Ozone / Oxygen ip. in a preclinical cancer study with an outlock in human- and veterinary medicine

Schulz Siegfried → University of Marburg Germany

Schulz et al., 2008: Int. J. Cancer: 122, 2360-2367

Pneumoperitoneum

- The presence of air or gas (eg. CO2) in the abdominal (peritoneal) cavity
  - Injection of gas into the peritoneal cavity as a diagnostic or therapeutic measure
  -insufflated for laparoscopic surgery or
  - occuring pathological (many causes)

- O3/O2- pneumoperitoneum - ozonized oxygen in the intraperitoneal cavity

Zorraquin G. et al., 1947

Simpaticectomias Distónicas, Etiopatogéni - cas, Viscerales, al Ozono y Octozono Intraperitoneal,

en Lugar de Simpaticectomias Operatorias

•En La Semana Médica Buenos Aires, 1947

Presentado al Congreso Interamericano de Cirugia de Montevideo, 1946 y realizado en la Asistencia Pública de Buenos Aires, Hospital Fernandez, Servicio de Cirugia General y Intestinal

Zorraquin, Dr. 1947

Ozono contra dolorosas abdominales

Casuisticas:

- 35 años 200ml (x 2) = 400ml
- 20 años 100ml, 200ml, 400ml (x3) = 700ml
- 17 años 200ml (x2) = 400ml
- 30 años con ozono (x8) = ?????

Concentration : µg O3 / ml **?**

Ozone generator: aparatos italianos de Gambarotta, el aparato de Payr de Stutgart

Zorraquin, Dr. 1947

„Las inyecciones de ozono intraperitoneales son sin embargo sensiblemente dolorosas y paresian transitoriamente al músculoso diafragma. Estos inconvenientes se previenen con una inyección doble de morfina“

Zorraquin, Dr. 1947

„ En veinte años nunca hemos tenido un accidente por inyección de gas dentro del peritoneo y tan familiarizados a esto estamos, que no recurrimos más a nuestro aguja manométrica, de 20 años atrás, para neumotórax y neumoperitoneo, más difundida en Europa que aqui „

Zorraquin, Dr. 1947

„ Inicialmente debemos declarar que no hemos visto en nuestras inyecciones de ozono intraperitoneal en cavidad cerrada, ninguna acción cáustica corrosiva o citolitica de importancia. En una epidemia de chanchitos de India sólo sobrevivieron los inyectados con ozono y oxigeno intraperitoneal en volúmenes parecidos a sus pesos“

INTRAPERITONEAL INJECTIONS

maximum acceptible volumes

	Volumes of liquids		gas (e g. CO_2) Ozone/O_2
	ml	ml/kg	?
Mice (30gr)	2-3	83	
Rats (250gr)	5-10	40	
Rabbits (2.5kg)	50-100	40	80 mlO_3/O_2/kg
Dogs (20kg)	200-500	25	IAP < 5mbar
Humans (70kg)	?????	??	> 4-6 L pressure-control IAP

O3/O2-PP vs O3-AHT

- large volumes — relative small volumes
- large dosis — relative small dosis
- no blood contact — ozonized blood

O3/O2-PP vs O3-AHT

cies	µg/ml	mg/kg	x days	total dosis mg/kg	application	diseases etc.	literature
man	1- 50	0.01 - 0.07	> 10	0.1 – 0.7	O3-AHT	various	Bocci (book)
bit	50	4	5	20	O3/O2-PP	cancer (VX2)	Schulz,2004 Schulz,2008
	10	0.8	5 (sepsis)	40	O3/O2-PP	prevention	Schulz,2003
	50	4	5	20	O3/O2-PP	basic research	Sch. +N.
	50	1.6	45	72	O3/O2-PP	toxicol. research	B. + Sch.
use	50	4.0	5	20	O3/O2-PP	basic research	N. + Sch.
n	50	?	4-18	?	O3/O2 –PP	cancer (various)	Kleef , Brazil,
als)							Olmedo,
orts							Germany
esults							Swiss

Ozone therapy in a cancer model

Vx2 carcinoma: head and abdomen in rabbits.

A pilot study

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In cooperation with

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Supported
by the Philipps-University of Marburg

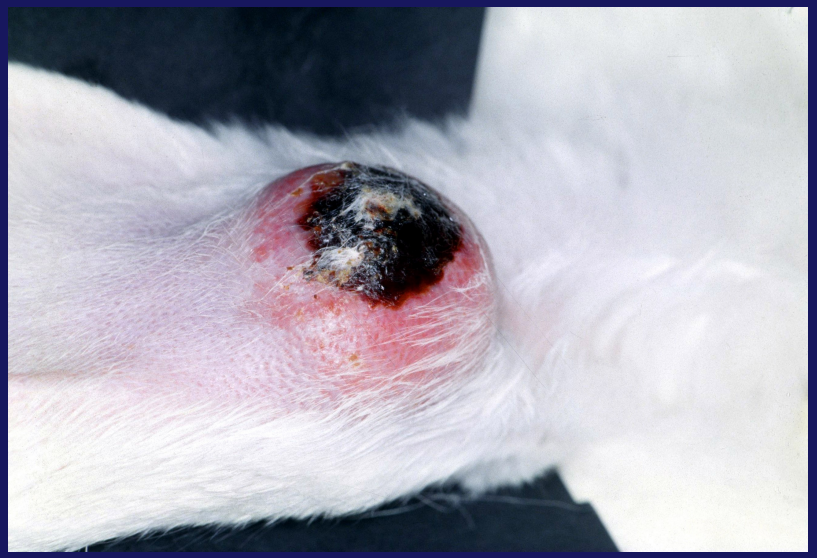
Vx2 tumor development in the **ears** of rabbits: Influence of O_3/O_2-PP

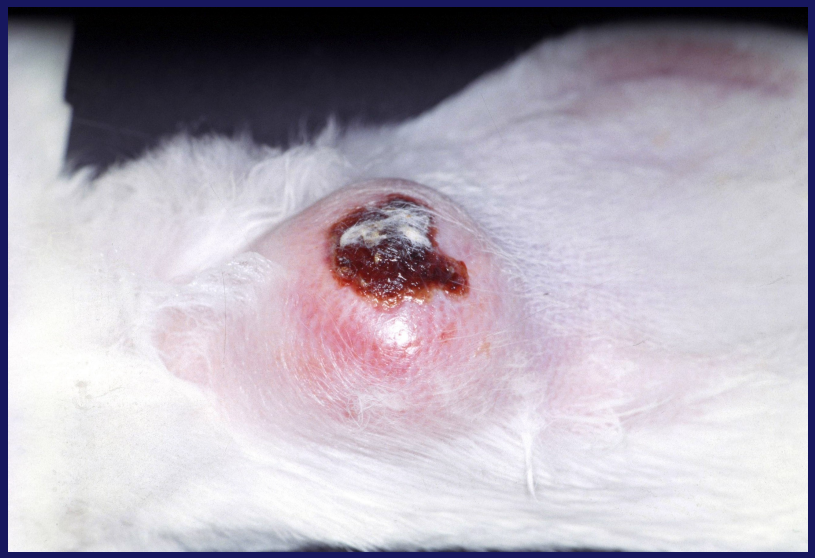
		height x width (mm)	**changes of tumor size** (%)			
		day +18	**day +23**	**day +33**	**final day +39-83)**	
	ear					
Case 1	**left 1**	**24.4 x 21.9**	**+15.4 x +15.0**	**n.d.**	**+24.4 x +64.0**	**+47**
ctrl NZ	**right 1**	**27.7 x 24.9**	**+11.4 x +15.0**	**n.d.**	**+71.0 x +48.0**	
Case 2	**left 1**	**22.1 x 17.8**	**-27.4 x -22.9**	**no tumor**	**no tumor**	**+47**
NZ	**right 1**	**17.3 x 17.7**	**-2.1 x -6.6**	**-57.4 x -64.7**	**no tumor**	
Case 3	**left 1**	**30.7 x 15.8**	**n.d.**	**n.d.**	**- 2.0 x -20.0**	**+39**
NZ	**right 1**	**20.8 x 17.2**	**n.d.**	**n.d.**	**+5.4 x +14.0**	
		15.6 x 14.3	**n.d.**	**n.d.**	**+38.0 x -24.0**	
Case 4	**left 2**	**17.5 x 15.5**	**n.d.**	**n.d.**	**no tumor**	**+83**
NZ	**right 2**	**20.2 x 15.0**	**n.d.**	**n.d.**	**+30.0 x -26.6**	
		18.1 x 17.4	**n.d.**	**n.d.**	**+17.7 x +20.9**	
Case 5	**left 1**	**25.6 x 21.5**	**n.d.**	**- 6.2 x -2.3**	**no tumor**	**+43**
Chinch.	**right 1**	**19.5 x 19.0**	**n.d.**	**no tumor**	**no tumor**	

right ear

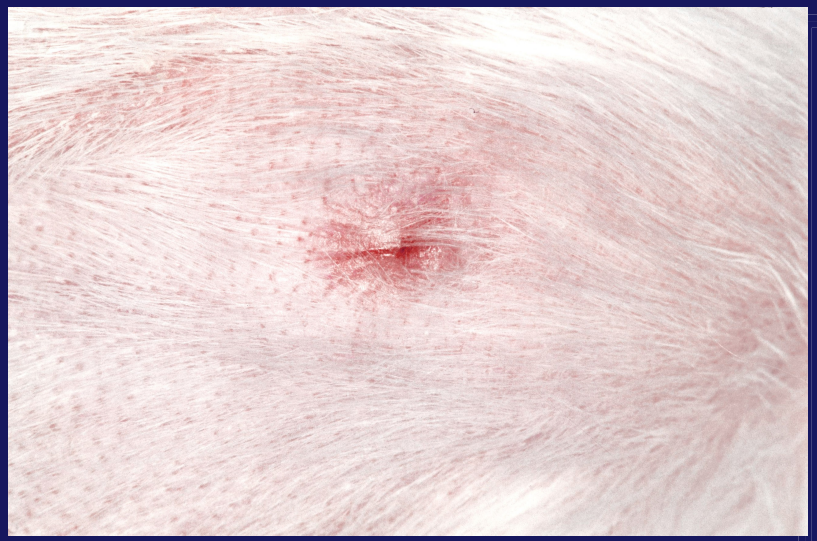
left ear

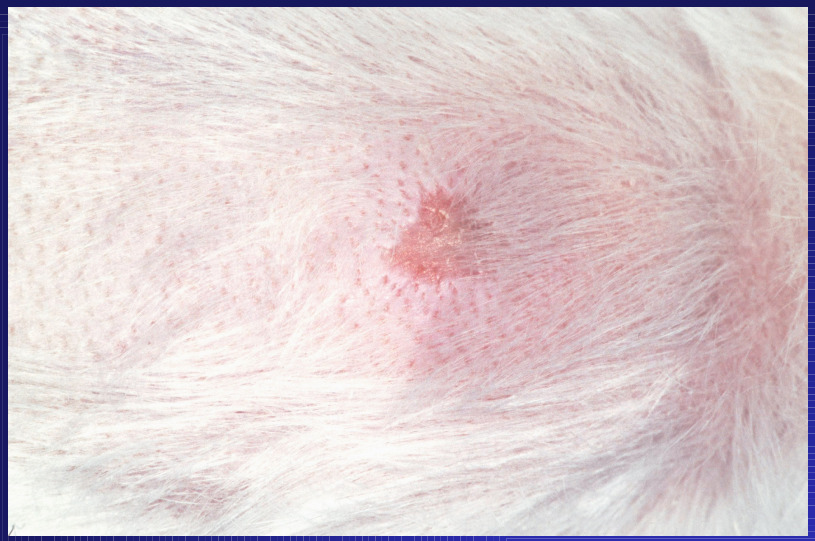
tumor +day 19





cured +day 47





Total tumors: s = 10

dissapearance to tumors after O3/O2 treatment s = 5 (50%)

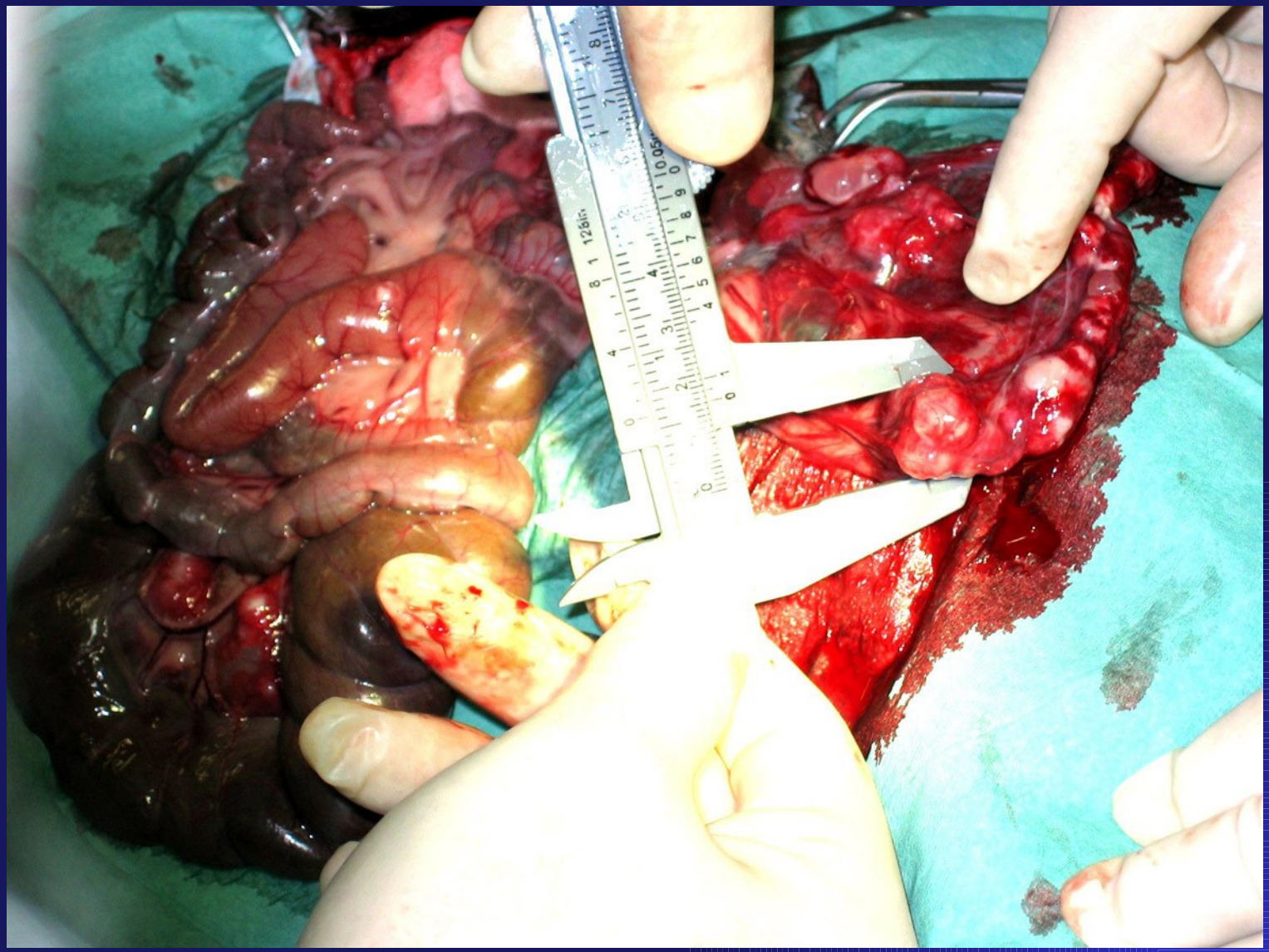
Histopathological analysis of cranial and thoracical organs of Vx2 tumor infected rabbits: Influence of O_3/O_2-PP

	ear	necropsy	ear tumor	lymph nodes parotid	caud.	rost.	lung
Case 1 ctrl NZ	left 1	+47 days	solid	metastasis	neg.	neg.	multiple
	right 1		ulcerated	metastasis	metastasis	neg.	metastasis
Case 2 NZ	left 1	> 229 days	disappeared	-	-	-	neg.
	right 1		disappeared	-	-	-	X-ray
Case 3 NZ	left 1	+39 days	ulcerated, necrotic	metastasis	neg.	n.d.	neg.
	right 1		ulcerated, necrotic	metastasis	neg.	n.d..	
Case 4 NZ	left 2	+83 days	ulcerated, necrotic	metastasis + perinodal spread	neg.	neg.	multiple metastasis
	right 2		disappeared				
			ulcerated, necrotic	metastasis + perinodal spread	neg.	neg.	
			ulcerated, necrotic				
Case 5 Chinch.	left 1	> 139 days	disappeared	-	-	-	neg.
	right 1		disappeared	-	-	-	X-ray

Vx2 tumor development in the abdomen of rabbits: Influence of O_3/O_2-PP

	Initial weight	treatment day +5 to +9	laparotomy day +21	necropsy	final weight	changes in weight (%)	Survival days
Case 1 ctrl Bast.	3.17	control	8 tumors	8 tumors	2.49 kg	-21.5	+ 21
Case 2 Bast.	2.96	ozone	1 tumor	1 tumor	3.69 kg	+27.1%	>84
Case 3 Bast.	2.91	ozone	2 tumors	no tumor	3.32 kg	+18%	>84
Case 4 Bast.	3.04	ozone	1 to tumor	no tumor	3.31 kg	+8.2%	>76

Measurement of Vx2-tumor size in the omentum



Case 1 ctrl necropsy at day 21 post inocculation

A new Medozon^{ip} generator for intraperitoneal insufflation of O_3/O_2-pneumoperitoneum.

The efficacy and safety of therapeutical O_3/O_2 gas in a lethal ear carcinoma (VX2) model in rabbits.

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In cooperation with M. Bette, A. Dünne, A.A. Häussler, R. Mandic, B. Watzer, E. Weihe, JA. Werner, J.T. Heverhagen , H. Schweer,

The Medozon^{ip} generator

If you want to start a treatment, press the following key.

Start course of treatment

If you want to change the settings or if you need any information, press the following key.

Information / Service Settings

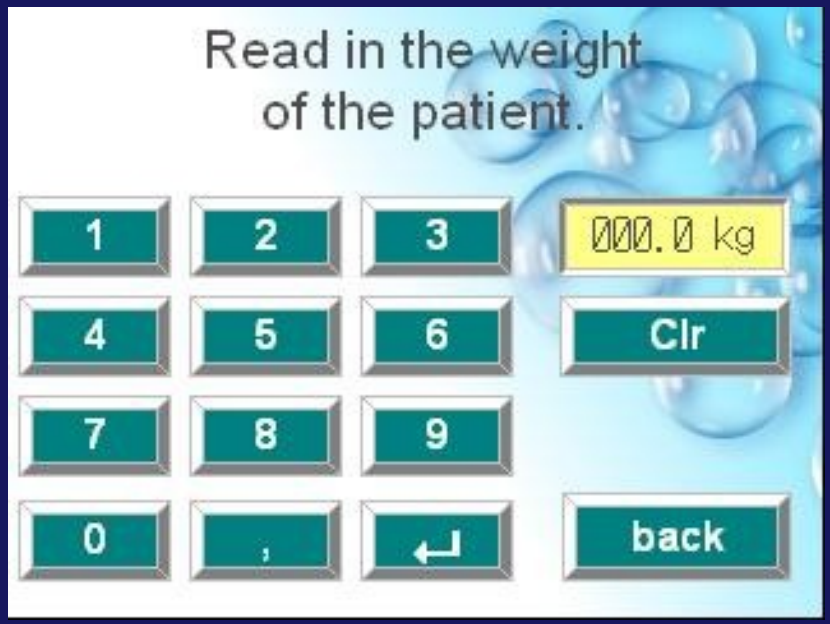
Connect the disposable material to both of the connections and open the three-way tap.

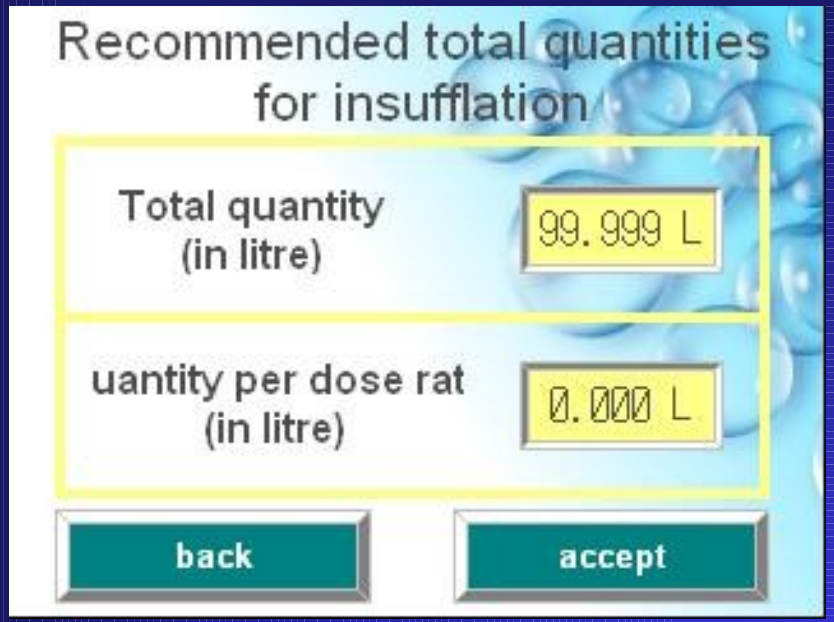
After that press the key -rinse Ozone-kit ip-

back

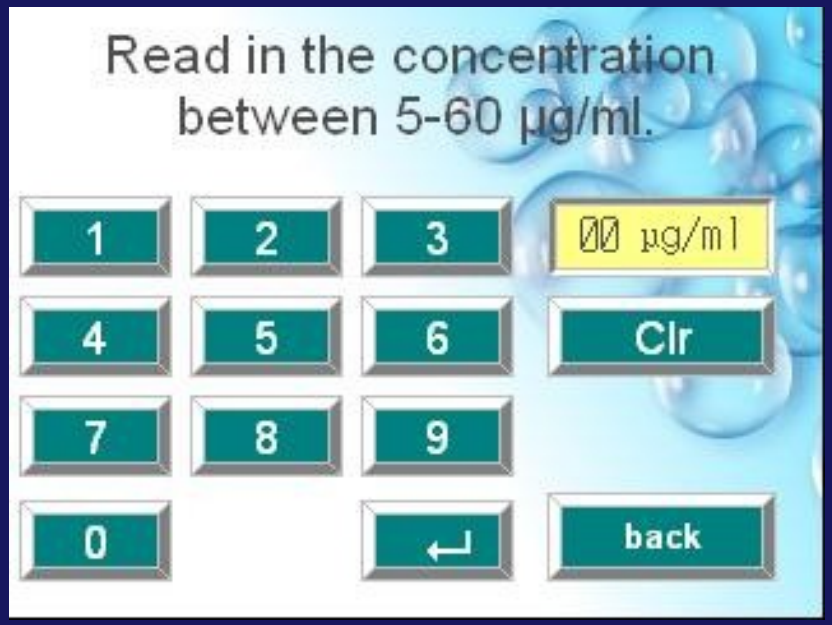
Rinse Ozone-kit ip

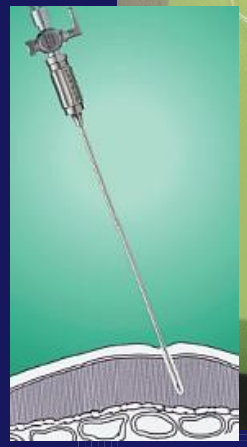
The Medozon^{ip} generator

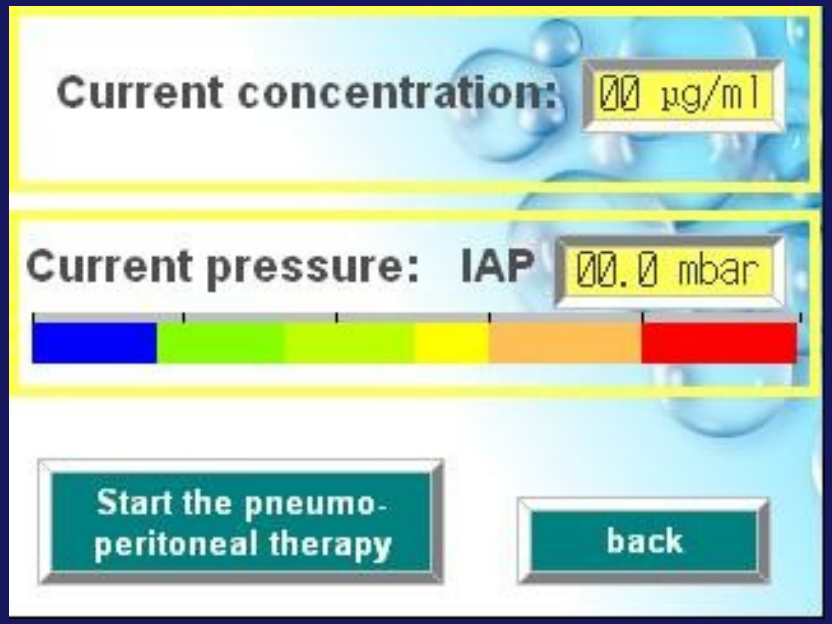




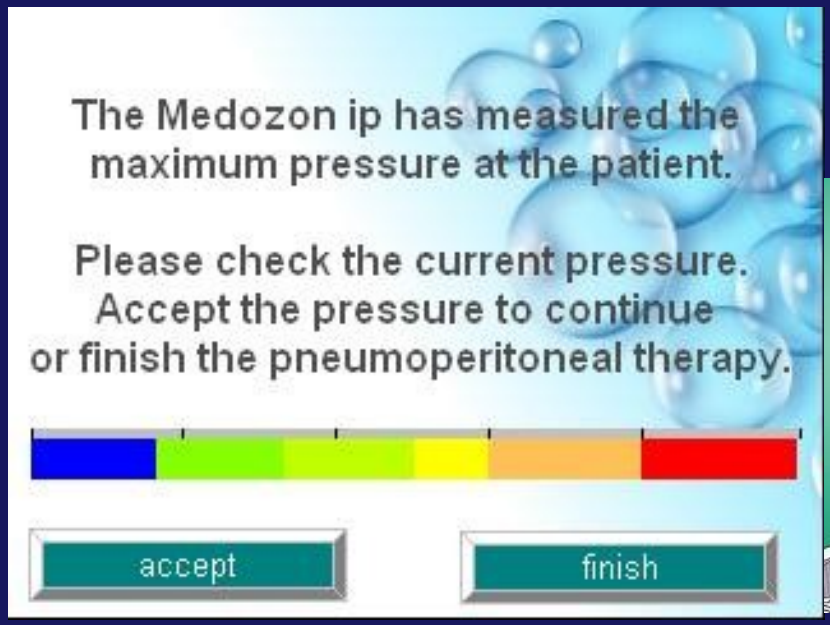
The Medozon^{ip} generator

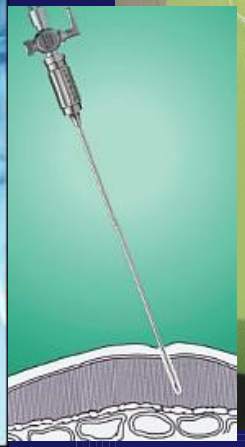






The Medozon^{ip} generator






Peking 2007, Military Hospital

The therapeutical impact of O3/O2-pneumoperitoneum

head and neck squamous carcinoma cell (HNSCC)

363 000 new cases per year

200 000 deaths annually worldwide

* Parkin et al 1999, in Global cancer statistics; A Cancer J. Cl.

Squamous cell carcinoma in skin

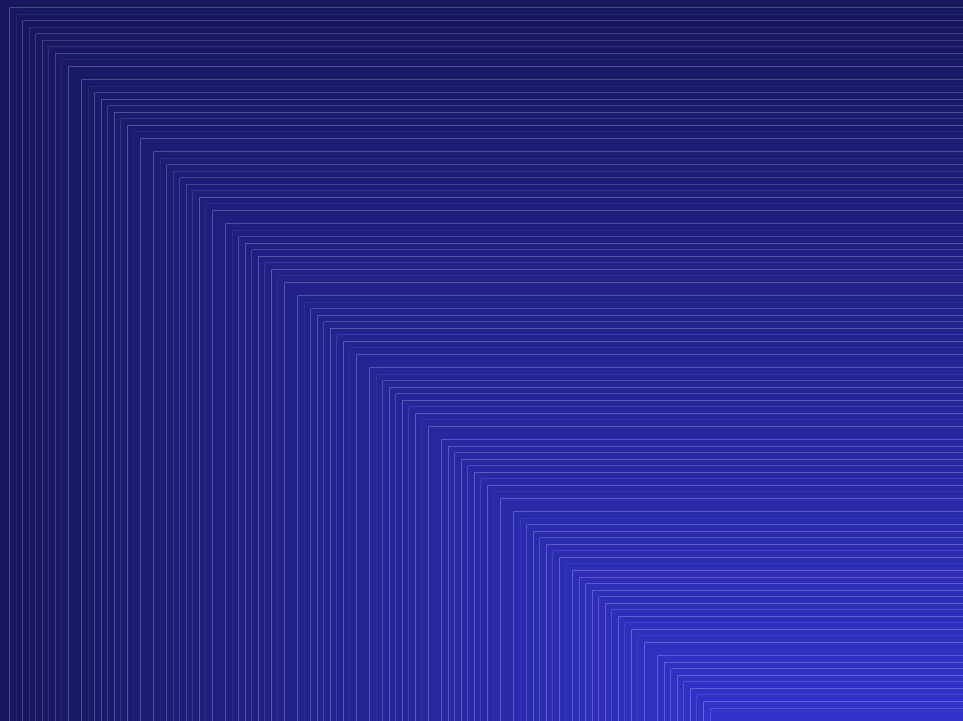
- High mortality rate > 50 % in man and animals
- Most malignant neoplasma in head and neck region
- Aetiologies: eg. Epstein-Barr Virus
  Human Papilloma Virus
  hereditary factors
- VX2-Carcinoma cells: Rabbit Shope Virus

Conventional treatment

Surgery

Radiotherapy

Chemotherapy



Alternative treatment modalities

1. Intra-arterial chemotherapy
2. Immune-stimulation (Biological response modifiers
   e.g.Ribi-vaccine, cytokine Interleukin-2)
3. Gene therapy technology (Anti-oncogenes, replacement –genes,
   genes enhancing immune surveillance)
4. Photodynamic therapy (oxygen radicals)
5. Anti-angiogenetic therapy
6. Herpes simplex virus thymidine kinase
7. Ozone therapy ?

Animal (models) for novel therapies against head and neck cancer: squamous cell carcinoma (SCC) in man

1. Spontaneously squamous cell carcinomas:

   sheep, cat: **ear;** dog, horse: **skin**; bovine: **eye** rabbit**: skin**

2. Topical application of a carcinogen (4-nitro-quinoline-1-oxide):

   mouse, rat: **skin;** hamster: **check pouch carcinoma** (3- 6 month)

3. Transplanted carcinoma cell lines:

   rabbits, rats, nude-mice: **skin and organs**

In vivo VX2 models and tumor transplantations

A. head : (bi)-auricular model (rabbit) used in the study

tongue (nude-mice)

used in the study

B. abdomen: systemic (i.p.)

organic **e.g. liver, uterus, kidney, bladder**

way of VX2 transplantaion

- solid tumour pieces
- tumour cell suspension

used in the study

Tumor transplantations

- 0.15-0.25 ml suspension containing 10-20 x 10^6 vital tumor cells from a donor rabbit (hind leg or lung)

- Injected into area between central auricular artery and caudal margin at the dorsal middle-third of both auricles

Mortality rates in aggressive VX2-tumor model of rabbits

Organs	inoculation cells/ml	days of survival	mortality (%)	literature
Renal	0.1-0.3 x10^6	42.5 ± 14	100	Lee et al. 2003, Eur.Radiol.
Bladder	0.1 x 10^6	within 40	100	Yang et al. 2001, Urol. Res.
Uterus	1 x 10^8	within 60	>80	Harima et al. 1996, Cancer Chemother. Pharmacol.
Liver	1.5 mm solid	61 ± 7	100	Taburo et al. 2001, Cancer Chemother. Pharmacol.
Liver	1.5 mm solid	within 90	100	Miao et al. 2000, Eur. Radiol.
Ear	10-20 x 10^6	within 75	100	Van Es, 2000, J. Craniomacillofac. Surg.

Aim of our investigation

Analysis of possible anti-tumorgenic and anti-metastatic influences of O_3/O_2-pneumoperitoneum on VX2 tumor:

a. tumor growth (on primary tumors)

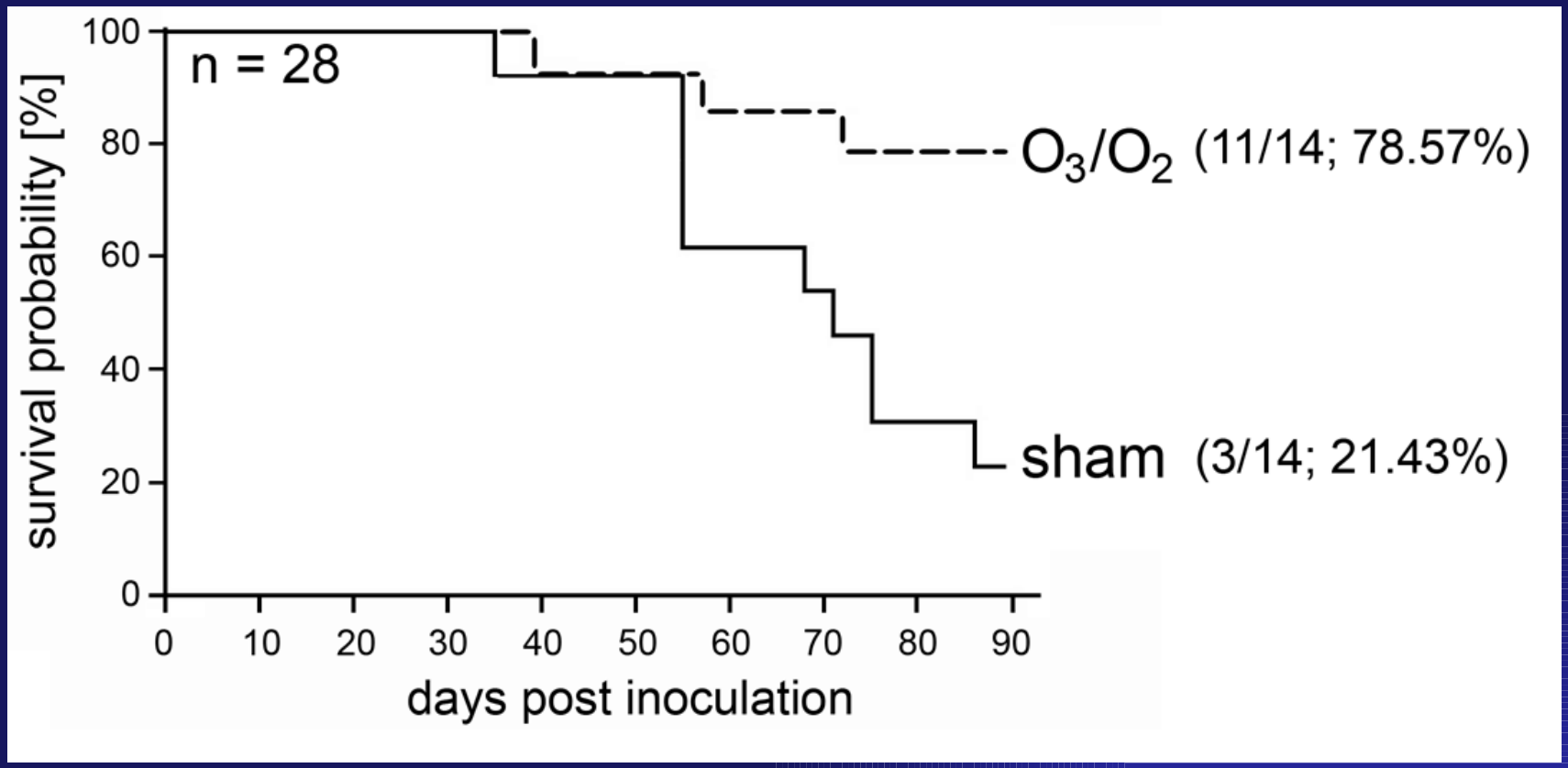
b. occurrence of metastasis in cervial lymph nodes, lung

c. multiplicity* of growing tumors in the lung

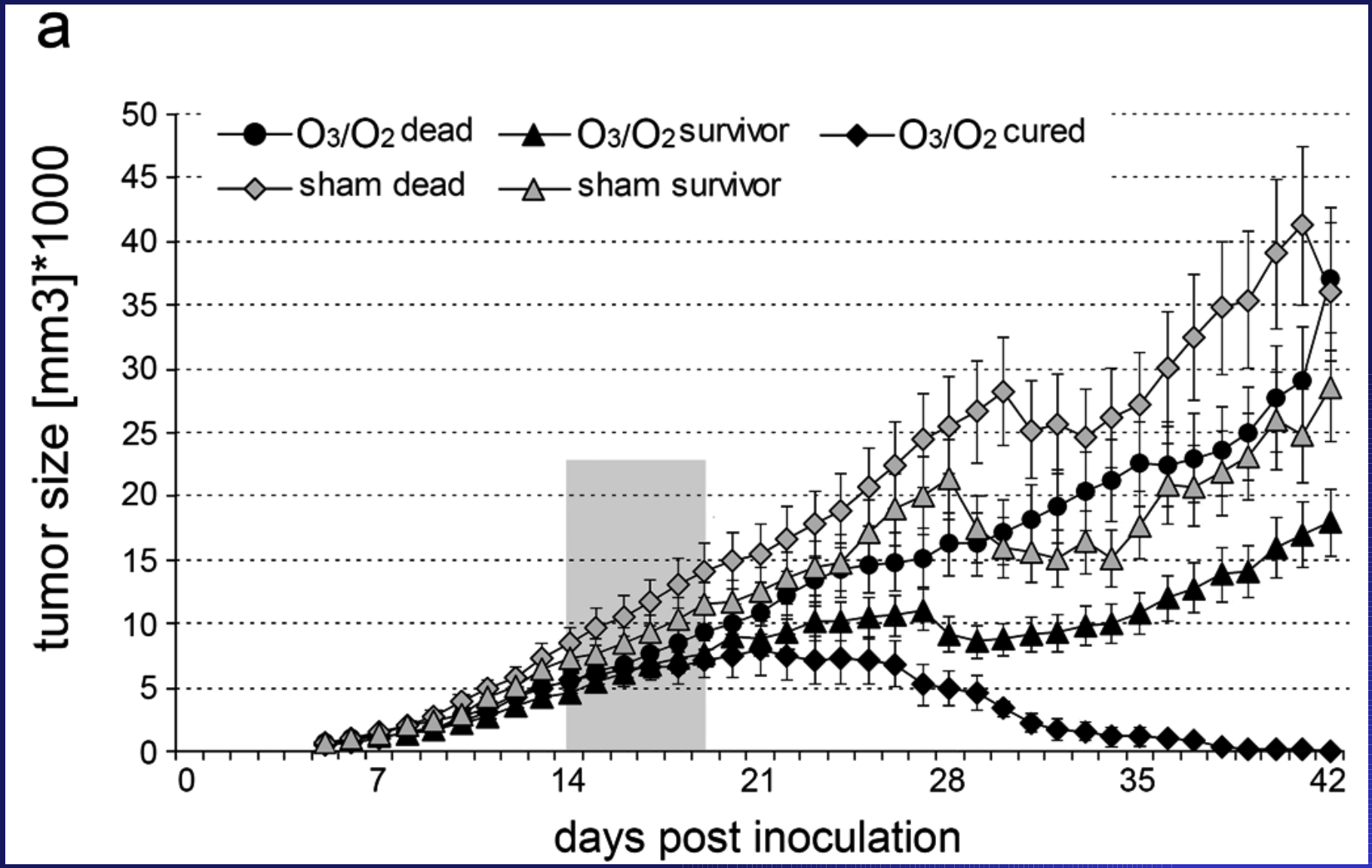
d. body weight

e. survival rate

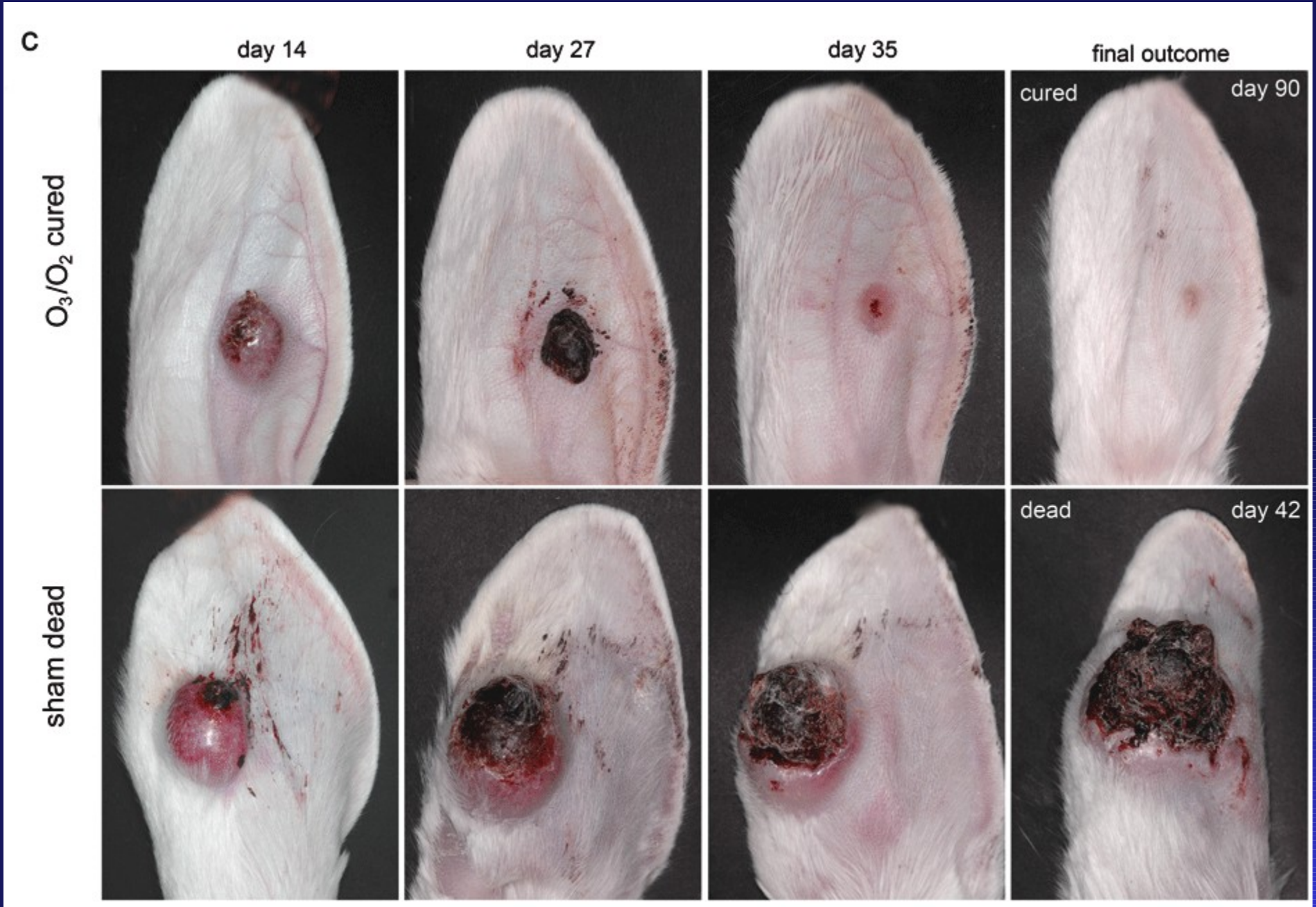
Survival probability

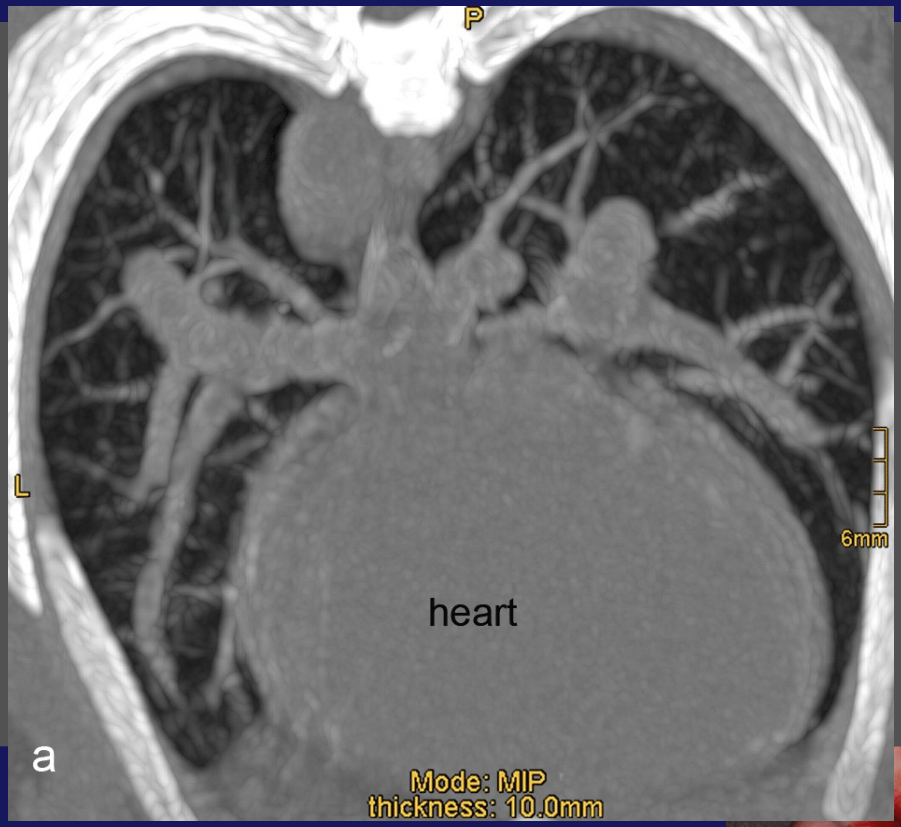


Tumor development



Tumor development

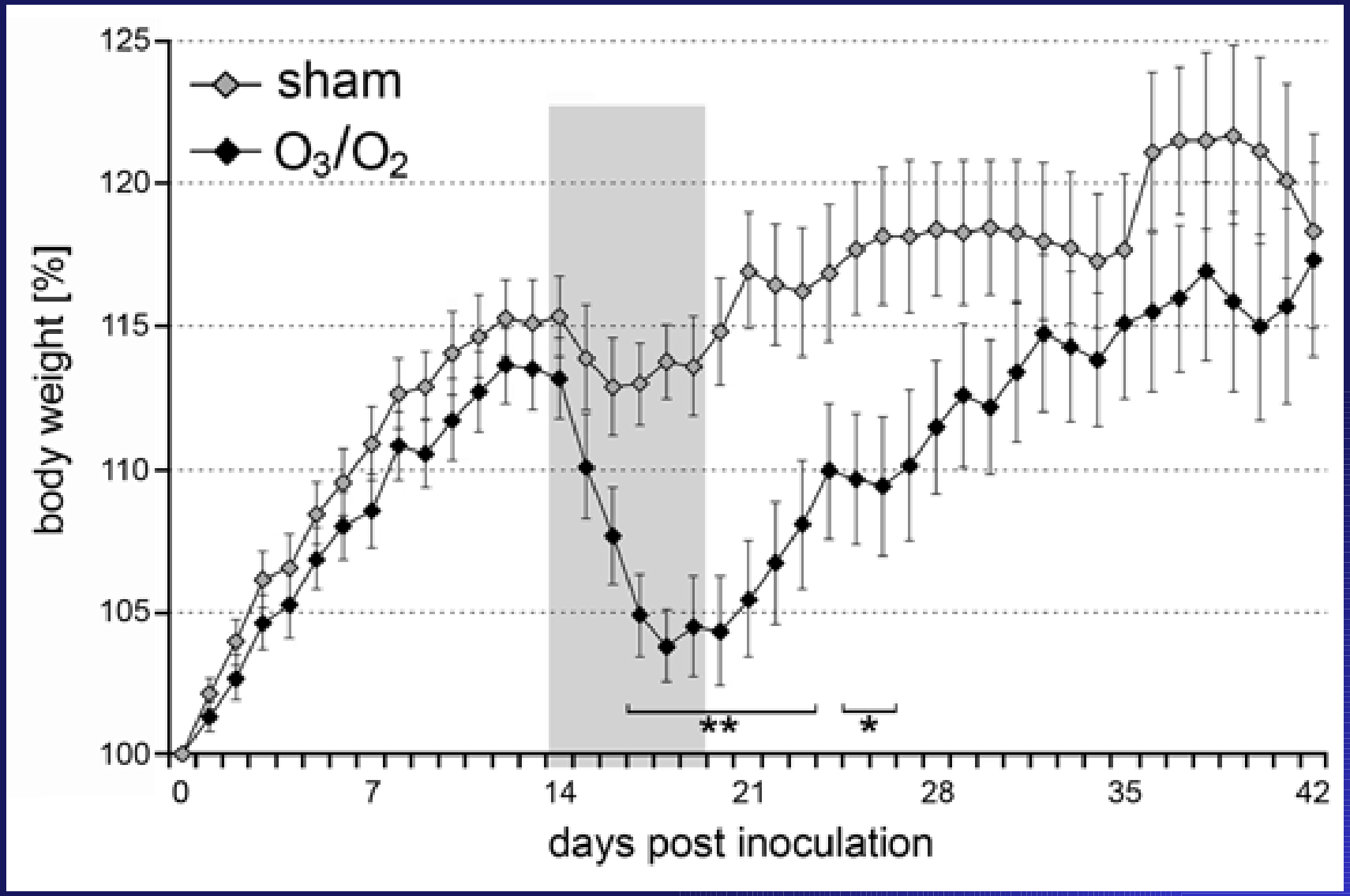




CT scan of the thorax of a O3/O2-cured rabbit

Necropsized lung of a sham rabbit after death

Adverse effects: Body weight



Adverse effects :
Hematological and clinical chemistry parameters

parameter	O_3/O_2 (n = 14)		Sham (n = 14)		O_3/O_2 cured (n = 6)	sham dead (n = 11)	typical value[30]
	d 14	d 19	d 14	d 19	d 90	at death	
WBC (total)	8.6	11.4***	8.6	10.7*	7.6	20.9	2.5-9.8 (10^3/mm^3)
granulocytes	3.4	5.5***	3.5	4.9*	1.8	14.9	1.6-3.7 (10^3/mm^3)
lymphocytes	4.9	5.7*	4.9	5.6*	5.6	5.4	3.3-7.0 (10^3/mm^3)
monocytes	0.2	0.3**	0.2	0.3	0.1	0.6	0.0-0.4 (10^3/mm^3)
RBC	5.85	5.55	5.64	5.59	5.91	6.08	5.20-6.80 (10^6/mm^3)
hemoglobin	11.7	11.6	10.0	11.5	12.9	9.0	9,8-14.0 (g/dl)
HCT	38.4	36.4	36.7	36.1	40.2	33.5	36.0-47.0 (%)
creatinine	0.736	0.863**	0.787	0.800	0.848	n.d.	0.5-2.6 (mg/dl)
GOT	17.39	13.73	15.15	13.72	29.72	n.d.	8.0-56 (U/l)
GPT	34.7	27.3**	22.9	21.0	74.9	n.d.	18.0-123.0 (U/l)

Bi-auricular re-implantation of VX2 tumor cells in O_3/O_2 treated cured rabbits

experimental group	animals [n]	tumors* [n]	mean tumor volume [mm^3]
O_3/O_2 cured + Dex/CSA	3	4/6 (66.7 %)	3089
O_3/O_2 cured + sham	3	0/6 (0 %)	< 200#
control + Dex/CSA	1	1/2 (50%)	1466
control + sham	1	2/2 (100%)	5657

Changes in prostanoid values from blood plasma after O_3/O_2-pneumoperitoneum

Arachidonic acidic metabolites	mean basal value [ng/ml]	mean maximum value [ng/ml]	x-fold increase	time post insufflation* [h]
dinor-6-k-F1α	0.014 (0.002-0.037)	1.182 (0.281-1.935)	**84.5**	4.0
6-keto-PGF1α	0.028 (0.013-0.036)	1.070 (0.406-1.568)	**40.0**	5.3
PGEM	0.023 (0.015-0.036)	0.342 (0.222-0.477)	**14,8**	4.0
dinor-TxB2	0.016 (0.003-0.023)	0.197 (0.049-0.470)	**9.4**	0.5
11-dinor-TxB2	0.058 (0.043-0.078)	0.522 (0.183-1.063)	**9.0**	0.5
PGF2α	0.054 (0.047-0.063)	0.171 (0.102-0.278)	**3.2**	5.6
Isoprostane	0.386 (0.307-0.477)	1.082 (0.742-1.696)	**2.9**	4.0
PGE2	0.127 (0.103-0.158)	0.293 (0.136-0.583)	**2.3**	5.3
PGD2	0.008 (0.003-0.022)	0.014 (0.006-0.024)	**1.8**	4.0
Thromboxane B2	0.568 (0.007-1.160)	0.680 (0.210-1.641)	**1.2**	8.0

Summary

O3/O2-pneumoperitoneum during VX2 tumor disease:

enhances survival probability

leads to complete tumor remission and the cure of the animal

prevents for the appearance of distant metastases

induces tolerance against VX2 tumor cells

exhibits no major adverse effects

enhances blood levels of some arachidonic acid metabolites

Proposed mechanisms

O_3/O_2-PP may systemically activate leukocytes which combat the existing tumor and might protect tumor metastasis.

O_3/O_2-PP may increase the endogenous prostacycline levels and by this may increase tumor tissue oxygenation.

Cured since 6 years after 5 days treatment (O_3/O_2-Pneumoperitoneum)

La vida normal despues de la tratamiento con ozono (2007)

Outlook in human –and veterinary medicine

First therapeutical trials with O3/O2-PP in cancer patients from Brazil

Pat.	Volume (total ml)	x d	range (L.)	b.w. (kg)	mean volume/d (ml/kg O3/O2)	age/s. (y)
			Concentration of Ozone (**50 ug/ml)**			
• 1	14 800	4	(0.50-6.46)	81	45.6	71 m.
• 2	15 800	5	(1.76-4.30)	60	52.6	21 f.
• 3	7 800	5	(0.55-2.35)	62	25.1	64 f.
• 4	12300	5	(0.35-3.45)	83	37.3	83 m.

ad 1 cancer liver and metastasis

ad 2 cancer liver

ad 3 cancer head of pancreas and metastasis

ad 4 cancer intestinal and metastasis in liver and lung

$$x = \frac{\overline{40.2}}{} = \textbf{2 mg O3/kg}$$

First therapeutical trials with O3/O2-PP in veterinary medicine

dosis : 80ml O3/O2/kg x 50 ug/ml = 4 mg O3 / kg x 5 d = 20 mg O3/ kg

Case 1

- Malignant melanoma on nose **Yorkshire Terrier** **Schulz 2008**
  > 20 % reduction + surgery
-

Cases 7 (6 dogs and 1 cat) different races **Gräßer et al**

- Malignant melanoma in mouth
- Malignant melanoma on the paw
- Carcinoma on ear (cat) 20 % reduction of primary tumor
- Mamma carcinoma after 5 days of treatment
- Skin tumor (mast cell)

- Sarcoma on leg non-response
  Osteosarcoma (Femur) 10 % reduction

Scientific challenge in ozone/oxygen research cancer, inflammation and infection

a. More efficacy – and risk studies from more suitable animal models in comparison of different froms of applications (O3/O2 PP , O3-AHT and rectal)

b. Complete dose-response curves ; eg. Therapeutical versus toxicological concentrations (finding of effective dosis)

c. Therapeutic schemes (bolus and repetitive applications); sessions

d. Risks and adverse effects (early and late effects with O3)

e. Indications/contraindications (in cancer , inflammation and infection)

Scientific challenge in ozone research

a. Pain research (nociception, suitable analgo-sedativa, anaesthetics) before, during and after ozone therapy)

a. Co-medication of ozone with established therapies ; complementary medicine ?

a. Insufflation and desufflation (O3/O2-PP); role of oxygen ?

a. Local and systemic effects and mechanisms with O3/O2 –PP an other methods

I Ethical considerations (therapeutical trials, eg. Cancer patients – tumor stage ?; case reports/pilot studiesand preclinical and clinical studies

a. Cost-benefit analyses ; financial support for basic research and clinical studies

a. Ozone and biomarkers etc.

Hypothesized mechanism of ozone therapy (O_3/O_2-pneumoperitoneum on Vx2 tumor development)

O_3/O_2-PP may increase the endogeneous prostacycline levels and by this may increase tumor tissue oxygenation.

O_3/O_2-PP may systemically activate leukocytes which combat the existing tumor and might protect tumor metastasis.

Local ozone/oxygen may exhibit direct cytotocic effects or might stimulate production of radicals (e.g. NO, endogenous O_3)*

* Babior et al. 2003 PNAS