

Research and Policy Needs for Environmental Health

January 23-26, 2017

The Israel Institute for Advanced Studies

Jerusalem

"It pays for more to prevent than to treat"

Bernardino Ramazzini, physician

Carpi 1633 - Padova 1714



Cesare Maltoni Cancer Research Center

Update on the Ramazzini Institute bioassays regarding ELFMF and RFR/MW

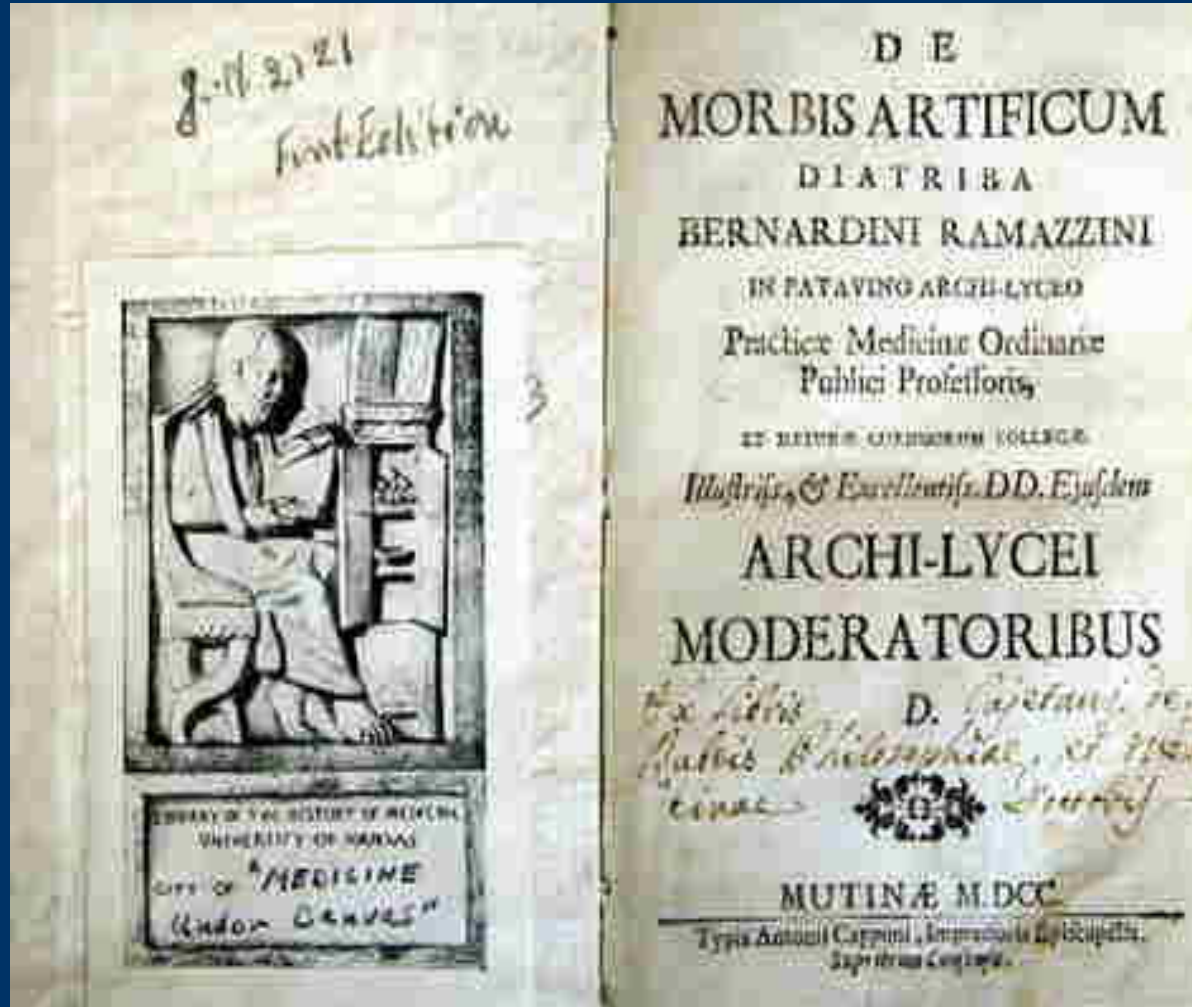
Fiorella Belpoggi
Director
Research Department
Ramazzini Institute
Bologna, Italy

My presentation deals with:

- Bernardino Ramazzini and the Institute
- SD rat: a human equivalent model
- ELF/MF results
- RFR/MW preliminary results



Carpi, 1633 – Padua, 1714



De morbis artificum diatriba, (1700)

The CMCRC (1969)

HISTORY...



Cesare Maltoni
(1930-2001)



Castle built in 1475
Domus Jucunditatis
of Bentivoglio family



...and
science



Ramazzini Institute

- The **Ramazzini Institute (RI)** is a **non-profit independent cooperative**; it is located in Bologna, Italy
- Our work principally involves strategies based on prevention of **environmental diseases**, in particular cancer
- The RI depends on support from more than **27,000 associates**, each year covering 30% of the RI budget
- The **budget is annually published on our website** and incomes include funds from national and international public institutions, liberal donations and contributions from private citizens and NGOs such as Environmental Health Trust



Ramazzini Institute: the aims

The **aims** of the Ramazzini Institute are:

- to implement schemes of tumour prevention by a strategy based on promotion of **scientific research**
- to **train** specialised staff
- to **circulate information** on environmental and work-related cancer risks, and
- to set up clinical programmes of **early tumour diagnosis**

Ramazzini Institute: the support of the Scientific Committee



Professor **PHILIP J. LANDRIGAN**, President, Professor of Pediatrics, Director, Children's Environmental Health Center Icahn School of Medicine at Mount Sinai, NY, NY, USA
<http://www.mountsinai.org/profiles/philip-j-landrigan>



Dr. **KRISTINA THAYER**, EPA, IRIS Division Director



Dr. **PIETRO COMBA**, Director, Unit of Environmental Epidemiology, National Institute of Health, Italy
<http://www.iss.it/chis/index.php?lang=1&tipo=13&chis=&pid=621>



Dr. **MELISSA McDIARMID**, Professor of Medicine and Director of the University of Maryland School of Medicine's Occupational Health Program, USA
<http://findadoctor.umm.edu/Detailes/23375>



Professor **ELLEN SILBERGELD**, Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MA, USA
http://www.jhsph.edu/research/centers-and-institutes/johns-hopkins-center-for-a-livable-future/research/inves_profiles/profiles/silbergeld.html



Dr. **LENNART HARDELL**, Professor of Oncology and Epidemiology, Orebro, Sweden.
<http://www.lennarthardellenglish.wordpress.com/>



Dr. **MARIE-NOEL BRUNE DRISSE**, Uruguay. Children Health Department, WHO <http://www.excellence-in-paediatrics.org/content/page/292/childrens-environmental-health>

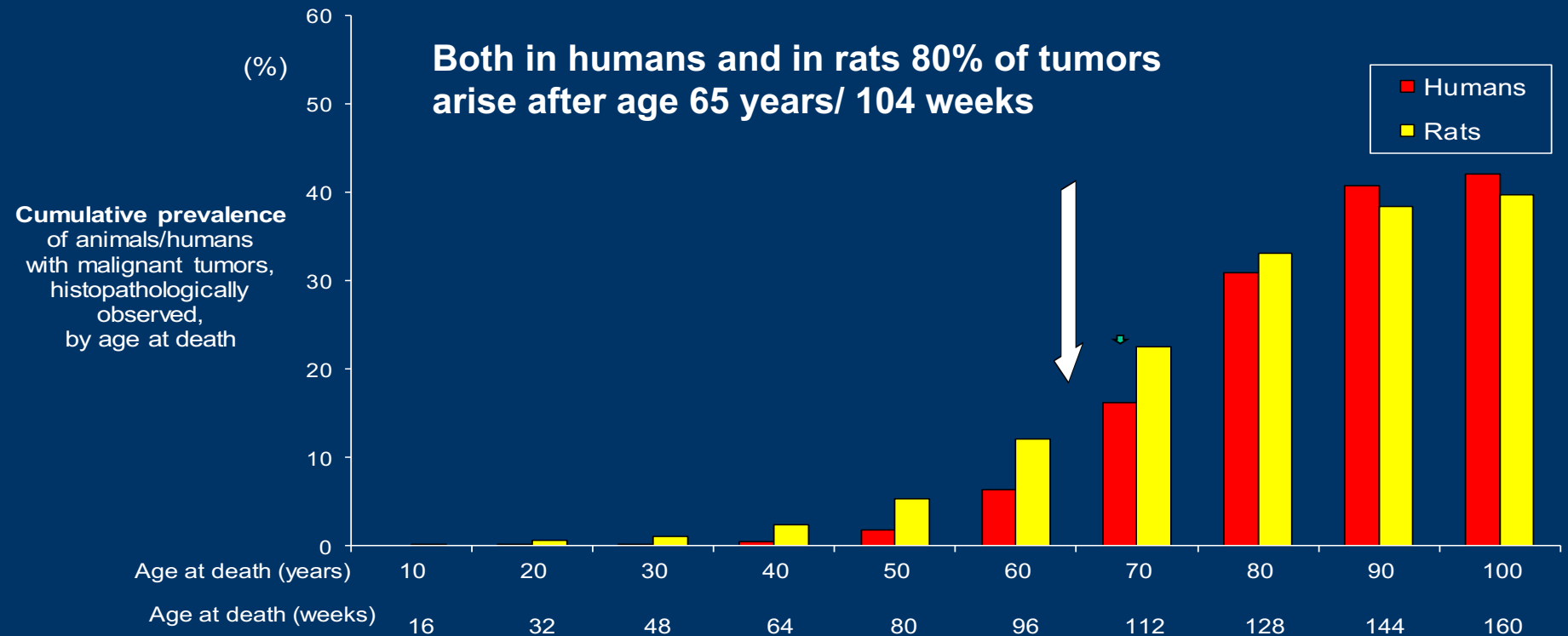
Peculiarity:
The human equivalent model

The Animal Model of The Ramazzini Institute



Sprague-Dawley rats

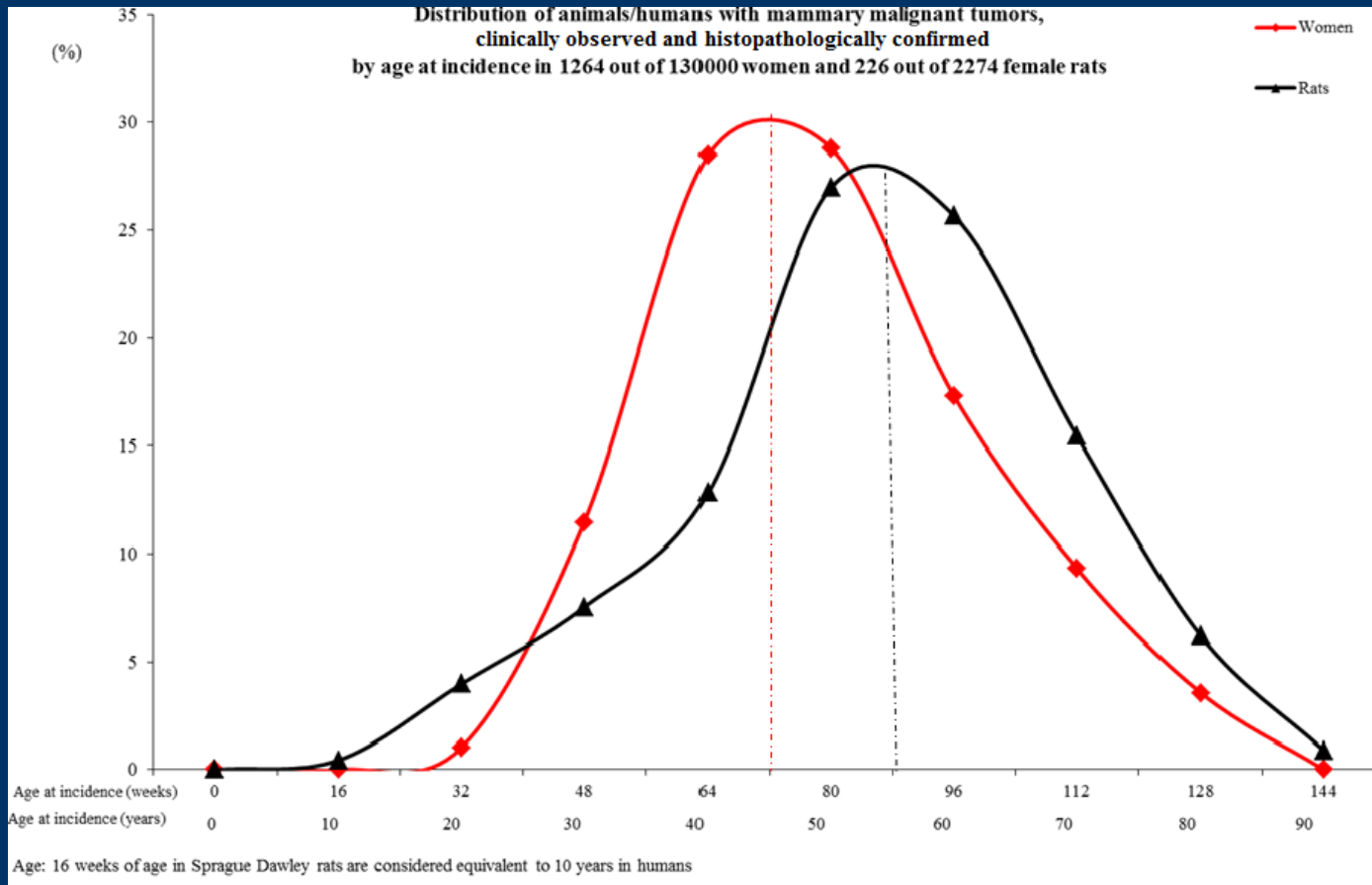
A HUMAN-EQUIVALENT MODEL



Age: 16 weeks of age in Sprague Dawley rats are considered equivalent to 10 years in humans

Data from the Hospital of Trieste were kindly made at our disposal by Professor Luigi Giarelli

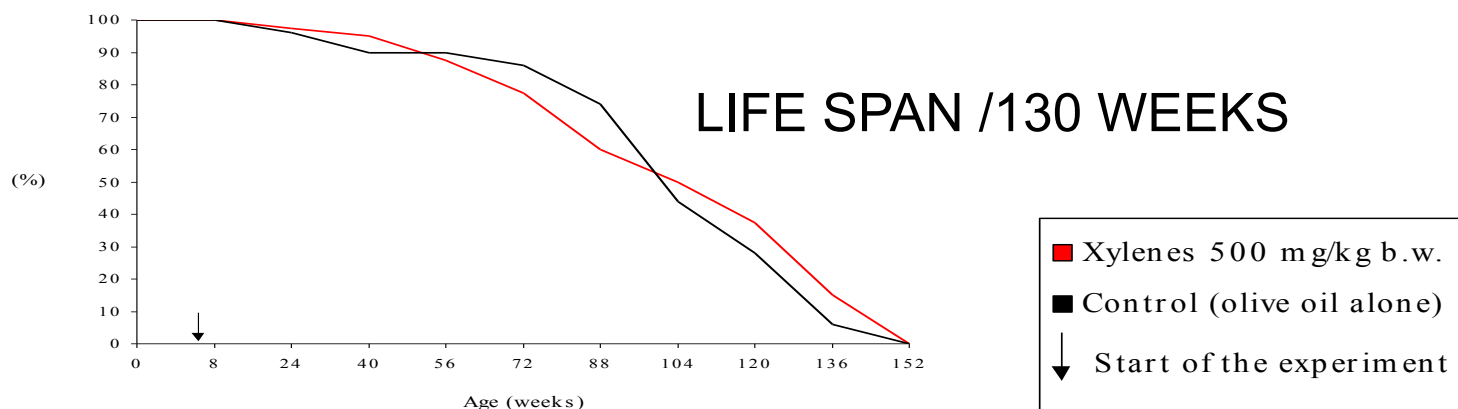
Human-equivalent model: Malignant Mammary Tumors



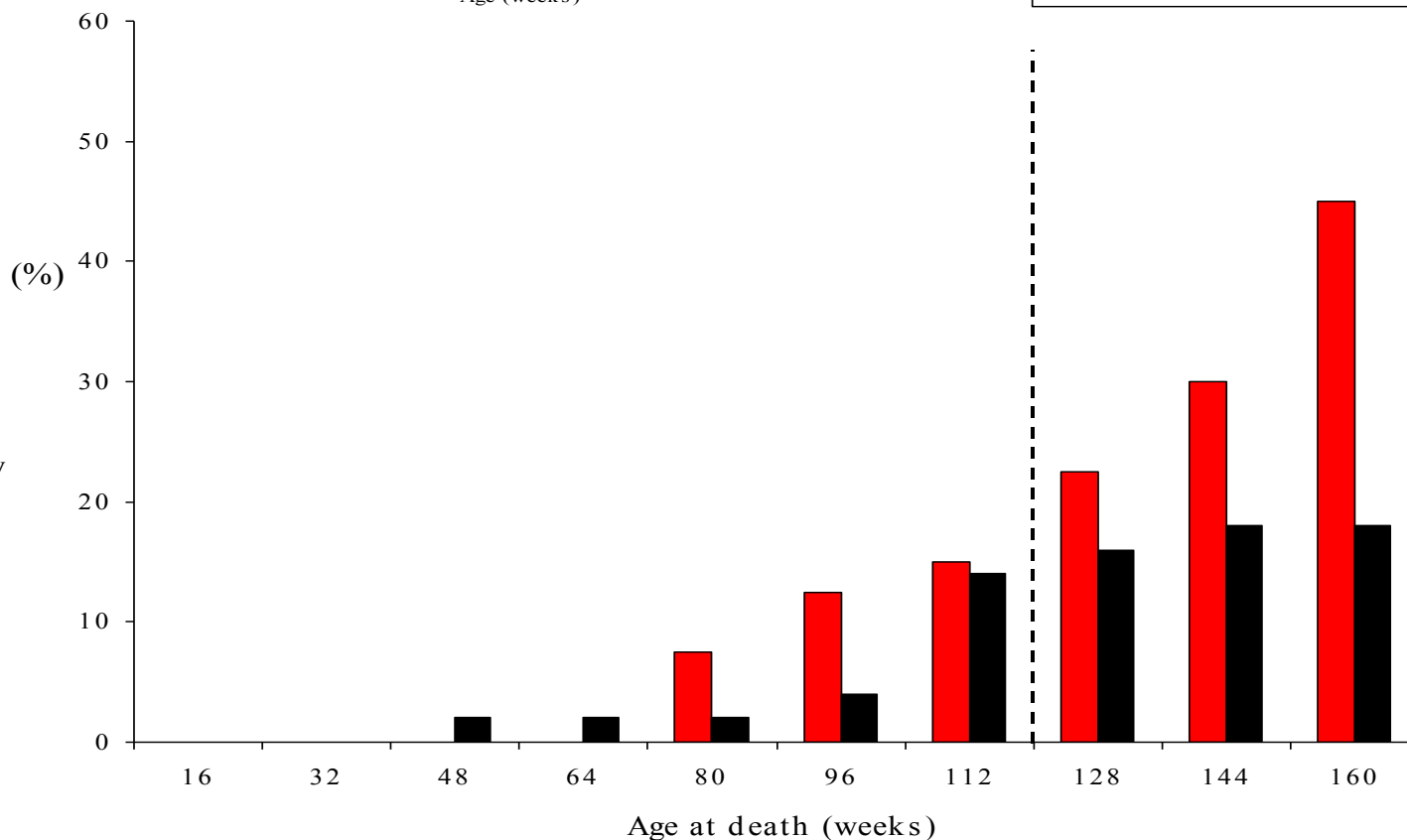
The curves follow the same pattern, the most frequent age of onset has a peak around 80-90 weeks in the female rat, corresponding to around 50-60 years in women.

- Results from the screening for breast cancer early detection, performed for women in the Bologna Province, compared to female SD rats of our historical controls groups
- The data are distributed by age at the time of the first clinical observation; diagnosis was histopathologically confirmed

Survival



Cumulative prevalence of total malignant tumors histopathologically observed, by age at death



Total malignant tumours in Sprague-Dawley, male rats

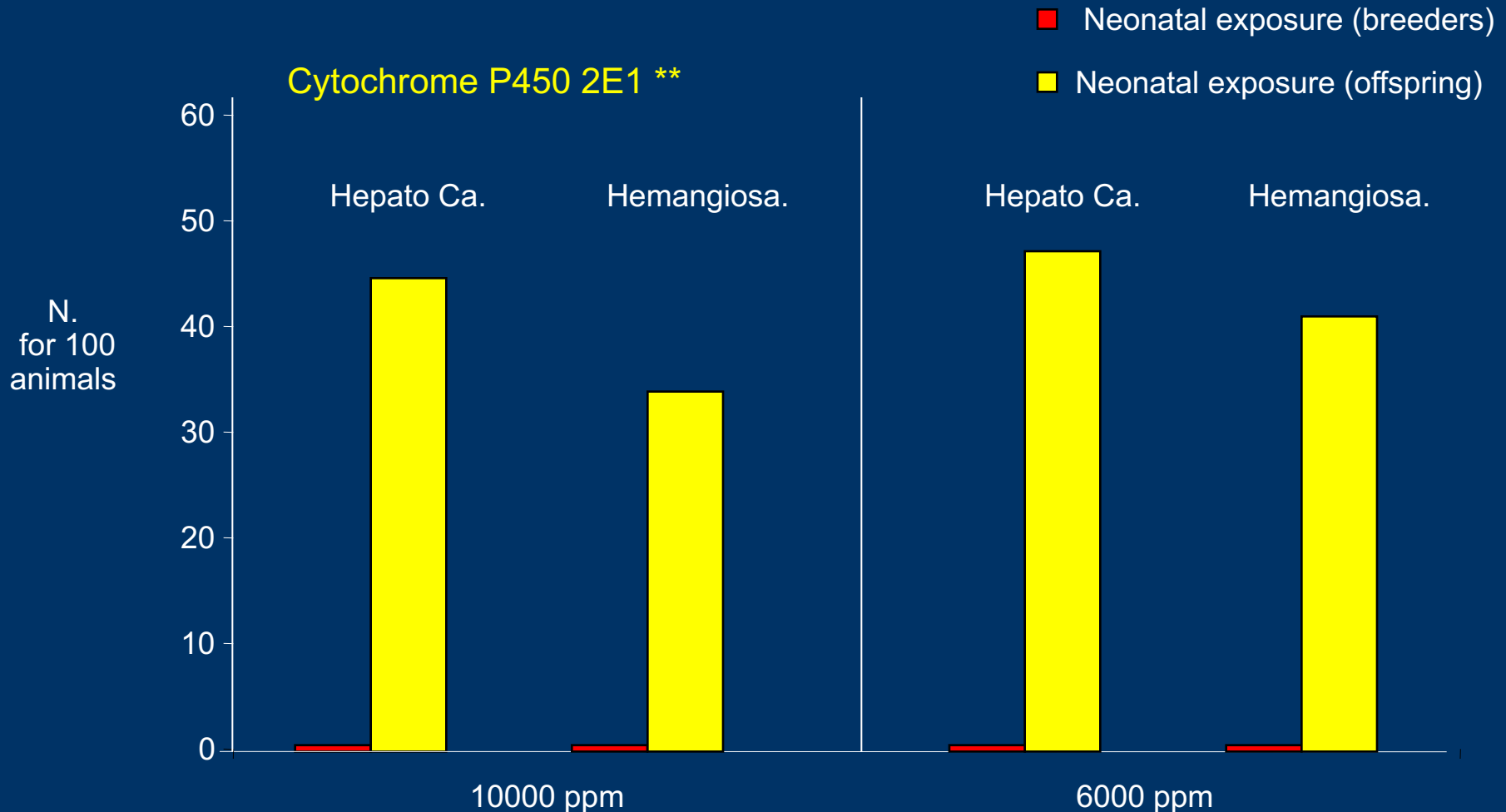
Xylenes

VINYL CHLORIDE: comparison of neonatal exposure in breeders and offspring

SPRAGUE-DAWLEY RATS (Exp. BT14, 1976)*

Treatment: 4hd/ 5dw/ 5w

Cytochrome P450 2E1 **



* Maltoni et al, 1981

** El Ghissassi,, Barbin, and Bartsch, 1998; Novak and. Woodcroft ,2000

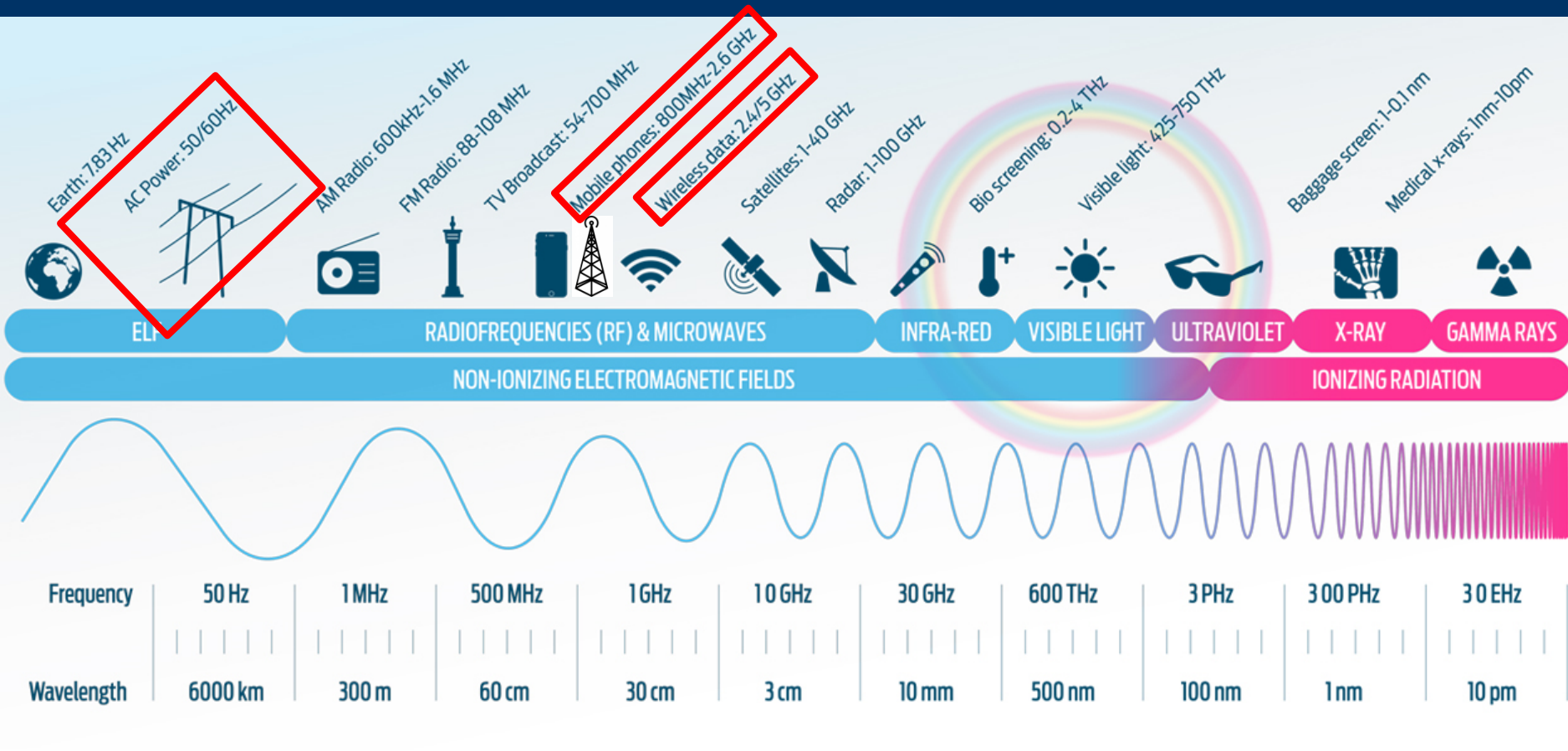
Predictivity of the RI study results

Agent/Compound	CMCRC-RI*	IARC Group/year	Predictivity
Vinyl Chloride	1974	1 /1979	5 years
Formaldehyde	1989	1 /2012	23 years
Trichloroethylene	1986	1 /2014	28 years
Benzene	1979	1 /2012	33 years
MTBE	1995	3 /1999	?
Aspartame	2005	?	?

***first evidence as multipotent carcinogen**

What studies has the
Ramazzini Institute
performed on ELFMF and
RFR?

RFR/MW in vivo studies



Integrated experimental project on Extremely Low Frequency Magnetic Fields (ELFMF)

ELFMF: exposure system



The **ELFMF exposure system** was represented by several independent toroidal-shaped magnetic devices with a wood support structure inside to support animal cages. Electricity flows in the wires all around the toroids and generates a MF inside the structure, avoiding any interference problems between the single device.

ELFEMF: exposure system



The probe of each device was linked to a dedicated personal computer and was able to show and register the magnetic field continuously

ELFMF: Long-term bioassays experimental project

All experiments started at the 12th day of pregnancy and continued life-span

Experiment	Number of animals (M+F)	Treatment	State of the art
BT 1 CEM	5,029	ELFMF S-50 Hz	Publication in progress <u>First results today</u>
BT 2 CEM	805	ELFMF S-50 Hz + formaldehyde	Published
BT 3 CEM	657	ELFMF S-50 Hz + γ - radiation (0.1 Gy)	Published
TOTAL	6,491		

Effects of ELFMF alone (BT1 CEM)



ELFMF alone (BT1CEM): experimental plan

Group	Animals		Treatment	
	Sex	No.	ELFMF S-50 Hz (μ T)	Schedule ^a
I	M+F	523	1000	C
II	M+F	500	1000	O/O
III	M+F	1000	100	C
IV	M+F	1003	20	C
V	M+F	1002	2	C
VI	M+F	1001	0 ^b	-
Total		5029		

^a Treatment for 19 hours/day, continuous (C) or intermittent On/Off (O/O)

^b The control group is common among the experiments BT 1-2-3 CEM

ELFMF alone (BT1CEM): preliminary results

- No increased incidence of benign and/or malignant tumours was observed in Sprague Dawley rats treated with ELFMF alone compared to control
- Statistical evaluation is still ongoing
- Publication of data is planned for 2017

CONCLUSIONS:

The results of our study show that, in our experimental conditions,
ELFMF alone are not carcinogenic

synergic effects of
ELFMF + formaldehyde exposure
(BT2CEM)

Published in 2016

ELFMF + formaldeide

AMERICAN JOURNAL OF INDUSTRIAL MEDICINE

Synergism Between Sinusoidal-50 Hz Magnetic Field and Formaldehyde in Triggering Carcinogenic Effects in Male Sprague–Dawley Rats

Morando Soffritti, MD,^{1*} Eva Tibaldi, PhD,¹ Michela Padovani, MPH,¹ David G. Hoel, PhD,²
Livio Giuliani, PhD,³ Luciano Bua, MD,¹ Michelina Lauriola, PhD,¹ Laura Falcioni, DMV,¹
Marco Manservigi, PhD,¹ Fabiana Manservigi, PhD,¹ and Fiorella Belpoggi, PhD¹

Background *Experimental rodent bioassays performed up to now have failed to provide conclusive confirmation of the carcinogenicity of extremely low frequency magnetic fields (ELFMF).*

Objectives *To evaluate the potential synergistic carcinogenic effects of concurrent exposure to ELFMF and formaldehyde in four groups of male and female Sprague–Dawley rats.*

Methods *One group was exposed from prenatal life until natural death to S-50 Hz MF and to formaldehyde in drinking water from 6 weeks of age for 104 weeks, two groups were treated only with formaldehyde or only with MF and one group served as untreated control.*

Results *Compared to untreated controls, exposure to MF and formaldehyde causes in males a statistically significant increased incidence of malignant tumors ($P \leq 0.01$), thyroid C-cell carcinomas ($P \leq 0.01$), and hemolymphoreticular neoplasias ($P \leq 0.05$). No statistically significant differences were observed among female groups.*

Conclusions *Life-span exposure to MF and formaldehyde induces statistically significant carcinogenic effects in male rats. Am. J. Ind. Med.*

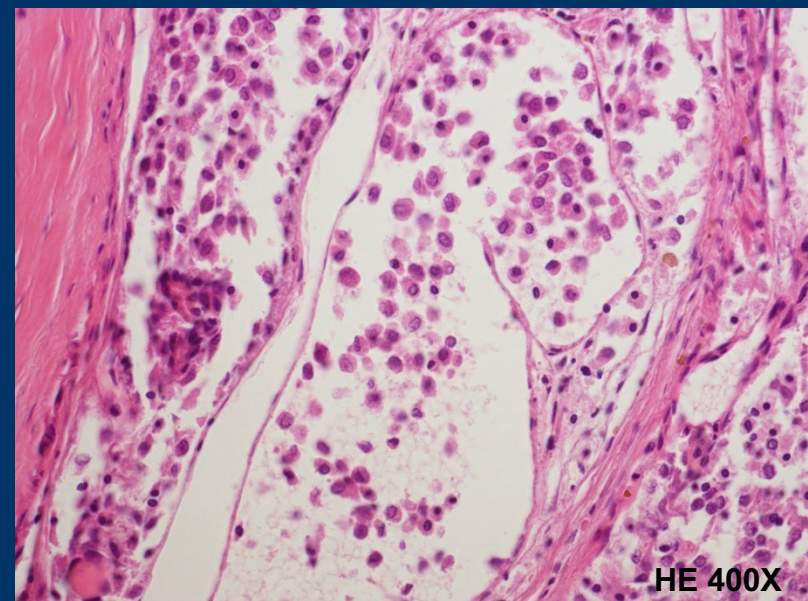
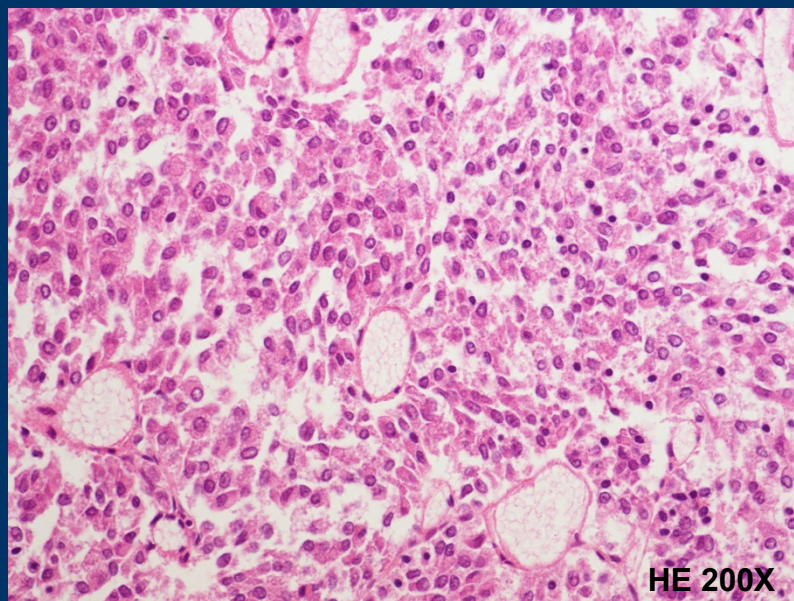
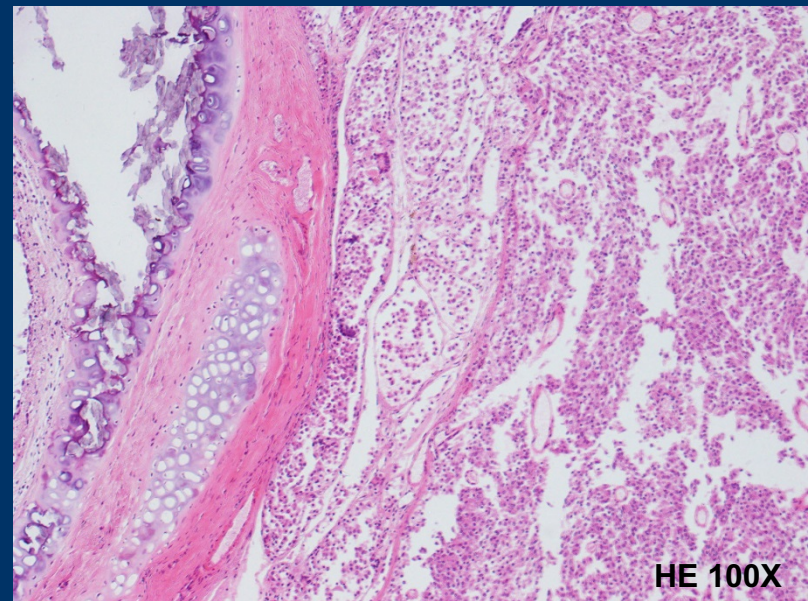
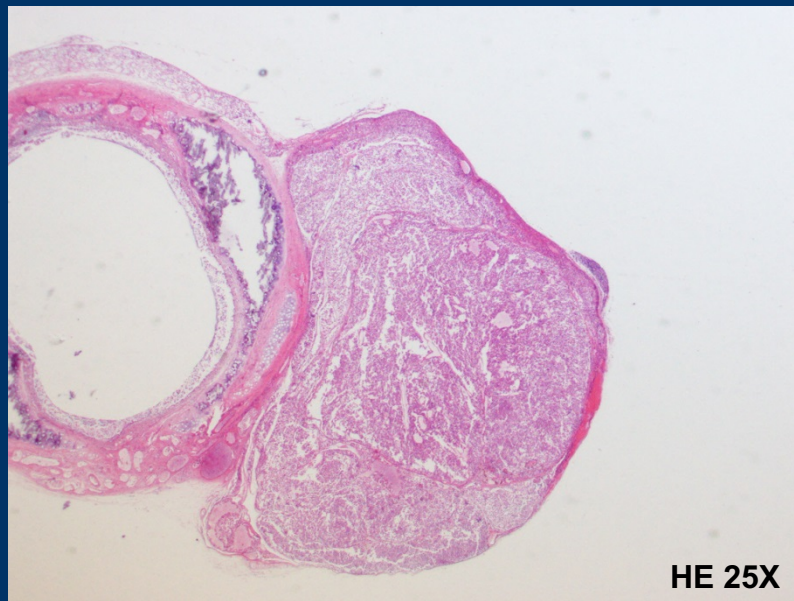
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ELFMF + formaldehyde: synergistic effects

- Statistically significant increase of carcinogenic effects in male Sprague-Dawley rats, namely in **total malignant tumors**, **C-cell carcinomas** and **lymphomas/leukemias**, compared to negative controls;
- Statistically significant increase of **C-cell carcinomas** and **lymphomas/leukemias**, compared to male Sprague-Dawley rats exposed to MF alone (positive control);
- Concerning C-cell carcinomas, our results **reinforce the biological significance** of the same increased incidence observed by US/NTP and classified as an equivocal finding (Boorman et al, 1999)

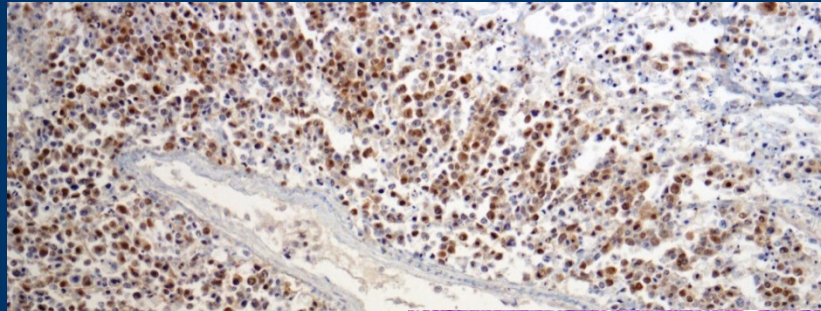
ELFMF + Formaldehyde: results

Thyroid C-cell Carcinoma

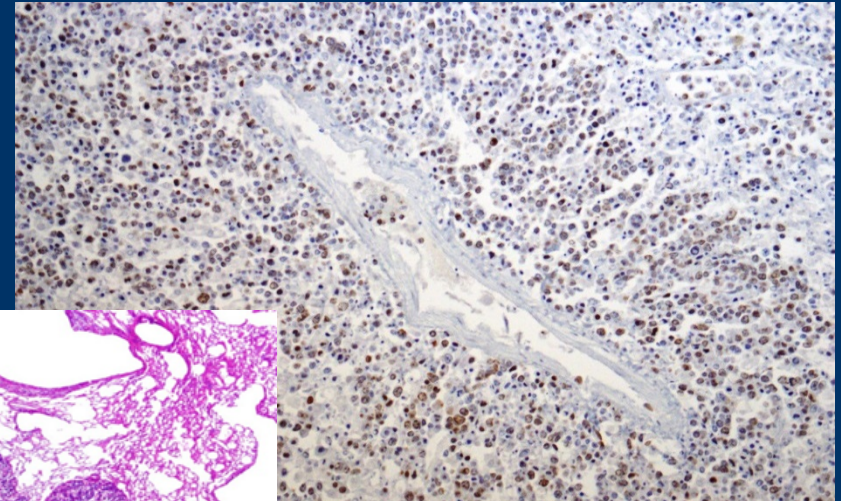


ELFMF + Formaldehyde: published results

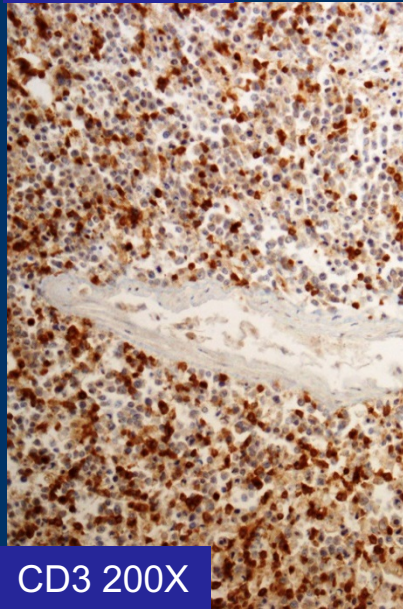
IHC characterization of Lymphomas



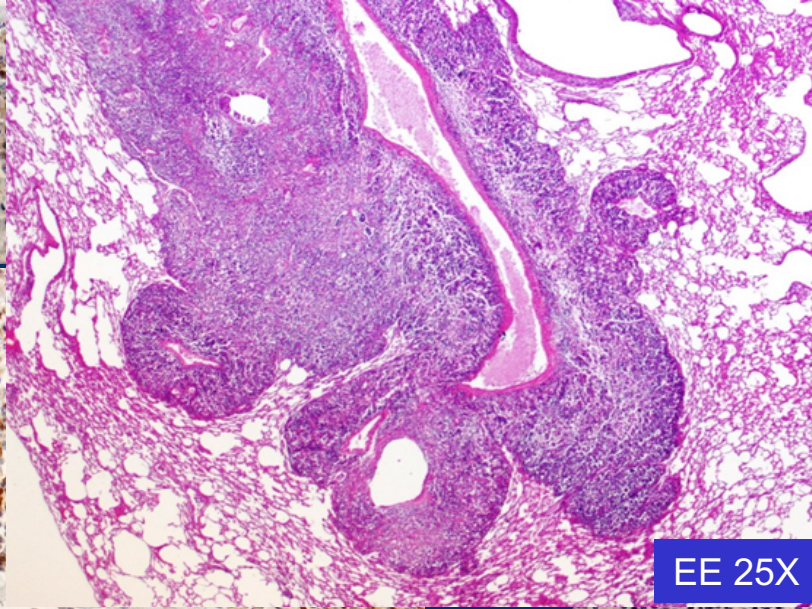
PAX5 200X



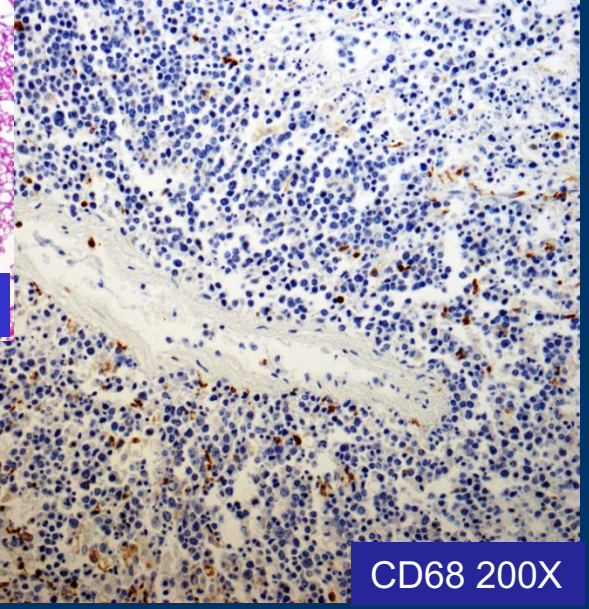
Ki67 200X



CD3 200X



EE 25X



CD68 200X

ELFMF + formaldehyde

CONCLUSIONS:

Our results confirm that in Sprague Dawley rats the carcinogenic potential of a known chemical carcinogen is enhanced by the interaction with ELFMF

Synergic effects of ELFMF +
 γ radiation
(BT3CEM)

Published in 2016

RESEARCH ARTICLE

Life-span exposure to sinusoidal-50 Hz magnetic field and acute low-dose γ radiation induce carcinogenic effects in Sprague-Dawley rats

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^aCesare Maltoni Cancer Research Center, Ramazzini Institute, Castello di Bentivoglio, Bentivoglio, Bologna, Italy; ^bMedical University of South Carolina, Charleston, SC, USA; ^cNational Institute for Insurance Against Injuries at Work (INAIL), Firenze, Italy

ABSTRACT

Background In 2002 the International Agency for Research on Cancer classified extremely low frequency magnetic fields (ELFMF) as a possible carcinogen on the basis of epidemiological evidence. Experimental bioassays on rats and mice performed up to now on ELFMF alone or in association with known carcinogens have failed to provide conclusive confirmation.

Objectives To study the carcinogenic effects of combined exposure to sinusoidal-50 Hz (S-50Hz) magnetic fields and acute γ radiation in Sprague-Dawley rats.

Methods We studied groups of male and female Sprague-Dawley rats exposed from prenatal life until natural death to 20 or 1000 μ T S-50Hz MF and also to 0.1 Gy γ radiation delivered as a single acute exposure at 6 weeks of age.

Results The results of the study showed significant carcinogenic effects for the mammary gland in males and females and a significant increased incidence of malignant schwannomas of the heart as well as increased incidence of lymphomas/leukemias in males.

Conclusions These results call for a re-evaluation of the safety of non-ionizing radiation.

ARTICLE HISTORY

Received 19 June 2015

Revised 5 November 2015

Accepted 8 January 2016

KEYWORDS

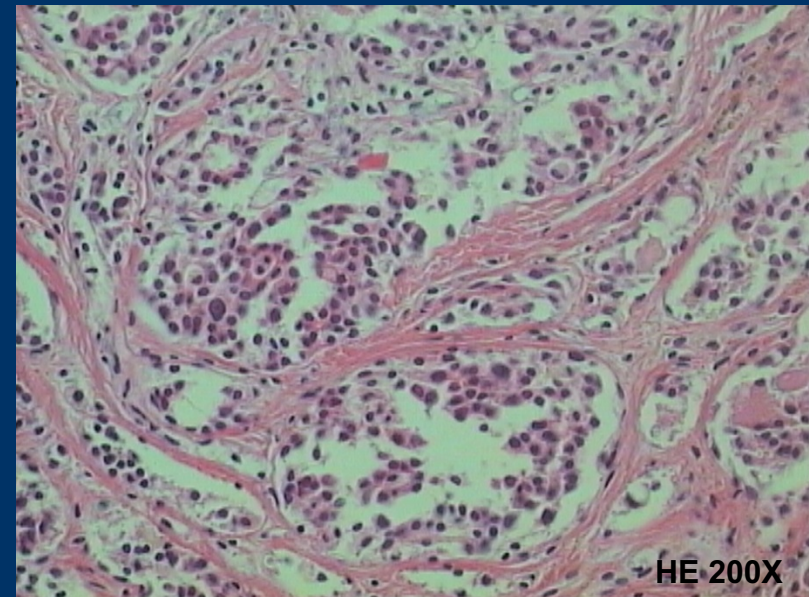
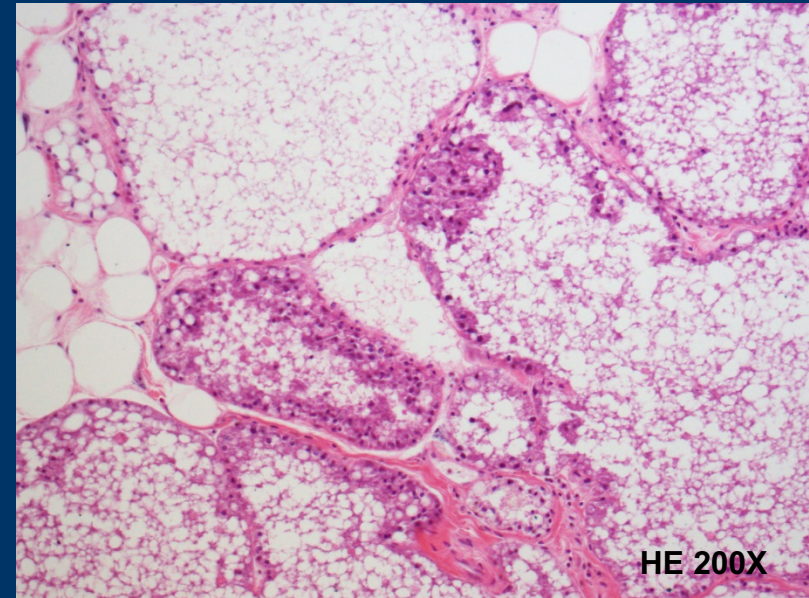
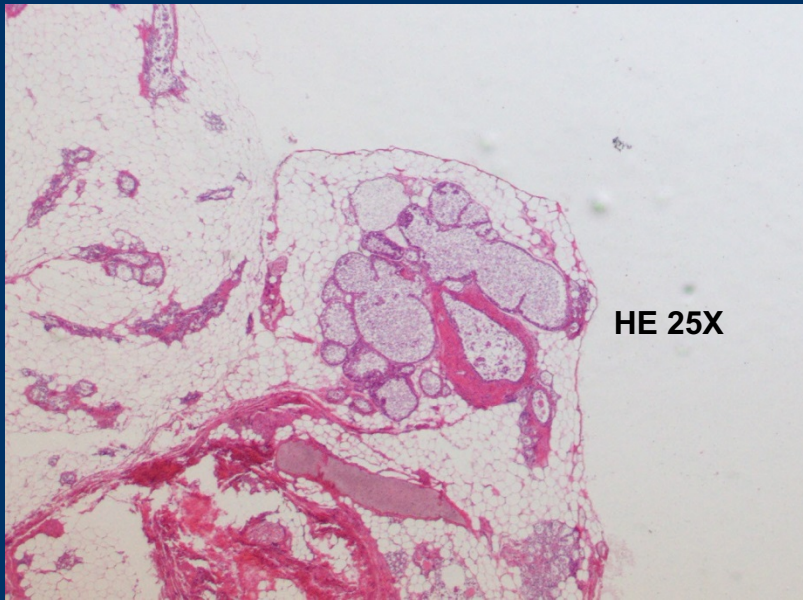
S-50Hz MF carcinogenicity; cancer promotion; Sprague-Dawley rats; life-span bioassays; breast cancer; hemolymphopoietic neoplasias; heart malignant schwannomas

ELFMF S-50 Hz + γ radiation exposure: synergistic effects

- a significant dose-related increased incidence of **mammary adenocarcinomas** in male and female rats compared to negative control
- a dose-related increased incidence of **heart malignant Schwannomas** in male rats compared to negative control
- a significant increased incidence of **lymphomas/leukemias** in male compared to negative control
- a significant increased incidence of **mammary adenocarcinomas** in female and **lymphomas/leukemias** in male compared to positive control (γ -radiation)

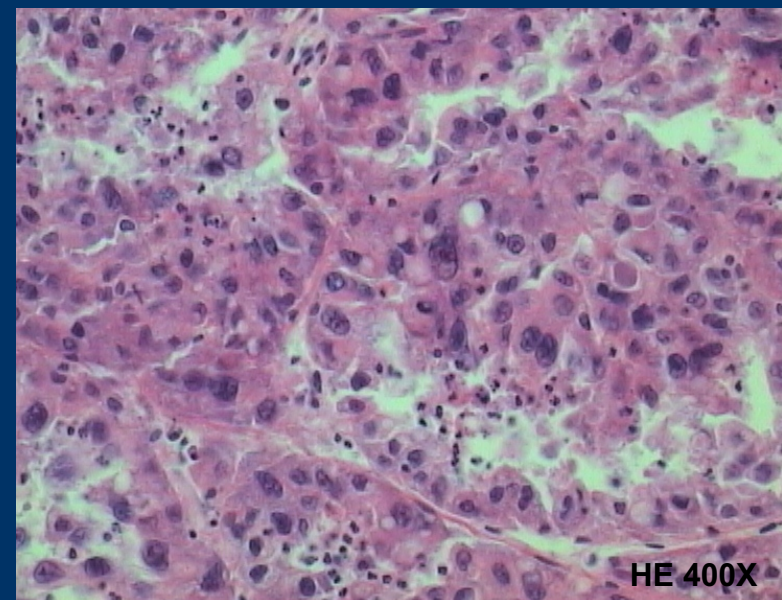
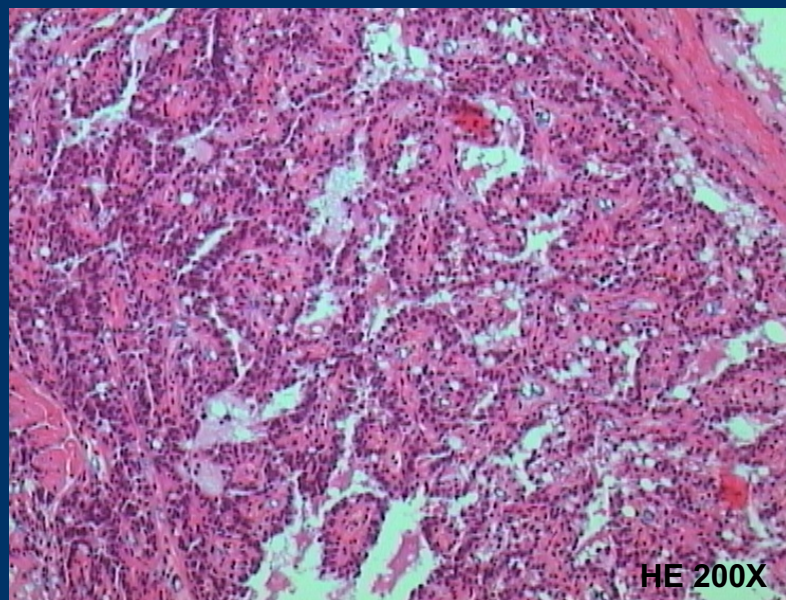
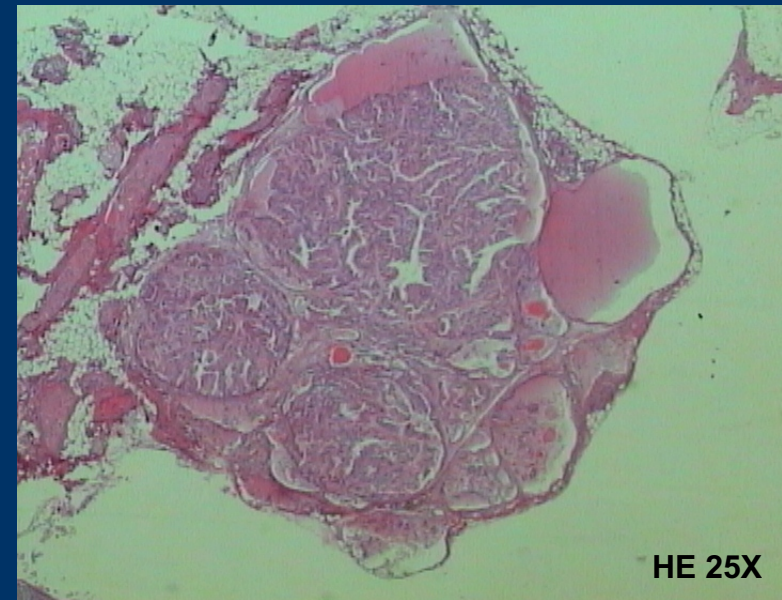
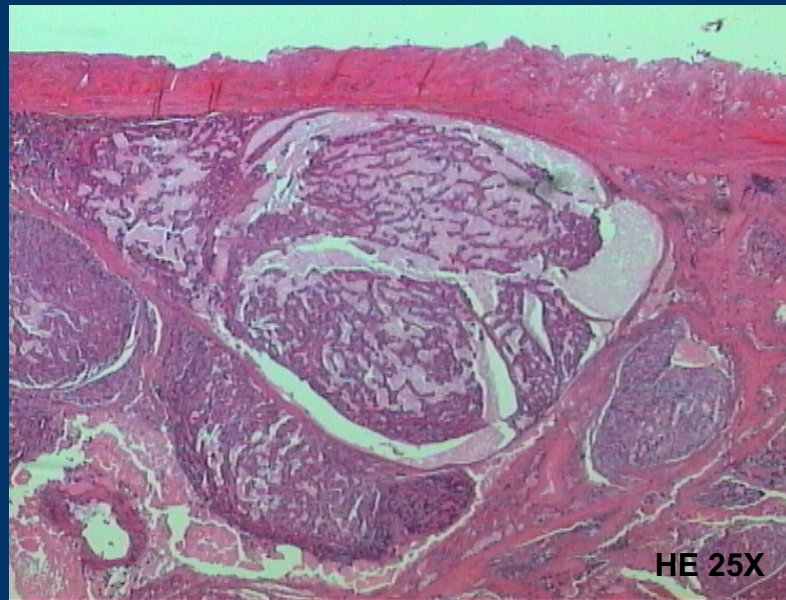
ELFMF + γ radiation: published results

Mammary gland hyperplasia with atypia



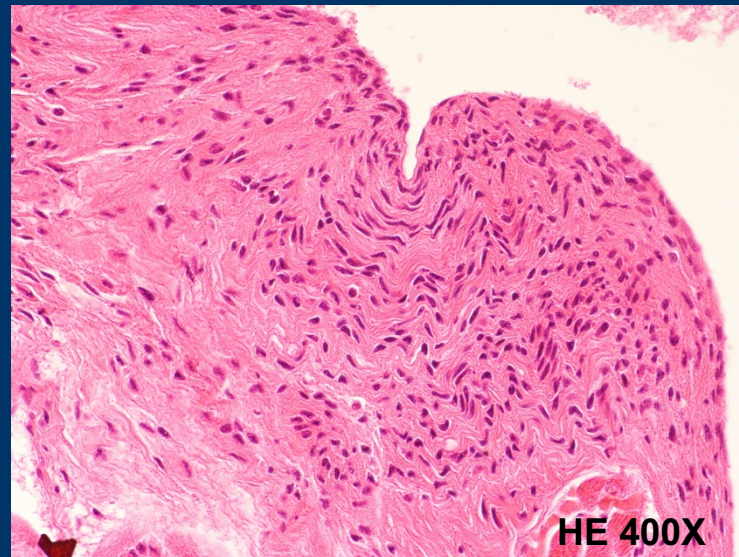
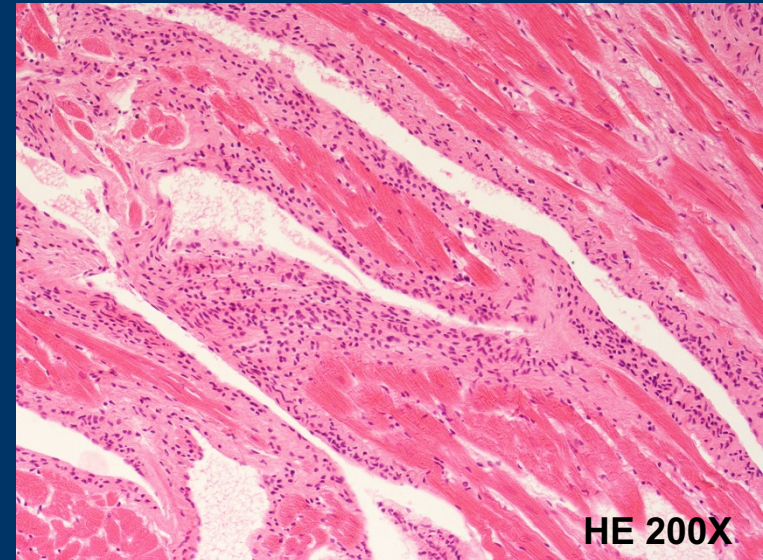
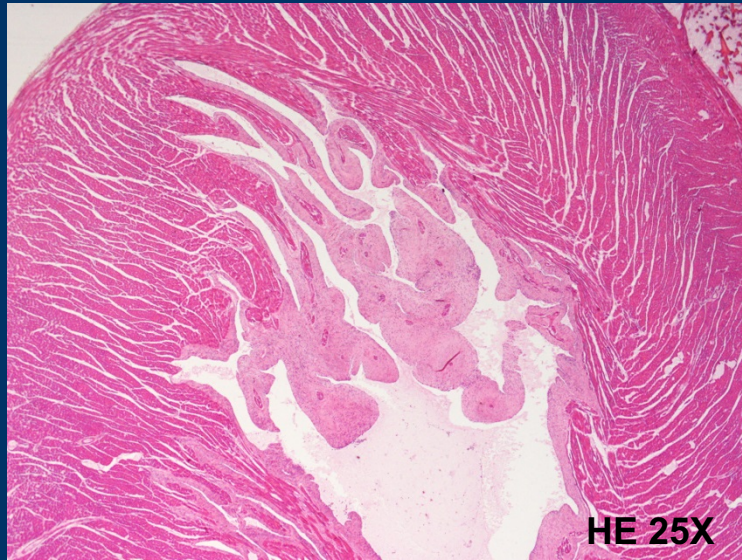
ELFMF + γ radiation: published results

Mammary gland Adenocarcinoma



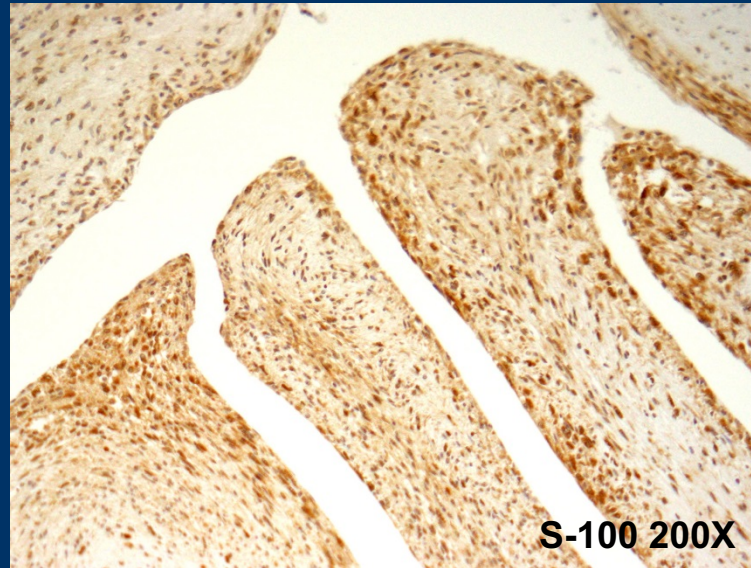
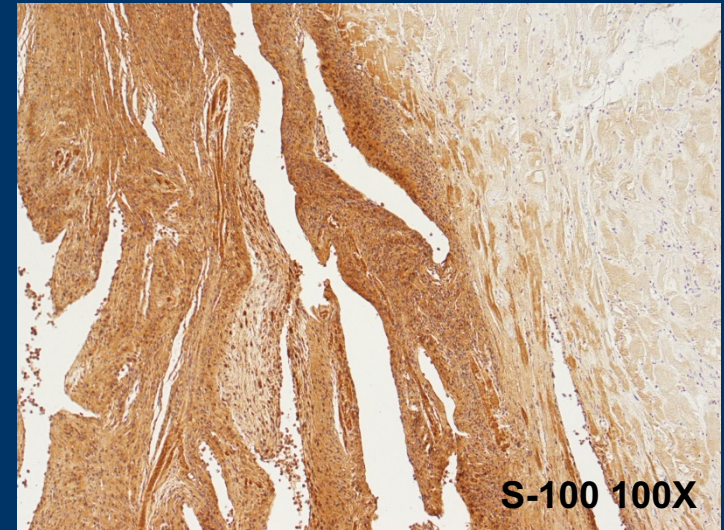
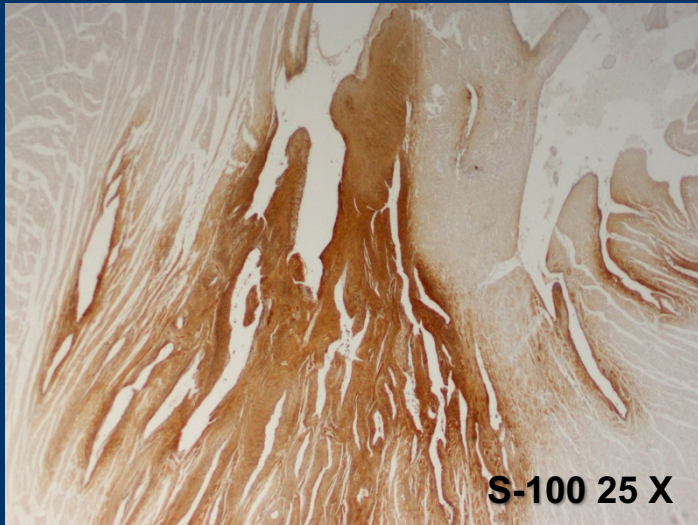
ELFMF + γ radiation: published results

Heart Malignant Schwannoma

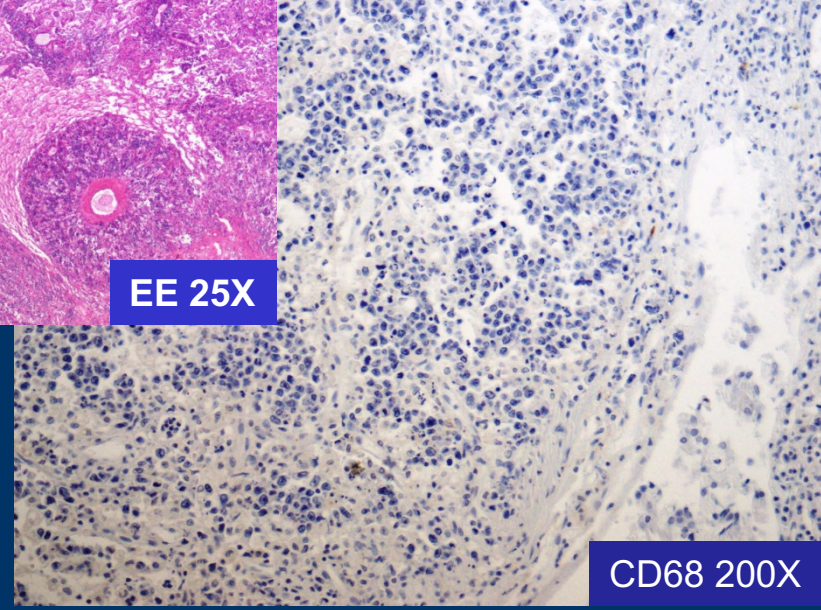
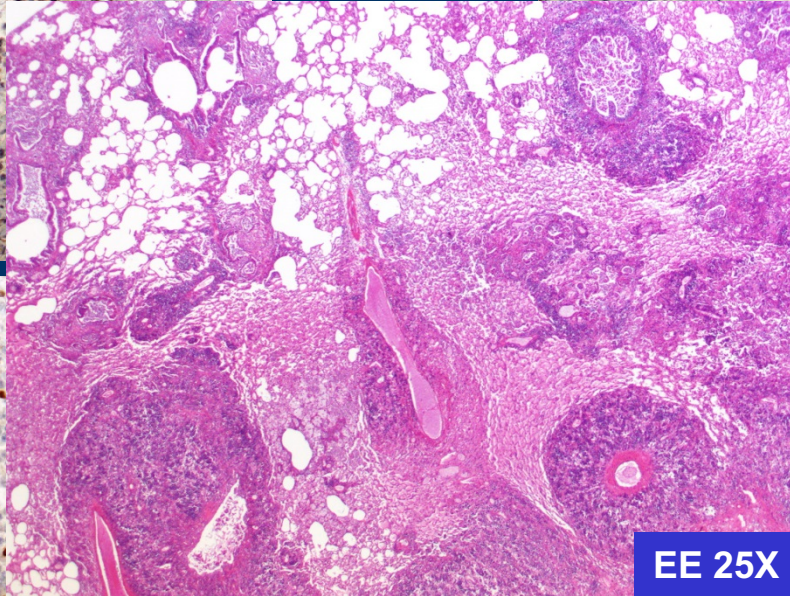
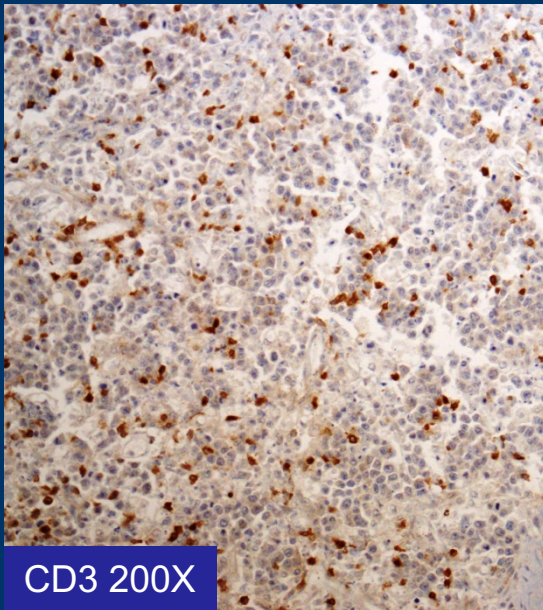
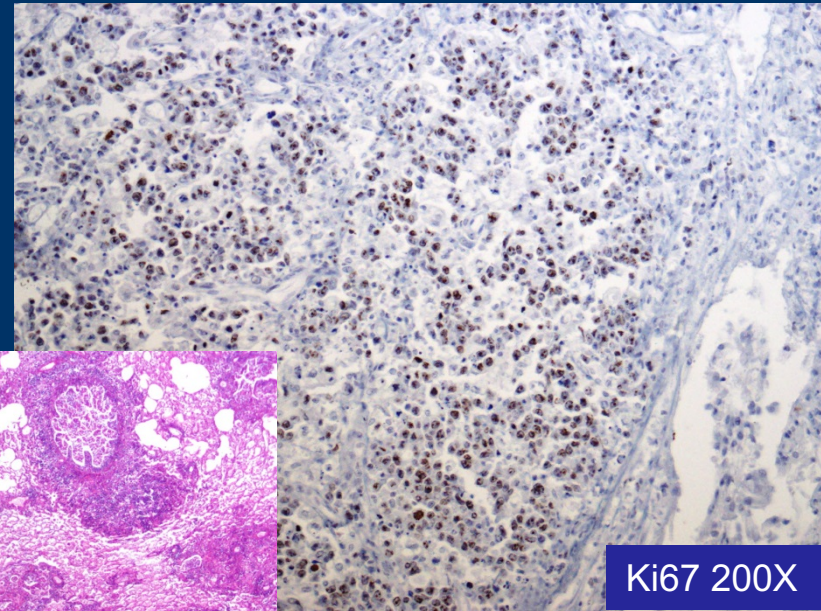
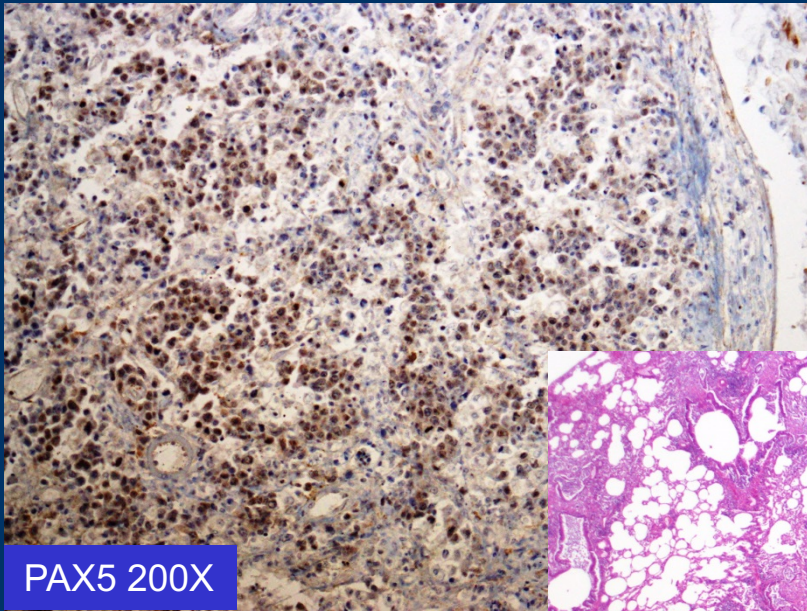


ELFMF + γ radiation: published results

IHC characterization of Schwannoma



ELFMF + γ radiation: IHC characterization of Lymphomas



ELFMF S-50 Hz + γ radiation exposure: Synergic carcinogenic effects

CONCLUSIONS:

The results of our study demonstrate, for the first time, that exposure to ELFMF S-50 Hz, from prenatal life until natural death, enhances the carcinogenic effects of γ -radiation in male and female Sprague-Dawley rats

Integrated experimental
project on Radiofrequency
Radiation
(RFR/MW)

First presentation of results

Radio Frequency radiation (RFR/MW) *in vivo* studies

NOMINATION



The nomination for NTP to study cell phone RF radiation was made by the U.S. Food and Drug Administration

Animals were exposed to frequencies and modulations currently used in cellular communications in the United States



The nomination for the RI study was performed on the basis of the number of people exposed and the inadequacy of information on the possible hazards

Animals were exposed to frequencies and modulations that mimic the common human population exposure to radiobase-station antennas in the world

RFR/MW *in vivo* studies



NTP

National Toxicology Program

U.S. Department of Health and Human Services



**GSM (Global System for Mobile Communication)
& CDMA (Code Division Multiple Access)
for both mice and rats
 \approx *Near Field***



Istituto Ramazzini

COOPERATIVA SOCIALE ONLUS



GSM (Global System for Mobile Communication)

Far Field

RFR/MW *in vivo* studies

Whole body
SAR values (W/Kg)
(base-10 log scale)



RFR/MW: exposure system



To expose the animals to a mobile phone radiofrequency field representative of a 1.8 GHz base station, a specific radiation system, totally representative of the real environmental situation present in geographic areas close to GSM base station radiation emissions, was designed and constructed by TESEO S.P.A. Company, Turin, Italy.

RFR/MW: exposure system



- The rats were located in 4 rooms with the same environmental conditions (i.e. temperature of $22\pm 3^{\circ}\text{C}$, a relative humidity of 40-60% and 12 h/day homogeneous diffusion of light).
- The rat cages were located in wooden circular-shaped devices.

RFR/MW: exposure system



- The exposure rooms were totally shielded in order to minimize the effect of field non-uniformity due to reflections and consequent interference caused by the walls.
- Each single exposure devices served at least 400 rats.
- All devices were identical and a different intensity of RFR was provided in compliance with the experimental design.

RFR/MW: exposure system

Monitoring probe



The method applied for the measurement of the RFR was completely in compliance with the standards generally applied during “on-the-site” GSM measurement and evaluation.

The above mentioned standard in Italy was defined in detail in D.L. 381 which outlines the timing and procedures to apply during such measurements. The probe used to measure the field was the TESI2001 field sensor.

RFR/MW: exposure system



The probe was linked to a dedicated personal computer and was able to show continuously the field intensity value updated every 10 seconds. The TESI2001_WIN software was able to control the probe, to download the recorded information and produce diagrams of the three field values by day, week or month.

RFR/MW: experimental project

All experiments started at the 12th day of pregnancy and continued life-span

Experiment	Number of animals (M+F)	Treatment	State of the art *
BT 1 CEMRF	2,448	RFR/MW 1.8 GHz	Ongoing
BT 3 CEMRF	617	RFR/MW 1.8 GHz + γ -radiation (0.1 Gy)	Ongoing
TOTAL	3,065		

* STATE OF THE ART:

- RFR/MW alone: all tissues embedded, all slides of the brain reviewed at microscope, heart slide evaluation at microscope is still ongoing, complete evaluation at end 2017– PWG has to be organized; publication of results early 2018
- RFR/MW + γ radiation: all tissues embedded, all slides to be prepared

RFR/MW alone: experimental plan (BT1CEMRF)

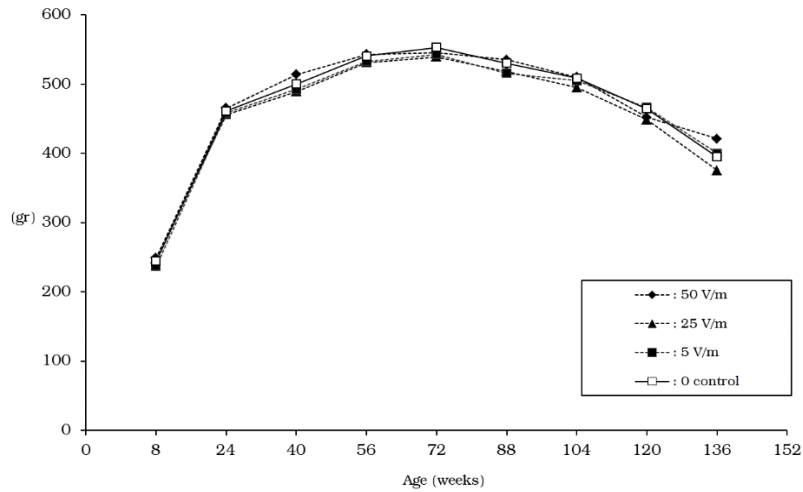
Group	Animals		Treatment	
	Sex	No.	RFR/MW 1.8 GHz ^a (V/m)	Other
I	M+F	409	50	-
II	M+F	411	25	
III	M+F	811	5	-
IV	M+F	817	0	-
Total		2,448		-

^a : The treatment with **RFR/MW 1.8GHz** for 19 hr/day started on the 12th day of pregnancy and lasted until natural death

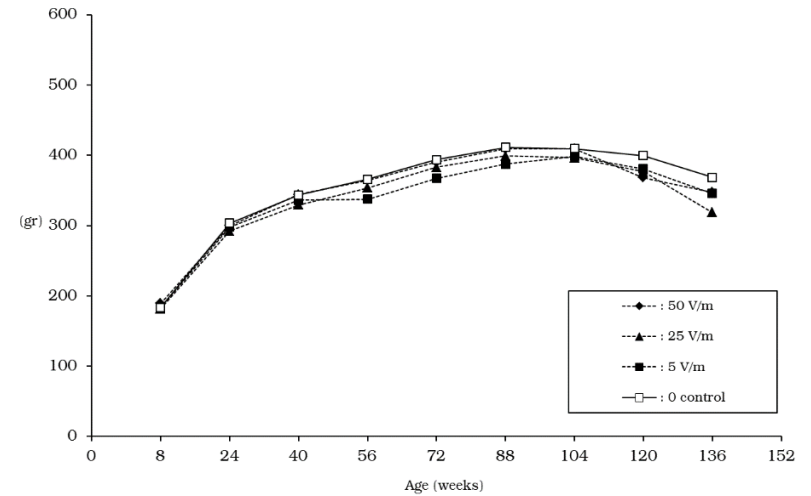
RFR/MW alone: all tissues embedded, all slides of the brain reviewed at microscope, heart slide evaluation at microscope is still ongoing, complete evaluation at end 2017–PWG has to be organized; publication of results early 2018

RFR/MW alone (BTICEMRF)

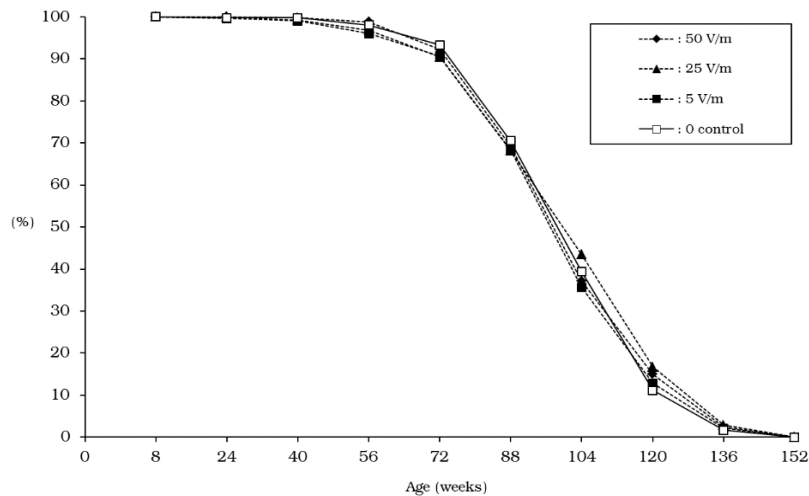
Males mean body weight



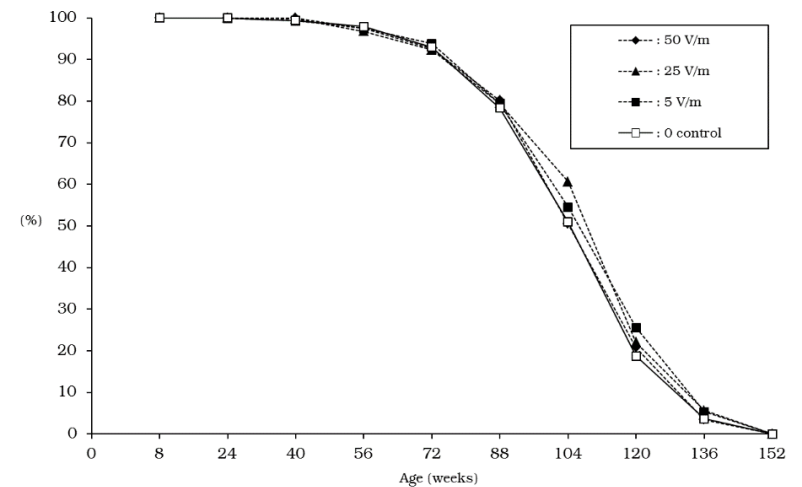
Females mean body weight



Males survival



Females survival



RFR/MW alone: mean body weight in newborns

Mean weight in litters

Group	PND					
	9	10	11	12	13	
1	13.4**	15.2**	16.5**	18.4**	17.8**	mean
	1.75	1.67	2.29	1.97	2.23	standard deviation
2	16.7**	17.9**	18.9	20.1	19.8	
	1.61	2.1	2.4	1.32	2.37	
3	16.2*	17.1	18.7	20.4	20.4	
	1.9	1.86	1.91	2.33	2.71	
Control (4)	16.5	16.7	18.4	20.9	20.5	
	1.66	1.77	2.33	1.16	2.77	

** p-value < 0.01

* p-value < 0.05

- PND: Post Natal Day;
- The p-values are related to the comparison of all means in group 1, 2, and 3 with respect to group 4 (control group) derived from linear regression models (one for each value of PND) that take into account the dimensions of the litters.
- Statistical analysis was performed by Professor Rossella Miglio
Department of Statistical Sciences "Paolo Fortunati", University of Bologna, Italy

RFR/MW + γ radiation: exp. plan (BT3CEMRF)

Group	Animals		Treatment	
	Sex	No.	RFR/MW 1.8 GHz ^a (V/m)	Other ^b
I	M+F	184	50	0.1 Gy
II	M+F	224	25	0.1 Gy
III	M+F	209	0	0
Total		617		

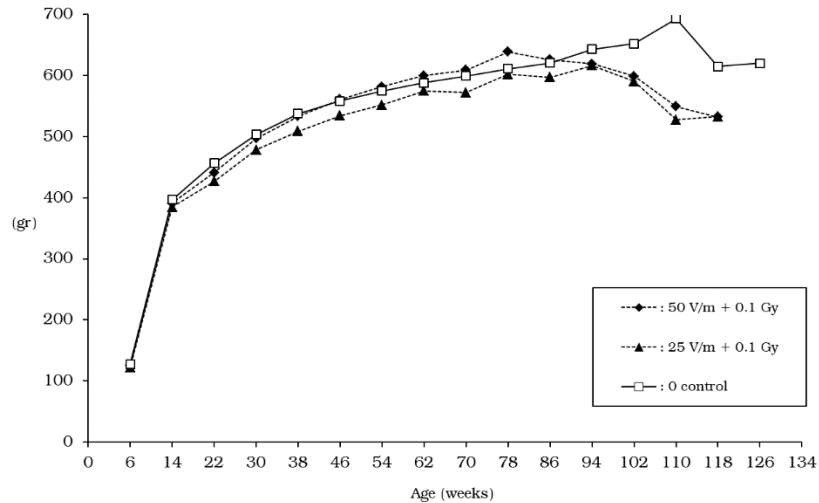
^a : The treatment with **RFR/MW 1.8 GHz** for 19 hr/day, started on the 12th day of pregnancy and lasted until natural death

^b : The γ radiation was administered *una tantum*, at the age of 6 weeks, as initial treatment.

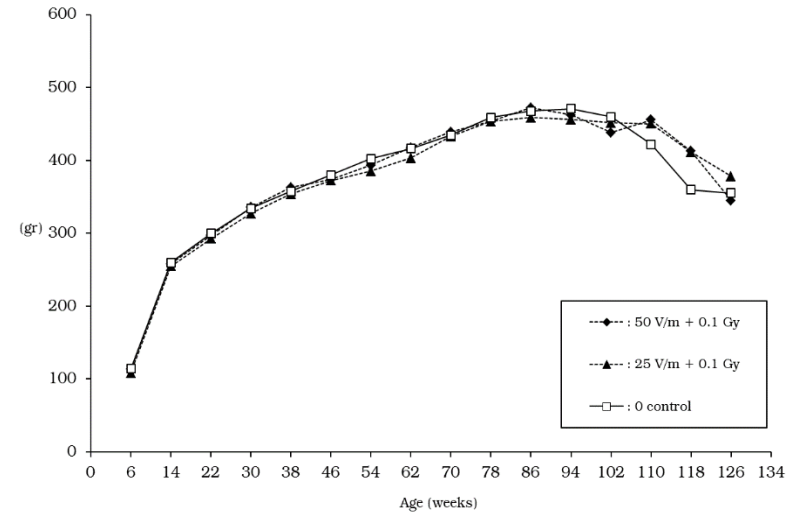
RFR/MW + γ radiation : all tissues embedded, all slides still to be prepared

RFR/MW + γ radiation (BT3CEMRF)

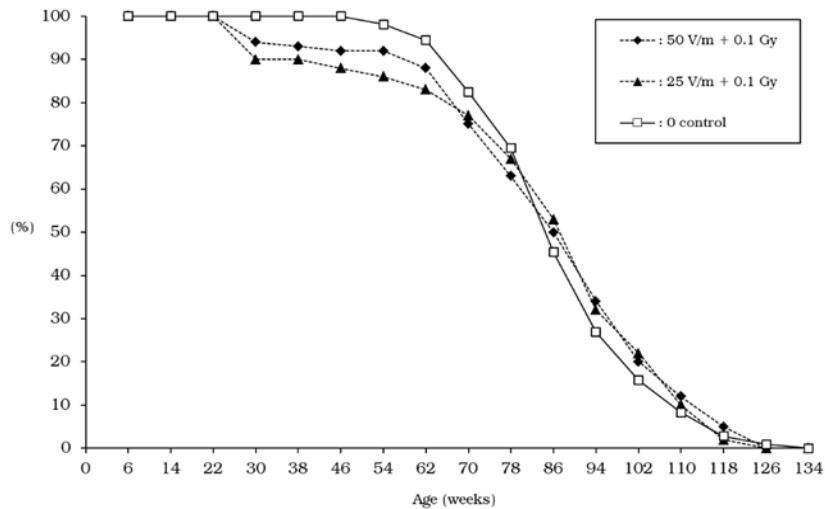
Males mean body weight



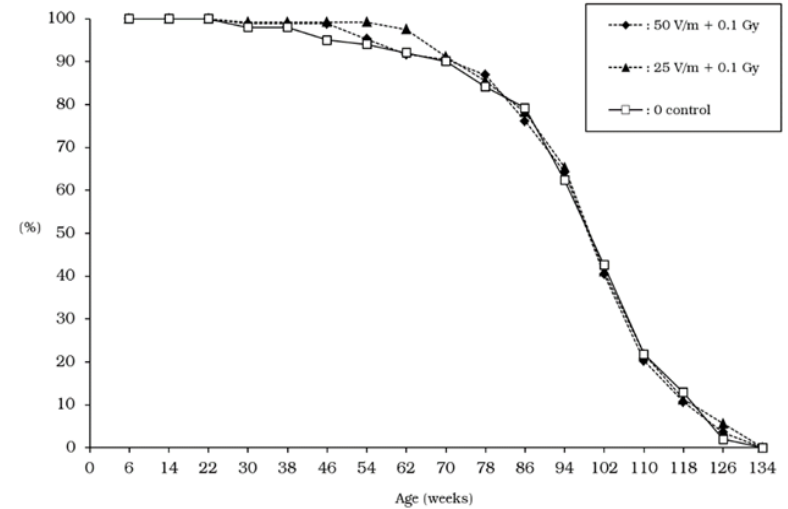
Females mean body weight



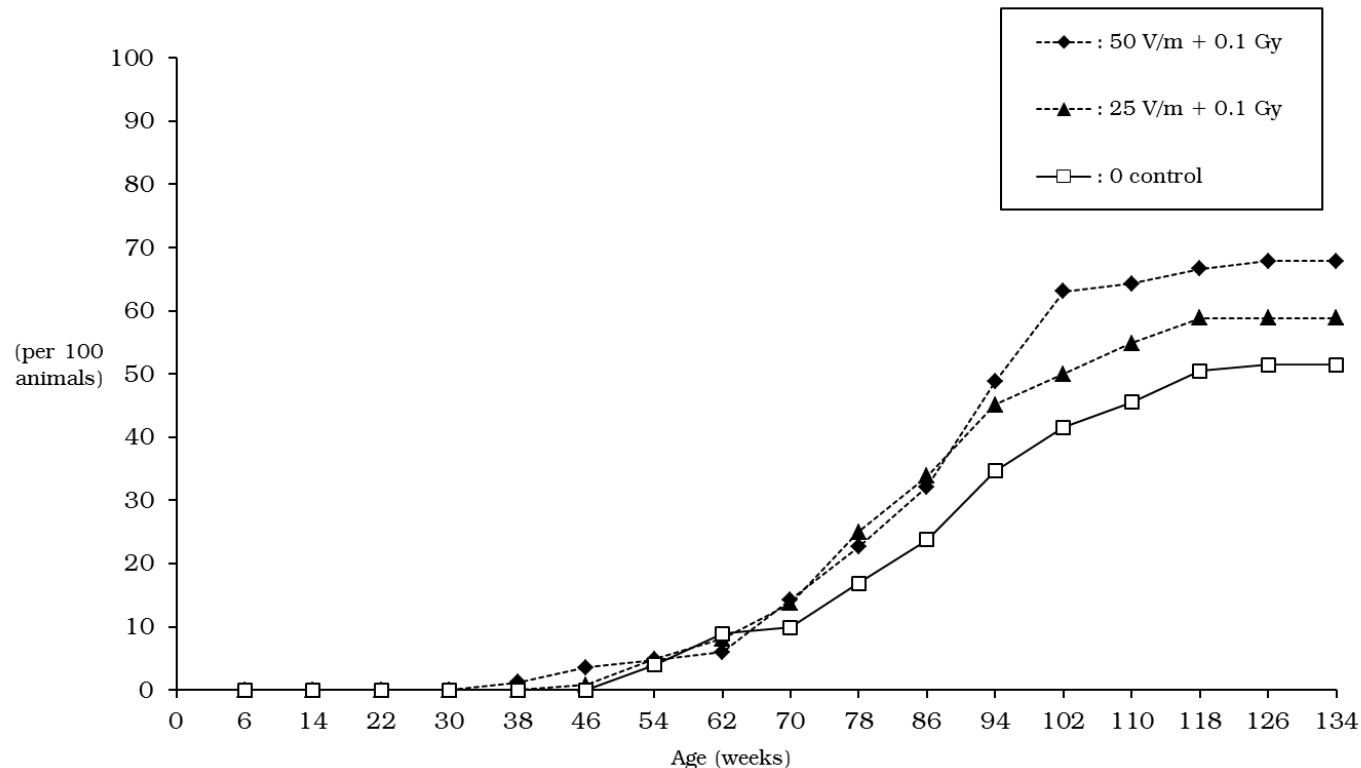
Males survival



Females survival



RFR/MW + γ radiation (BT3CEMRF): total mammary lumps (benign and malignant)



- Cumulative prevalence of MAMMARY LUMPS (benign and malignant tumours) in female Sprague-Dawley rats, clinically observed during the biophase

RFR/MW + γ radiation (BT3CEMRF): preliminary litter evaluations

Group	Treatment ^a (GSM-1,8 GHz)			Other exposure	Duration of the experiment	Animals (dams)	Litters	
N.	EMF ^b (V/m)	Start	Duration (weeks)			N. cohabitated	Mean size ^c	Postnatal body weight (PND~15)
I	50	P ^d	LS ^e	γ Radiations 10 rad ^f	LS ^e	25	12 \pm 3	21,5 \pm 2,5*
II	25	P	LS	γ Radiations 10 rad	LS	25	13 \pm 3	20,7 \pm 2,8*
III	0	-	LS	-	LS	25	13 \pm 3	22,3 \pm 3,1
TOTAL						75		

^a: The treatment with electromagnetic fields GSM- 1,8 GHz for 19h daily starts the 12° day of prenatal life (28/12/2011)

^b: EMF: Electromagnetic field

^c: The mean litter size is calculated at PND 7 using the formula: total number of pups delivered (live and stillborn)/ number of dams that delivered

^d: P = prenatal

^e: LS = Life span

^f: γ radiation are delivered as starting treatment, *una tantum*, at 6 weeks of age between February 15 and 16, 2012

*: Significant effects ($p < 0,001$) indicated by T Student test.

What are the next steps?

- Complete histopathology
- Perform an external PWG
- Study molecular events induced by ELFMF and RFR (e.g. oxidative stress)
- Study genetic toxicity assays (DNA repair processes of particular interest) in both ELFMF and RFR
- Share our biological materials with the NTP to examine early molecular changes and compare results

ELFMF and RFR/MW : Funding

The projects on ELMF and RFR/MW (about € 10,000,000) up to now are supported by:

- Ramazzini Institute Associates, Bologna, Italy ($\approx 60\%$)
- Ministry of Health, Italy
- National Institute for Insurance Against Injuries at Work (INAIL, ex ISPESL), Italy
- Regional Agency for Prevention and the Environment (ARPA) of the Emilia-Romagna Region, Italy
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Thank you from the RI research staff !