Fertility after childhood cancer: Who is at risk and what can be done

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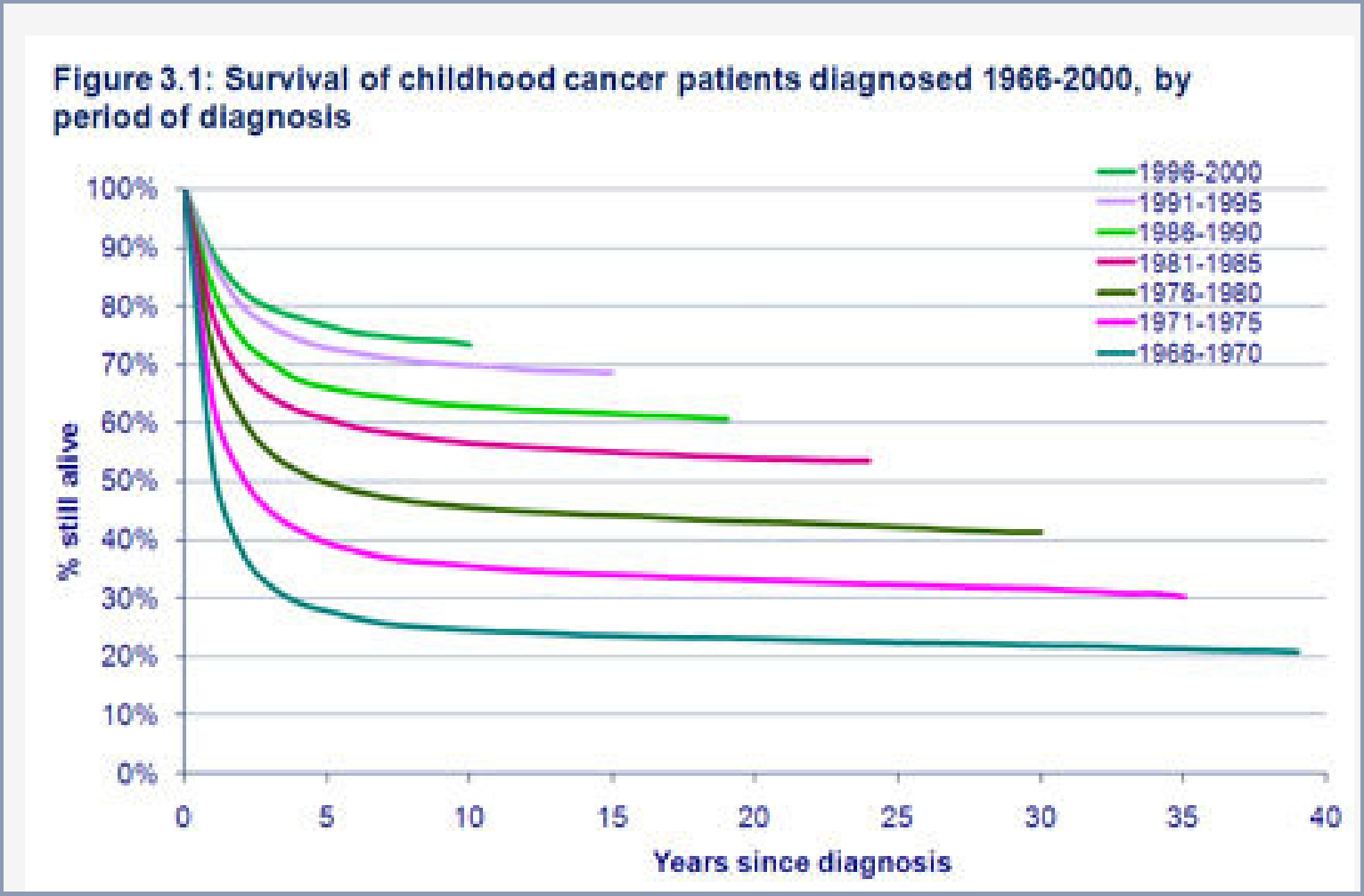
Scotland, UK

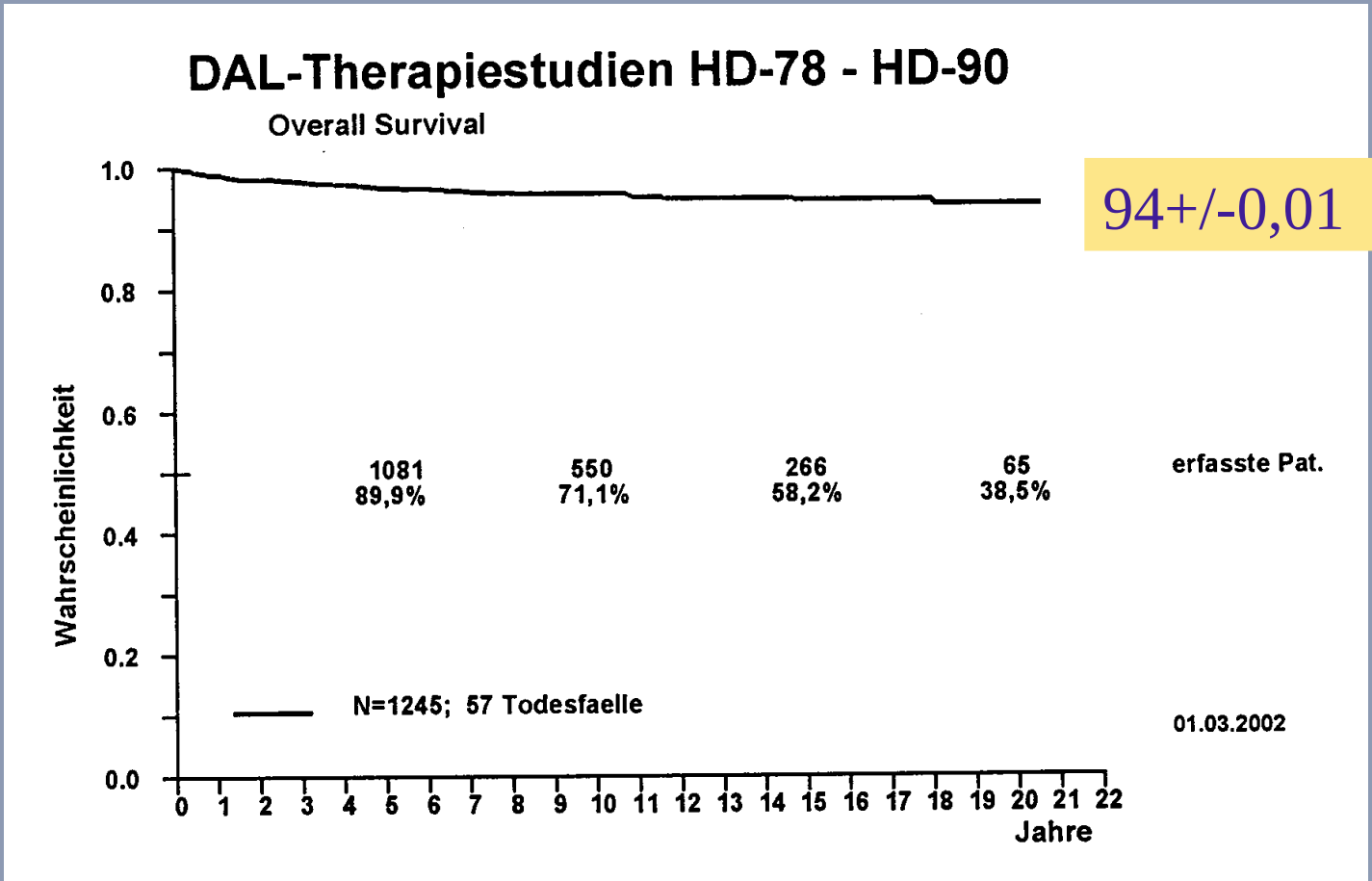
UCLH 26.02.14

Improved Five Year Survival (1966-2000)



Longterm survival after Hodgkin lymphoma

Results of the DAL78 - 90 studies



94+/-0,01

Prof. Schellong (late effects report)

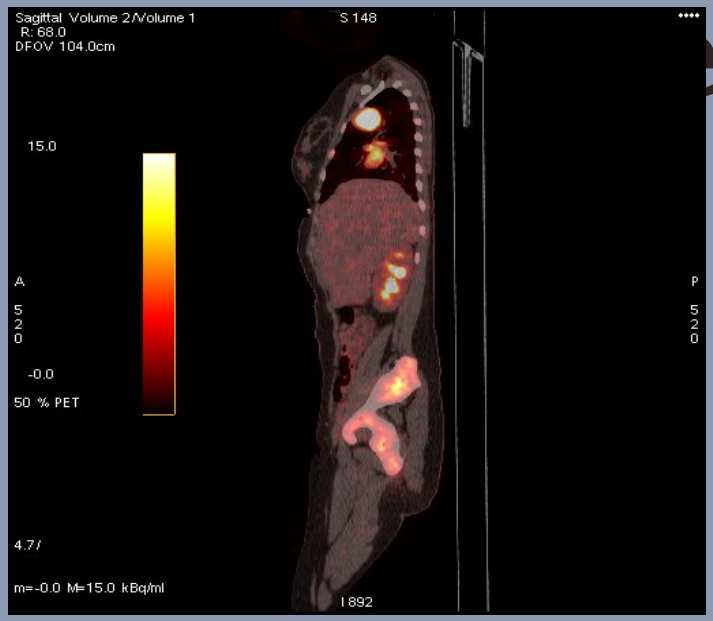
A Patient

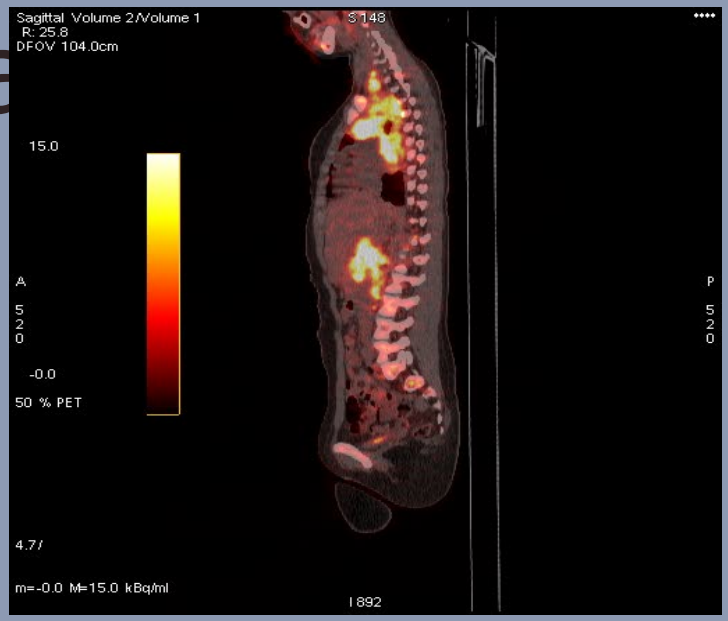
March 2011 (age 15 years)

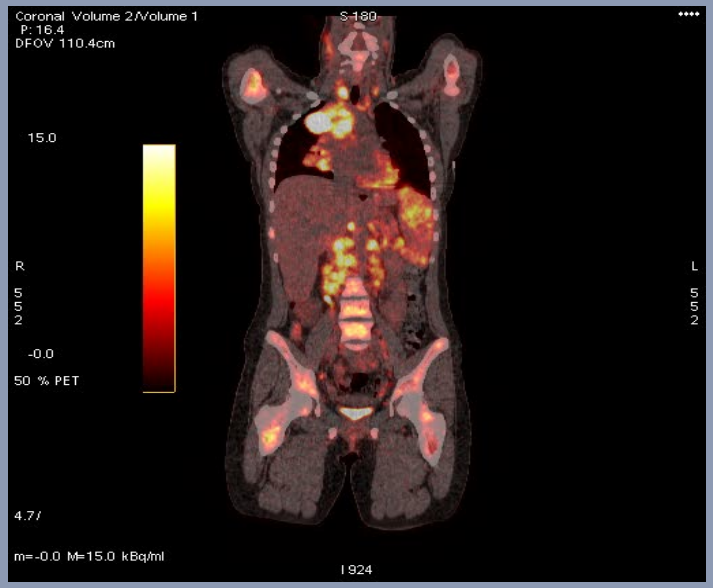
Six month H/O of intense pruritis of her feet

Three month H/O fever, night sweats, lethargy, pallor, poor appetite and weight loss

Widespread LN – lower cervical, mediastinum, abdomen


Sagittal Volume 2/Volume 1
R: 68.0
DFOV 104.0cm
S 148
15.0
A
P
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 892


Sagittal Volume 2/Volume 1
R: 25.8
DFOV 104.0cm
S 148
15.0
A
P
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 892


Coronal Volume 2/Volume 1
P: 16.4
DFOV 110.4cm
S 180
15.0
R
L
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 924


Coronal Volume 2/Volume 1
P: 7.0
DFOV 110.4cm
S 180
15.0
R
L
-0.0
50 % PET
4.7/
m=-0.0 M=15.0 kBq/ml
I 924

Laura

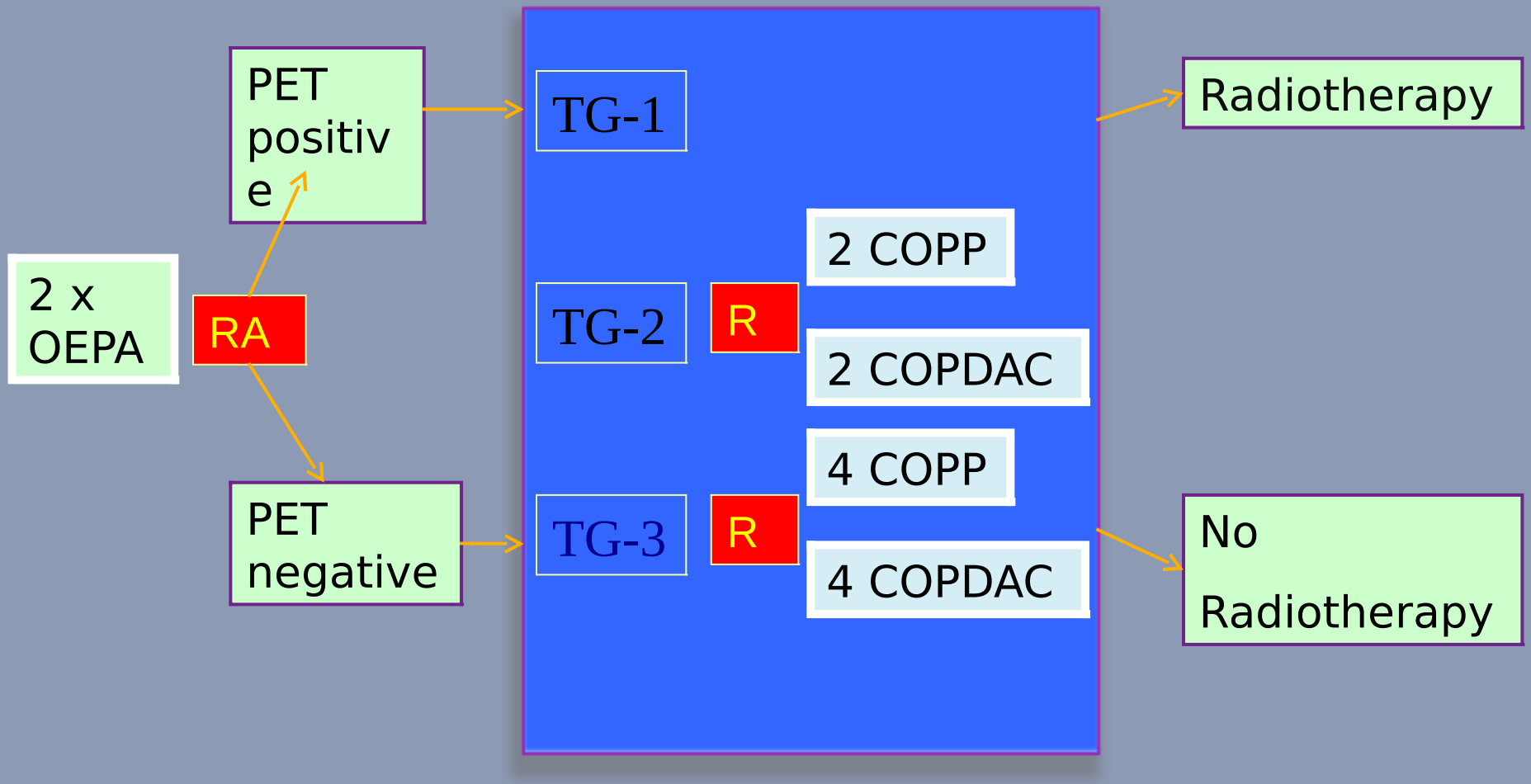
EuroNet-PHL-C1 Protocol:

Treatment Group 3 (TG3)

Two cycles of OEPA

Four cycles of COPDAC

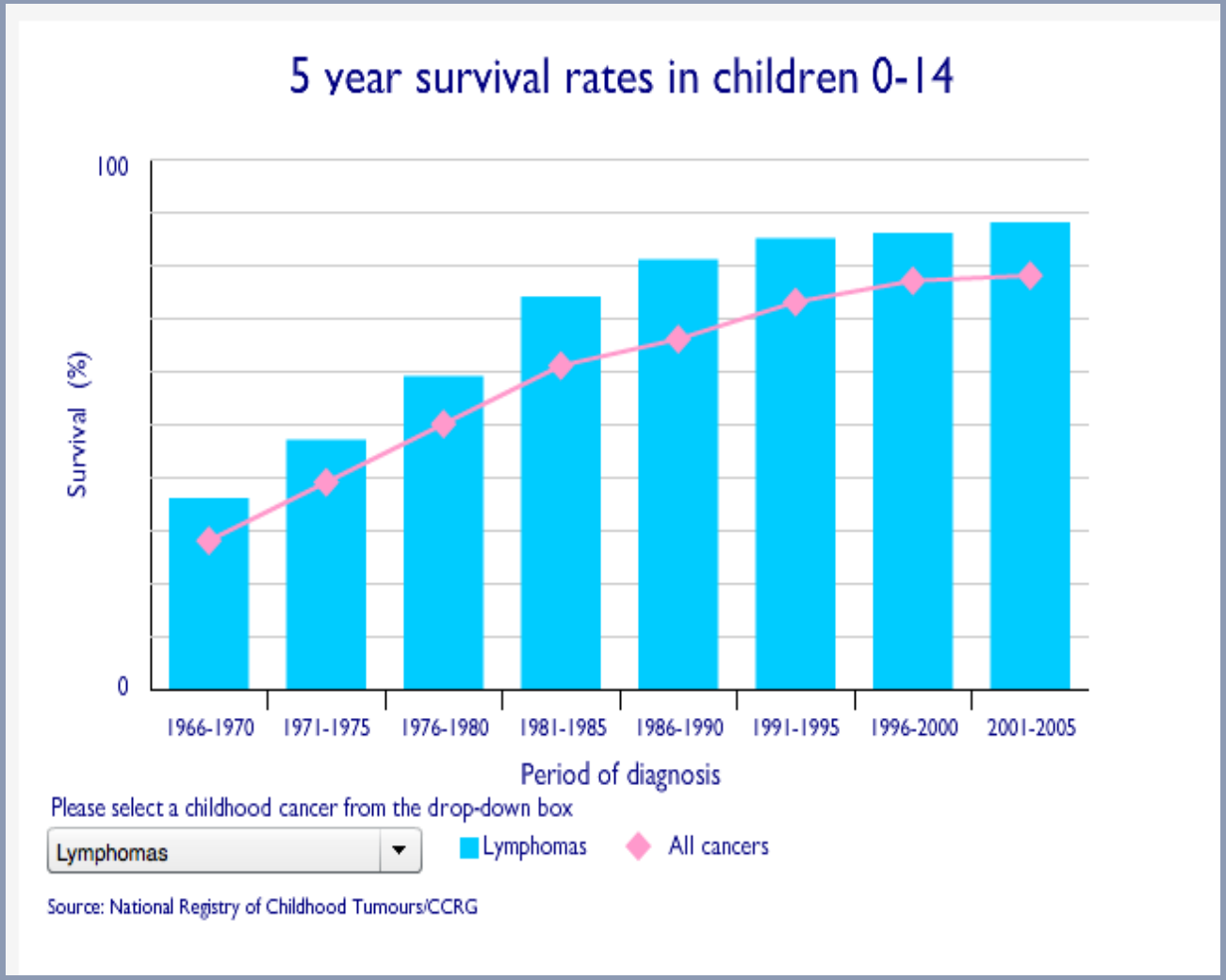
EuroNet-PHL-C-1



Wallace WH. UK Chief Investigator

CRUK support 400K

Stage IVB Hodgkin lymphoma



Risk assessment for Fertility preservation

Intrinsic factors

- Heath status of patient
- Consent (Patient/Parent)
- Assessment of ovarian reserve

Extrinsic factors

- Nature of predicted treatment
  - High/Medium/Low/Uncertain Risk
- Time available
- Expertise available

Wallace WH, Critchley HOD & Anderson RA. JCO, 2012

Infertility - Risk Factors

RT to HPA or a field that includes testes/ovaries

Busulphan

BCNU

CCNU

Cyclophosphamide

Ifosfamide

Melphalan

Mustine

Nitrogen mustard

Procarbazine

Thiotepa

Chlorambucil

Cytarabine

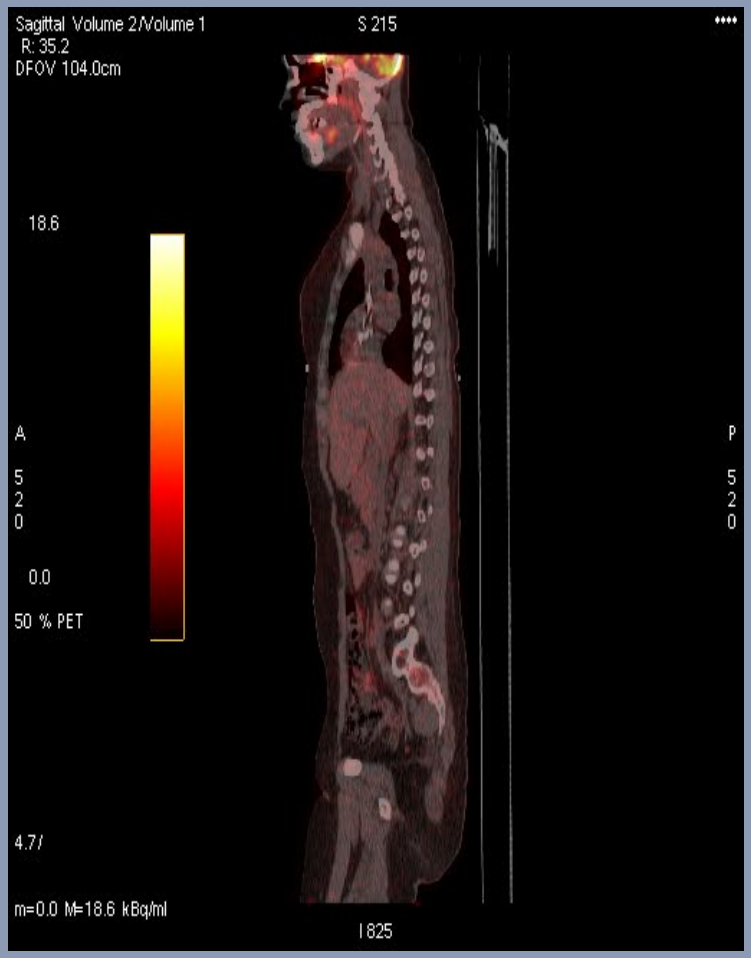
The pre-pubertal gonad is not protected

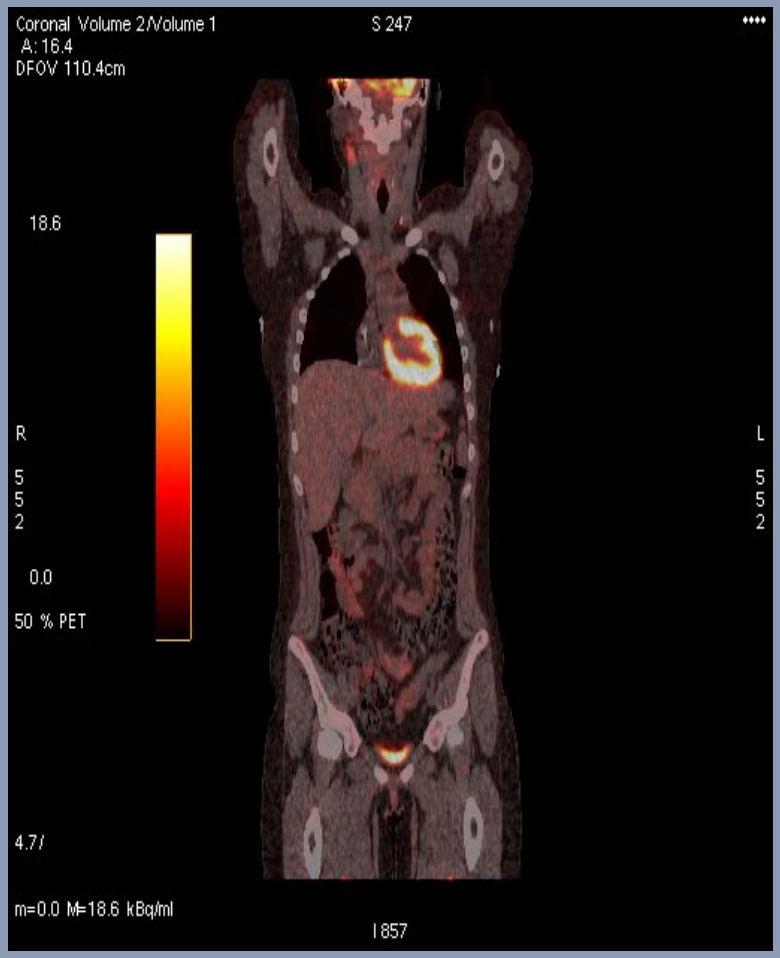
Risk of infertility

Low risk (<20%)	Medium risk	High risk (>80%)
ALL Wilms' tumour Brain tumour Sx, RT < 24Gy Soft tissue sarcoma (stage1) **Hodgkin's Lymphoma** **HL(Low stage)**	AML Osteosarcoma Ewing's sarcoma STS: stage II/III Neuroblastoma NHL Brain tumour RT>24Gy **HL (High Stage)**	Total Body Irradiation Pelvic/testes RT Chemo pre BMT Metastatic Ewing's **HL (Pelvic RT)**

Wallace, Anderson, Irvine. Lancet Oncology 2005

Early Response Assessment PET scan



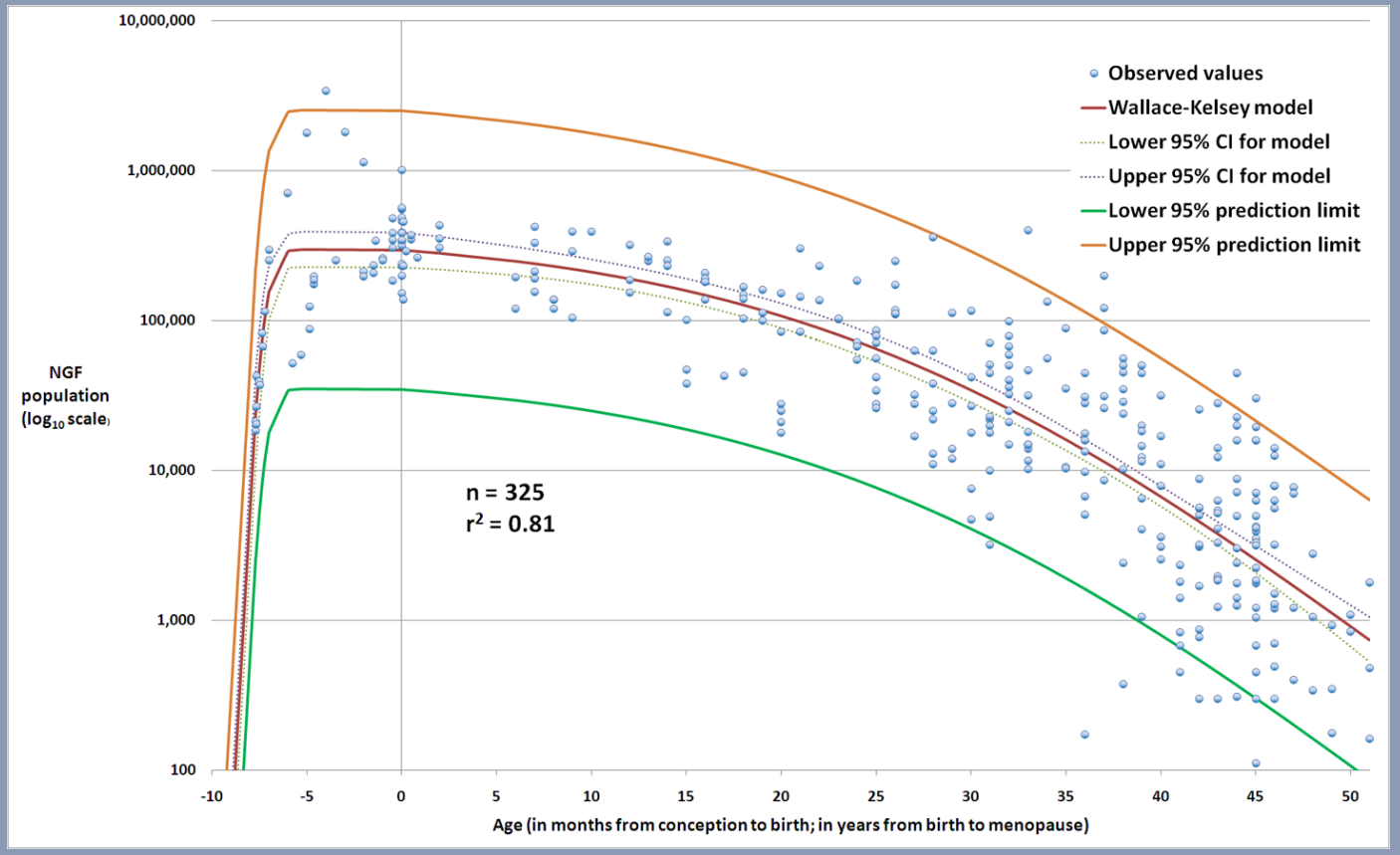


Radiotherapy Field and estimated doses to organs at risk

Organs at risk	Maximium dose received	Mean Dose
- spinal cord	2139.7 cGy	1916.2 cGy
- heart	2116.1 cGy	1701.4 cGy
- left kidney	2169.1 cGy	1439.8 cGy
- right kidney	2022.2 cGy	639.3 cGy
- lung	2148.5 cGy	1168.9 cGy
- right breast	2195.1 cGy	476.7 cGy
- left breast	2156.4 cGy	654.6 cGy
- liver	2153.4 cGy	830.2 cGy
- thyroid	2047.2 cGy	1999.0 cGy

Ovarian Reserve?

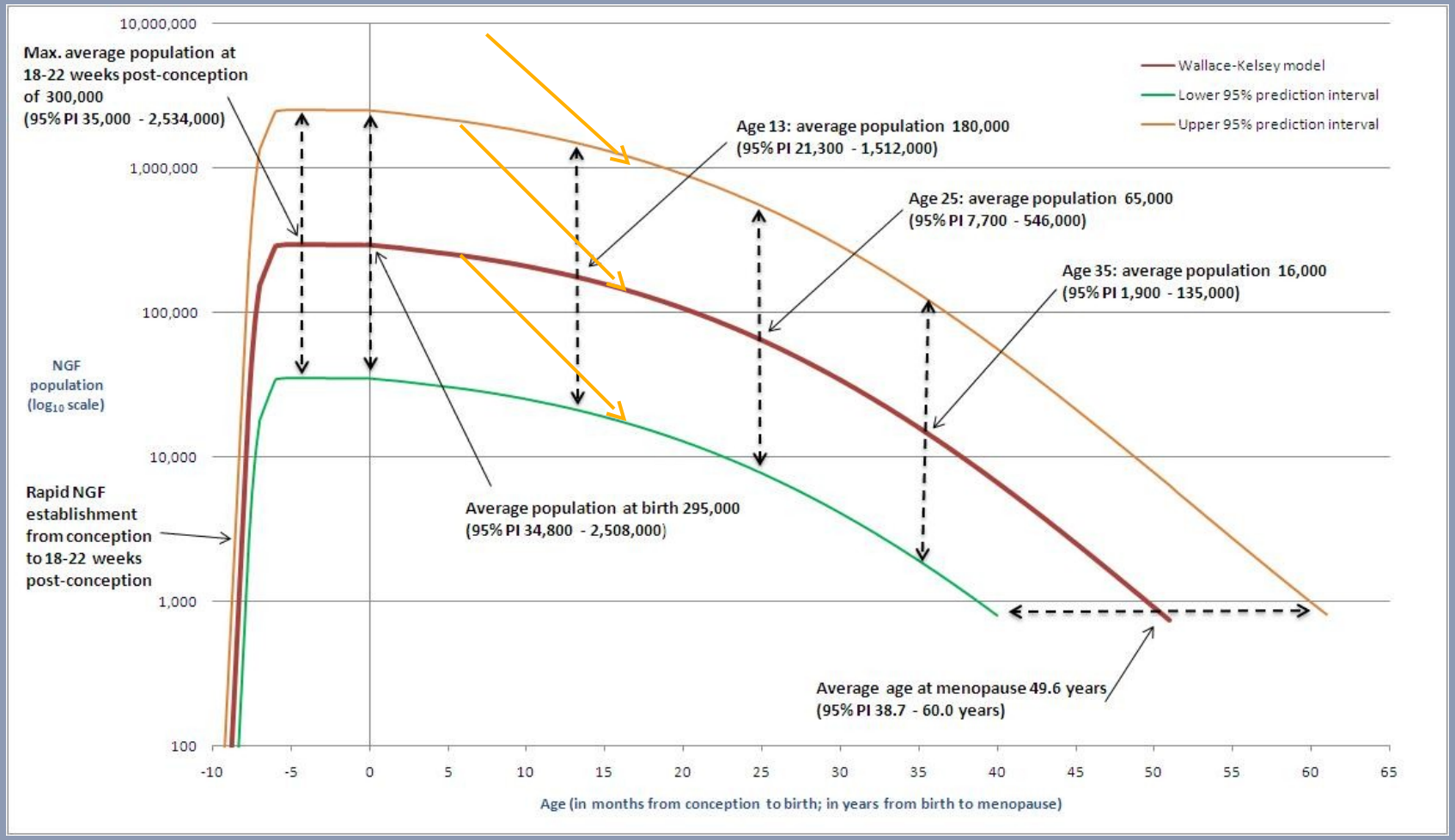
The Wallace-Kelsey Model
(Five parameter asymmetric double-Gaussian cumulative curve)



$$log_{10}(y) = \frac{a}{4}\left[1 + \mathrm{Erf}\left(\frac{x+b+\frac{c}{2}}{d\sqrt{2}}\right)\right]\left[1 - \mathrm{Erf}\left(\frac{x+b-\frac{c}{2}}{e\sqrt{2}}\right)\right]$$

Wallace &Kelsey (2010) PloS ONE

Ovarian reserve:
A Validated model from Conception to Menopause
(NGF population)



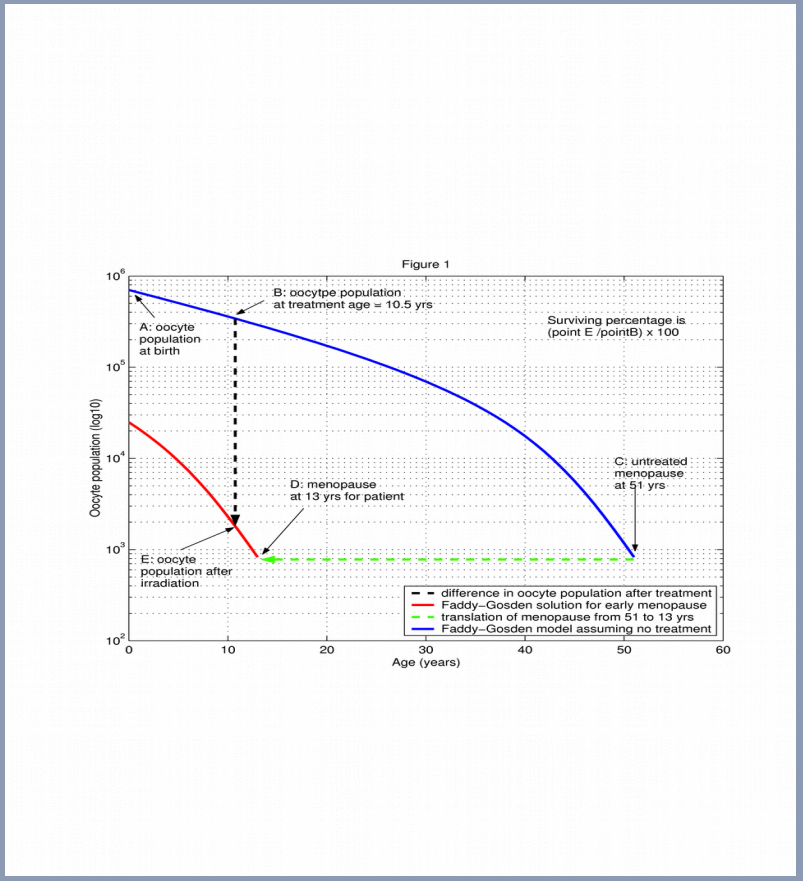
Wallace &Kelsey (2010) PloS ONE

Radiation-induced ovarian damage

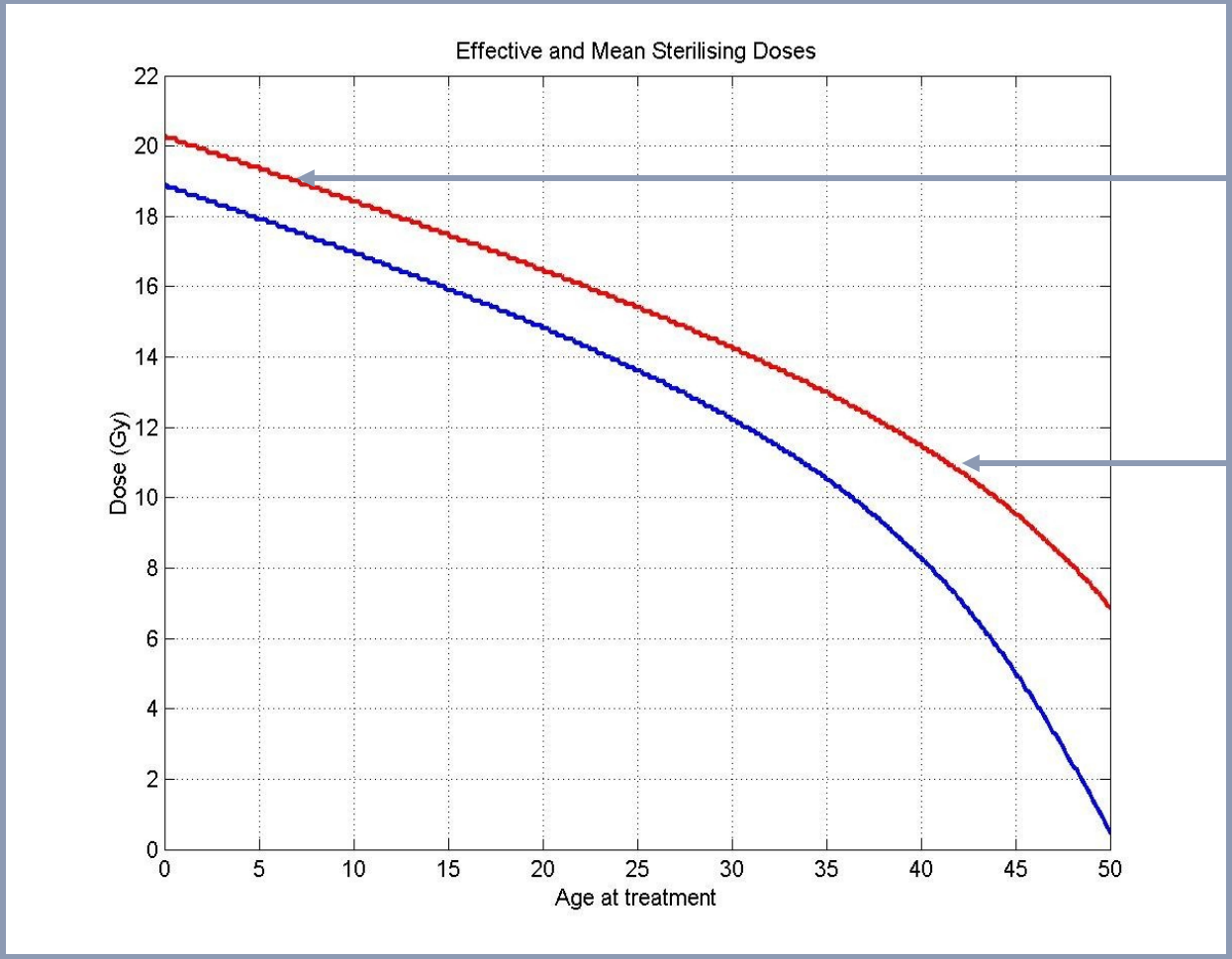
Human oocyte (Primordial follicle)

$LD_{50} < 2$ Gy

Wallace, Thomson, Kelsey. (2003) Hum Reprod.



Effective and mean ovarian sterilizing doses of radiotherapy at increasing age



19 Gy will sterilize at 7 years

11 Gy will sterilize at 42 years

Wallace WH et al. IJRBP (2005)

Prediction of Ovarian Reserve (AMH)

Anti Mullerian Hormone (AMH) is an important product of the adult ovary, produced by the granulosa cells of small growing follicles

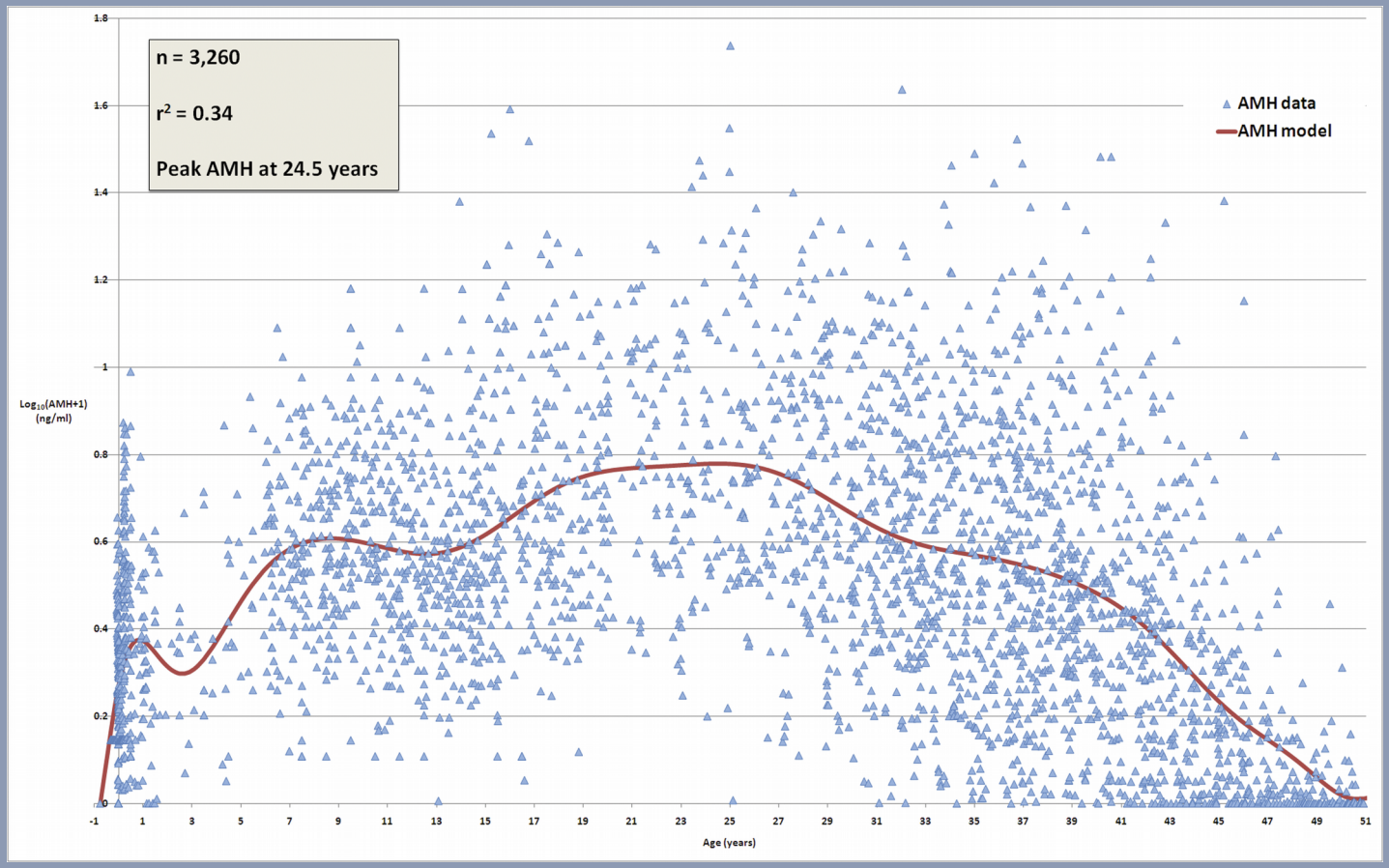
AMH has little variation across and between menstrual cycles

AMH is the best currently available marker of the number of small-growing follicles in the ovary

But there was no validated reference model for AMH available

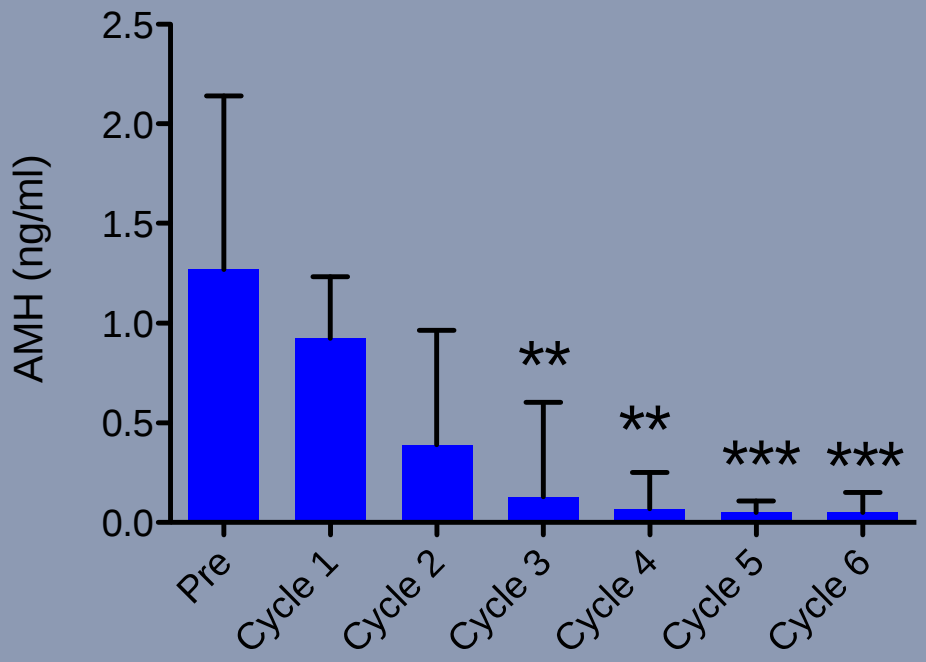
Anderson, Nelson, Wallace (2011) Maturitas

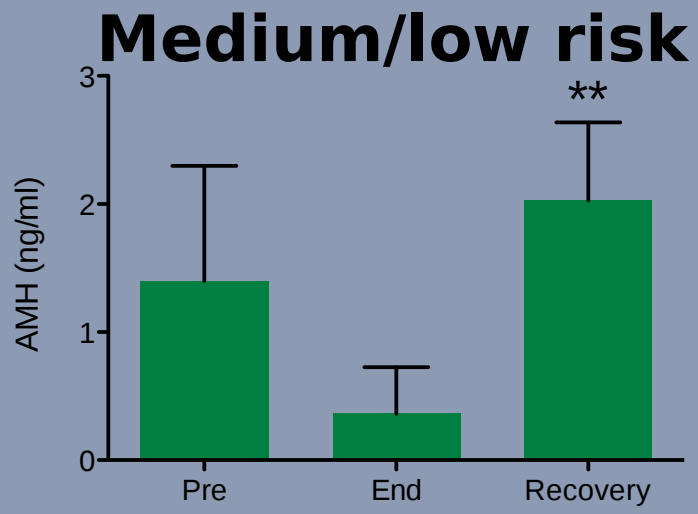
A validated model of serum anti-Mullerian hormone (AMH) from conception to menopause

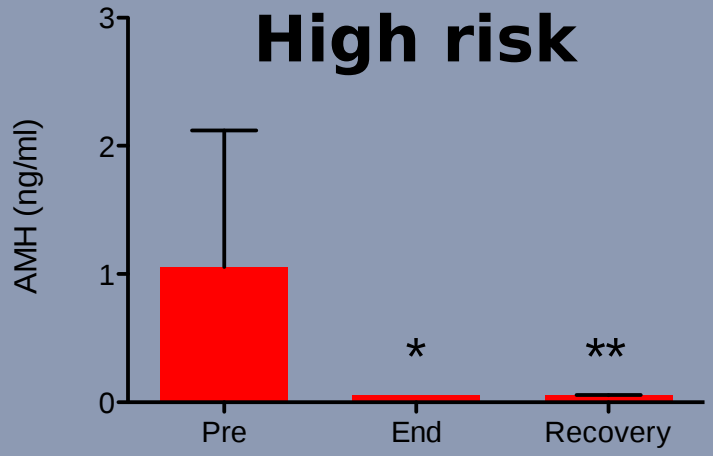


Kelsey et al. PLoS ONE 2011

AMH in childhood cancer



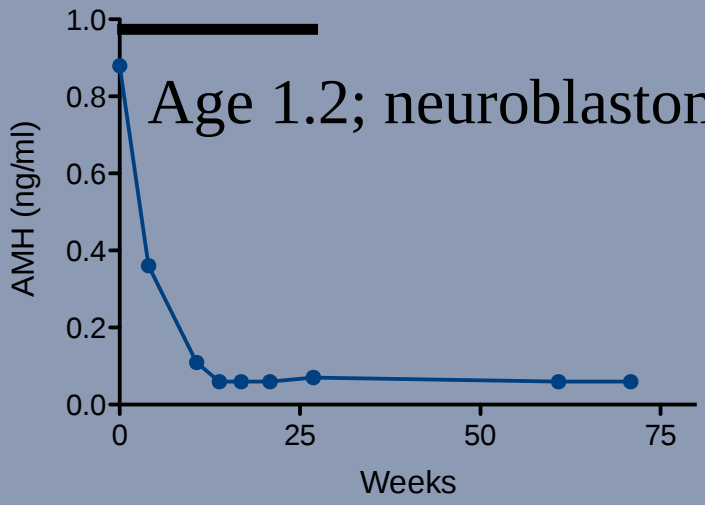


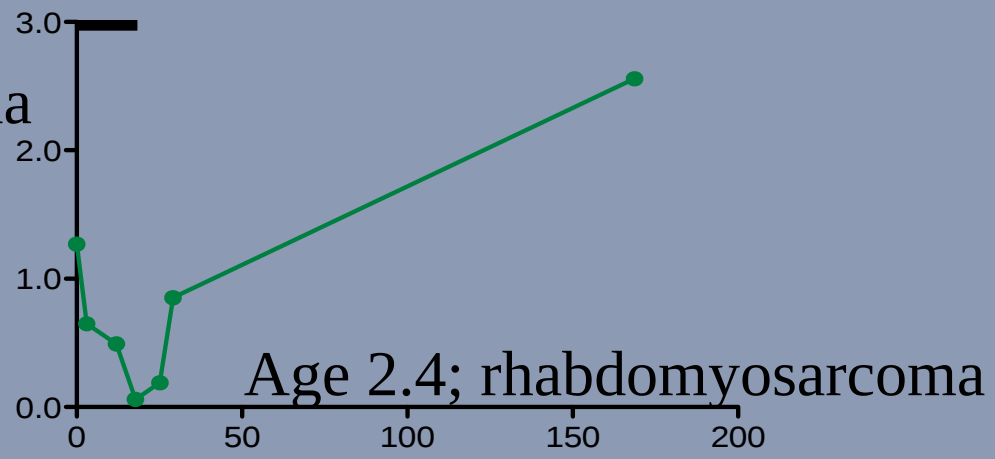


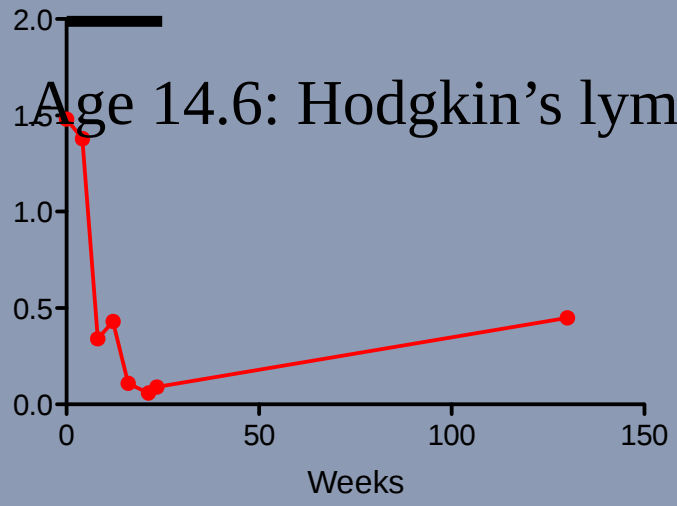
22 girls age 0.3-15yr
17 prepubertal

Brougham et al 2012 JCE&M

AMH in 3 girls with cancer







Brougham et al 2012 JCE&M

Summary

AMH is detectable before puberty

AMH falls rapidly during cancer treatment in both pre-pubertal and pubertal girls

AMH levels recover in those patients at low/medium risk of gonadotoxicity

AMH fails to recover in those at high risk. This could be indicative of future reproductive impairment

Brougham et al 2012 JCE&M

Fertility preservation options: established and experimental

Key features of the 3 options for fertility preservation for women

Embryo cryopreservation

- Established but require time and a partner

Oocyte cryopreservation

- Established but require time and hormone stimulation (success rate per oocyte low)

Ovarian tissue cryopreservation

- Minimal delay
- No lower age limit
- Surgical procedure
- Allows for future developments

Cryopreservation: World-wide experience

- At least 30 pregnancies worldwide after othotopic reimplantation of frozen–thawed ovarian cortex
- Success rate is unclear as the denominator is unknown
- No pregnancies reported following the reimplantation of ovarian tissue harvested pre-pubertally
- Young children are potentially ideal candidates

Donnez, J. & Dolmans, M.-M. *Nat. Rev. Endocrinol.* 9, 735–749 (2013)

Cryopreservation: European experience

Three centres (Denmark, Spain and Belgium)

60 cases of orthotopic reimplantation.

Of these women, 11 (21%) became pregnant

Six have delivered 12 healthy babies.

Restoration of ovarian activity was observed in 93% of the patients between 3.5 months and 6.5 months after grafting

The mean duration of ovarian function after trans-plantation is ~4–5 years but can persist for up to 7 years.

Donnez, J. *et al.* *Fertil. Steril.* 99, 1503–1513 (2013).

Ovarian Cryopreservation & Ovarian Function

Edinburgh experience in children (< 18 yrs) 1996-2012

Cryopreservation of ovarian cortical tissue – Edinburgh criteria

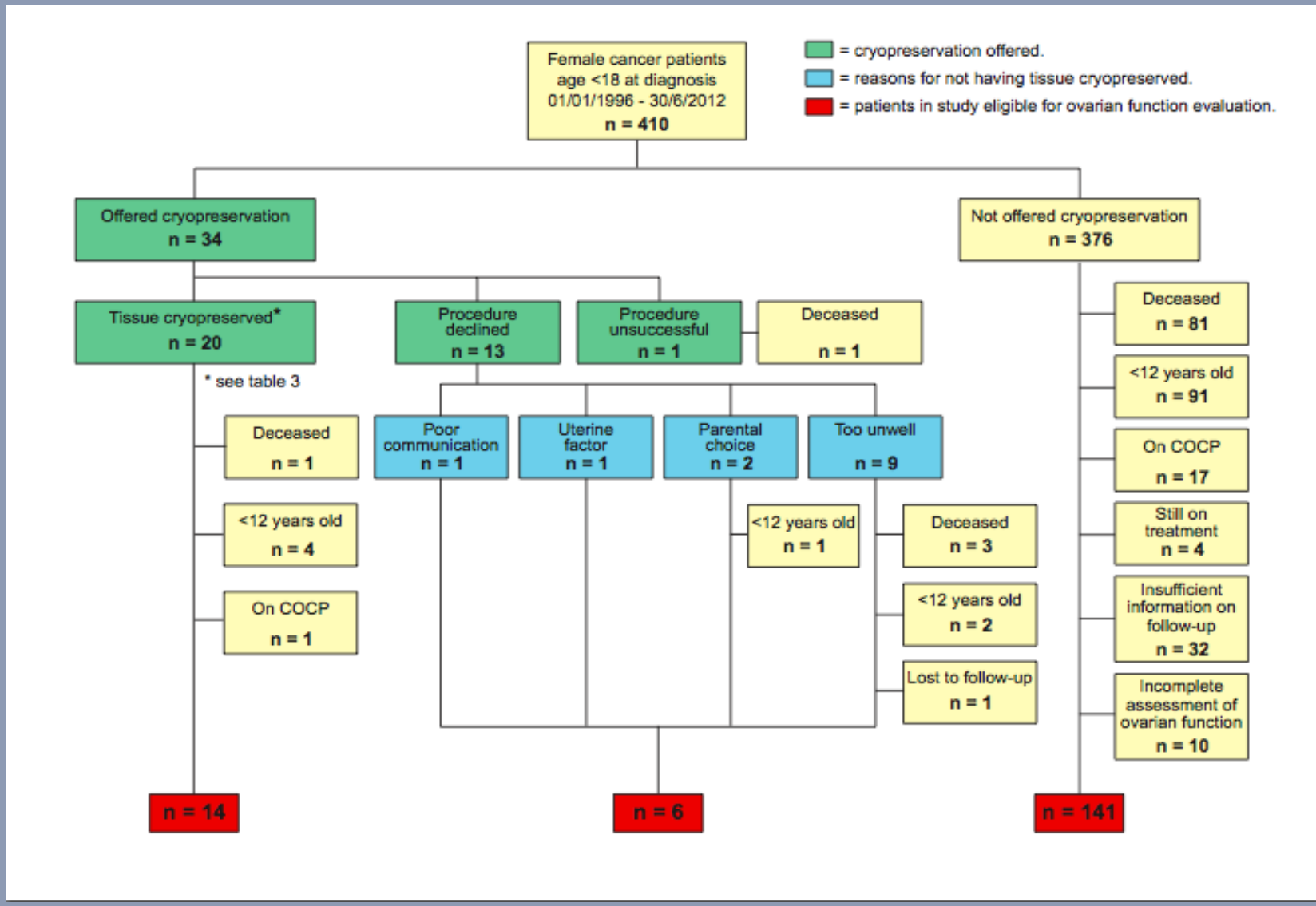
Selection criteria (1995, modified 2000)

- Age < 35 years
- No previous chemotherapy/radiotherapy if age >15 years
- Mild, non gonadotoxic chemotherapy if < 15 years
- A realistic chance of surviving five years
- A high risk of ovarian failure
- Informed consent (parent and where possible patient)
- Negative HIV and Hepatitis serology
- No existing children

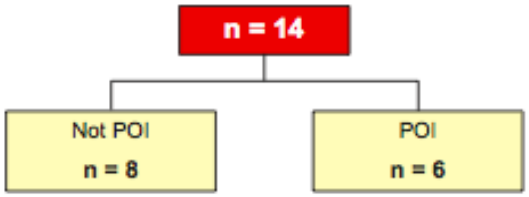
Edinburgh Paediatric Experience

Table 3: Patients that had ovarian tissue cryopreserved

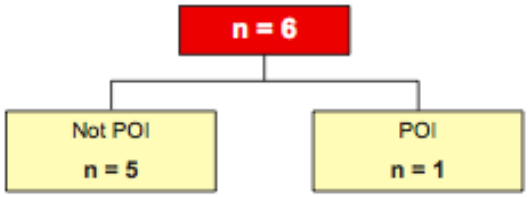
Patient No.	Diagnosis	Age at procedure	Method	Complications
1	Hodgkin's lymphoma¤	14.9	Laproscopic Cortical Strip	None
2	Ewing's sarcoma of pubic bone	14.9	Laproscopic Cortical Strip	None
3	Sacral ependymoma	11.3	Laproscopic Cortical Strip	None
4	Hodgkin's lymphoma	13.7	Laproscopic Cortical Strip	None
5	Hodgkin's lymphoma	11.0	Laproscopic Cortical Strip	None
6	Chronic granulocytic leukaemia	9.9	Laproscopic Cortical Strip	None
7	Rhabdomyosarcoma	5.3	Laproscopic Cortical Strip	None
8	Ewing's sarcoma (pelvic)	9.8	Laproscopic Cortical Strip	None
9	Uterine Cervix Rhabdomyosarcoma*	16.5	Laproscopic Cortical Strip	None
10	Hodgkin's lymphoma°	14.1	Laproscopic Cortical Strip	None
11	Abdominal embryonal Rhabdomyosarcoma	7.9	Laproscopic Cortical Strip	None
12	Ewing's sarcoma	12.1	Laproscopic Cortical Strip†	None
13	Hodgkin's lymphoma	12.7	Laproscopic Cortical Strip	None
14	Metastatic Medulloblastoma	8.1	Laproscopic Cortical Strip	None
15	Hodgkin's lymphoma	15.2	Laproscopic Cortical Strip	None
16	Alveolar Rhabdomyosarcoma	10.5	Laproscopic Cortical Strip	None
17	Embryonal Rhabdomyosarcoma	3.0	Oophorectomy	None
18	Ewing's Sarcoma	12.0	Laproscopic Cortical Strip	None
19	Undifferentiated Sarcoma	12.3	Laproscopic Cortical Strip†	None
20	Wilm's Tumour	1.2	Oophorectomy	None


= cryopreservation offered.
= reasons for not having tissue cryopreserved.
= patients in study eligible for ovarian function evaluation.
Female cancer patients age <18 at diagnosis 01/01/1996 - 30/6/2012 n = 410
Offered cryopreservation n = 34
Not offered cryopreservation n = 376
Tissue cryopreserved* n = 20
* see table 3
Deceased n = 1
<12 years old n = 4
On COCP n = 1
n = 14
Procedure declined n = 13
Procedure unsuccessful n = 1
Deceased n = 1
Poor communication n = 1
Uterine factor n = 1
Parental choice n = 2
Too unwell n = 9
<12 years old n = 1
Deceased n = 3
<12 years old n = 2
Lost to follow-up n = 1
n = 6
Deceased n = 81
<12 years old n = 91
On COCP n = 17
Still on treatment n = 4
Insufficient information on follow-up n = 32
Incomplete assessment of ovarian function n = 10
n = 141

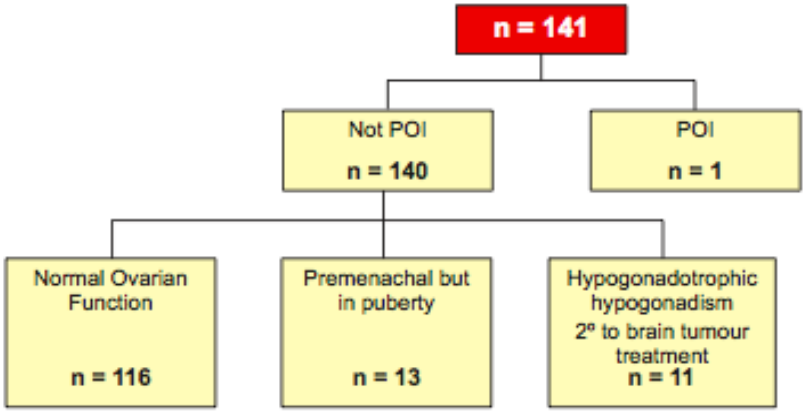
CRYOPRESERVED



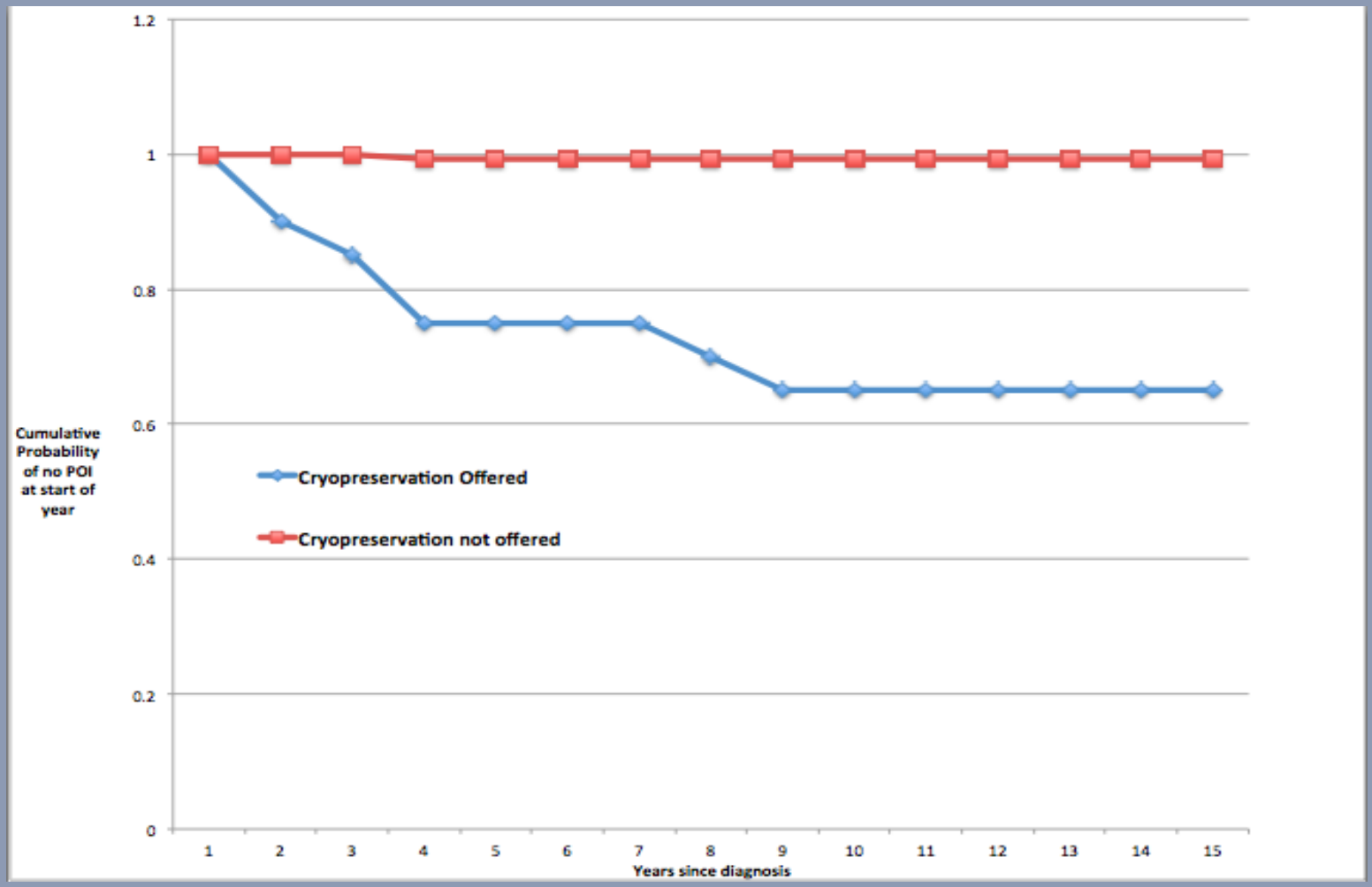
OFFERED CRYOPRESERVATION - procedure declined



NOT OFFERED CRYOPRESERVATION



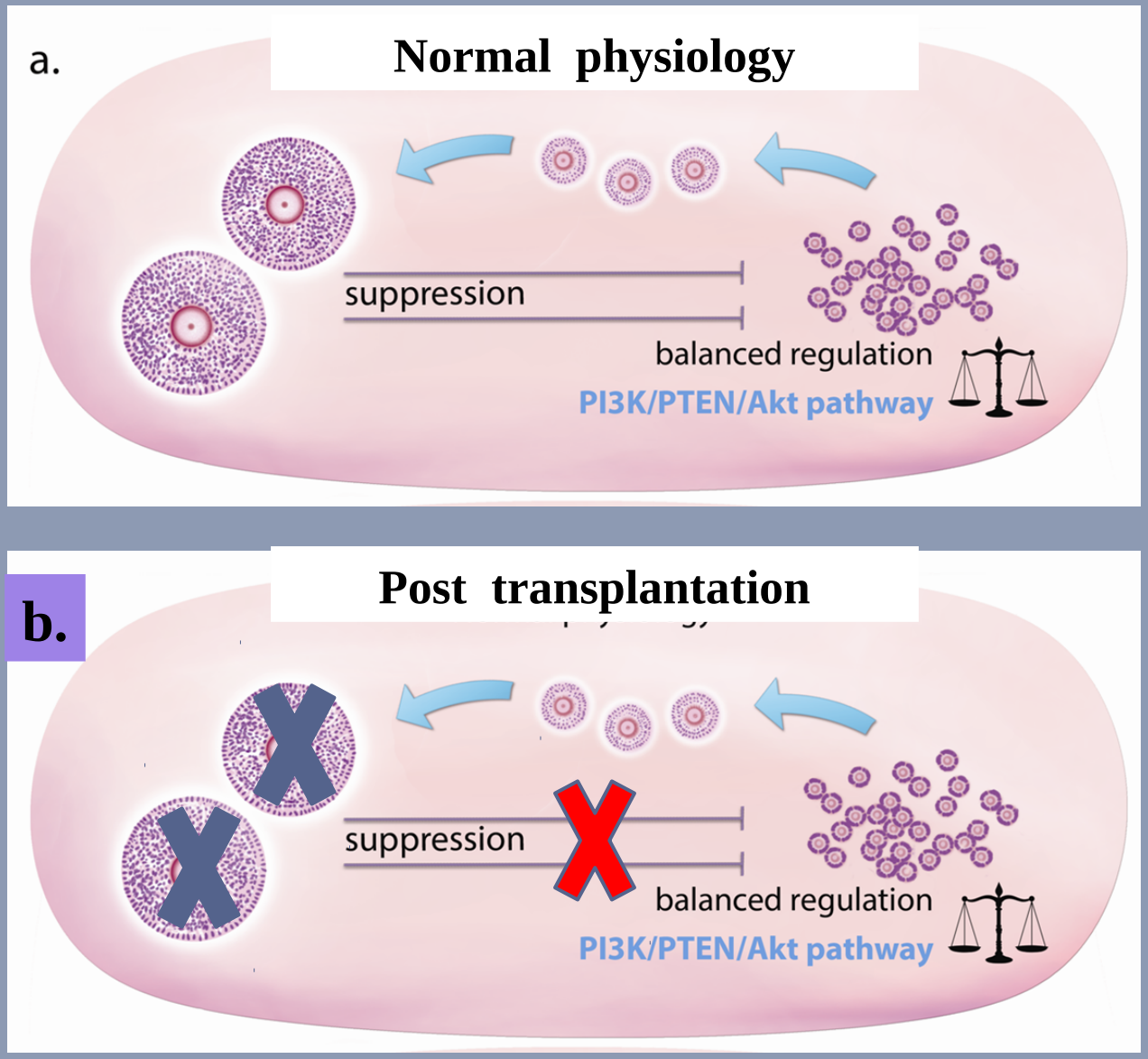
Life Table Analysis of POI



Summary

- Ovarian cryopreservation was offered to 8% of our patients, and performed in 5%
- The procedure was safe and without complications
- No patients have asked for re-implantation of their tissue – to date (15.7 [1.3-30.9] yrs)
- All patients who have thus far (bar one) developed premature ovarian insufficiency were identified
- The Edinburgh Selection Criteria have proved to be helpful and accurate in determining the correct patients for ovarian cryopreservation

The “Burn-Out” mechanism post transplantation

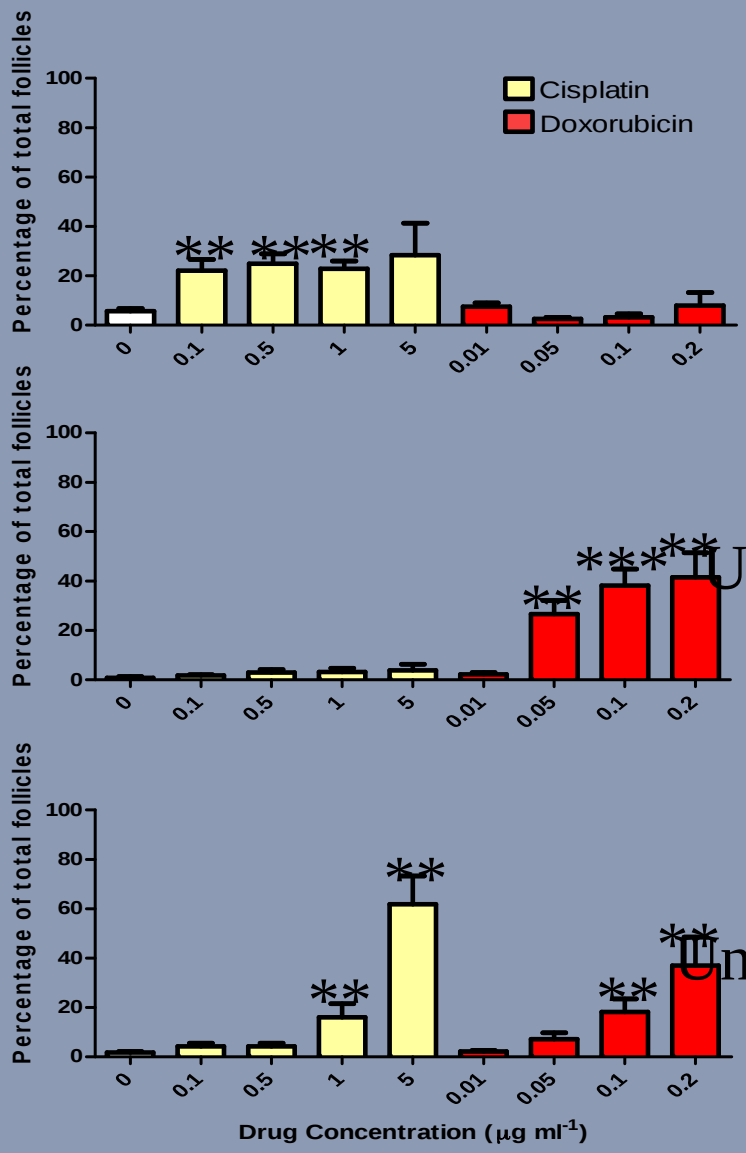


Oocyte or granulosa cells?

Newborn mouse ovary culture system

Morgan et al. 2013, PLoS ONE

Cisplatin and doxorubicin: a mouse ovary culture system



Unhealthy oocytes

Unhealthy granulosa cells

Unhealthy oocytes and granulosa cells

Morgan et al, 2013, PlosOne

Cisplatin and Doxorubicin (Mouse ovary)

Cisplatin showed oocyte-specific damage

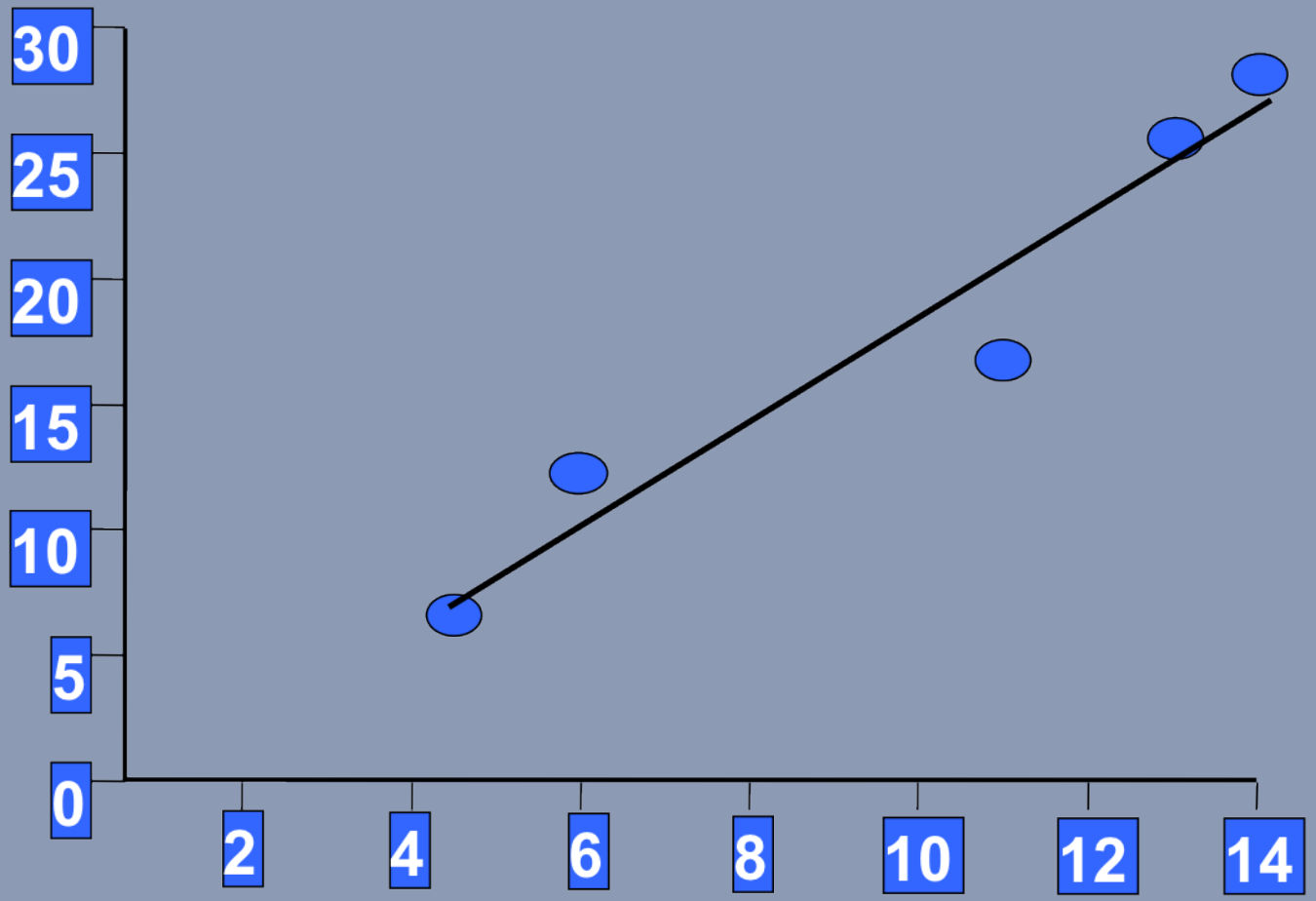
Doxorubicin preferentially caused damage to the granulosa cells

Suggestion:

Imatinib protected the mouse ovary against damage by cisplatin but not doxorubicin

Morgan et al, 2013, PlosOne

Uterine volume and age at irradiation (TBI)



Bath et al. BJOG (1999)

Uterine function after cancer treatment

No reports of uterine damage due to chemotherapy

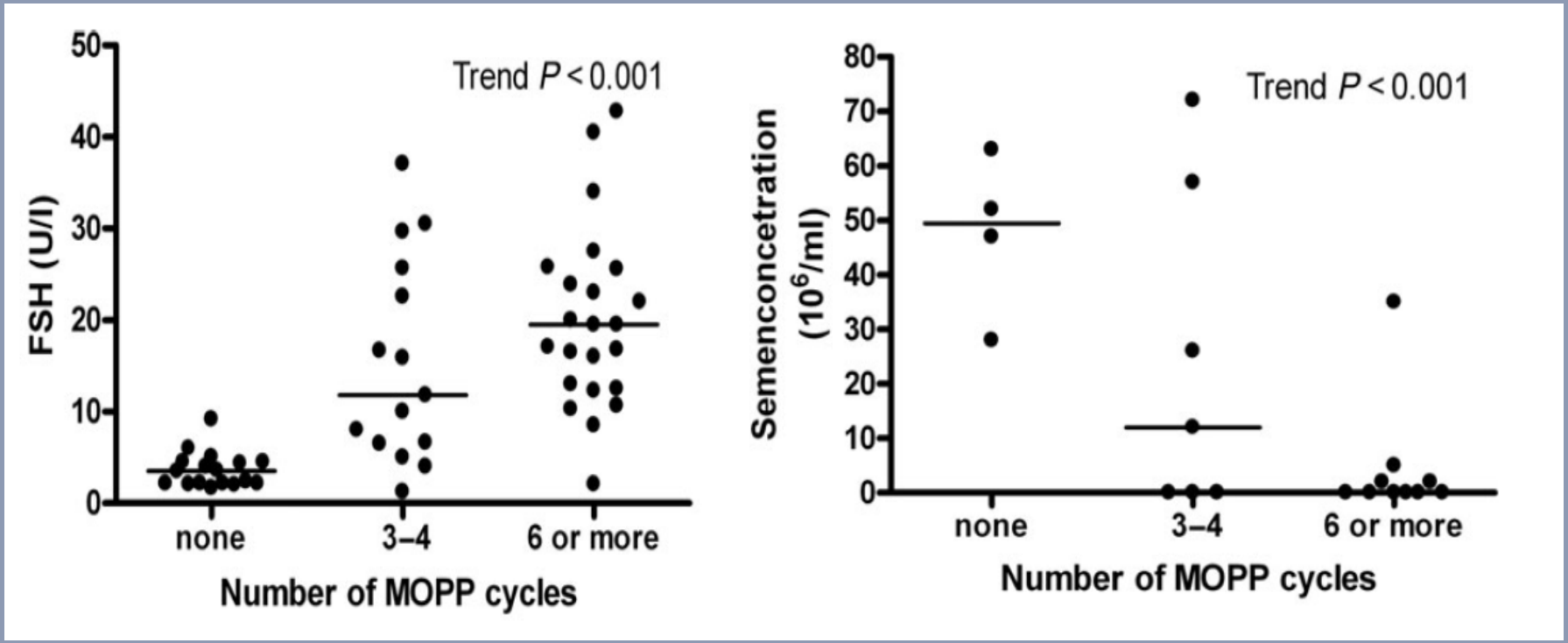
Radiotherapy:

Uterine damage, manifest by impaired growth and blood flow.

Uterine volume correlates with age at irradiation.

Exposure of the pelvis to radiation is associated with an increased risk of miscarriage, mid-trimester pregnancy loss, PPH, pre-term birth and low birth weight.

FSH and semen concentration by # of MOPP cycles in paediatric HL



Van Beek 2007

,

Radiation-induced testicular damage

Germinal epithelium

- >1.2Gy azoospermia

Leydig cells

- >20Gy pre-pubertal
- >30Gy post-pubertal

Leydig cell function after radiotherapy

Preliminary multivariate analysis of studies that report Age at RT, Dose to testes, Interval between RT and follow up measurement of Testosterone - suggests that the most important factors affecting testosterone after radiation exposure to the testes are:

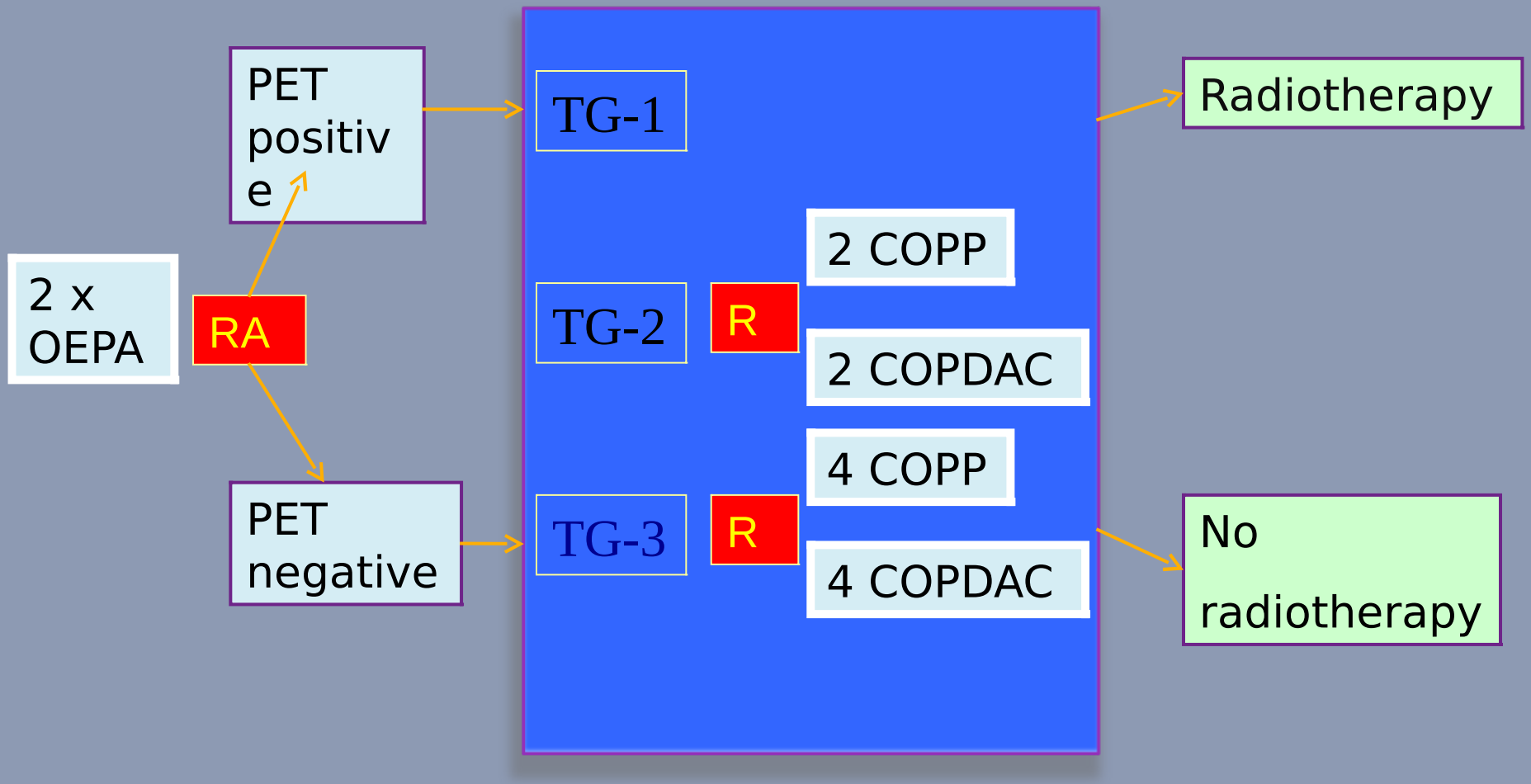
Dose received by testis $P < 0.05$

Time Interval after radiotherapy $P < 0.05$

Age at treatment NS

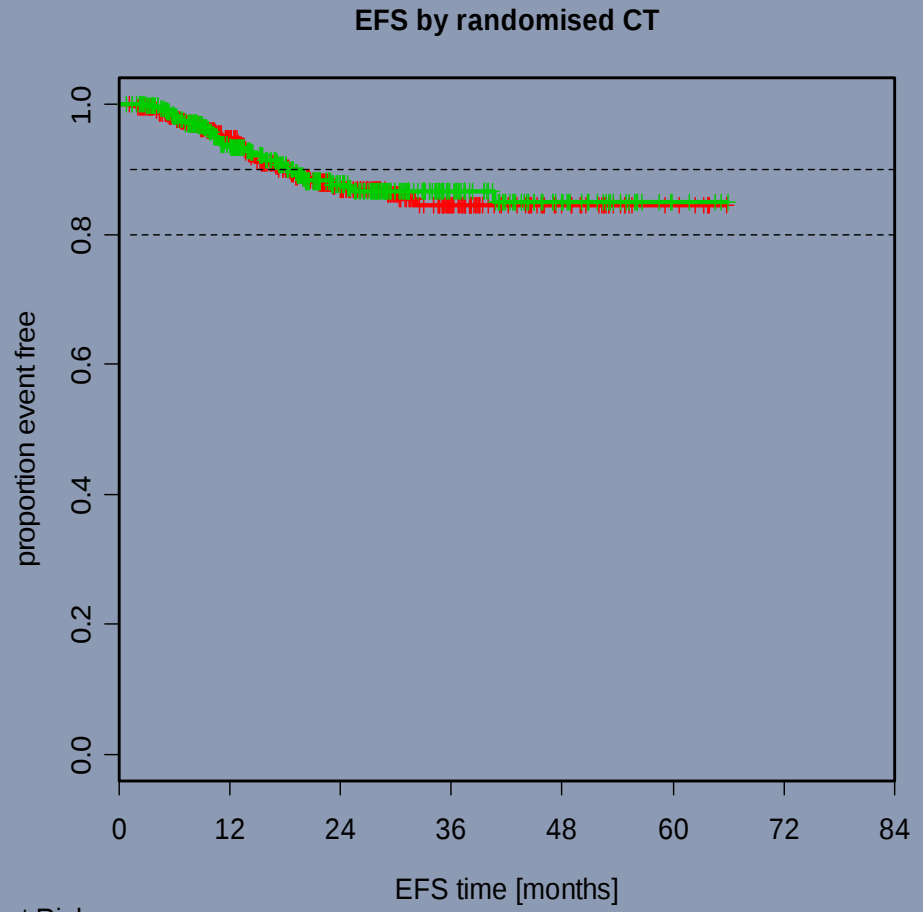
Li, Kelsey, Wallace (unpublished data)

EuroNet-PHL-C-1



CRUK support 400K

EuroNet-PHL-C1
Chemotherapy question



Curves	Events	N
COPDAC	42	444
COPP	43	447

Rate estimates at 36 months (95% CI)

COPDAC	0.865 [0.826 0.906]
COPP	0.844 [0.799 0.893]

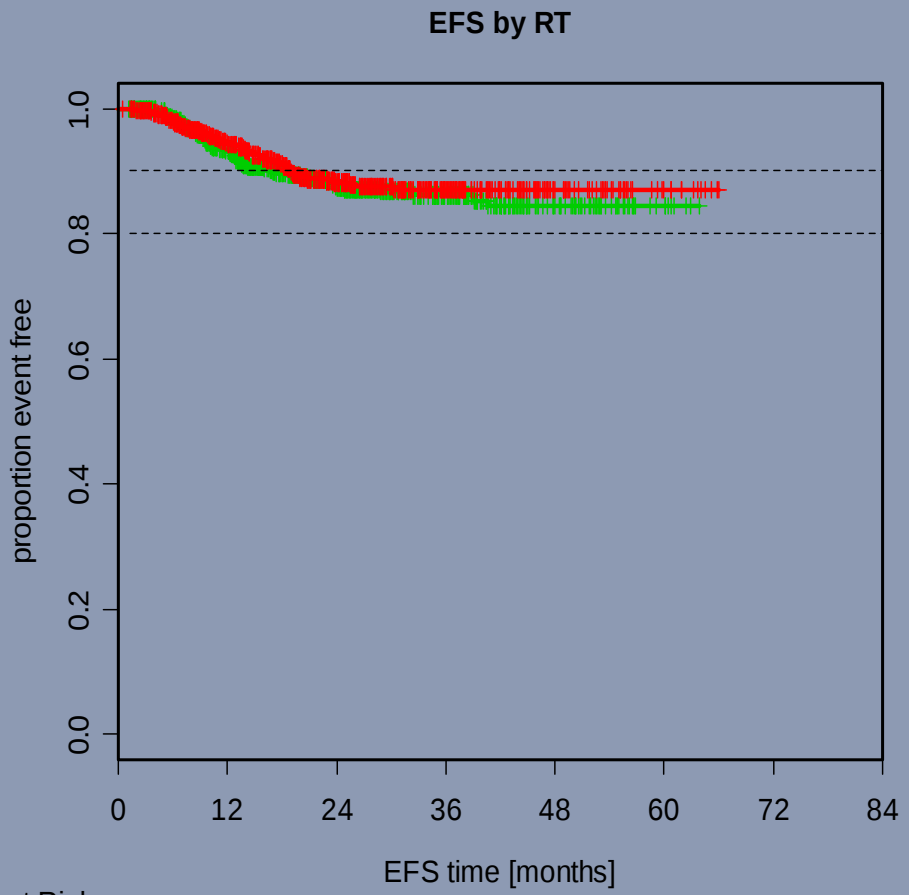
LogRank-Test: p= 0.8473

logHazardRatio:	-0.042 [-0.476 0.392]
HazardRatio:	0.959 [0.622 1.48]

N at Risk							
COPDAC	444	303	165	69	27	4	0
COPP	447	294	163	74	28	8	0

EuroNet-PHL-C1 DH 2012-10

EuroNet-PHL-C1
Radiotherapy question



Curves	Events	N
noRT	73	737
RT	73	856

Rate estimates at 36 months (95% CI)

noRT	0.861 [0.83 0.894]
RT	0.871 [0.842 0.901]

LogRank-Test: p= 0.4925

logHazardRatio:	-0.114 [-0.445 0.218]
HazardRatio:	0.893 [0.641 1.243]

N at Risk

noRT	737	510	303	140	49	8	0
RT	856	562	306	135	46	11	0

EuroNet-PHL-C1 DH 2012-10

Data in follow-up

Boys in DB: N=920

Boys and past puberty: (age≥16 or Tanner>3) N=479

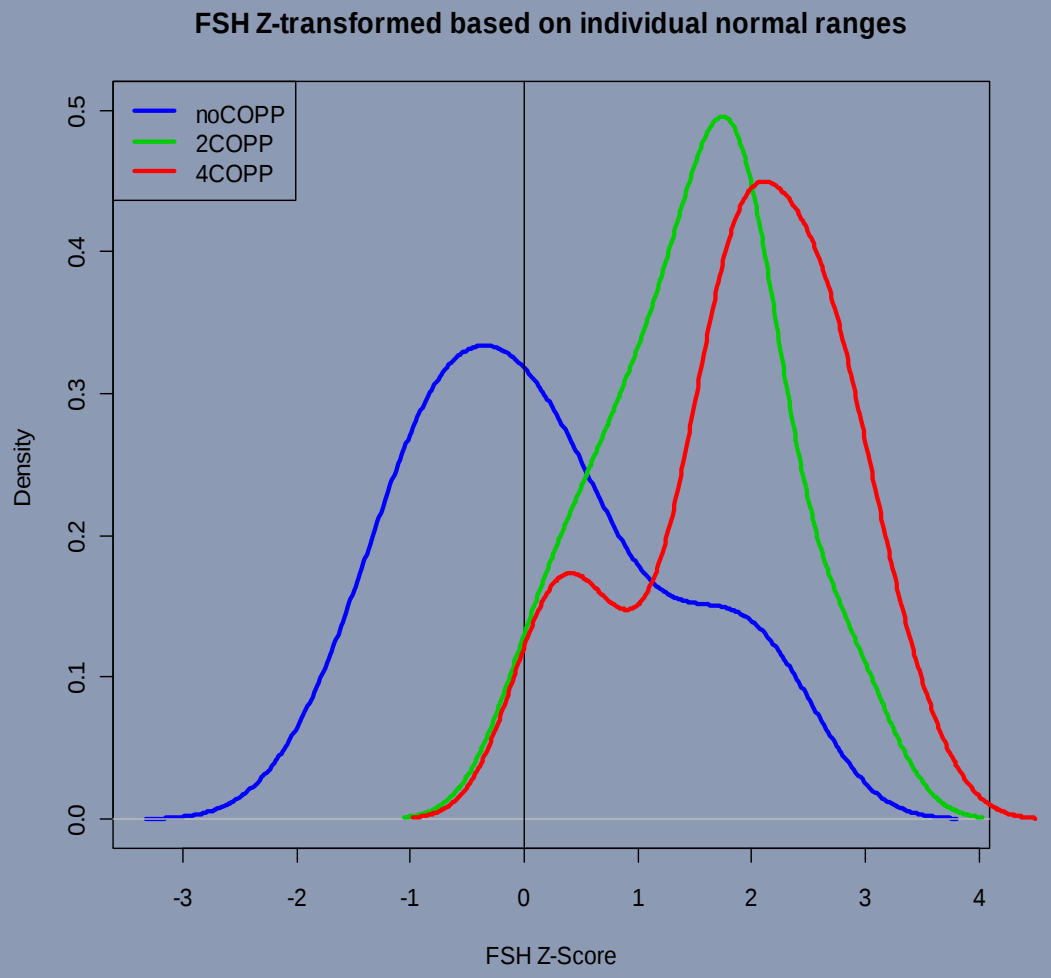
Boys, past puberty, > 6 months off chemo: N=335

Boys, past puberty, > 6 months off chemo and FSH: N= 67

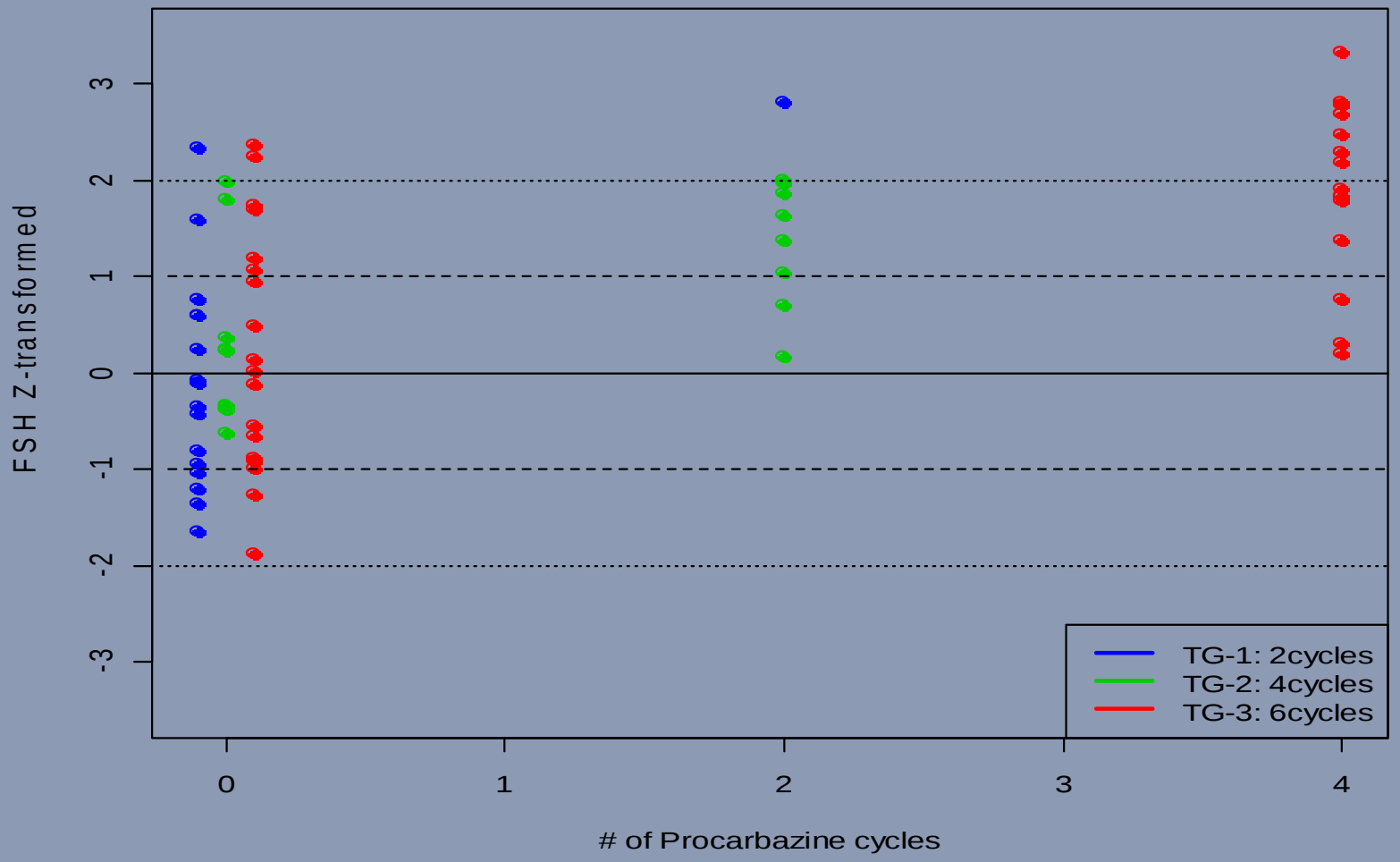
Evaluable on FSH: 67/335= 20%)

	TG-1	TG-2	TG-3	Sum
noCOPP	16	9	18	43
COPP	1*	8	15	24
Sum	17	17	33	67

Density plots suggest COPP effect



FSH Z score and exposure to procarbazine



Azoospermia rates

COPP 2/3

COPDAC 0/4

Males: Fertility preservation

Young men who can produce semen should have the opportunity of sperm banking before treatment begins

Sperm retrieval should be considered if the chances of infertility are high and the testes are >10mls

- Storage of gametes is governed by the HFE act 1990
- Written informed consent from a competent male is required

There is currently no established option to preserve fertility in the pre-pubertal boy….

Cryopreservation of pre-pubertal testis tissue prior to cancer treatment

Boys undergoing cancer treatment with >80% risk of infertility

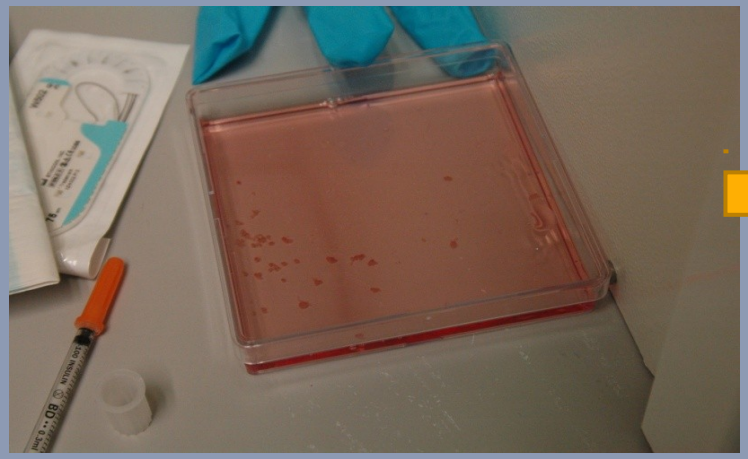
Biopsy to be taken with routine procedure

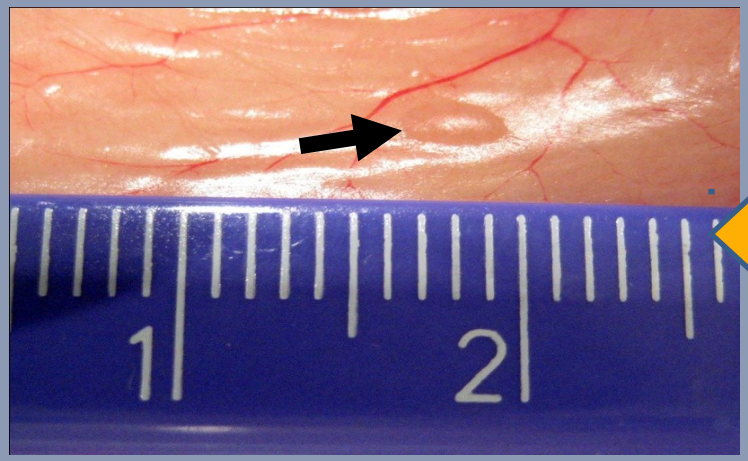
Storage by Tissue Services according to ‘mature’ or ‘immature’ protocol

Small piece of tissue to be used for research

Ethical Approval Granted – September 2013

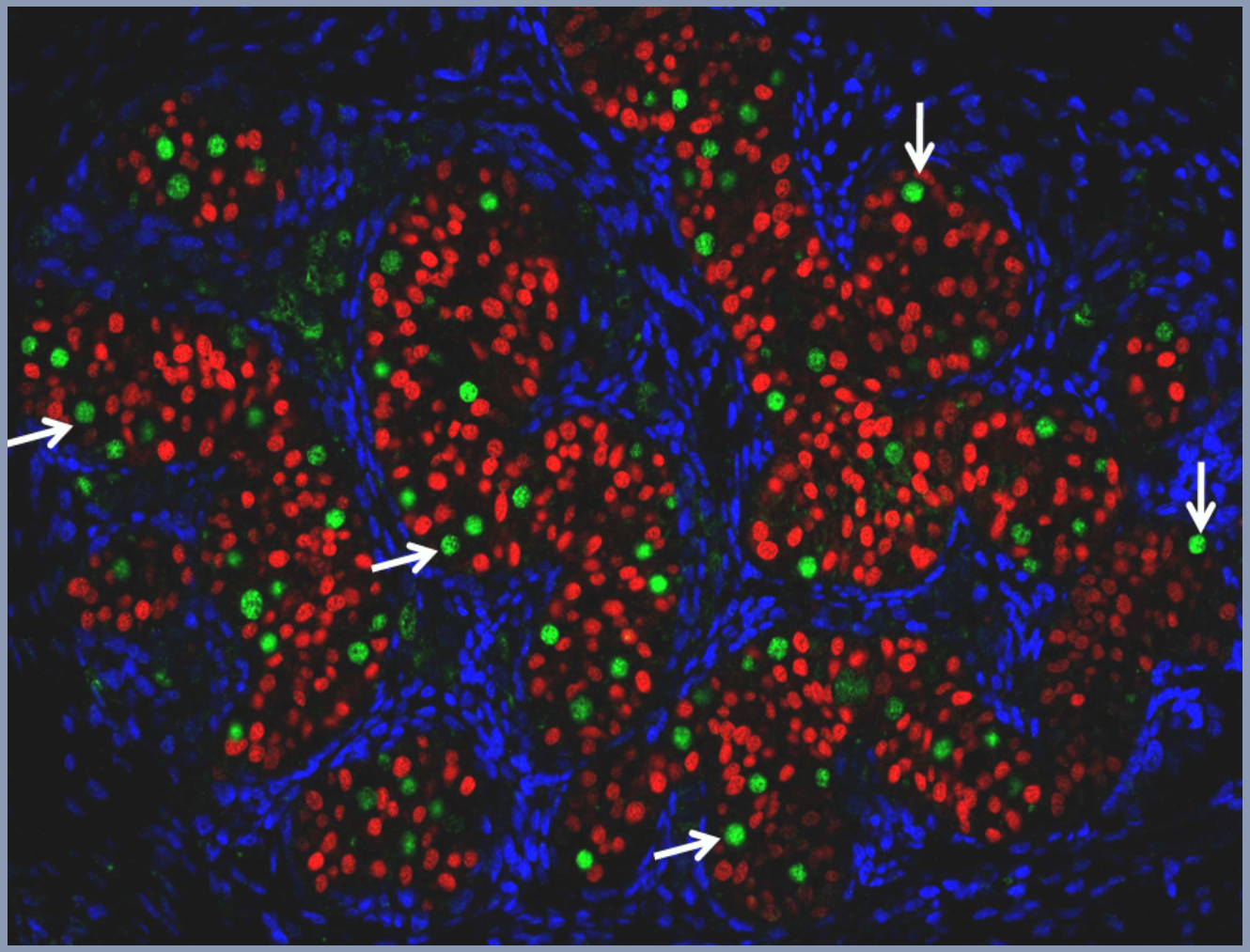
Human Testis Xenografting





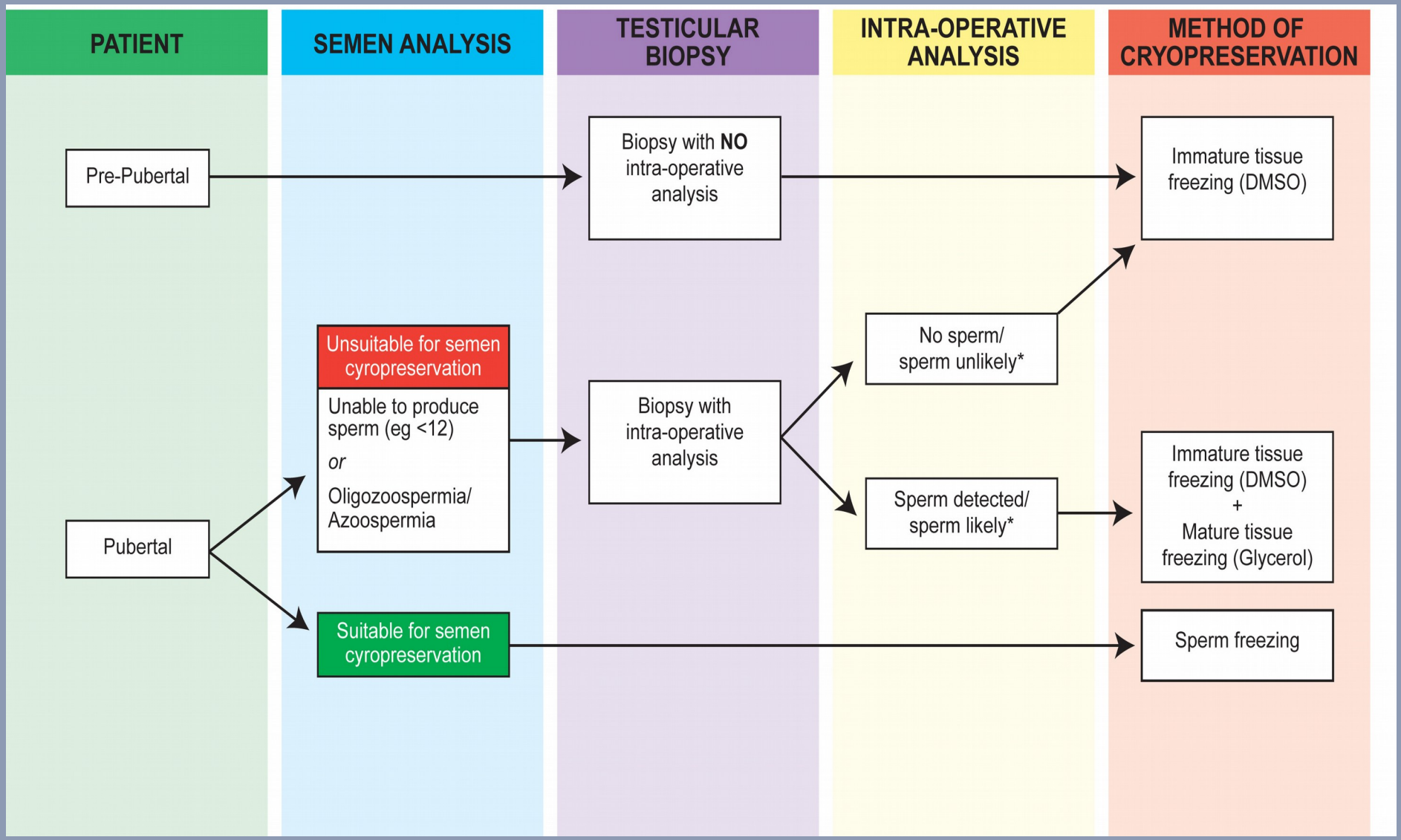
Human Testis Xenografts

Grafting Period – 6wks



Green = germ cells (arrows) Red = Sertoli cells

Algorithm for Tissue Cryopreservation



Challenges

Provide fertility counseling to all young patients with cancer

Cryopreserve ovarian and pre-pubertal testicular tissue from the right (high risk) patients

Define the success rate of the procedures

Develop IVG/M as a safe alternative to re-implantation through basic research

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Thank You

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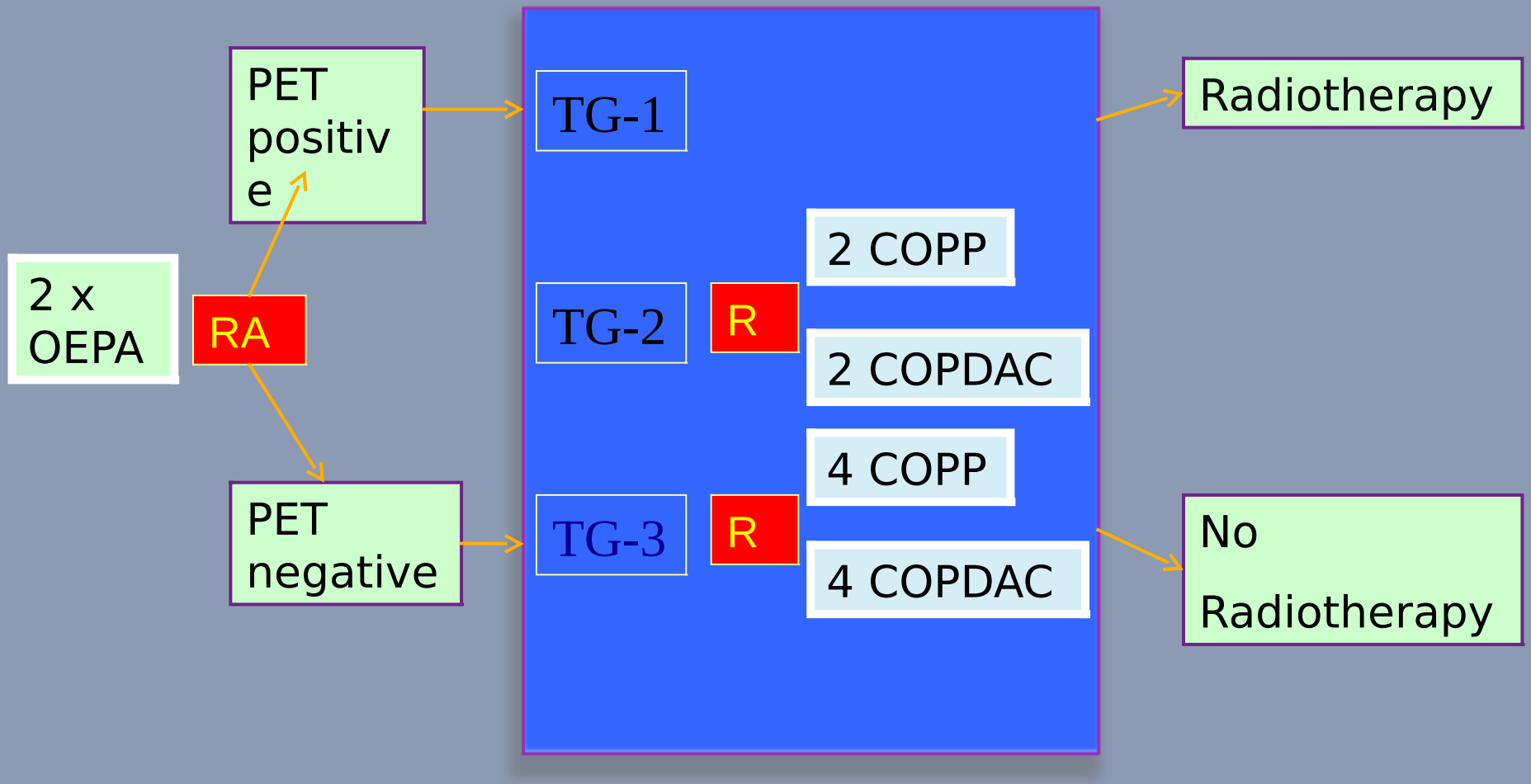

Wallace WH. UK Chief Investigator

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