Ozone / Oxygen ip.

in a preclinical cancer study with an outlook in human- and veterinary medicine (pilot-results)

and other pre-clinical studies in discussion of human diseases

Dr. Siegfried Schulz

Philipps-University of Marburg, Germany

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

Pneumoperitoneum

The presence of air or other gases (eg. CO_2) 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)

O_3/O_2-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:			
age	dose	sessions	total
35 años	200ml	(x2)	400ml
20 años	100ml, 200ml, 400ml	(x3)	700ml
17 años	200ml	(x2)	400ml
30 años	???ml	(x8)	???ml

Concentration : µg O_3 / 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

Volumes of liquids or gases (eg. CO_2, O_3/O_2) maximum acceptible volumes			
	ml	**ml/kg**	**?**
Mice (30gr)	2-3	83	
Rats (250gr)	5-10	40	
Rabbits (2.5kg)	50-100	40	80 ml O3/O2 /kg
Dogs (20kg)	200-500	25	IAP < 5mbar
Humans (70kg)	?	?	4-6l pressure control IAP

O_3/O_2-PP vs. O_3-AHT

O_3/O_2-PP	**O_3-AHT**
large volumes	relative small volumes
large dosis (1-4mg O_3/kg)	relative small dosis (0.07-0.14mg O_3/kg)
no blood contact	ozonized blood

O3/O2-PP vs O3-AHT

Species	µg/ml	mg/kg	x days	total dosis mg/kg	application	diseases etc.	literature
human	1- 50	0.01 - 0.07	> 10	0.1 – 0.7	O3-AHT	various	Bocci (book)
rabbit	50	4	5	20	O3/O2-PP	cancer (VX2)	Schulz,2004 Schulz,2008
rat	10	0.8	5	40	O3/O2-PP	prevention (sepsis)	Schulz,2003
rat	50	4	5	20	O3/O2-PP	basic research	Sch. +N.
rat	50	1.6	45	72	O3/O2-PP	toxicol. research	B. + Sch.
mouse	50	4.0	5	20	O3/O2-PP	basic research	N. + Sch.
human (first trials) Case reports Pilot- results	50	?	4-18	?	O3/O2 –PP	cancer (various)	Austria Brazil, Spain Germany Swiss

Ozone therapy in a cancer model

Vx2 carcinoma: head and abdomen in rabbits.

A pilot study

Dr. Siegfried Schulz

Veterinary Services and Laboratory Animal Medicine,
Philipps-University of Marburg, Germany

In cooperation with

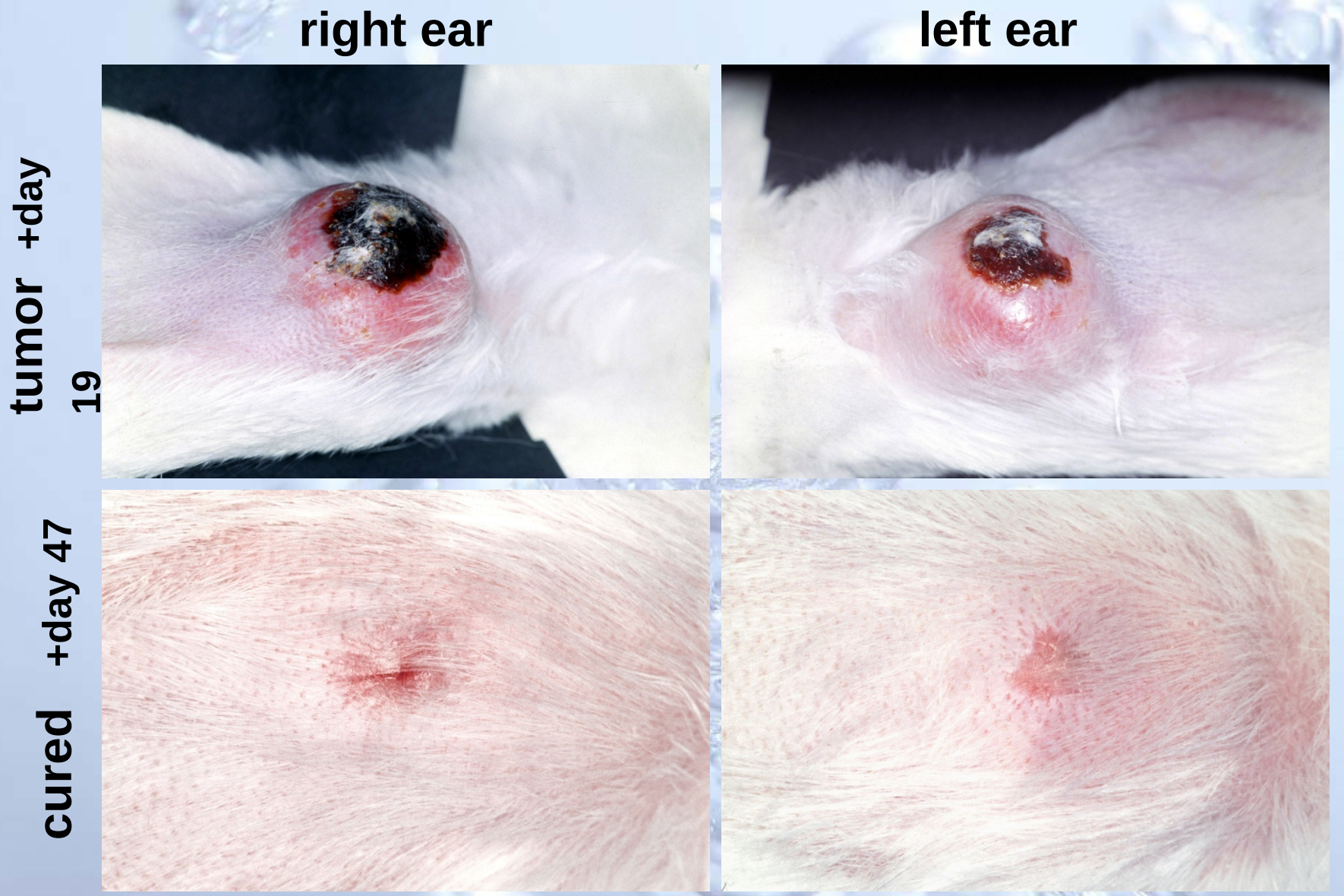
N. Sapundzhiev, A. Dünne, A. Ramaswamy,

R.Nüsing, R. Moll, JA. Werner, M.Bette

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 (%)			
	ear	day +18	day +23	day +33	final day +39-83)	
Case 1 ctrl NZ	left 1	24.4 x 21.9	+15.4 x +15.0	n.d.	+24.4 x +64.0	+47
	right 1	27.7 x 24.9	+11.4 x +15.0	n.d.	+71.0 x +48.0	
Case 2 NZ	left 1	22.1 x 17.8	-27.4 x -22.9	no tumor	no tumor	+47
	right 1	17.3 x 17.7	-2.1 x -6.6	-57.4 x -64.7	no tumor	
Case 3 NZ	left 1	30.7 x 15.8	n.d.	n.d.	- 2.0 x -20.0	+39
	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 NZ	left 2	17.5 x 15.5	n.d.	n.d.	no tumor	+83
	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 Chinch.	left 1	25.6 x 21.5	n.d.	- 6.2 x -2.3	no tumor	+43
	right 1	19.5 x 19.0	n.d.	no tumor	no tumor	



Total tumors: s = 10
disappearance to tumors after O3/O2 treatment s = 5 (50%)

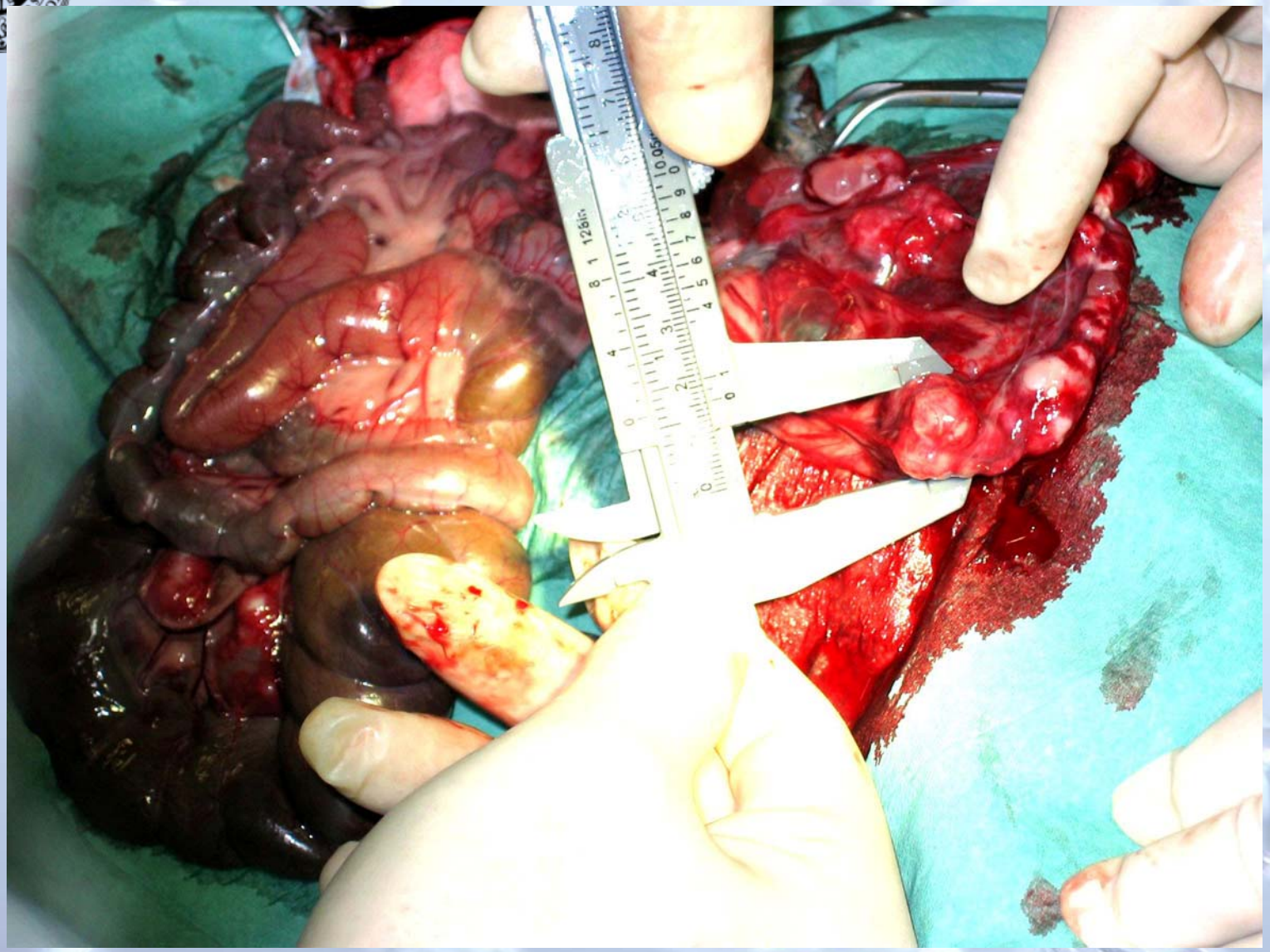
Histopathological analysis of cranial and thoracical organs of Vx2 tumor infected rabbits: Influence of O_3/O_2-PP

				lymph nodes			
	ear	necropsy	ear tumor	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 +			
			disappeared	perinodal spread	neg.	neg.	multiple
	right 2		ulcerated, necrotic	metastasis +	neg.	neg.	metastasis
			ulcerated, necrotic	perinodal spread			
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.

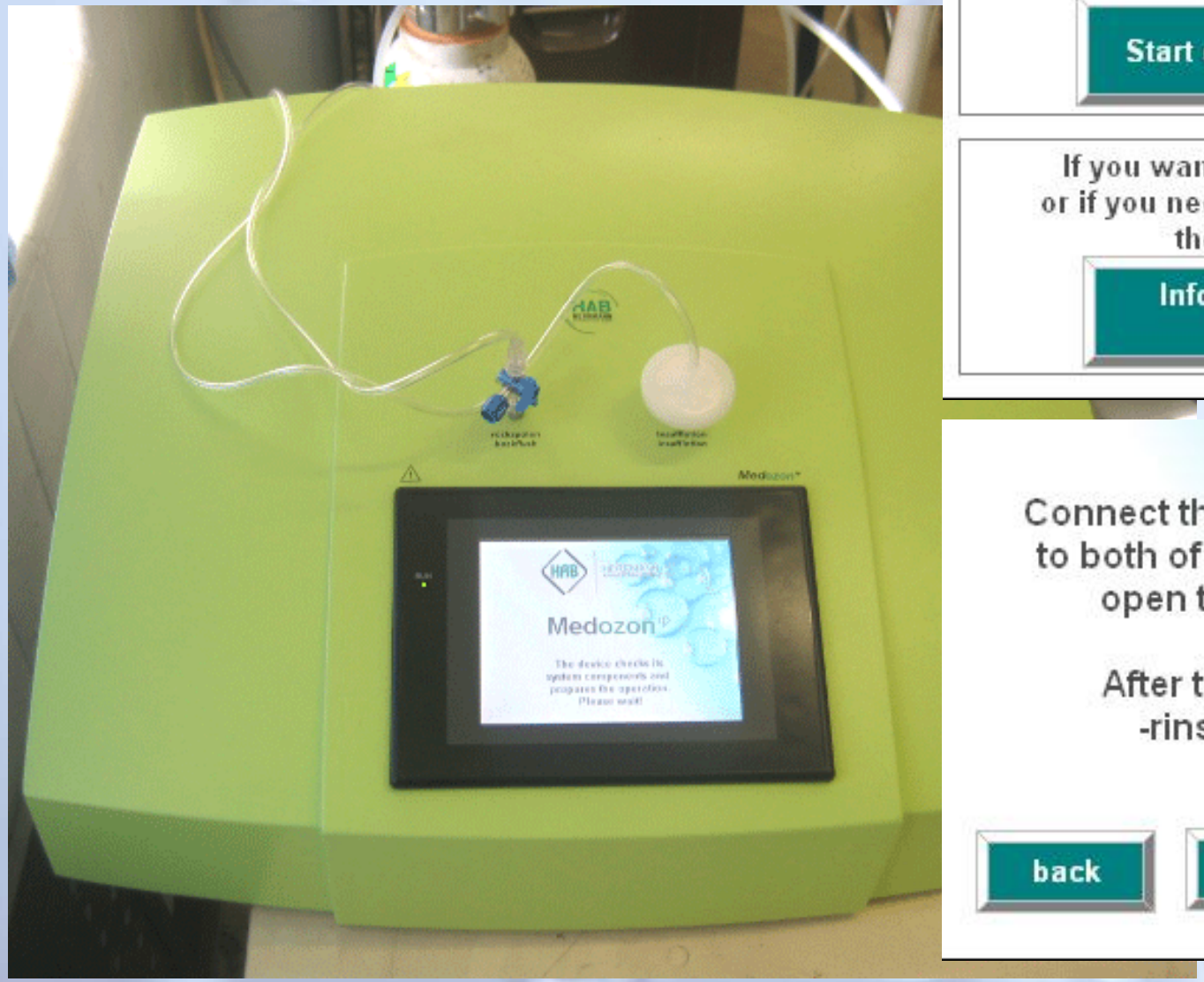
Dr. Siegfried Schulz

Veterinary Services and Laboratory Animal Medicine,

Philipps-University of Marburg, Germany

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

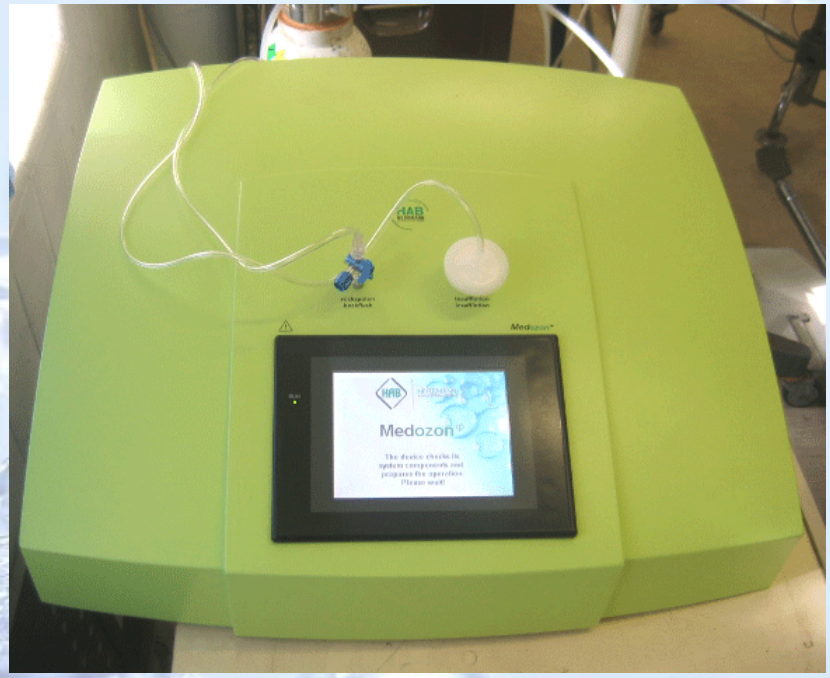
Connect the disposable material to both of the connections and open the three-way tap.

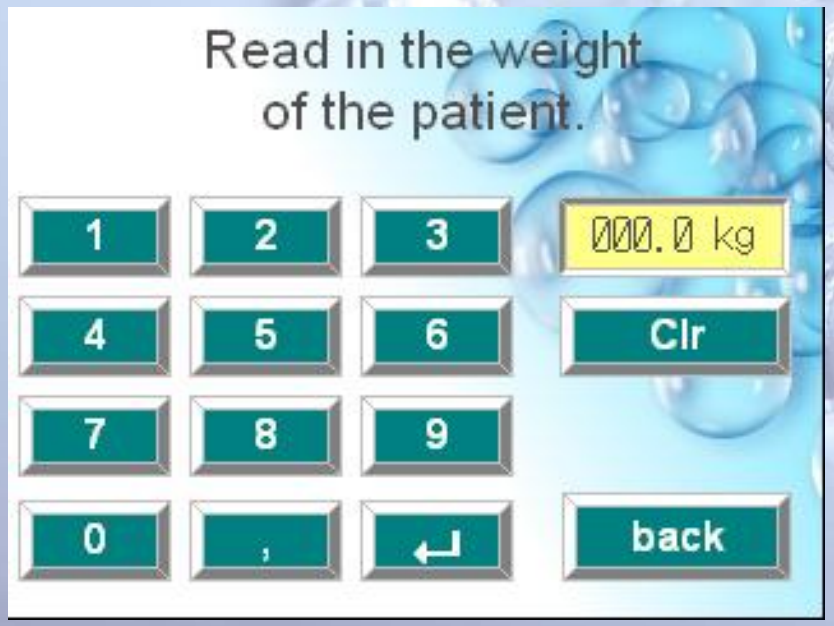
After that press the key -rinse Ozone-kit ip-

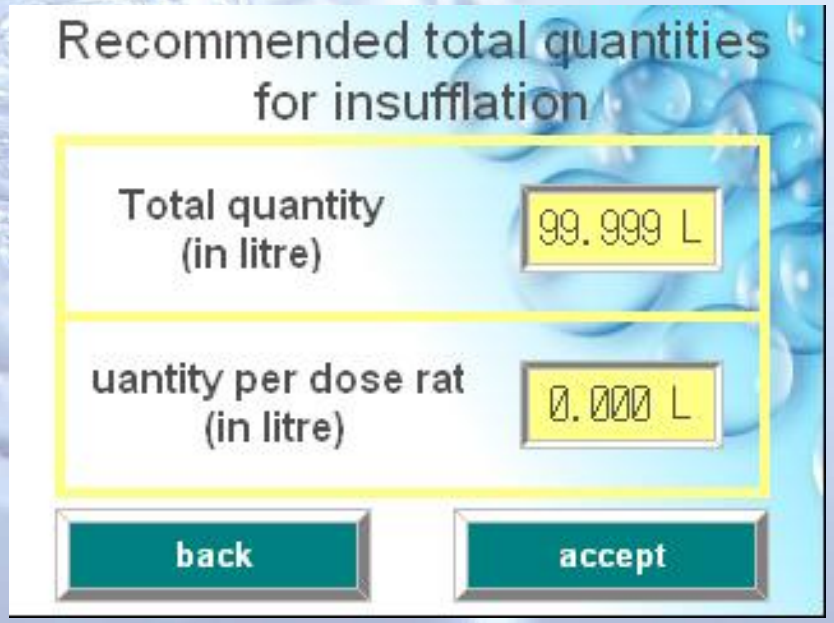
back

Rinse Ozone-kit ip

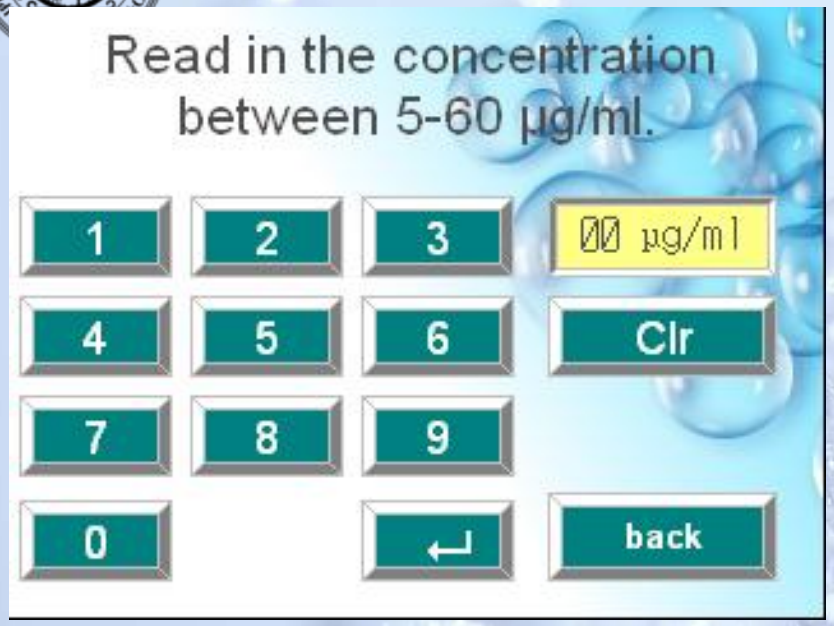
The Medozon^{ip} generator

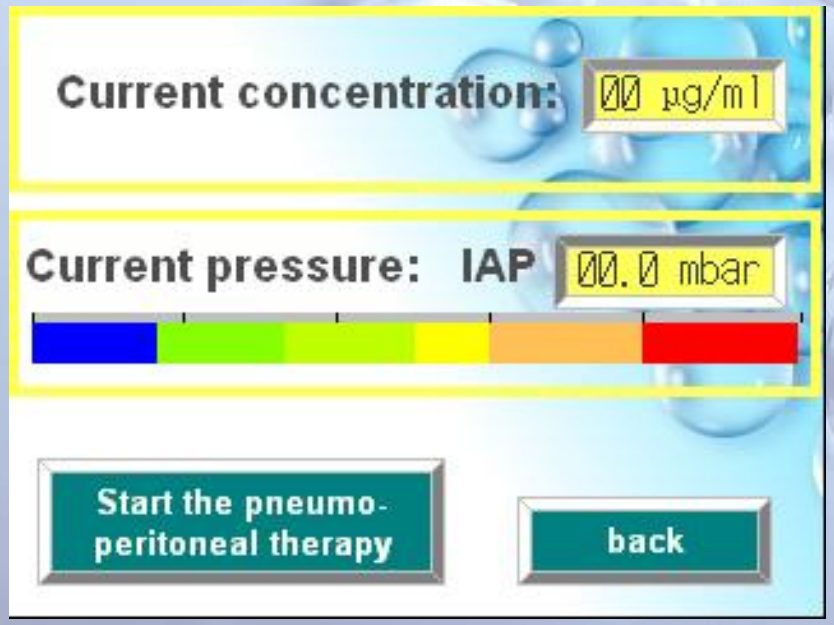


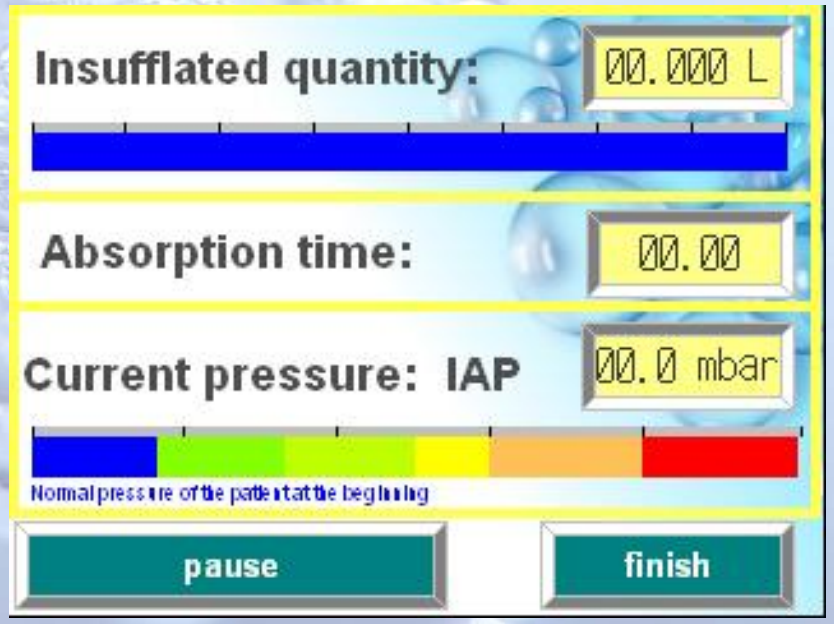




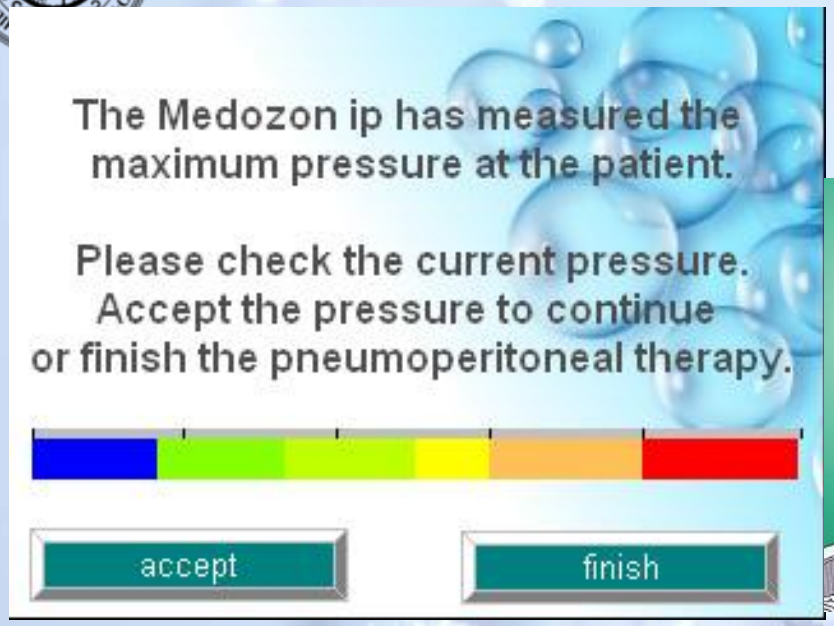
The Medozon^{ip} generator

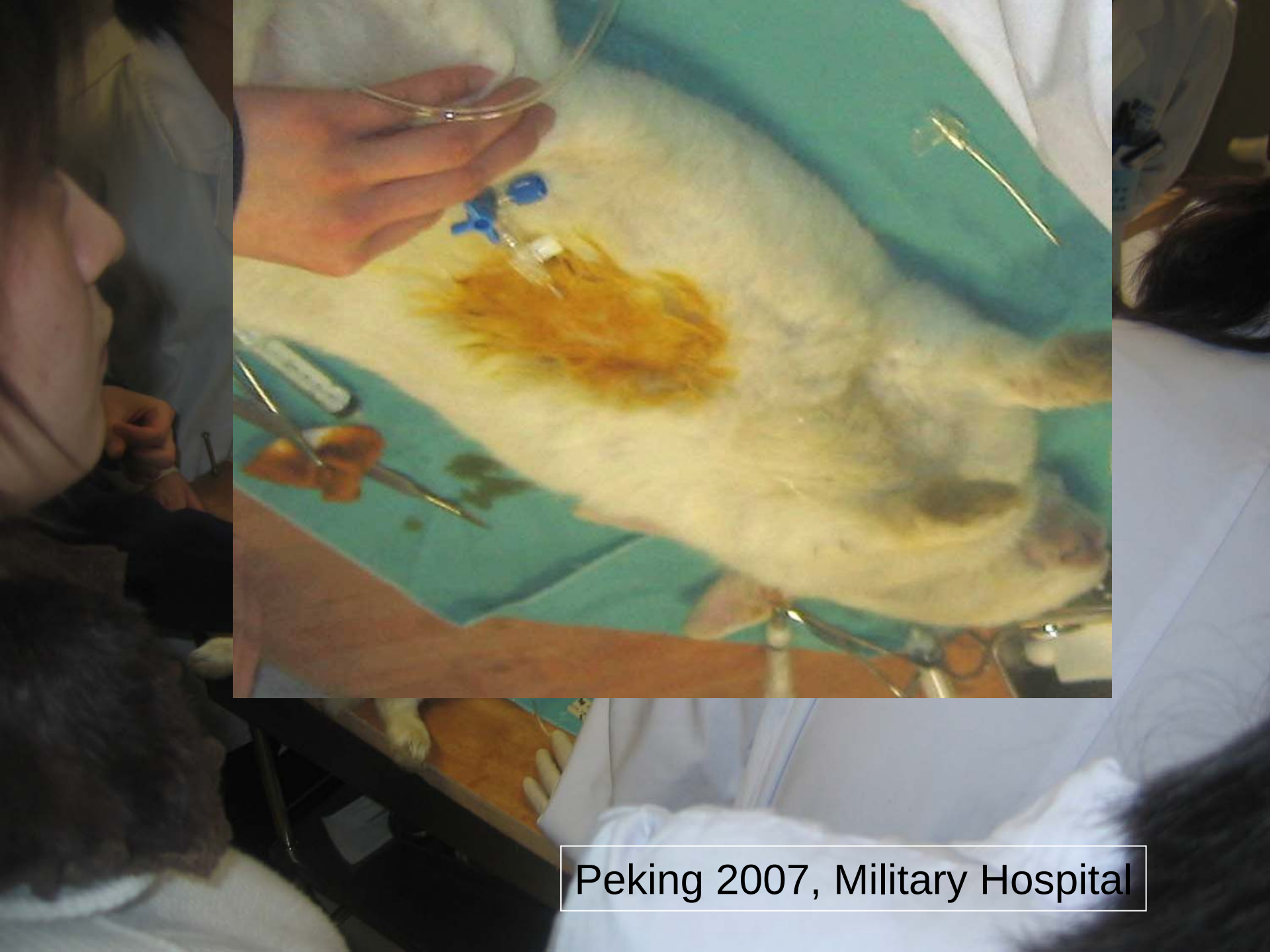






The Medozon^{ip} generator





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)

tongue (nude-mice)

used in the study

B. abdomen: systemic (i.p.)

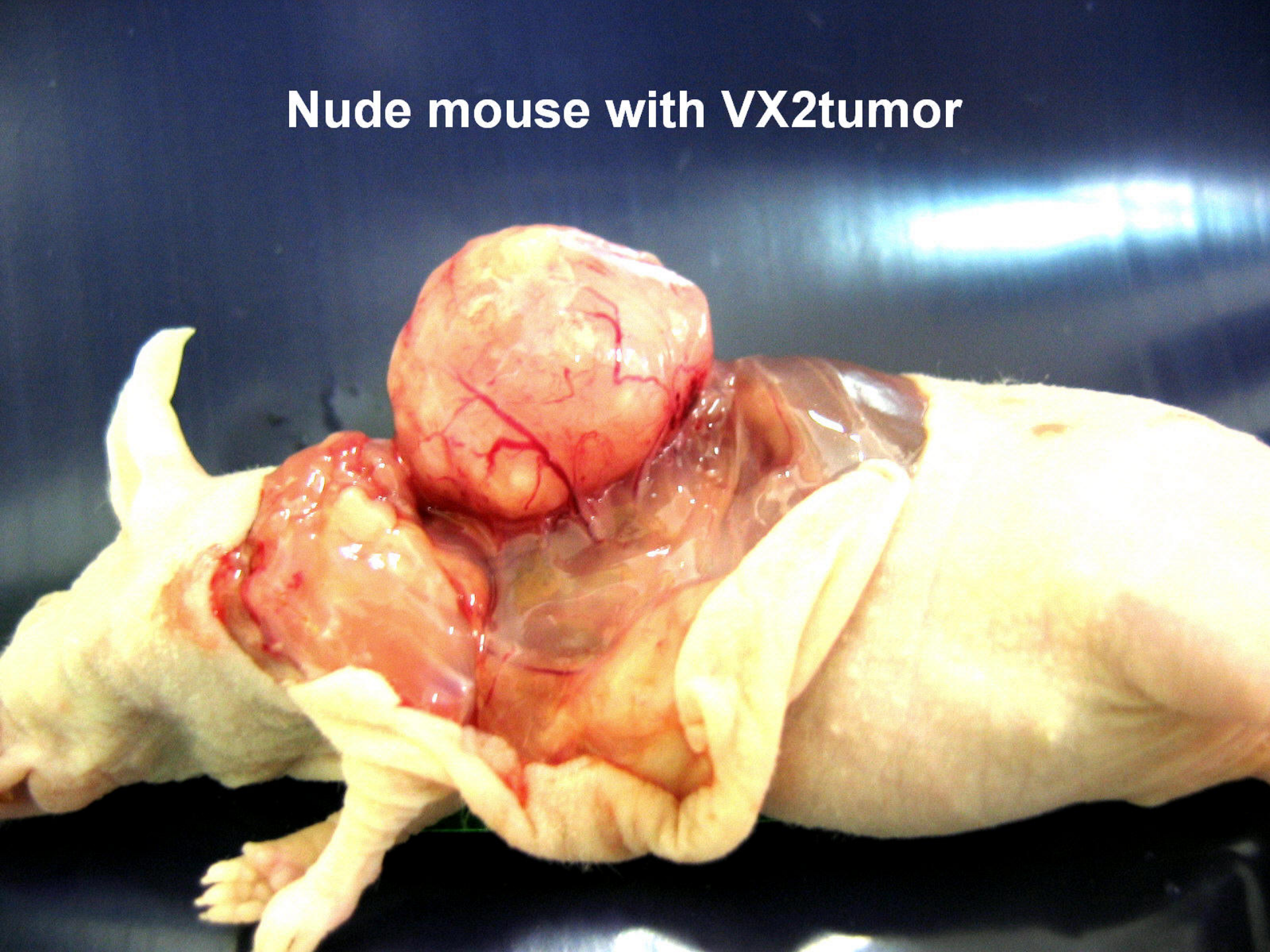
used in the study

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

way of VX2 transplantaion

- solid tumour pieces
- tumour cell suspension

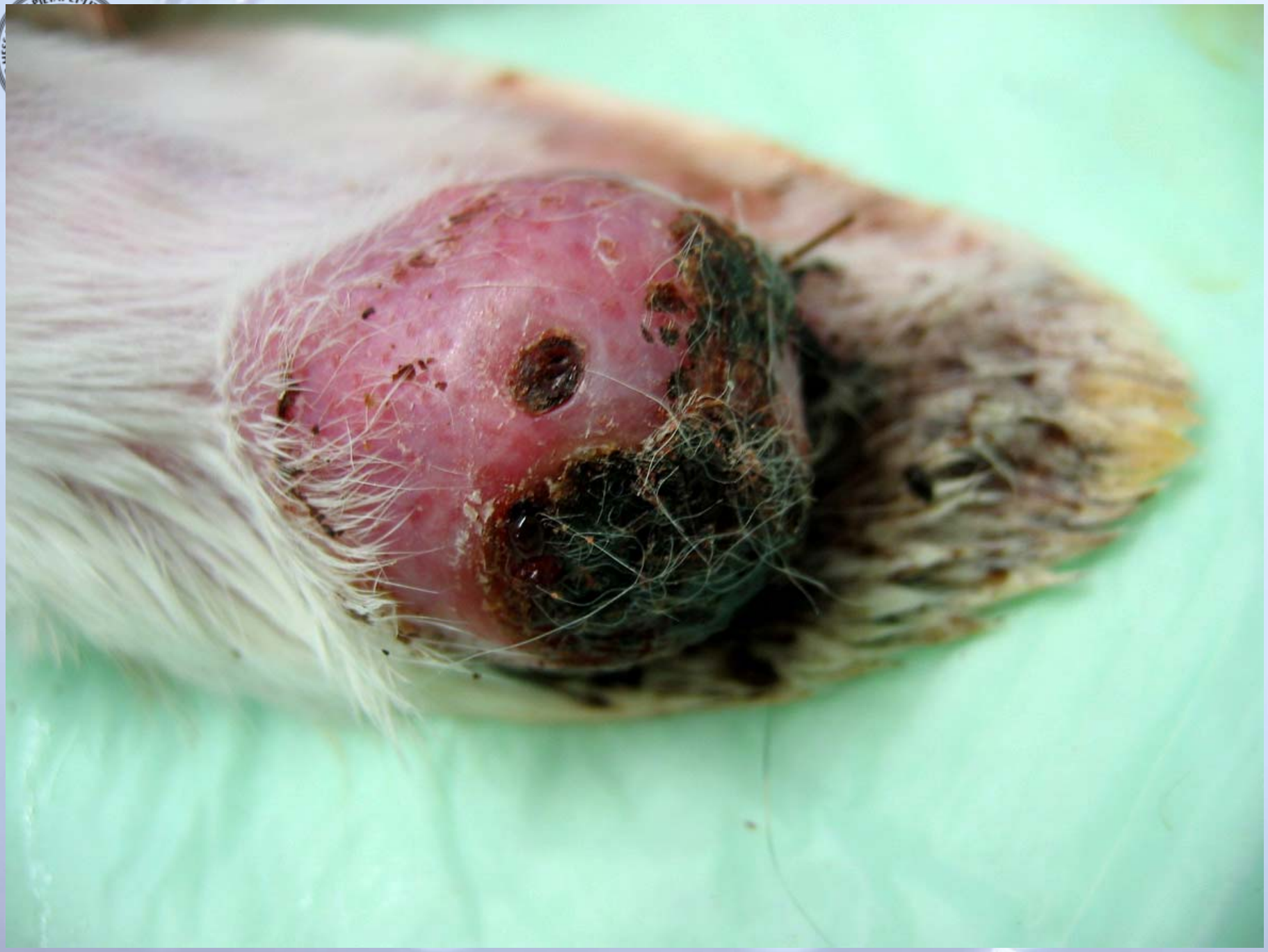
used in the study


Nude mouse with VX2tumor

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)

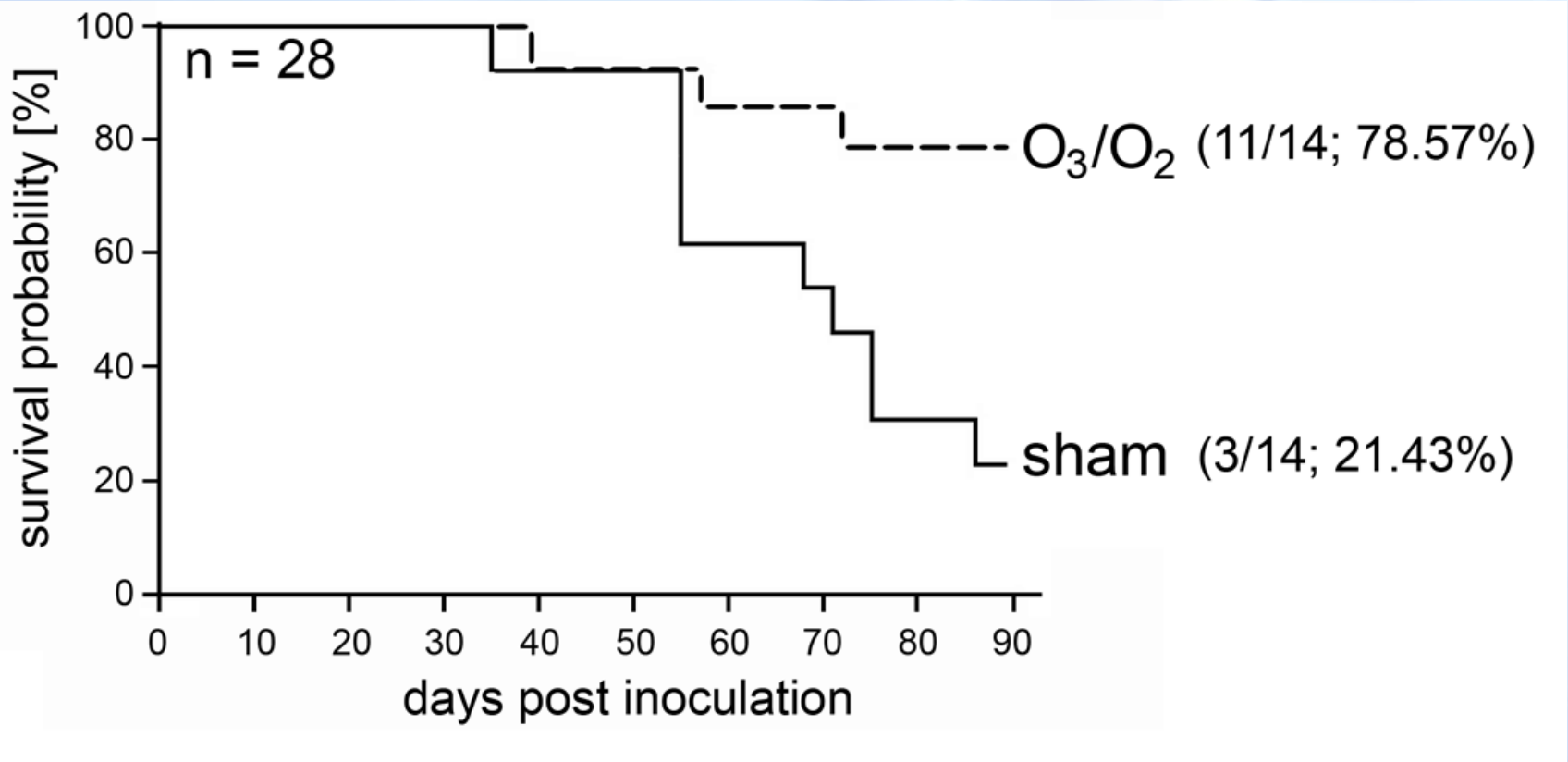
b. occurrence of metastasis in cervial lymph nodes, lung

c. multiplicity* of growing tumors in the lung

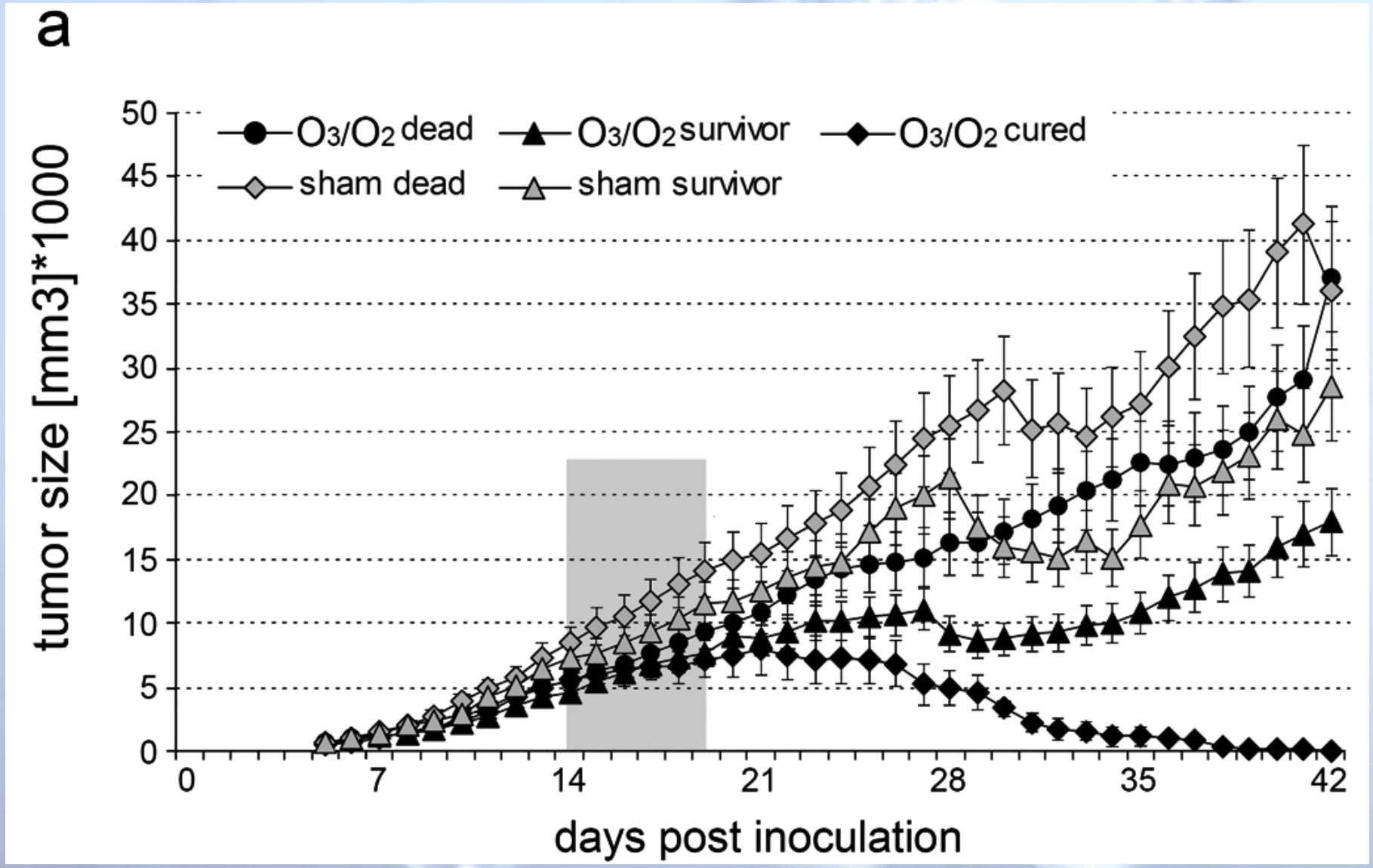
d. body weight

e. survival rate

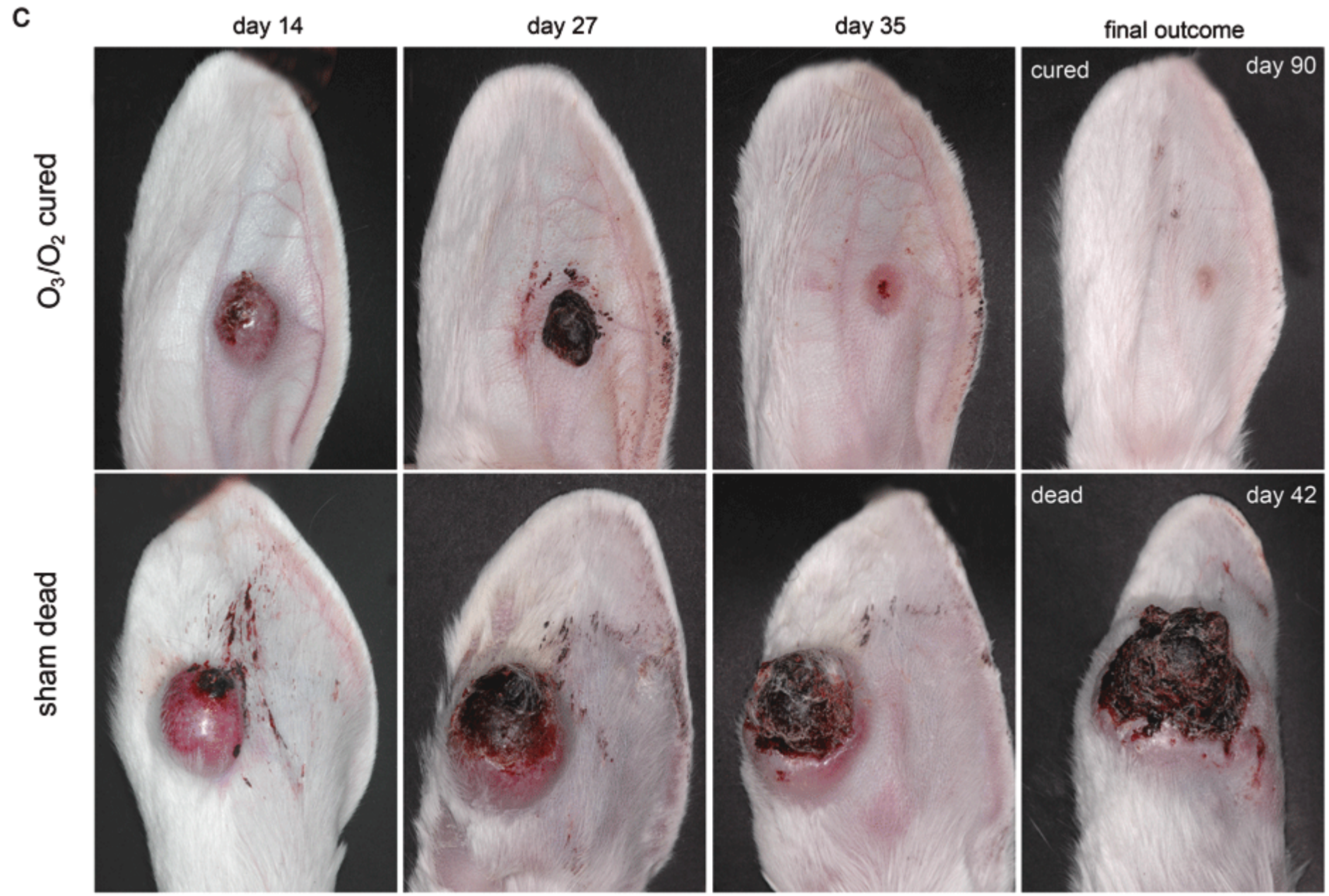
Survival probability

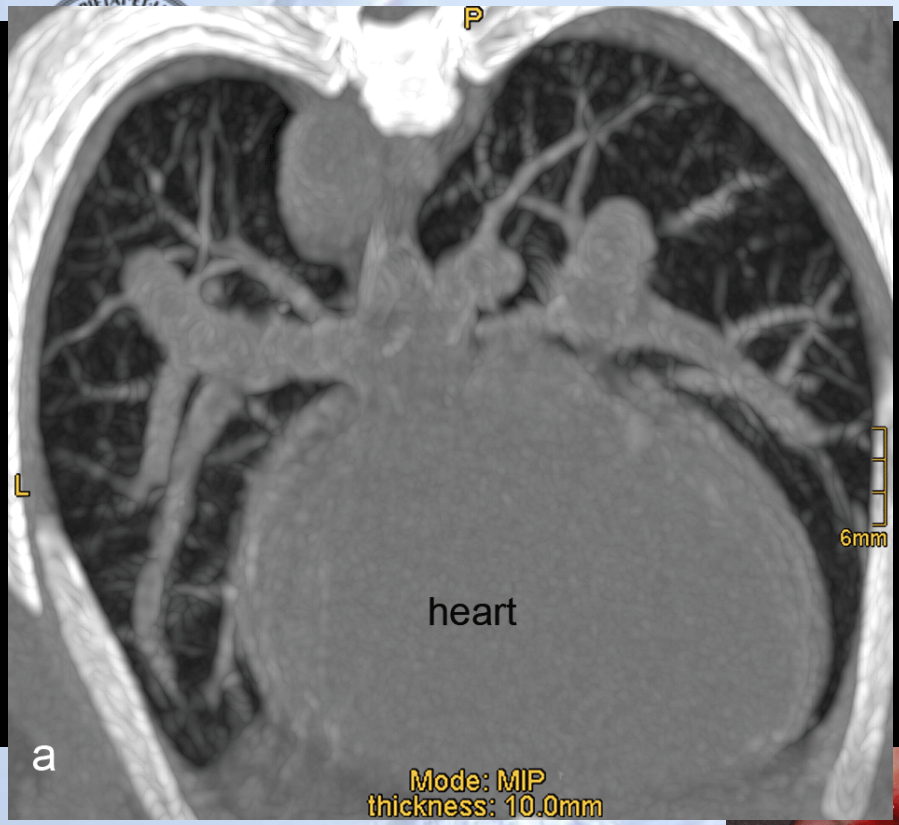


Tumor development



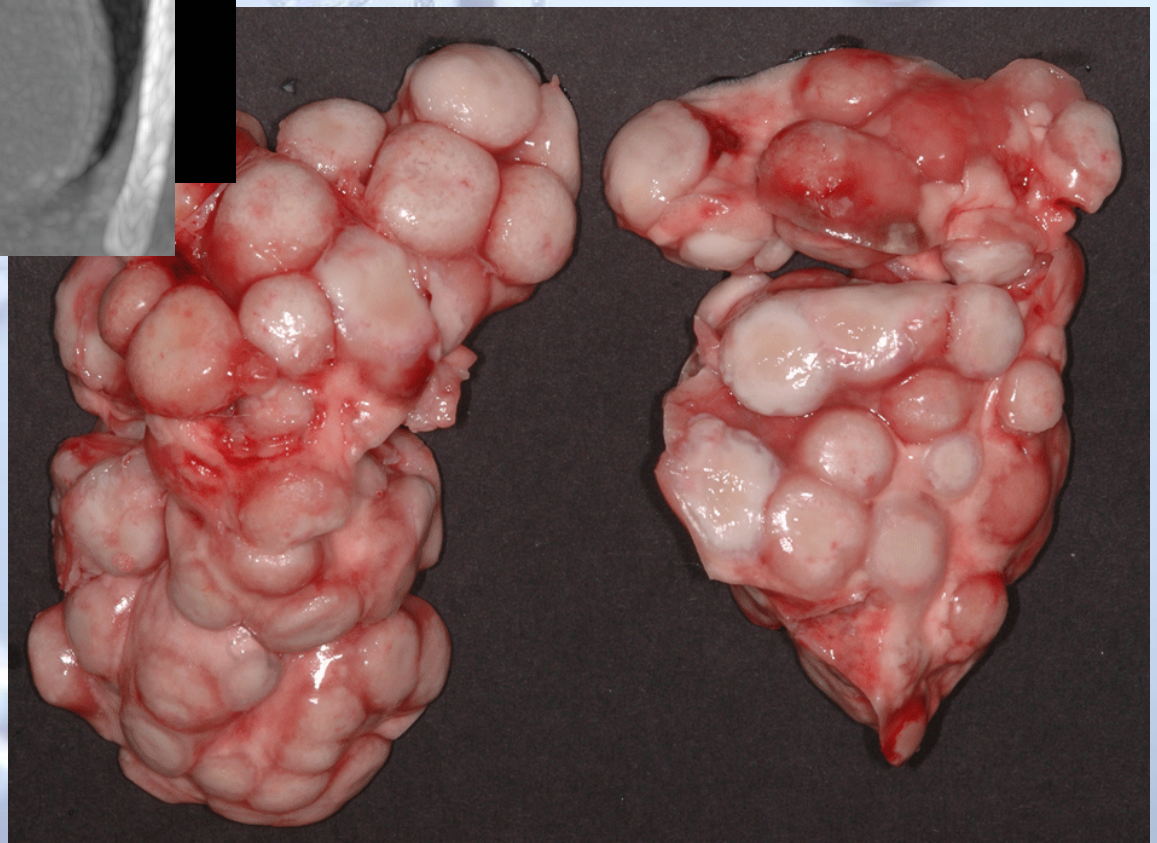
Tumor development



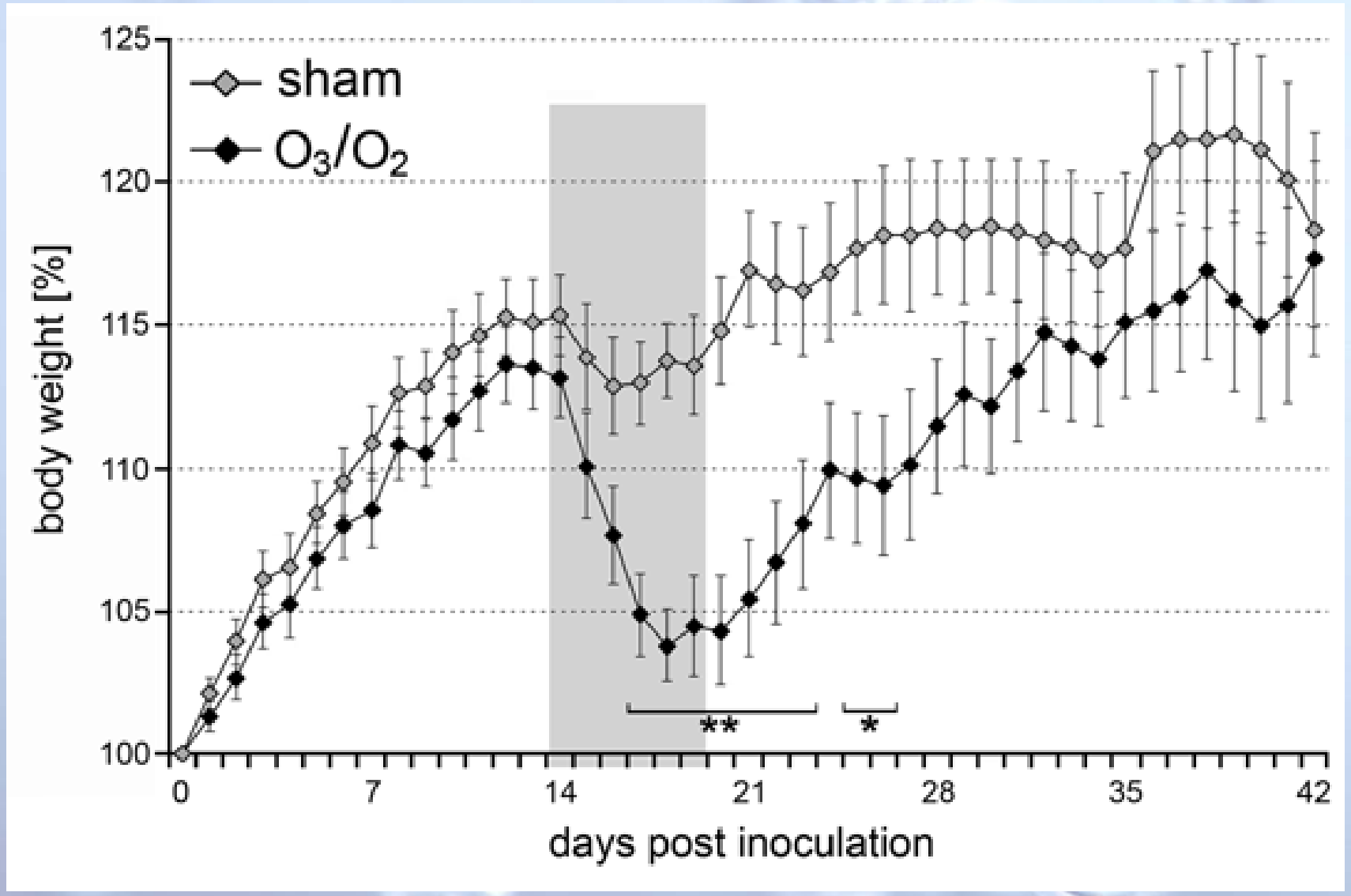


CT scan of the thorax of a O3/O2-cured rabbit

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-F1a	0.014 (0.002-0.037)	1.182 (0.281-1.935)	**84.5**	4.0
6-keto-PGF1a	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
PGF2a	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

O_3/O_2-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 O_3/O_2-PP in cancer patients from Brazil

Pat.	Volume (total ml)	x d	range (L.)	b.w. (kg)	mean volume/d (ml/kg O_3/O_2)	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	12 300	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

$$x = \frac{40.2}{} = 2 \text{ mg } O_3/\text{kg}$$

First therapeutical trials with O_3/O_2-PP in veterinary medicine

dosis : 80ml O_3/O_2/kg x 50 ug/ml = 4 mg O_3 / kg x 5 d = 20 mg O_3/ 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 (O_3/O_2 PP , O_3-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 O_3)

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

Scientific challenge in ozone research

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

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

h. **Insufflation and desufflation (O_3/O_2-PP); role of oxygen ?**

i. **Local and systemic effects and mechanisms with O_3/O_2 –PP and other methods**

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

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

l. **Ozone and biomarkers etc.**

A. head : (bi)-auricular model (rabbit) tongue (nude-mice)

B. abdomen: systemic (i.p.) organic **e.g. liver, uterus, kidney, bladder**

Hypothesized mechanism of ozon 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**

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