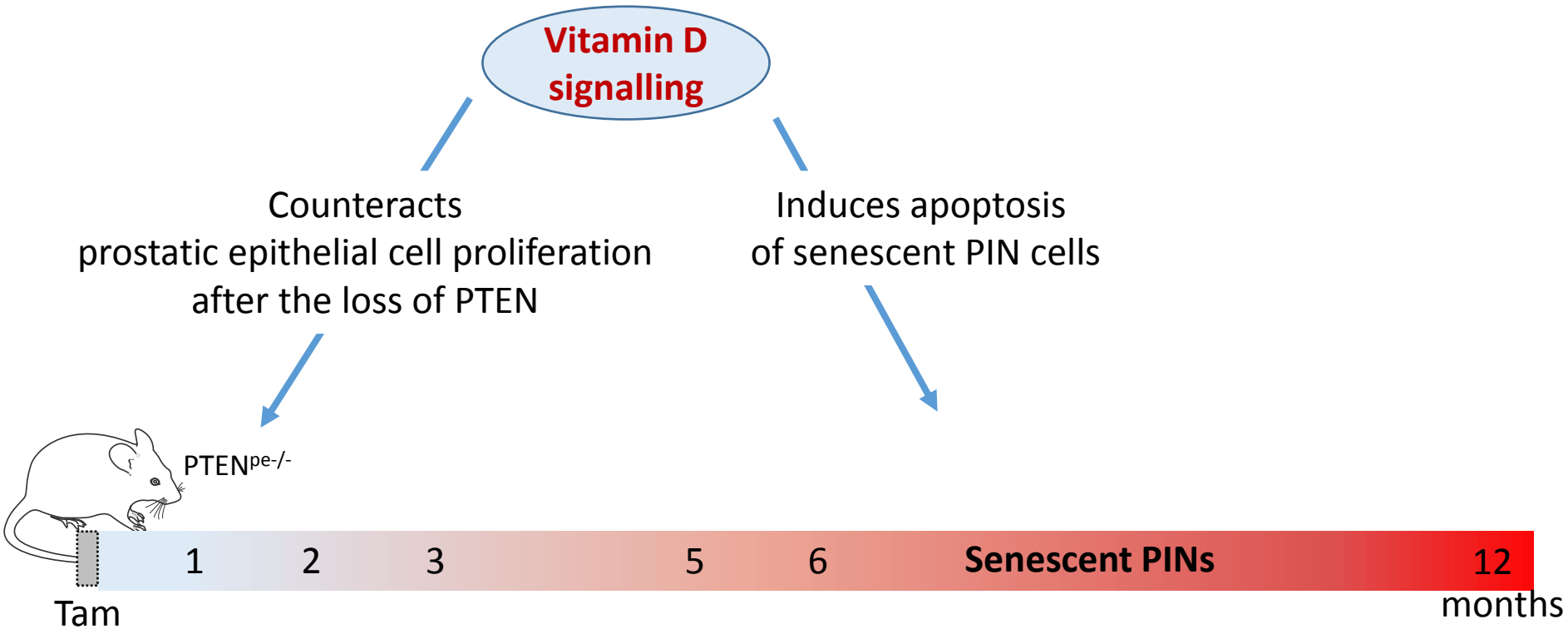
# Therapeutic potency of Gemini-72 for prostate cancer




**Gemini-72 decreases the severity of pre-cancerous lesions, by inducing apoptosis**

# Therapeutic potency of Gemini-72 for prostate cancer




**Vitamin D analogs represent**
**a promising preventive strategy for prostate cancer**

**→ Identification of additional potent VitD analogs**

# Prostate cancer

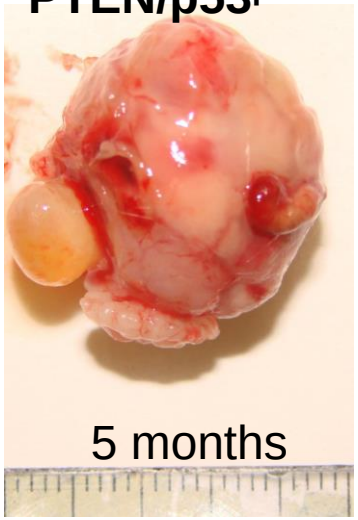
- p53 : mutated or deleted in advanced and metastatic prostate cancer

*(Chen et al., 2005)*

## Generation and characterisation of PTEN/p53$^{pe-/-}$ mice

- Increased cell plasticity
- Senescence bypass/escape
- Metastatic tumors
- Castration resistant

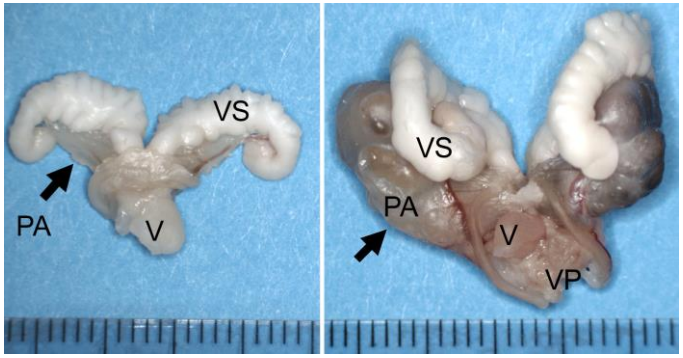
**PTEN/p53$^{pe-/-}$**



5 months

**prostate**

**WT** **PTEN$^{pe-/-}$**




1 cm

10 months

# Mouse models of cancer

- **Powerful tools**

  - to investigate tumor progression
  - to identify new markers
  - to develop new therapeutic strategies

**- Complementary to PDX, organoids …**

# Acknowledgements

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