Dutch Life Sciences
Leiden
7 December 2023

Womens and Mens Health Pipeline

Pantarhei Bioscience (PRB)

Pantarhei Oncology (PRO)

PANTARHEI ONCOLOGY

Conflict of Interest HCB

- Shareholder and President of Pantarhei Bioscience (PRB)
- Shareholder and President of Pantarhei Oncology (PRO)

Pipelines PRB and PRO

- Pantarhei Bioscience (PRB)
  - Androgen restored contraception (ARC)
  - Estetrol (E4) for hormonal contraception (HC) and hormone replacement therapy (HRT)
- Pantarhei Oncology (PRO)
  - High dose estetrol (HDE4) for the treatment of
    - Advanced breast cancer (BCa)
    - Advanced prostate cancer (PCa)
  - The ZP3Cancer (ZP3C) target for oncology

The ARC concept

(Androgen Restored Contraception)

Premenopausal women using hormonal contraception need testosterone replacement

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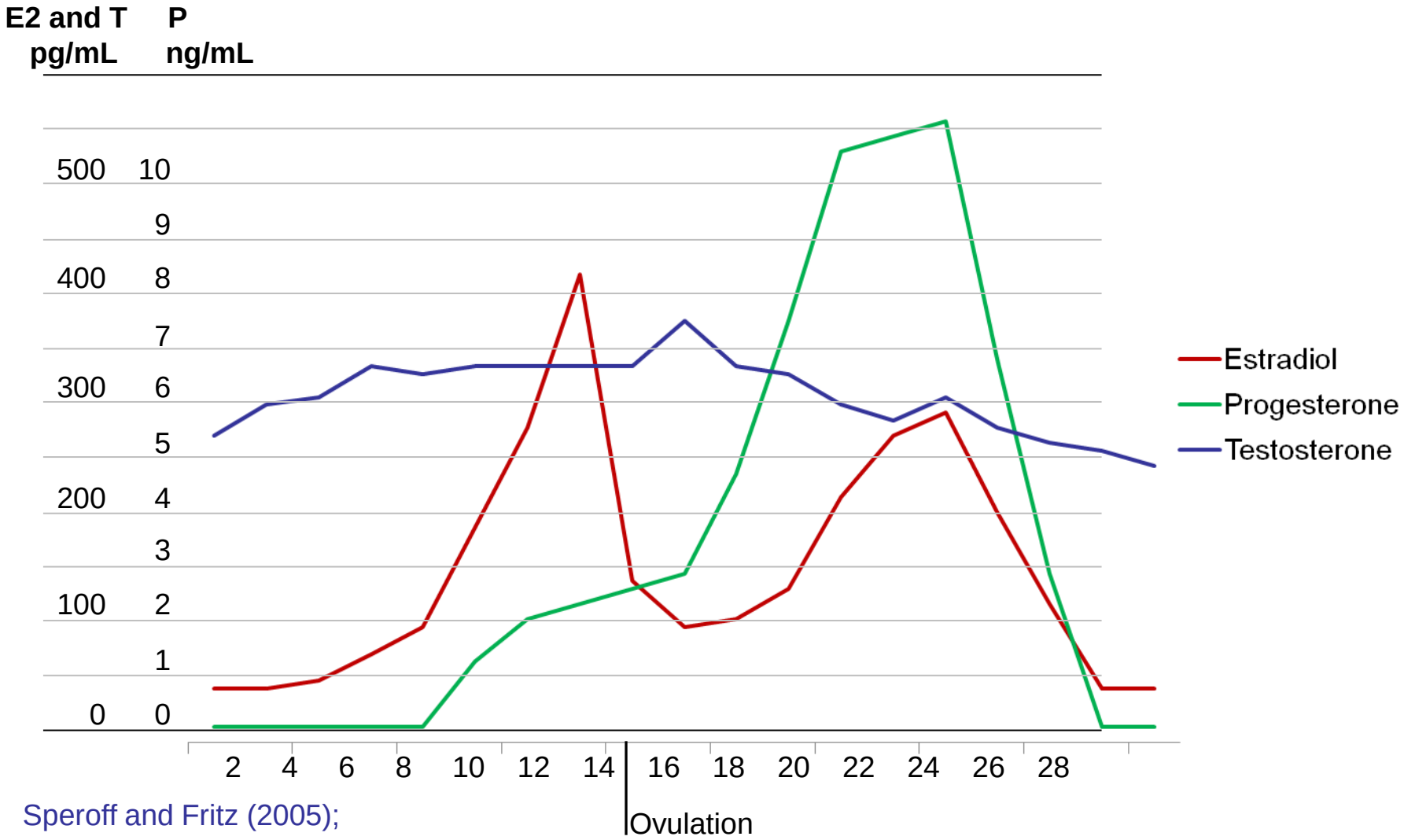
Five steps to understand the importance of T for premenopausal women and the concept of ARC

- 1) Premenopausal women have 5-10x more testosterone (T) than estradiol (E2)
- 2) With every combined oral contraceptive (COC) or progestin-only pill (POP) women loose most of their total and free bioactive T
- 3) Loss of T has many effects on Quality-of-Life (QoL) such as mood changes and depression and sexual function problems, esp. less desire and arousal
- 4) By adding 50 mg DHEA to a COC the T levels are normalised
  - (the liver metabolises DHEA to T and 50 mg DHEA restores the loss of T caused by the pill)
- 5) With DHEA, sexual function, mood and other quality of life aspects are improved without side effects

Step 1: women have up to 10x more testosterone than estradiol during the normal menstrual cycle



Speroff and Fritz (2005);
Bui et al., Steroids (2013)

Two phase II prospective, randomised, double-blind, placebo-controlled studies

ARC-AMC study van Lunsen and Laan, Amsterdam, NL	ARC-AMUSA study Foidart and Pintiaux, Liège, B
• **Participants:** 81 healthy COC users (20-35 yrs) and in steady relationship • **Treatment** (cross-over design): 1. One (1) month no hormonal contraception 2. Two COCs investigated; both 30 µg EE and either 150 µg levonorgestrel (LNG) or 3 mg drospirenone (DRSP) *combined with:* – Five (5) cycles placebo followed by 5 cycles 50 mg DHEA or the reverse	• **Participants**: 99 healthy and new COC users (18-35 yrs) • **Treatment** (parallel-group design): 1. Three (3) months no hormonal contraception 2. Three (3) cycles 30 µg EE/3 mg DRSP 3. Six (6) cycles EE/DRSP with 50 mg DHEA or placebo 4. Extension: Seven (7) cycles EE/DRSP with 50 mg or placebo (n=61)
Study objective	
To investigate the endocrine and clinical effects of the pill with and without co-administration of DHEA	

Step 2: Meta-analysis of pill effect on T and SHBG

(Zimmerman et al. Hum Reprod Update 2014;20:76-105)

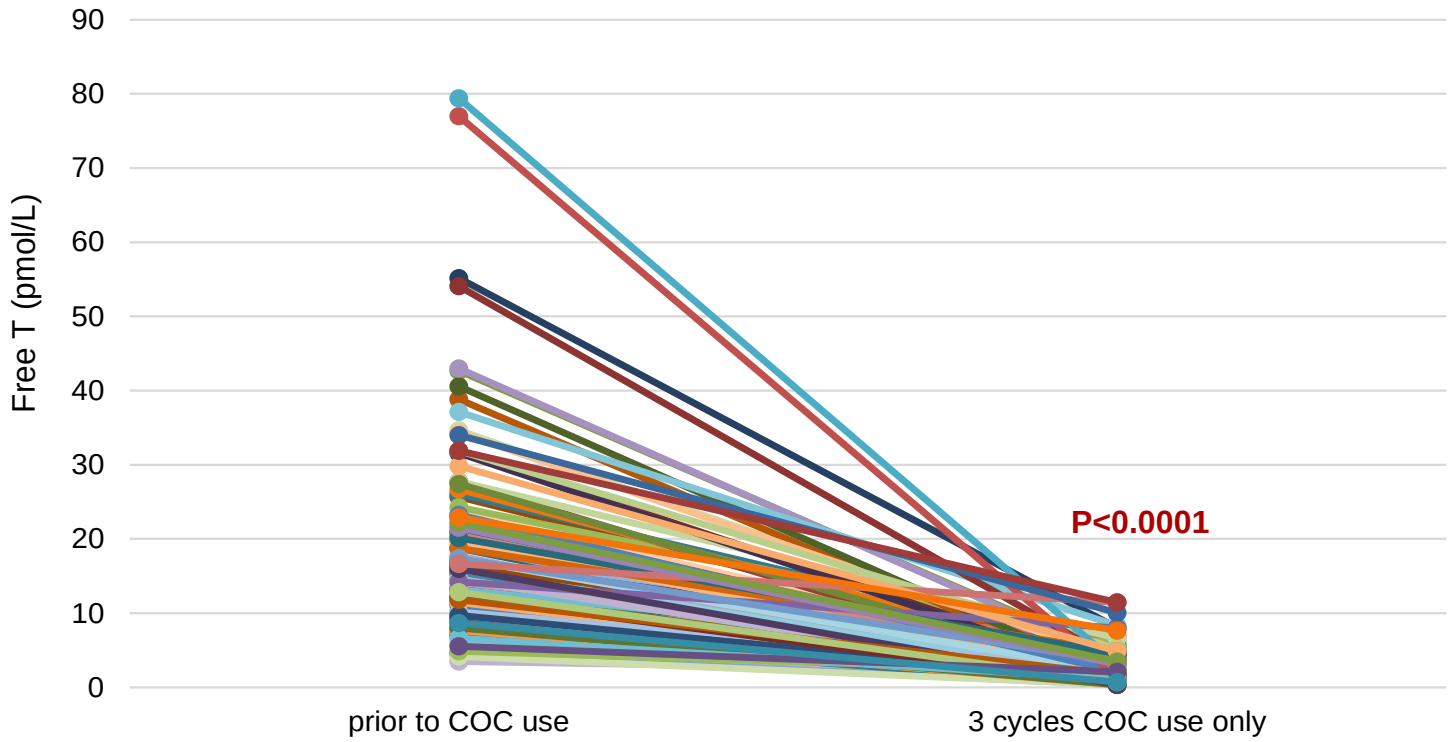
- Significant (P<0.0001) decrease T levels:
  - Total T: ↓ 31% (55% with LNG)*
  - Free T: ↓ 61% (68% for LNG and 76-86% for DRSP)*
  - No differences between LNG and DRSP
  - No differences between various COCs types
  - No relationship with SHBG increase (which ranged from 50-250%)
- Significant (P<0.0001) increase of SHBG levels
  - Dose-dependent estrogen (EE) effect
  - Related to the progestin (LNG 25%; DRSP 191%)*
- COCs also reduce levels of (precursor) androgens

* Pantarhei studies

ARC
a new generation of oral contraception that improves sexual health and mood
Women's Quality

Step 2: All COC users loose free T and the higher the baseline free T, the more complaints

1. Huge variation of free T levels during the normal menstrual cycle
2. COC reduces free T significantly and in all 99 women



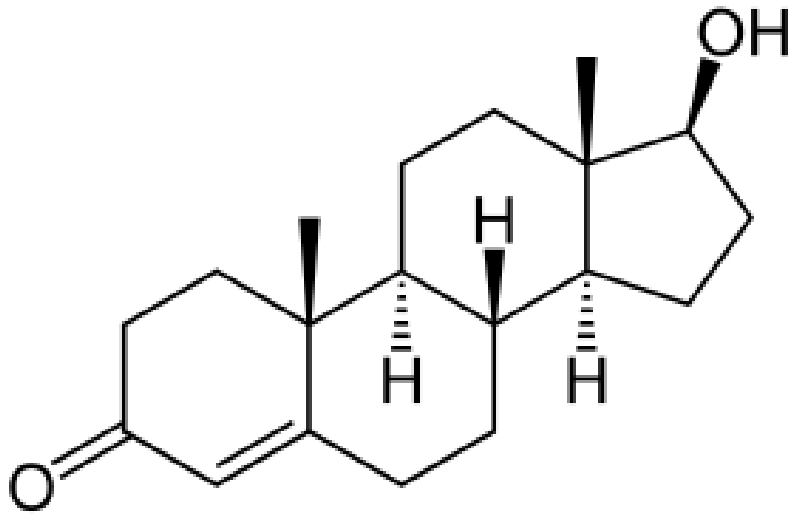
Step 3: subjective side effects due to loss of testosterone in women

- Fatigue
- Sleeping problems
- Loss of energy
- Apathy
- Mood changes and depression
- Cognition and memory problems
- Headache
- Sexual function problems (less desire, less arousal)

Step 3: the role of testosterone in sexual functioning in premenopausal women

Testosterone (T) enhances:

- Sexual arousability and responsivity
- Frequency of sexual thoughts and fantasies
- Genital sensitivity
- Correlates with sexual desire



Testosterone prepares the brain and the genitals for sex

1 Slob et al. (1995); Miller et al. (2007); Hermans et al. 2010); Warnock et al. (2006); Wåhlin-Jacobsen et al. (2014)

Step 3: Significant decline in sexual function
AMC study Contraception 98 (2018) 56–62

Sexual Function Diary (SFD):

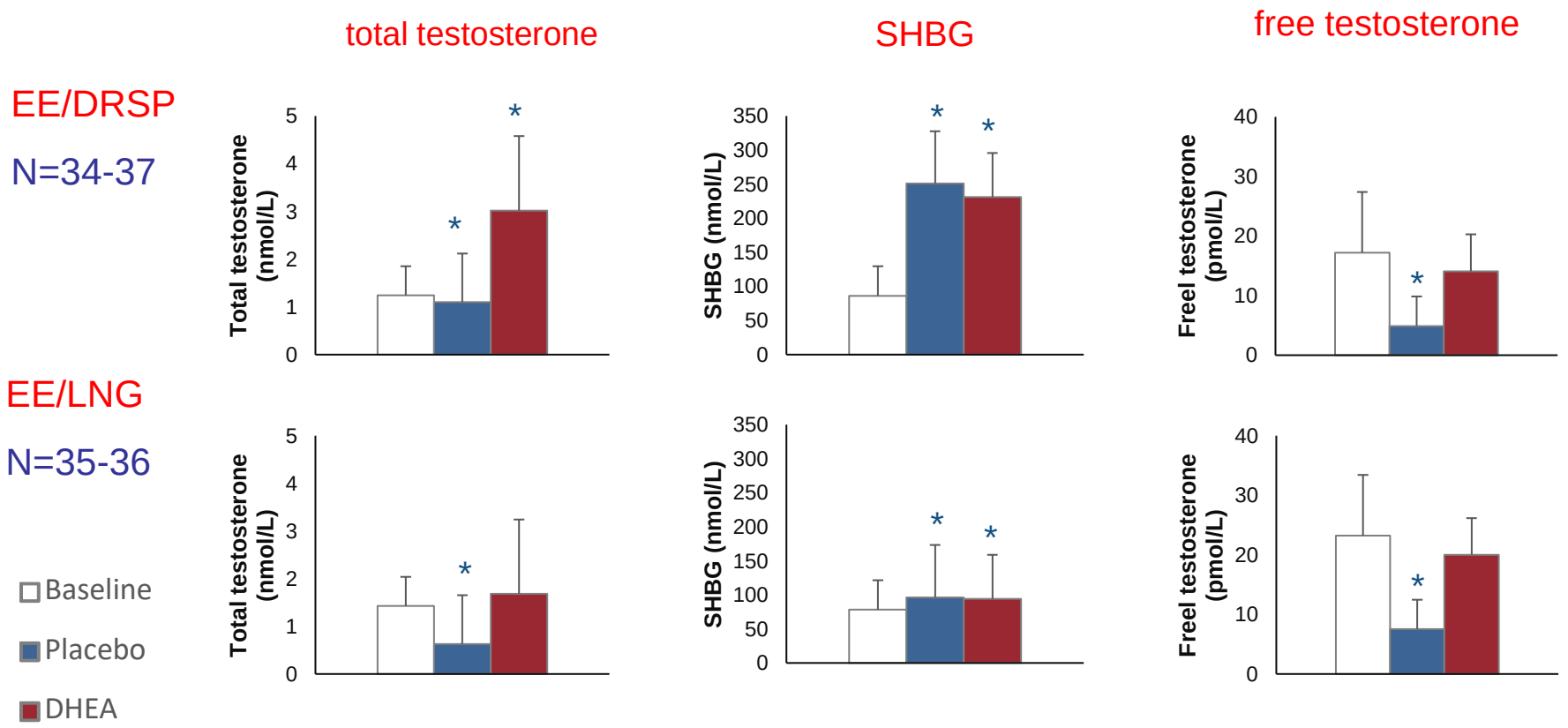
- Significant decrease of sexual desire and arousability (*P*<0.05) after 5 cycles COC; no difference between EE/LNG or EE/DRSP

Questionnaires:

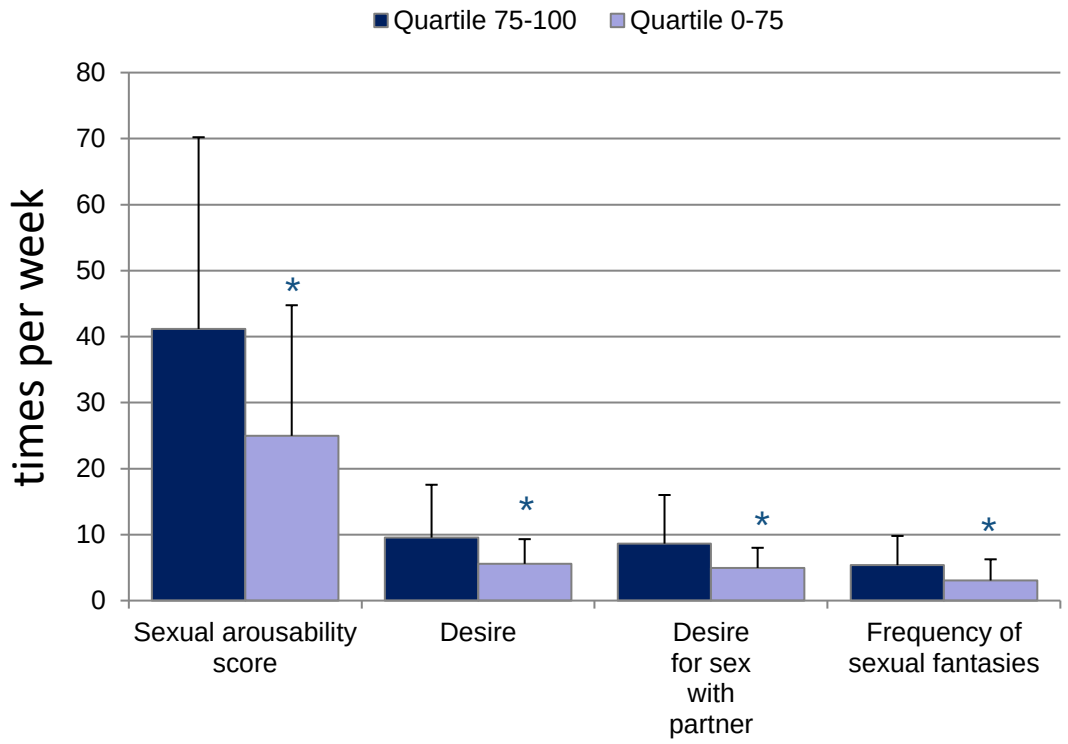
- Female sexual function index (FSFI): Total score and three FSFI domains (Arousal, Pain, Lubrication) indicated worse sexual function with EE/LNG use compared to baseline (*P*<0.05)
- MFSQ: Significant worsening for global score, sexual interest and orgasm (*P*<0.001) during use of EE/DRSP

Step 4: By adding 50 mg DHEA to a COC T and free T are normalised

With 50 mg DHEA, free T levels are restored to baseline with both pills
* significant difference versus baseline (P<0.05) (Contraception 2018)



Step 5: By adding DHEA to a COC sexual desire and arousability are improved



Sexual arousability, desire and fantasies significantly higher in women with highest levels of free testosterone with DHEA compared to placebo
* *P*<0.02 (Contraception 98 (2018) 56-62)

In this group (Q4):
14 women were using EE/LNG/DHEA and 4 women EE/DRSP/DHEA

Published Papers PCombi

- Estetrol Cotreatment of Androgen Deprivation Therapy in Infiltrating or Metastatic, Castration-sensitive Prostate Cancer: A Randomized, Double-blind, Phase II Trial (PCombi) (Herjan J.T. Coelingh Bennink, Jeroen A. van Moorselaar, E. David Crawford, Erik P.M. Roos, Diederik M. Somford, Ton A. Roeleveld, Tjard D. de Haan, Harm H.E. van Melick, Yacov Reisman, Yvette Zimmerman, Gonnie van Osta, Jan Krijgh, Neal D. Shore, Fred Saad, Andrew V. Schally, Frans M.J. Debruyne. EUR Urol Open Sci 2021;28:52-61)
- Estetrol Prevents Hot Flushes and Improves Quality of Life in Patients with Advanced Prostate Cancer Treated with Androgen Deprivation Therapy: The PCombi Study (Yvette Zimmerman et al, Eur Urol Open Sci 2022:45:59-67)
- Maintaining bone health by estrogen therapy in patients with advanced prostate cancer – a narrative review (Herjan J.T. Coelingh Bennink, Jan Krijgh, Jan F.M. Egberts, Maria Slootweg, Harm H.E. van Melick, Erik P.M. Roos, Diederik M. Somford, Yvette Zimmerman, Iman J. Schultz, Noel W. Clarke, R. Jeroen A. van Moorselaar, Frans M.J. Debruyne, Endocrine Connections 2023)

Summary design phase III study

- Randomised, cross-over, double-blind DHEA or placebo containing COC
- Duration of treatment: two periods of 6 COC cycles with either the COC/DHEA pill or the COC/placebo pill, both in a 24/4 regimen, followed by 7 COC/DHEA cycles to obtain one year safety data for the additional 50 mg DHEA
- Number of women participating: 300
- Age range: 18-40 years
- Key inclusion criterium: no earlier COC use
- Primary endpoint: sexual function diary (SFD)
- Secondary endpoints: other androgen related side effects of T loss
- Budget required: Euro 10-15 mln

Press Release Estetrol (E4) June 14, 2002

Zeist, Netherlands, June 14th 2002

Surprising discovery by Pantarhei Bioscience:

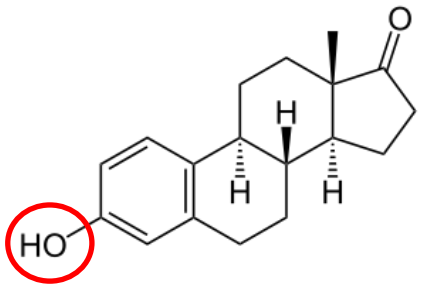
Neglected natural estrogen rediscovered; new alternative for contraception and climacteric complaints.

Estetrol is a so far totally neglected natural human estrogen, synthesized exclusively and in large quantities by the fetal liver during pregnancy. Pantarhei Bioscience (PRB) proved recently that this estrogen is unexpectedly potent. The estrogenic efficacy is demonstrated in a series of clinically relevant models. Since the safety of estetrol has been proven by Mother Nature during pregnancy, this rediscovered natural human estrogen is expected to have less side effects in estrogenic therapy for menopausal complaints and contraception. Today, June 14th, in the breaking news session of the 10th World Congress on the Menopause in Berlin, Prof. Herjan J.T. Coelingh Bennink, founder and managing director of PRB, will present these fascinating findings.

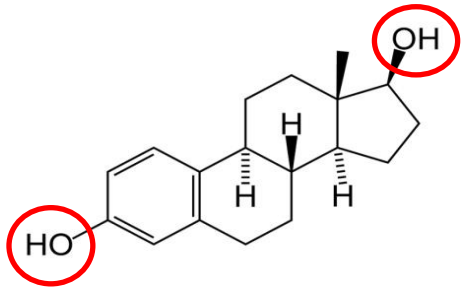
The four natural human estrogens

E1 (after menopause), E2 (during cycle) and E3 and E4 (during pregnancy)

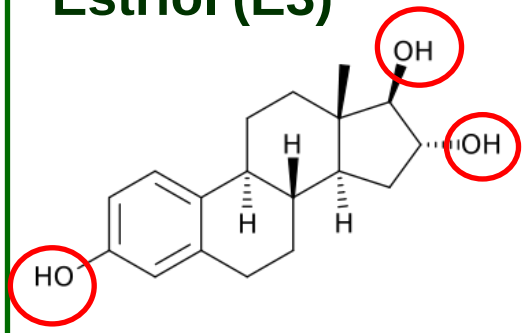
Estrone (E1)



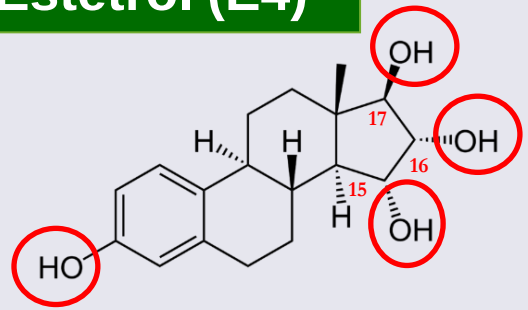
Estradiol (E2)



Estriol (E3)



Estetrol (E4)



ESTETROL – E4

Natural - A natural human fetal estrogen

Potentially safe - Produced by the fetal liver with high fetal exposure, therefore expected and confirmed to be safe

Oral bioavailability – Oral bio-availability 80% and oral elimination half life 24-32 hours allowing once daily oral dosing

Physiology of estetrol

- Estetrol is
  - exclusively produced by the human fetal liver during pregnancy,
  - is present at 9 weeks of gestation in maternal blood and
  - fetal plasma levels are 10-20x higher than maternal levels
- Fetal exposure at term is comparable to oral treatment with 50-60 mg E4 per day (kinetic simulation).
- Physiological function: nobody knows

4.500 – 14.000 pg/mL

400 – 1.200 pg/mL

Essential properties and Mechanism of Action (MoA) of Estetrol compared to other natural and synthetic estrogens

1. High oral bioavailability (80% for E4 vs 5% for E2),

   so very suitable as an oral drug

2. Little interference with the liver

   - Hardly any effect on liver function and coagulation
   - Slow liver metabolism of Estetrol
   - No active metabolites
   - Less DVT?

3. This explains why E4 is expected to be safer than other natural and synthetic estrogens and allows the use of high dosages of E4 for Women's Health (COC and HRT) and oncological applications

Estetrol for Reproductive Endocrine Oncology (REO)

High Dose E4 (HDE4) treatment in patients with advanced PC and advanced BC has shown (i) anti-tumor effects and (ii) strong estrogen treatment effects (Dual Efficacy)

Prostate cancer (PC)	Estetrol as combination treatment with Androgen Deprivation Therapy (ADT) with GnRH analogues for patients with advanced prostate cancer
Breast cancer (BC)	Estetrol for advanced breast cancer patients after failure of anti-estrogen therapy and/or in late (more than 5 yrs) postmenopausal patients

Treatment of Advanced Breast Cancer with High Dose Estetrol (HDE4)

Key message

Resistance to anti-E treatment or more than 5 yrs after menopause

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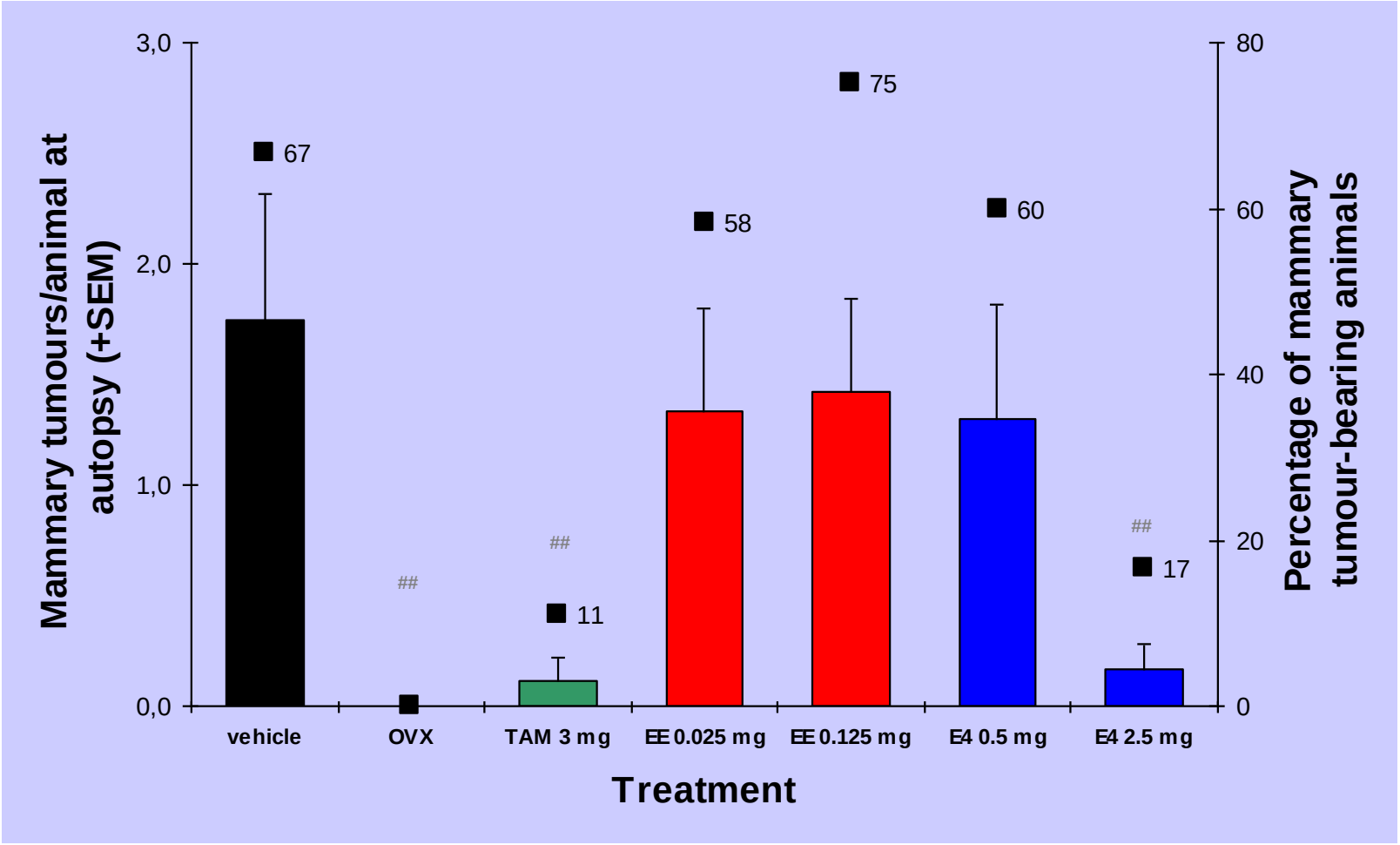
Breast tumors in DMBA exposed rats

co-treated 8 weeks orally with E4 (prevention study)

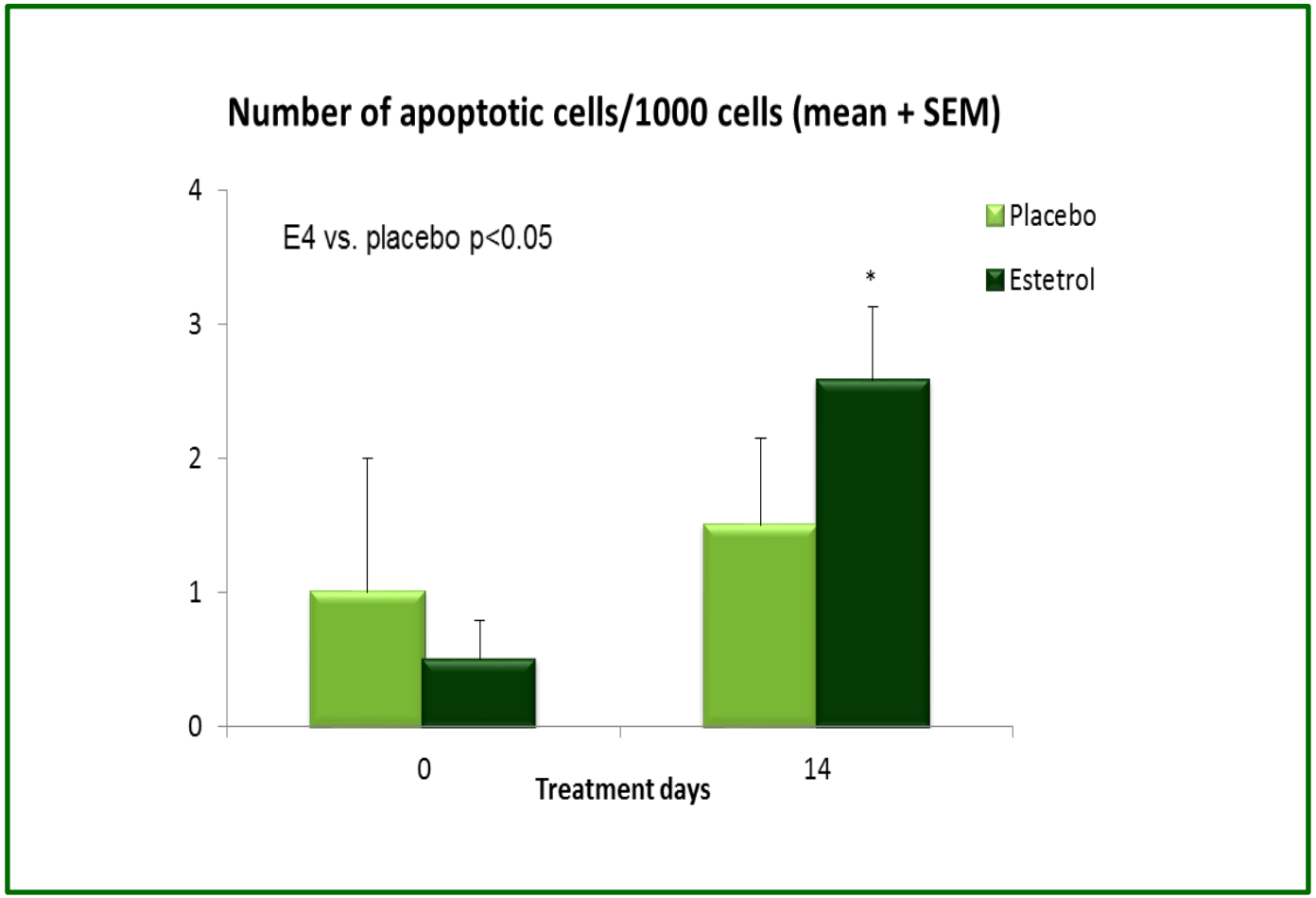
(Visser *et al* Horm Mol Biol Clin Investigation, 2012;9(1):95-103)

Significantly different from vehicle treatment p<0.05 (#), p<0.01 (##)



Increase of apoptosis in human ER+ BC tumor

Singer et al, Carcinogenesis, 2014



Proof-of-Concept HDE4 study in patients with advanced breast cancer

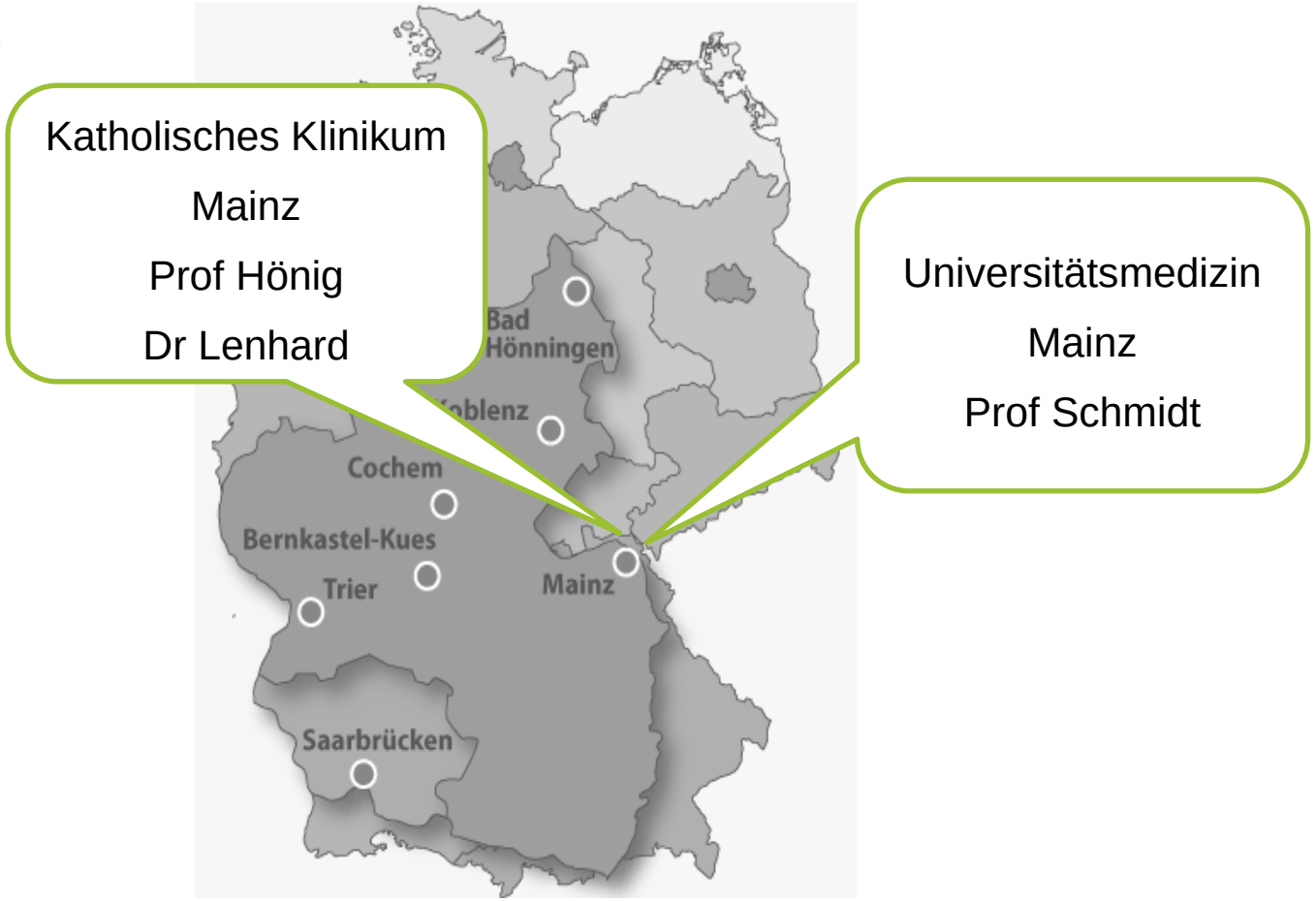
Anti Breast Cancer E4 (ABCE4) Study

Design:

Escalating E4 dose study with doses of 20, 40 and 60 mg E4 per day using the standard 3+3 design for oncology

Patients:

- End-stage advanced breast cancer
- Postmenopausal (at least 5 yrs)
- Anti-estrogen resistance



Anti-tumor response after 12 weeks of E4 treatment

RECIST criteria

Evaluation	20 mg E4 (n=3)			40 mg E4 (n=3)			60 mg E4 (n=3)		
Patients	102	103	106	107	108	109	110	111	112
Evaluation of Target Lesions	PD	SD	CR	SD	SD	SD	PD	PD	PD
Evaluation of Non-Target Lesions	PD	Non-CR/ Non-PD	Non-CR/ Non-PD	Non-CR/ Non-PD	Non-CR/ Non-PD	Non-CR/ Non-PD	PD		PD
New Lesions	Yes	No	No	No	No	No	Yes	Yes	No
Response Type	PD	SD	CR	SD	SD	SD	PD	PD	PD
Response	**No**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**No**	**No**	**No**

CR = complete response; SD = stable disease; PD = progressive disease

Co-treatment of Advanced Prostate Cancer with Androgen Deprivation Therapy (ADT) and High Dose Estetrol (HDE4)

Key message

no testosterone (T) means no estradiol (E2)

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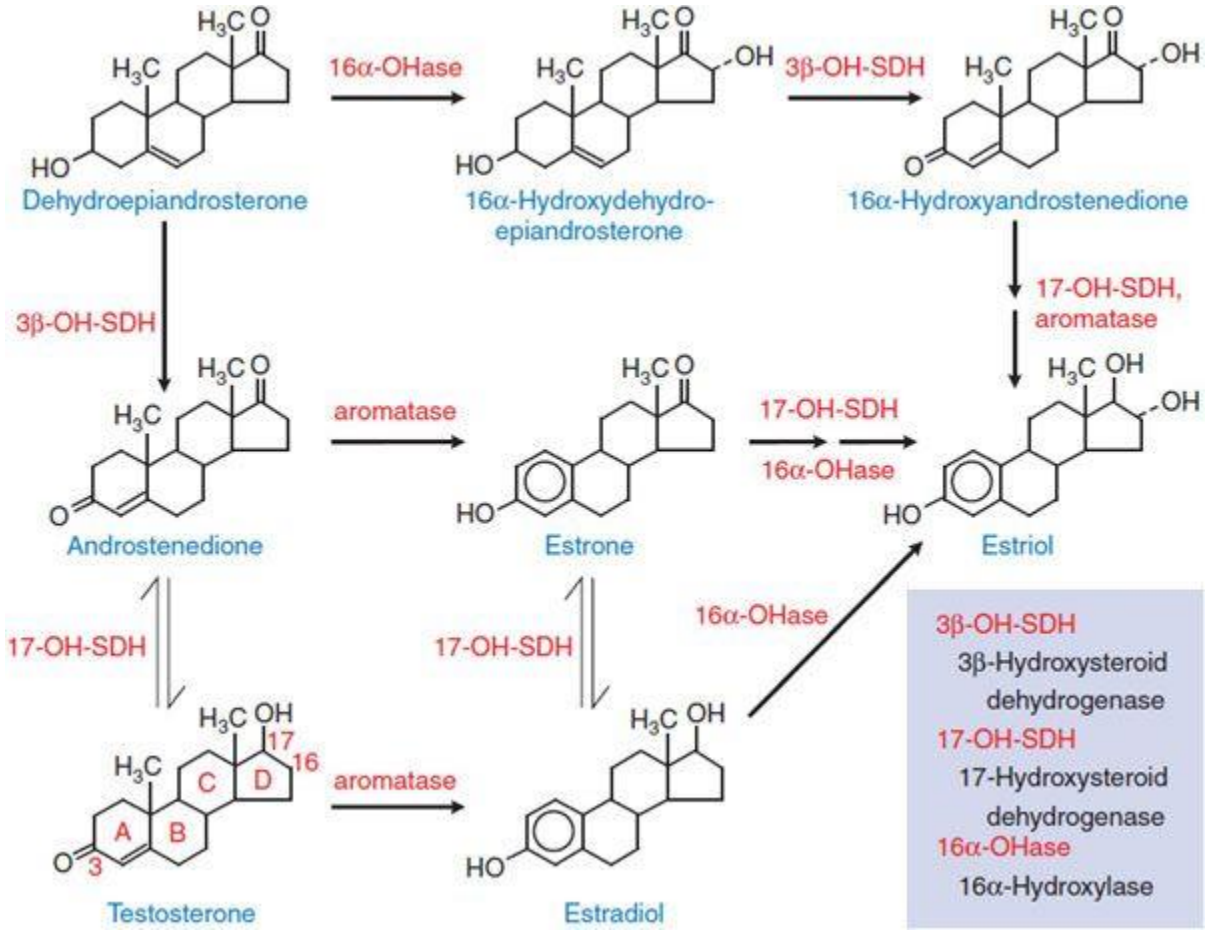
Testosterone is the precursor of Estradiol

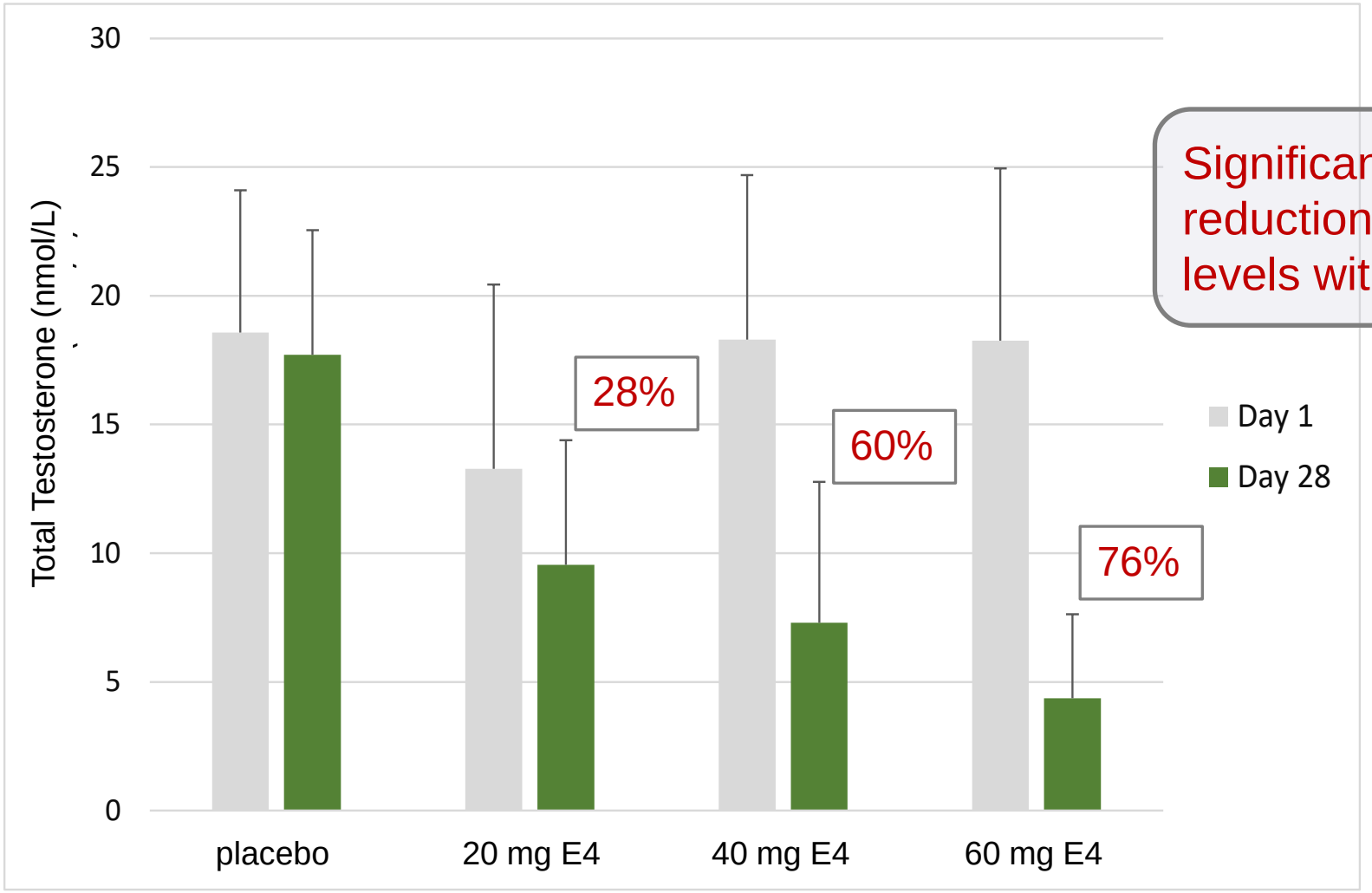


Side effects of ADT and whether it is due to the loss of Testosterone (T) or Estradiol (E2)

(Ref. HCB and YR, Endocrin Connect 2022)

'Big four'	What you see	What is not visible	What the patient feels
Libido loss **(T)**	Weight gain **(E)**	Loss of bone, decreased bone mineral density and increased fracture risk **(E)**	Fatigue (**T & E)**
Erection problems **(T)**	Gynecomastia **(T & E)**	Metabolic syndrome **(E)**	Sleeping problems **(T & E)**
Hot flushes & sweating **(E)**	Muscle atrophy (sarcopenia) **(T & E)**	Anemia **(T)**	Loss of energy **(T & E)**
Arthralgia (joint pain) **(E)**	Decreased size penis and testicles **(T)**	Increased arterial cardiovascular risk **(E)**	Apathy **(T & E)**
	Change hair pattern **(T)**		Mood changes and depression **(E)**
			Cognition and memory problems **(E)**

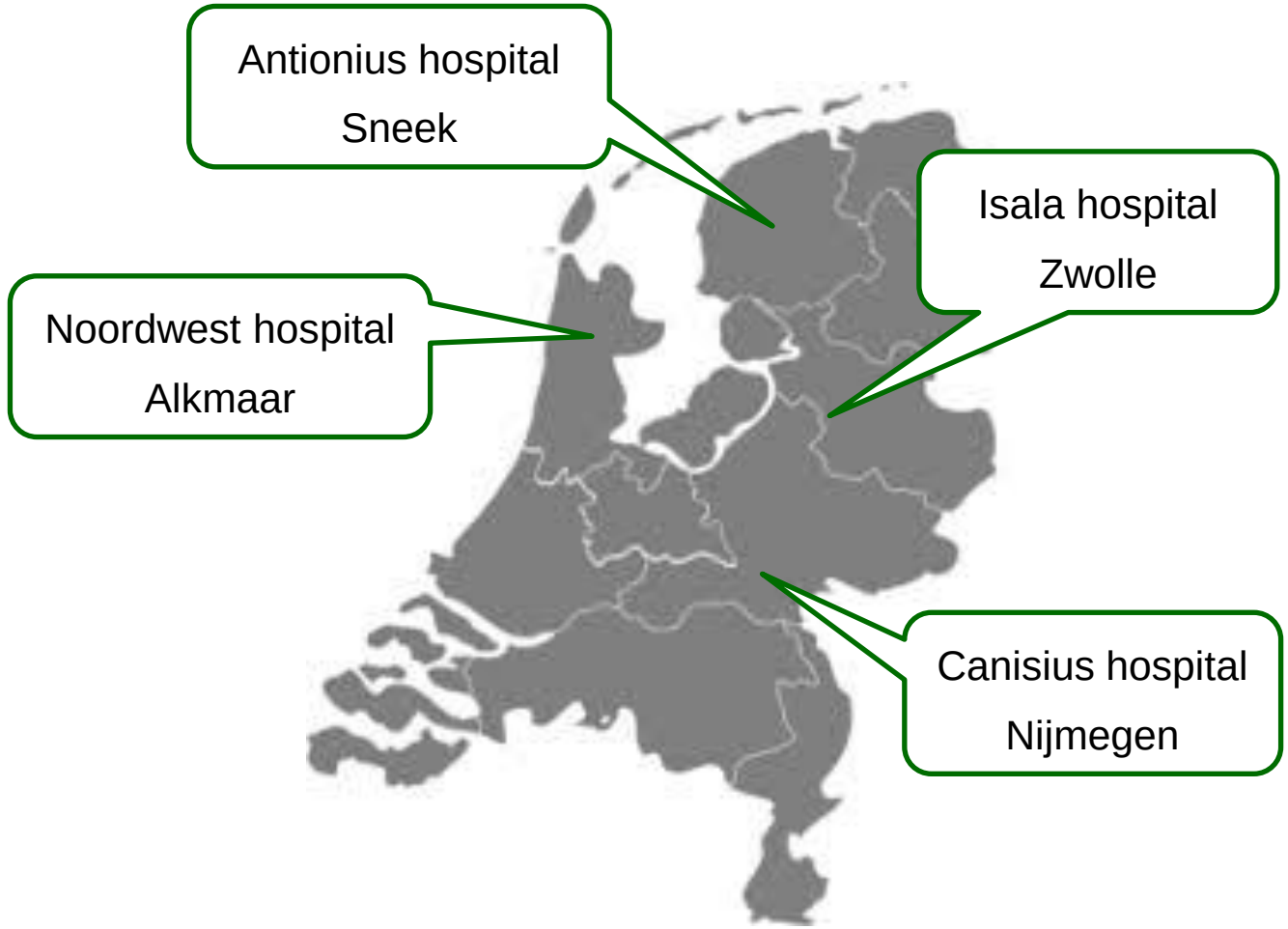
Effects of estetrol on testosterone;
the target hormone for effective prostate cancer treatment



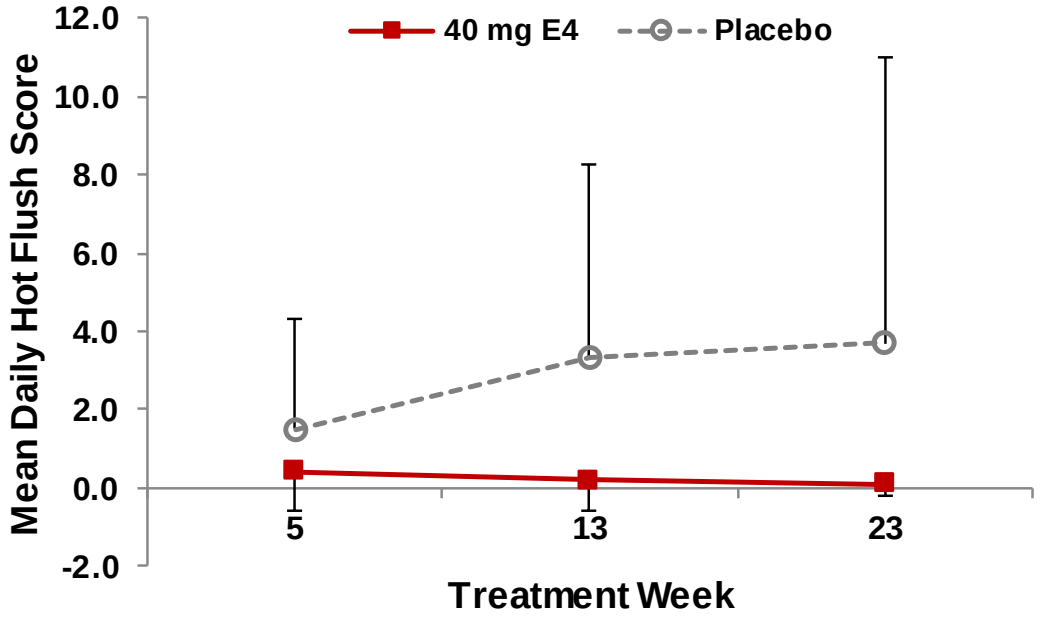
Significant dose-dependent reduction of testosterone levels with HDE4

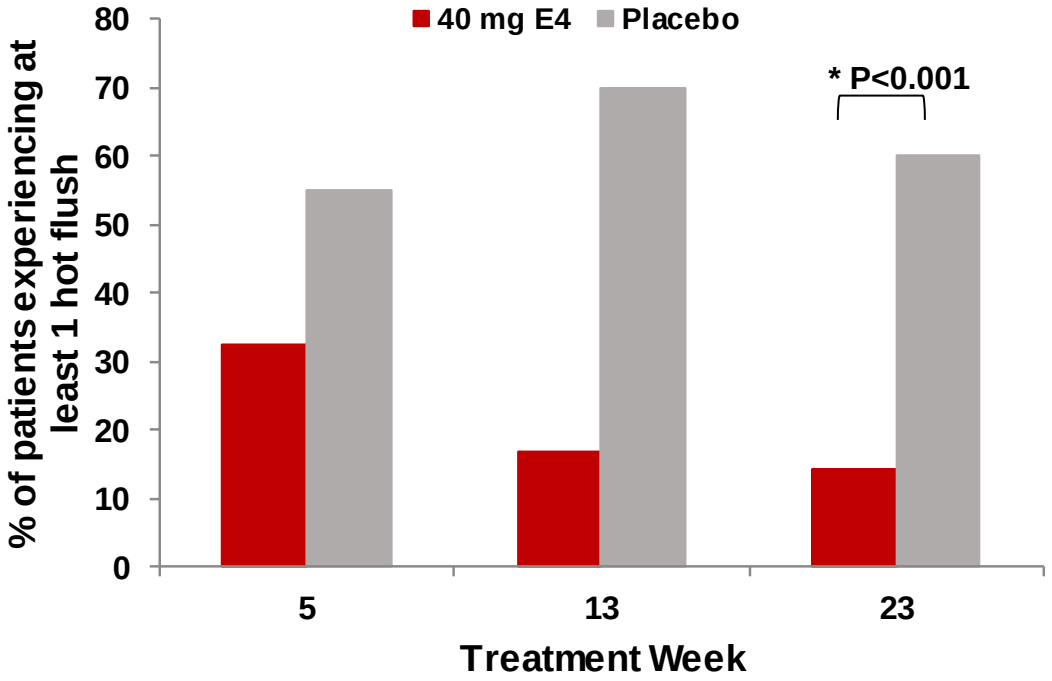
HDE4 plus ADT Proof-of-Concept study in patients with advanced prostate cancer

- Phase 2 PCa HDE4:
  - **Patients** with locally advanced or metastatic prostate cancer, qualifying for treatment with an LHRH agonist
  - **Design:** double-blind, randomised, placebo-controlled, multi-center study in four Dutch hospitals
  - **Treatment and no of patients** treated:
    1. LHRH agonist plus 40 mg E4 (n=41)
    2. LHRH agonist plus placebo (n=21)
  - **Treatment duration:** 6 months



C0-treatment with HDE4 “solves” the problem of hot flushes and other symptoms of estrogen deficiency

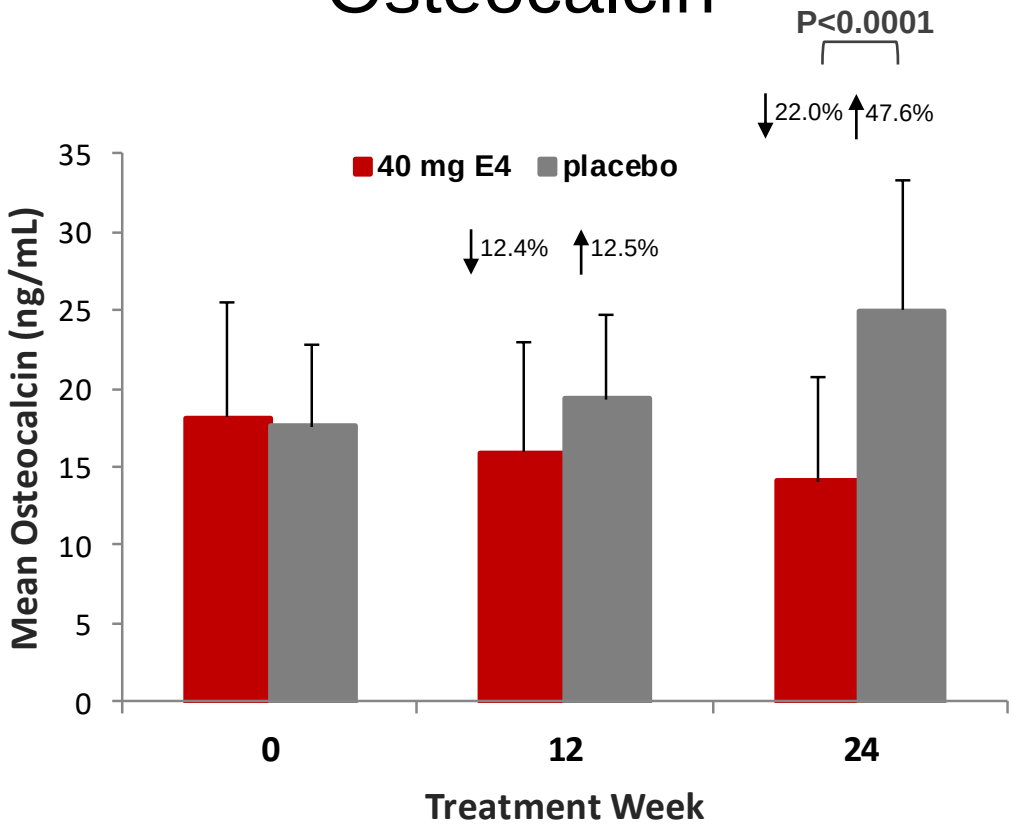




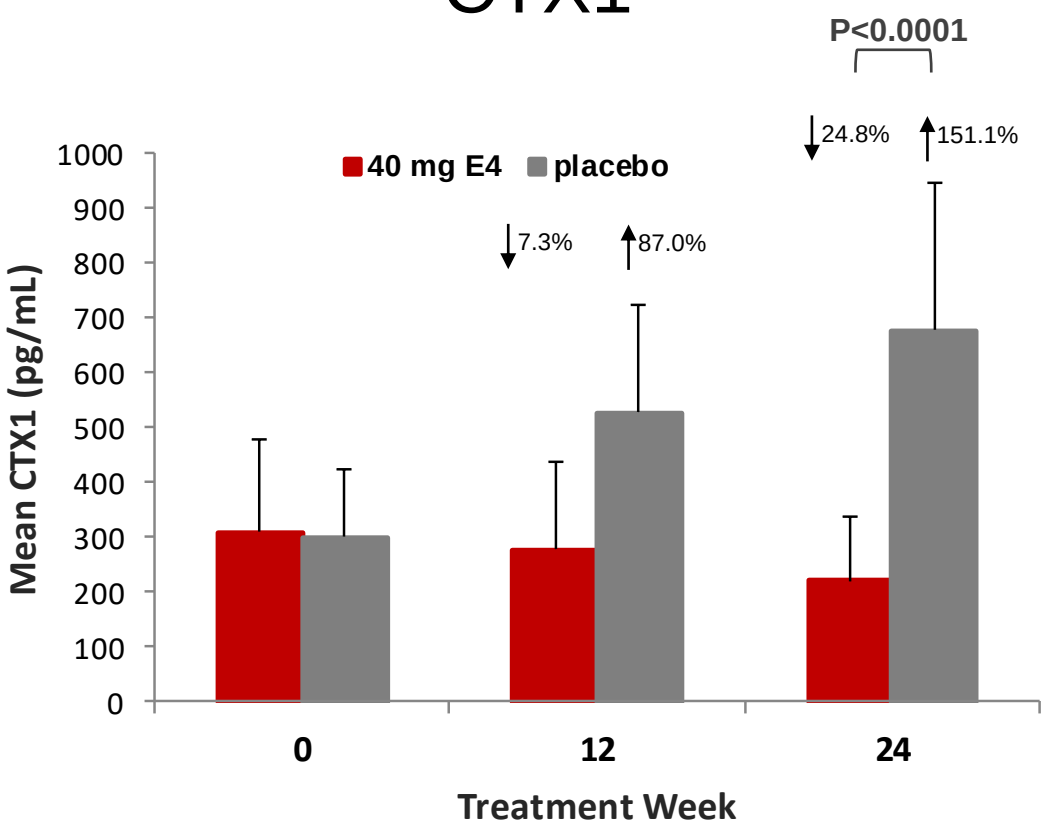
Patients without hot flushes after 23-24 weeks treatment: 87% (E4) vs 15% (placebo)

Strong effect of HDE4 on bone turnover parameters predicts prevention of bone loss and the potential to reduce the risk of fractures

Osteocalcin



CTX1

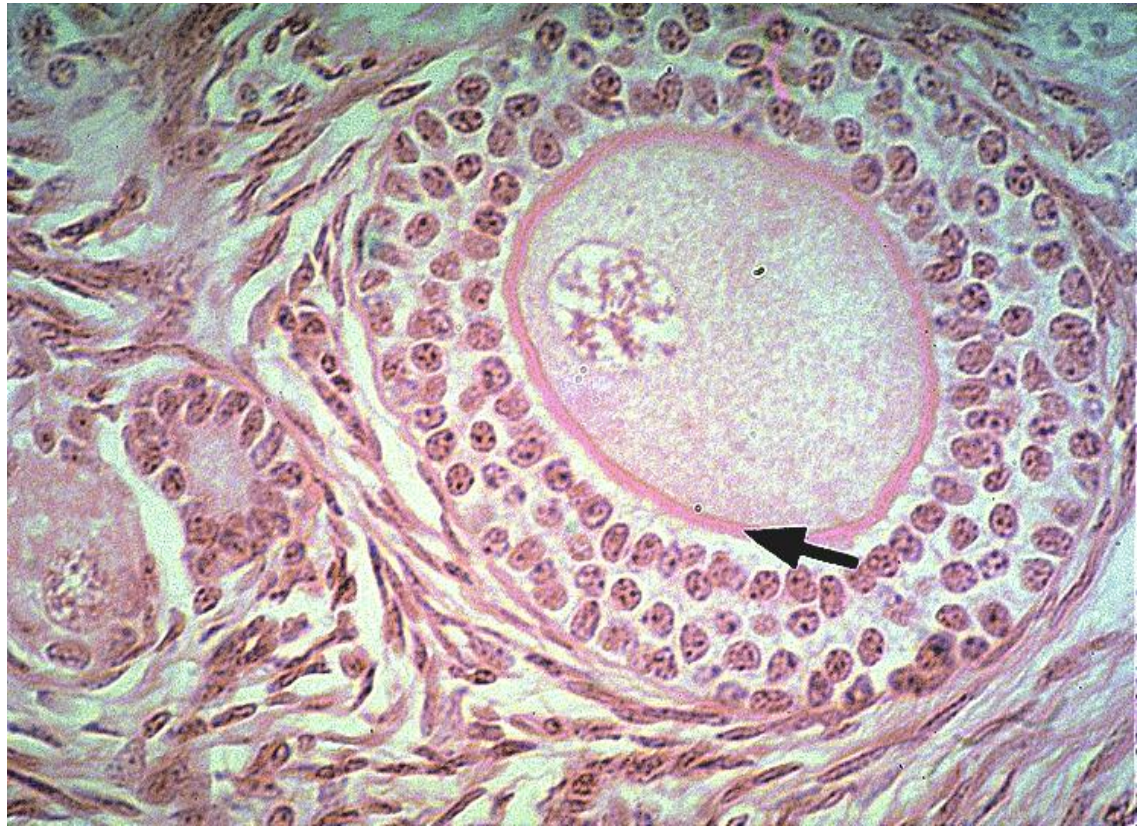


Summary
HDE4 plus ADT for advanced PCa

- Anti-tumor effects in advanced PCa on secondary endpoints
  - Earlier and better suppression of total and free T, PSA, FSH and IGF-1
- Strong estrogen replacement effects with a.o.
  - Highly significant reduction of HFs and arthralgia and improved QoL
  - Complete restoration of biochemical bone turnover (no need for bone drugs anymore)
- Potentially effective as co-treatment with all existing PCa medication (GnRH analogues, ARSIs. anti-androgens, chemotherapy, targeted treatments and immunotherapy)
- No serious AEs in Women's Health and Oncology study programs
  - Ready for phase II BCa development: budget required Euro 5-10 mln
  - Ready for phase III PCa development: budget required Euro 20-30 mln

The Zona Pellucida Glycoprotein 3 (ZP3) as Target for Cancer Immunotherapy

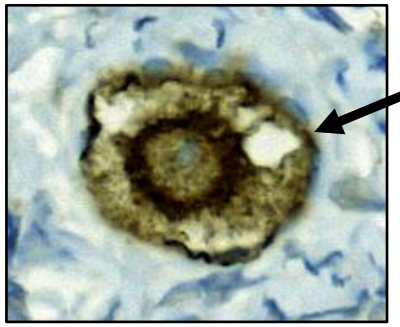
What is the Zona Pellucida (ZP) and What is its Function?



The ZP layer (arrow) contains four different ZP proteins (ZP1-4) and surrounds the egg cells in the ovaries to prevent multiple sperms from fertilising the egg and protects the early embryo before implantation

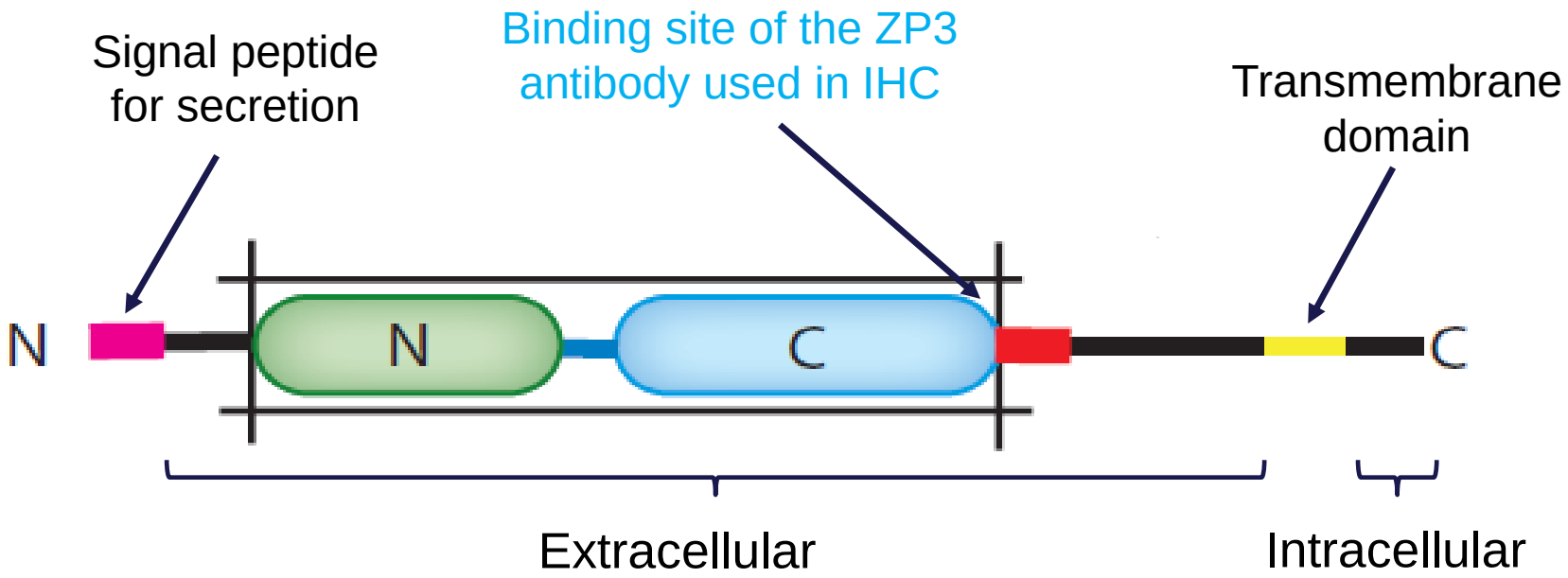
ZP3 is secreted from developing oocytes

- ZP3 is secreted from developing oocytes to become part of the Zona Pellucida:



Zona Pellucida

ZP3 protein domains:



Adapted from Litscher&Wassarman, Annu Rev Biochem 2020

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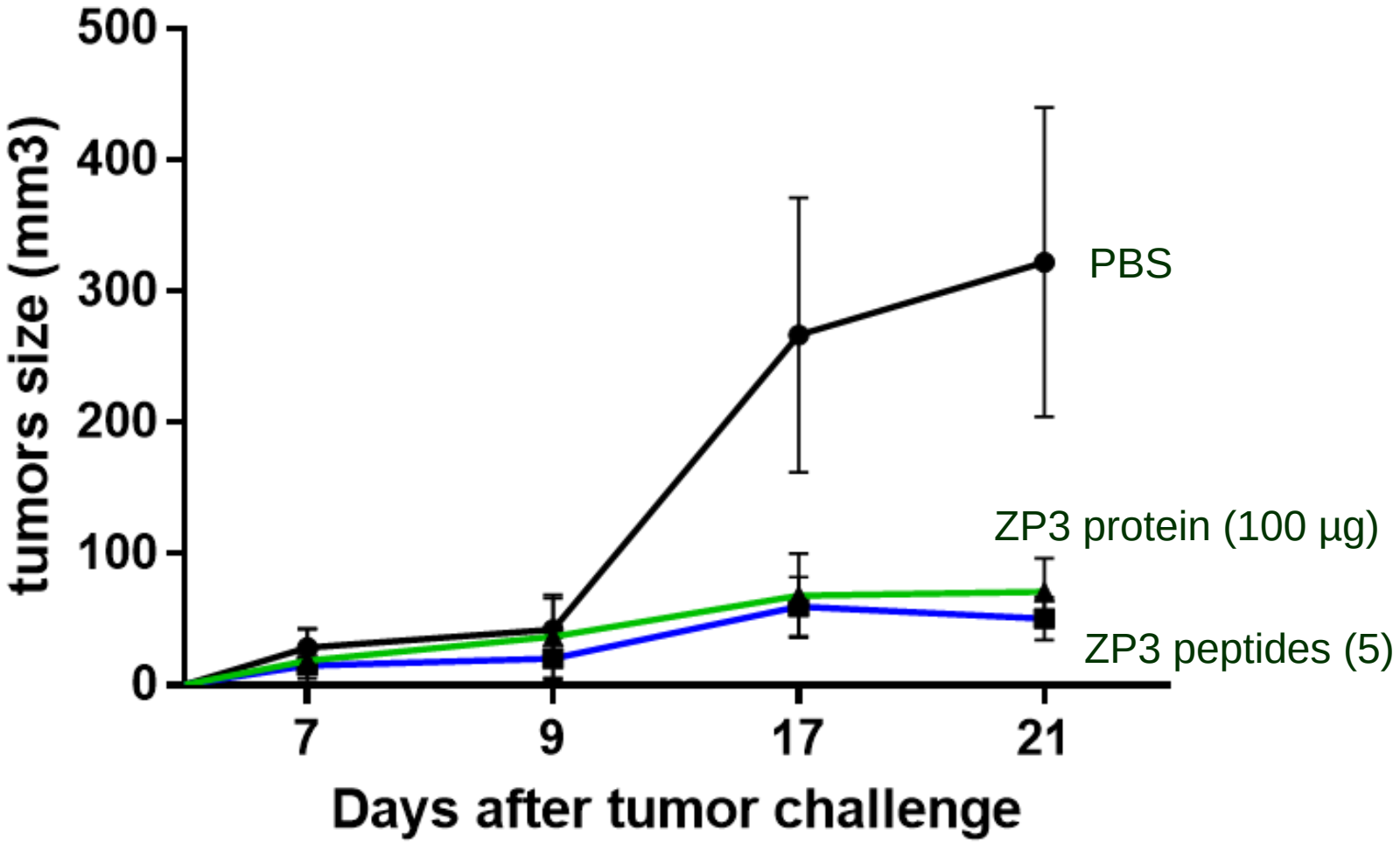
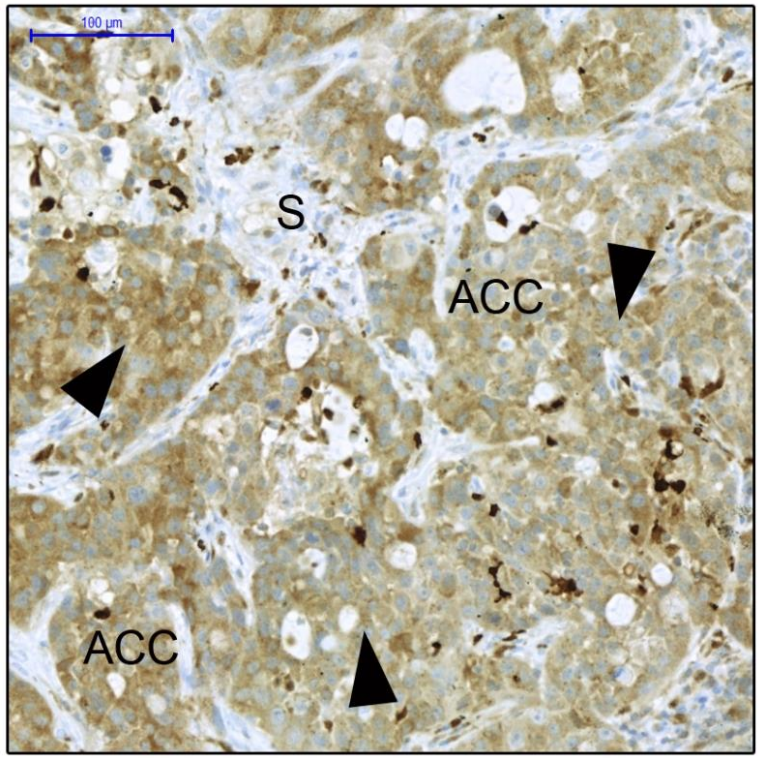
ZP3 active immunisation is able to stop tumour growth

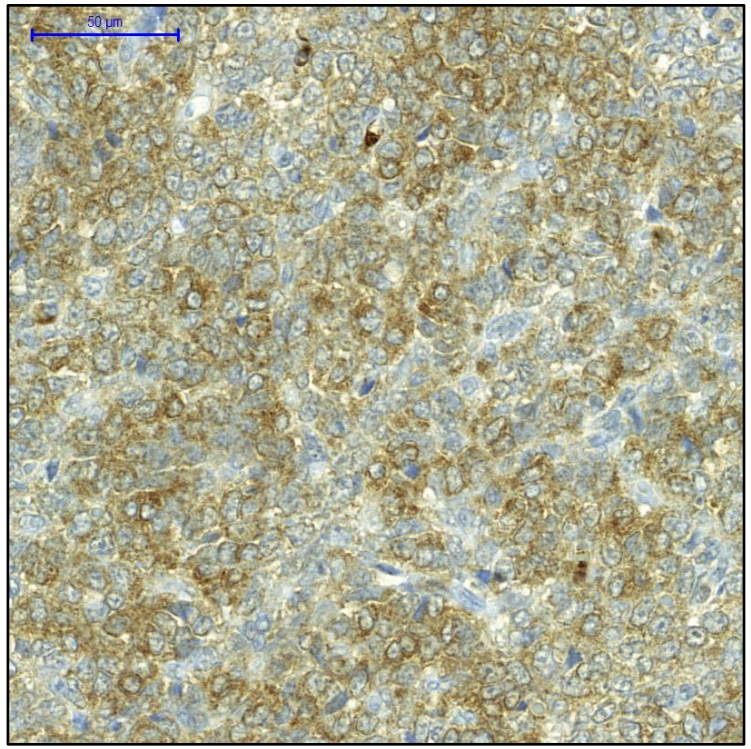


ZP3C protein expression in cancer tissue appears dominantly cytoplasmic

NSCLC tumour



Ovarian tumour



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ZP3C Protein Expression Overview

- IHC data scoring for a number of cancer types and some healthy tissues (scoring performed by expert pathologist prof. Paul van Diest, UMC Utrecht):

Cancer tissues:

ZP3 IHC score	LUSC (*n=39*)	OVCA (*n=53*)	ESCA (*n=60*)	BRCA (*n=70*)
Negative, n (%)	3 (7,7)	4 (7,5)	3 (5,0)	15 (21,4)
Low, n (%)	17 (43,6)	16 (30,2)	31 (51,7)	40 (57,1)
Medium, n (%)	12 (30,8)	25 (45,3)	24 (40,0)	14 (20,0)
High, n (%)	7 (17,9)	6 (17,0)	2 (3,3)	1 (1,4)

LUSC, lung squamous cell carcinoma (NSCLC); **OVCA**, ovarian cancer; **ESCA**, esophageal cancer; **BRCA**, breast cancer

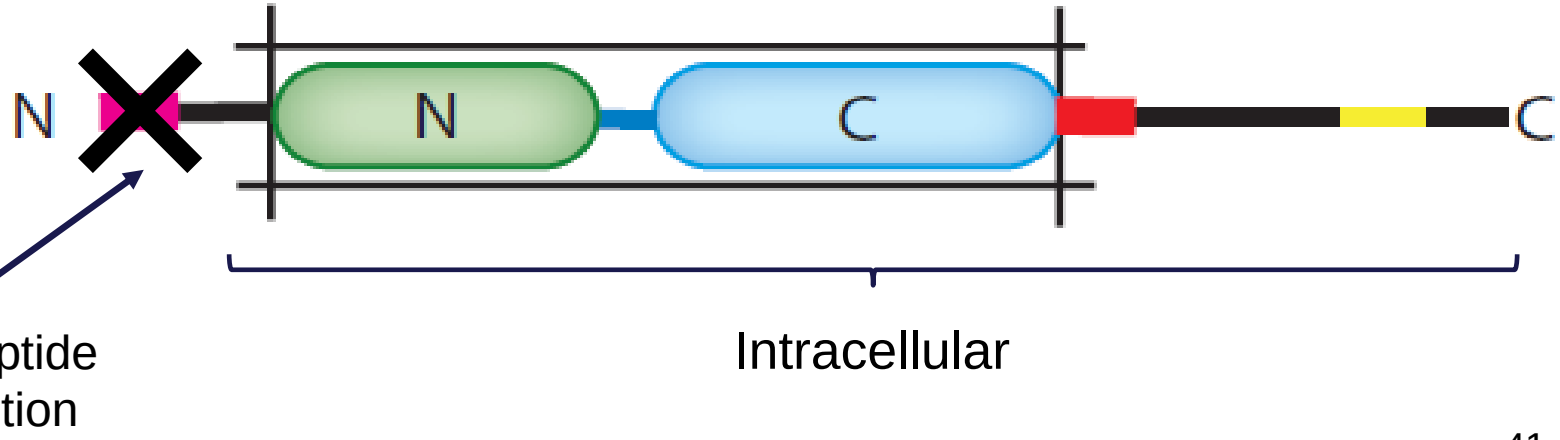
Healthy tissues:
(n=1/tissue)

Negative	lung, liver, small intestine, smooth muscle, lymphoid organ, placenta
Positive	ovary (follicles), testis (spermatogonia)

An alternative ZP3 mRNA isoform may explain the cytoplasmic localization observed in tumor cells

- A ZP3 mRNA isoform is annotated in the NCBI database that lacks the genetic information for the first 51 amino acids of the ‘Oocyte-ZP3’
- This results in the production of a ZP3 protein that lacks the signal peptide (first 21 amino acids) necessary for extracellular secretion:

‘ZP3-Intracellular’:



Confidential

Pantarhei ZP3C Ownership

- ZP3-Cancer (ZP3C) was discovered at Pantarhei
- The ZP3C antigen is present in high concentrations in tumors of patients with ovarian, colorectal and lung cancer
- Suitable for the mRNA technology:
  $\Rightarrow$ Same principle as the mRNA COVID vaccin (BioNTech/Moderna)
- Pantarhei has patent protected the use of ZP3C as mRNA-based cancer vaccine in 2022

ZP3C Development as Immunotherapy

1. Development of a ZP3-Cancer Cancer Vaccine using the mRNA technology:
   - Develop a ZP3-Cancer mRNA formulation for patient administration
   - Preclinical PK/PD and tox studies
   - Chemistry, Manufacturing and Control studies
   - GMP batch production for First-In-Human clinical trial
2. Generate more (pre)clinical evidence to attract Strategic Partner
   - Show an anti-ZP3 immune response in actual cancer patients
3. Proof-of-Concept study in OvCar or NSCLC
4. Budget required: Euro 5-10 mln

Summary status projects PRB and PRO

- PRB
  - Androgen restored contraception (ARC): ready for phase III
  - Estetrol (E4) for hormonal contraception (HC) and hormone replacement therapy (HRT): outlicensed to Mithra
- PRO
  - High dose estetrol (HDE4) for the treatment of
    - Advanced breast cancer (BCa): ready for phase II
    - Advanced prostate cancer (PCa): ready for phase III
  - The ZP3Cancer (ZP3C) target for oncology: ready for PoC