



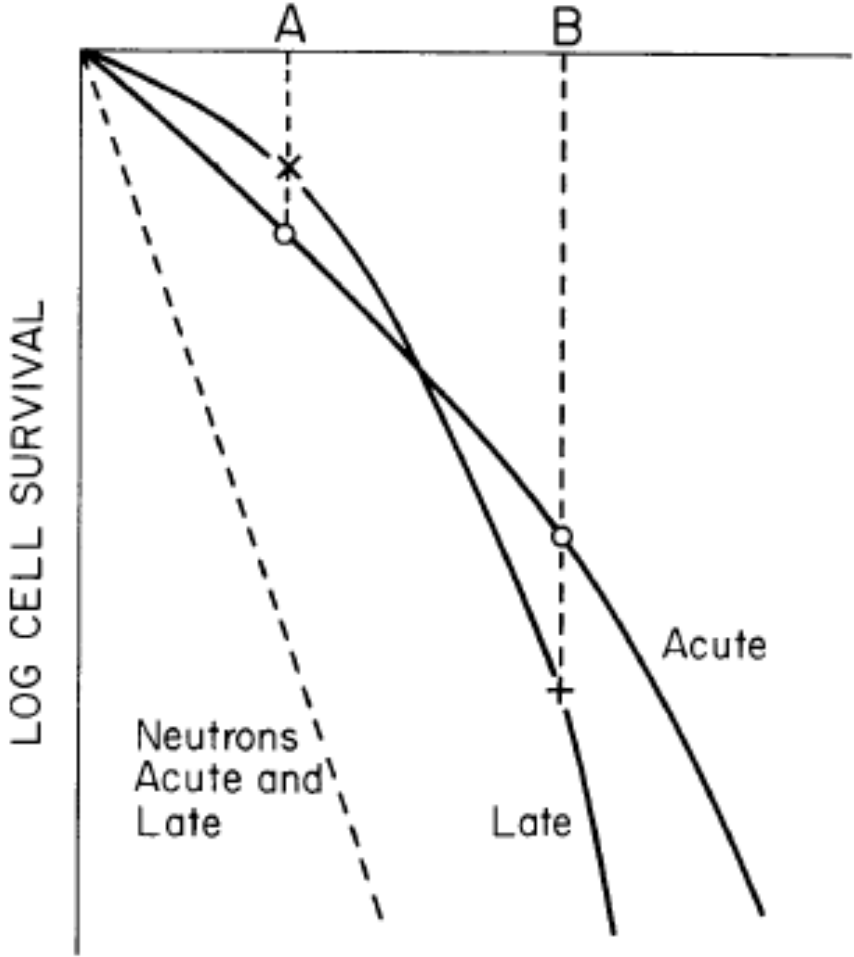
Update on IORT biology

Frederik Wenz
Department of Radiation Oncology
Universitätsmedizin Mannheim
University of Heidelberg

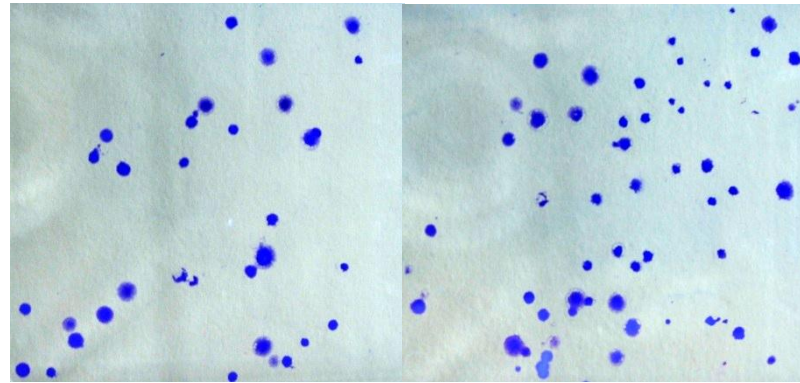
Radiobiology of high single doses

DOSE

more late effects in
late reacting tissue
e.g. breast, brain, liver, lung

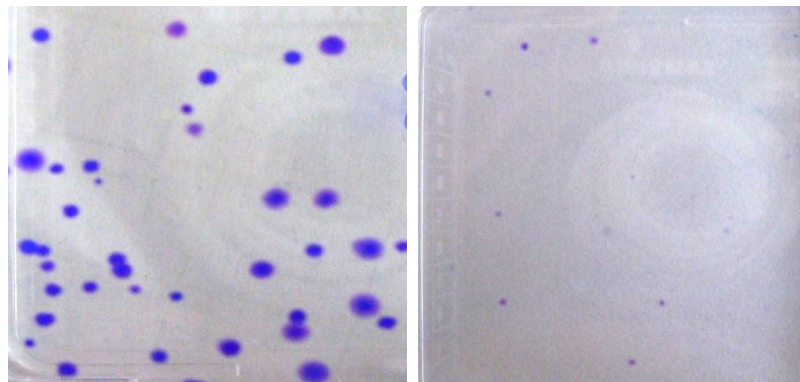


Radiobiology of high single doses



Unirradiated

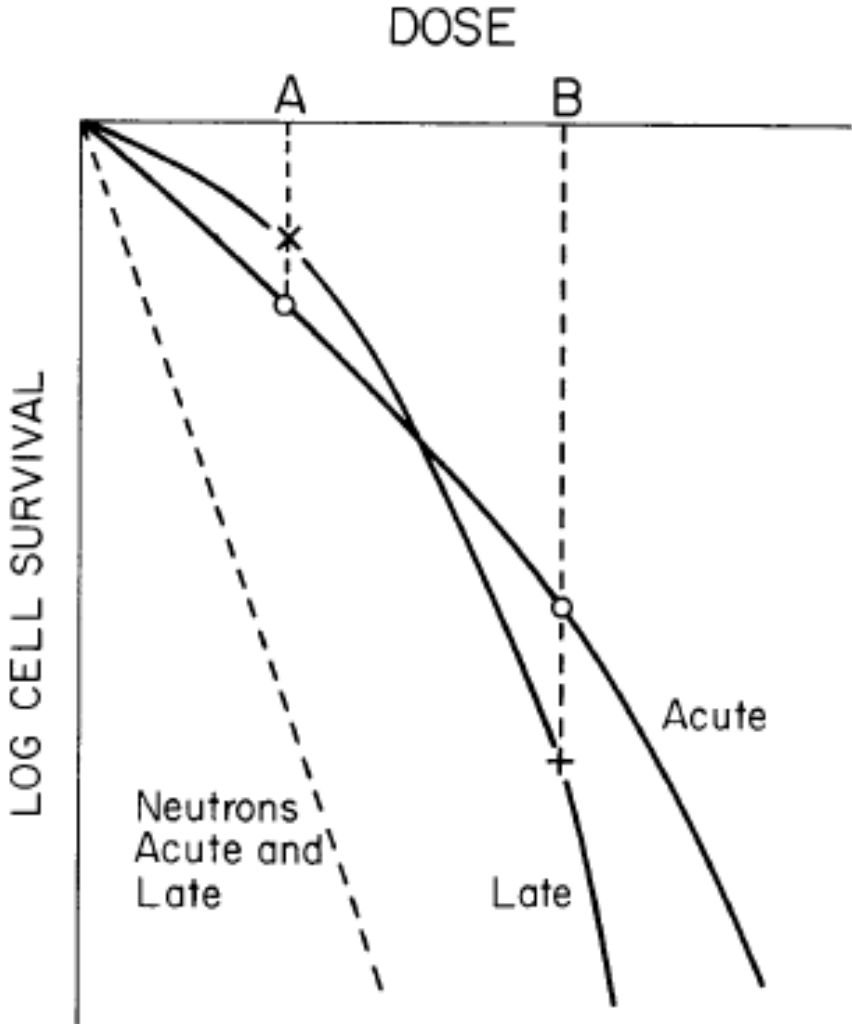
D=6 Gy



Unirradiated

D=15 Gy

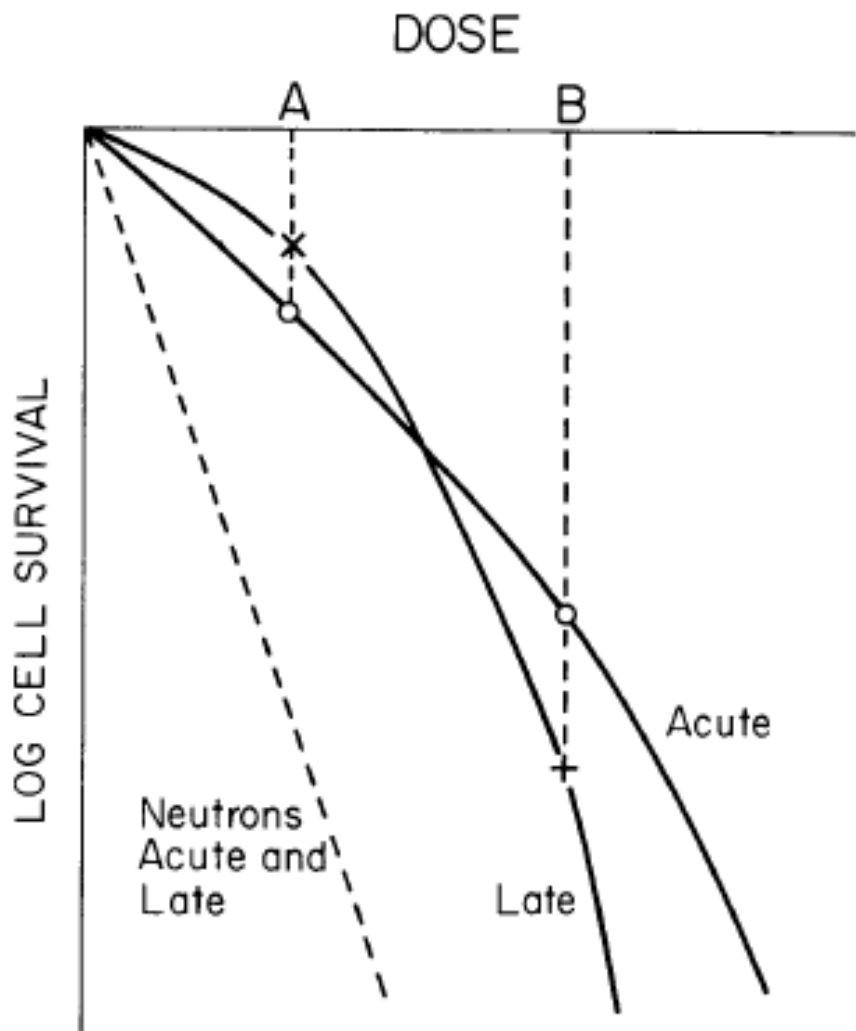
Radiobiology of high single doses



more late effects in late reacting tissue
e.g. breast, brain, liver, lung



Radiobiology of high single doses



more late effects in late reacting tissue
e.g. breast, brain, liver, lung



**BEWARE OF
HIGH SINGLE DOSES**

Radiobiology of high single doses

tumor $\alpha/\beta = 10 \text{ Gy}$
nl tiss $\alpha/\beta = 3 \text{ Gy}$

more late effects in
late reacting tissue
e.g. breast, brain, liver, lung

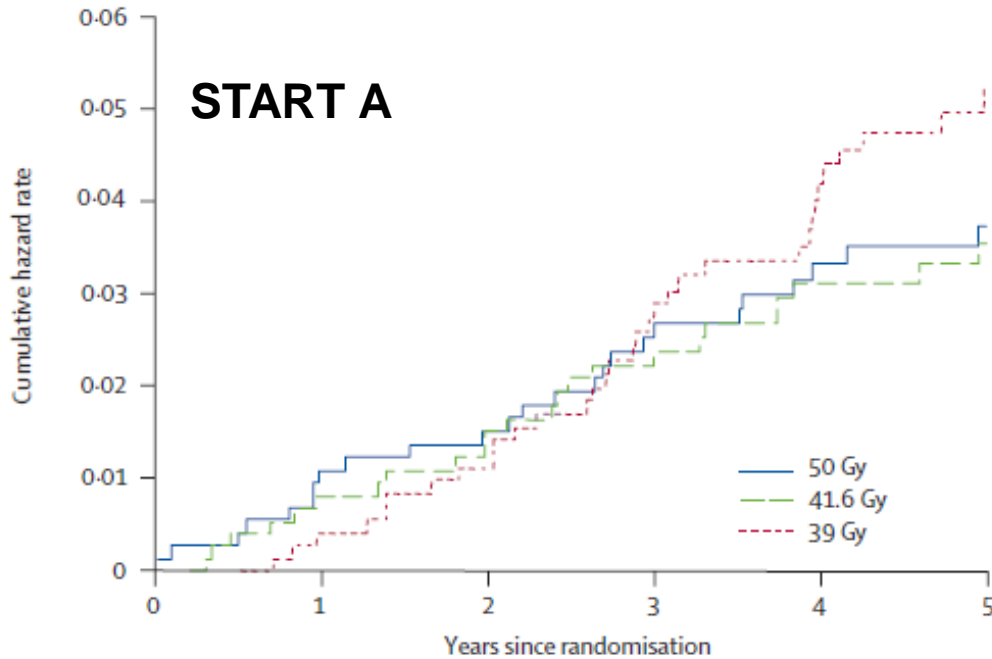


**BEWARE OF
HIGH SINGLE DOSES**

Radiobiology of high single doses

tumor $\alpha/\beta = 10$ Gy
nl tiss $\alpha/\beta = 3$ Gy

more late effects in
late reacting tissue
e.g. breast, brain, liver, lung



tumor $\alpha/\beta = 3$ Gy

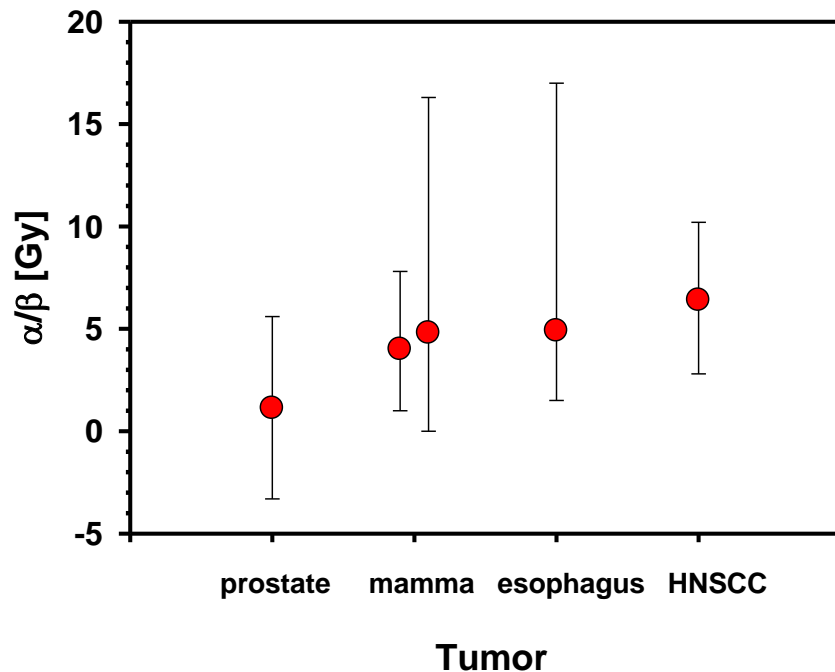


**BE CAREFUL WITH
HIGH SINGLE DOSES**

Radiobiology of high single doses

tumor $\alpha/\beta = 10$ Gy
nl tiss $\alpha/\beta = 3$ Gy

more late effects in
late reacting tissue
e.g. breast, brain, liver, lung



tumor $\alpha/\beta = 3-7$ Gy

prostate: Bentzen, Ritter, Radiother. Oncol. 76:1-3 (2005),
esophagus: Geh et al., Radiother. Oncol. 78:236-44 (2006)
HNSCC: Bentzen et al. ECCO 2007 (DAHANCA 5)



**BE CAREFUL WITH
HIGH SINGLE DOSES**

breast: Owen et al., Lancet Oncol 7:467-71 (2006)
START trialists group, Lancet Oncol 9:331-41 (2008)

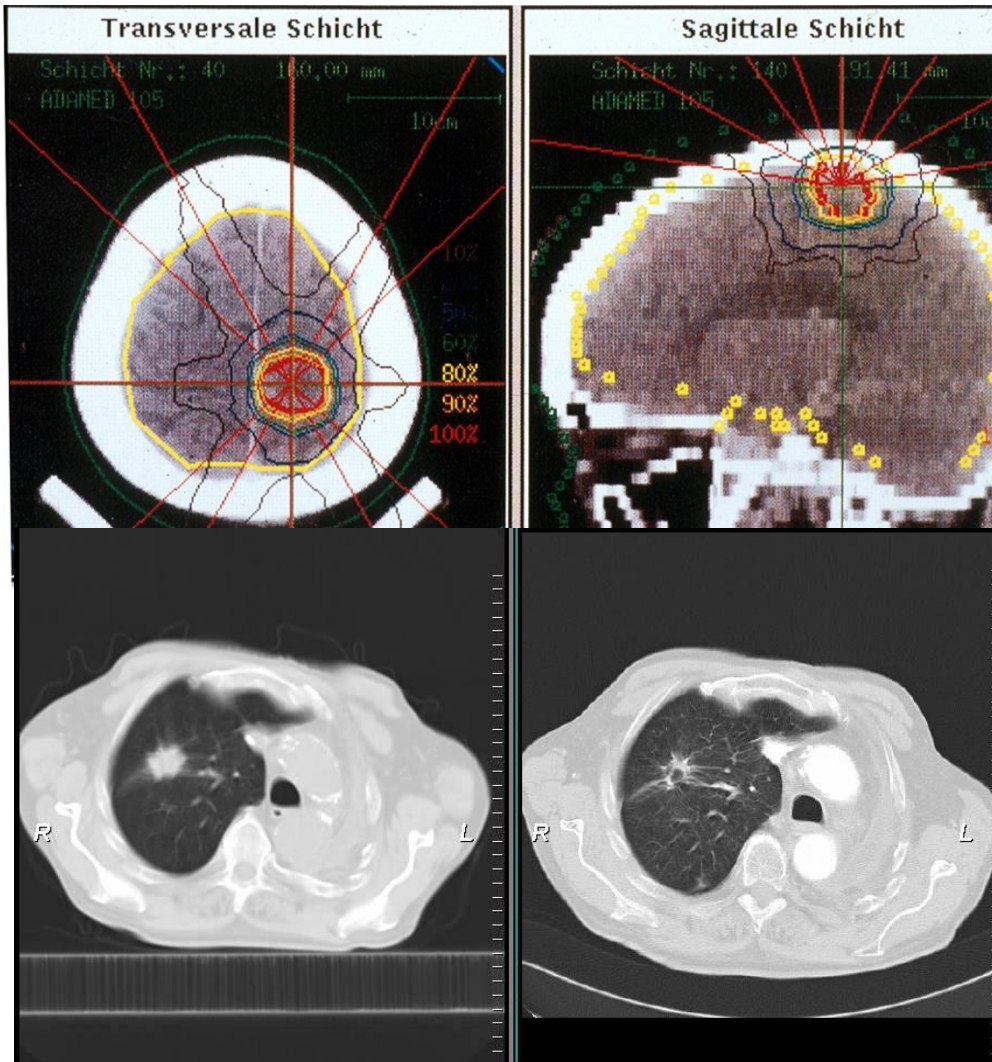
Radiobiology of high single doses

more late effects in
late reacting tissue
e.g. breast, brain, liver, lung



**BE CAREFUL WITH
HIGH SINGLE DOSES**

VOLUME MATTERS!! NO BENEFIT OF FRACTIONATION IN CASE OF SAME α/β !!



Why are high single doses more effective than expected?

Stereotactic Body Radiation Therapy in Multiple Organ Sites

J Clin Oncol 25:947-952. © 2007

Robert D. Timmerman, Brian D. Kavanagh, L. Chinsoo Cho, Lech Papiez, and Lei Xing

Table 1. Local Control in Early-Stage Non-Small-Cell Lung Cancer

Study	Year of Publication	Treatment	Local Control Rate (%)
North America/Europe			
Timmerman et al ¹⁰	2006	20-22 Gy × 3	95 at 2+ years
Bauman et al ⁴⁷	2006	15 Gy × 3	80 at 3 years
Fritz et al ⁴⁸	2006	30 Gy × 1	80 at 3 years
Nyman et al ⁴⁹	2006	15 Gy × 3	80, crude
Zimmermann et al ⁵⁰	2005	12.5 Gy × 3	87 at 3 years
Timmerman et al, ⁴⁵ McGarry et al ⁴⁶	2003, 2005	18-24 Gy × 3	90 at 2 years
Asia			
Xia et al ⁵²	2006	5 Gy × 10	95 at 3 years
Hara et al ⁵³	2006	30-34 Gy × 1	80 at 3 years
Nagata et al ⁵⁴	2005	12 Gy × 4	94 at 3 years

Why are high single doses more effective than expected?

INTRAOPERATIVE RADIOTHERAPY AS A BOOST DURING BREAST-CONSERVING SURGERY USING LOW-KILOVOLTAGE X-RAYS: THE FIRST 5 YEARS OF EXPERIENCE WITH A NOVEL APPROACH

Int. J. Radiation Oncology Biol. Phys., Vol. 77, No. 5, pp. 1309–1314, 2010

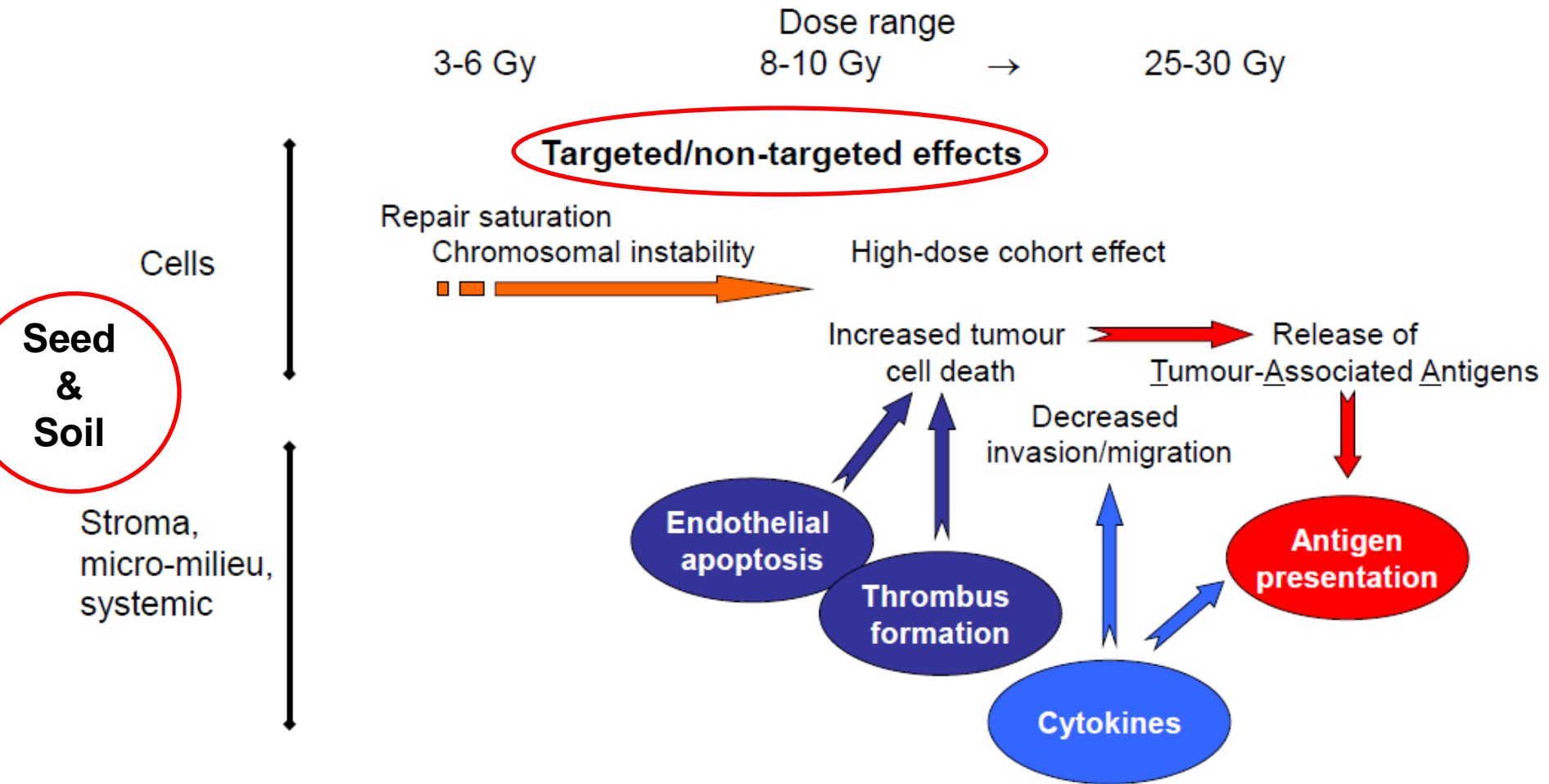
FREDERIK WENZ, M.D.,* GRIT WELZEL, M.Sc.,* ELENA BLANK, M.S.,* BRIGITTE HERMANN, M.D.,* VOLKER STEIL, M.Sc.,* MARC SÜTTERLIN, M.D.,† AND UTA KRAUS-TIEFENBACHER, M.D.*

Table 2. IORT as a boost followed by EBRT (including EORTC boost trial as benchmark)

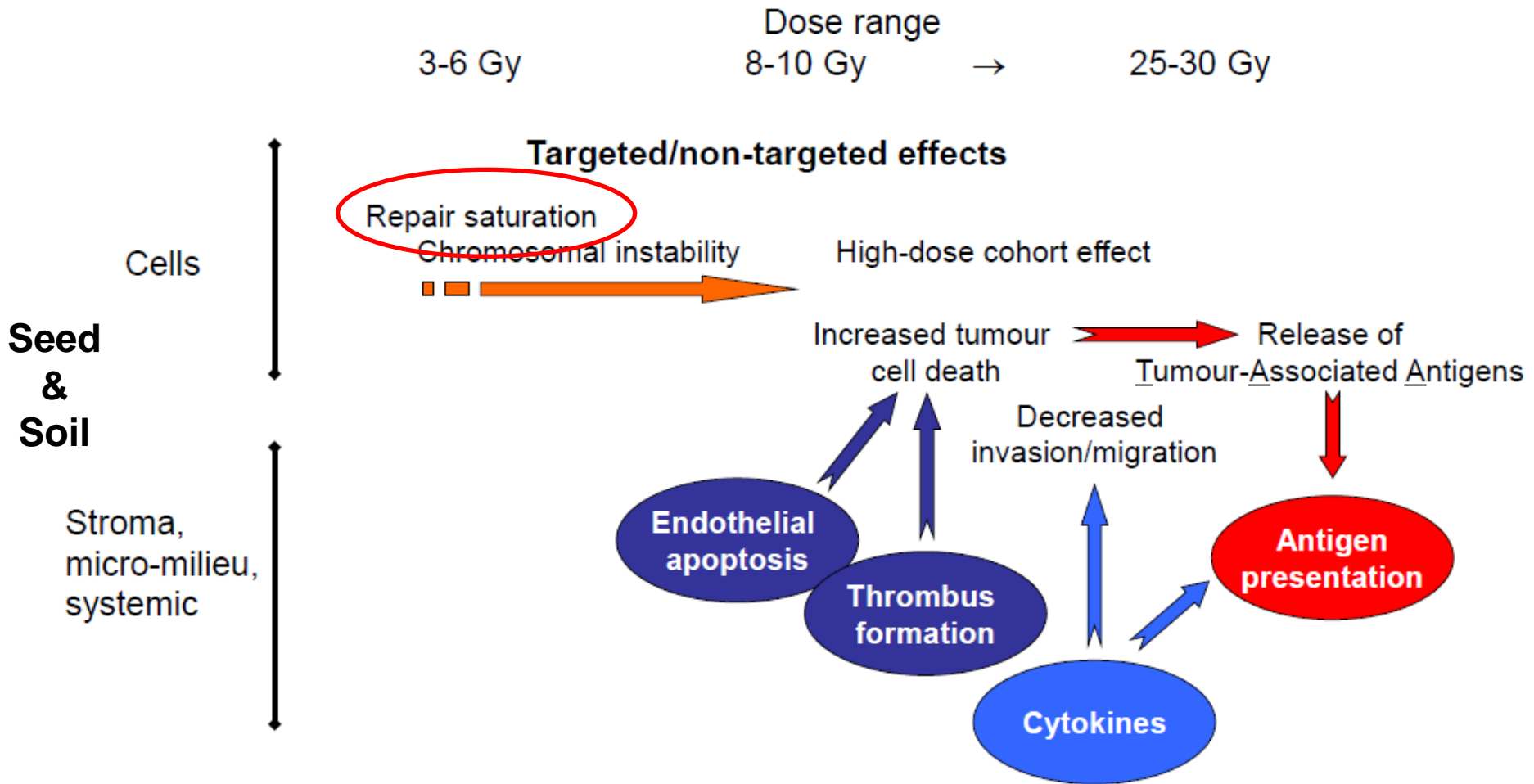
Authors, year (reference)	<i>n</i>	Method	5-y IBTR (%)	Late toxicity
Reitsamer <i>et al.</i> , 2008 (38)	1200	Electrons	0.5	Not reported
Vaidya <i>et al.</i> , 2006 (25)	301	50-kV X-ray	2.6	Not reported
Vaidya <i>et al.</i> , 2008 (39)	301	50-kV X-ray	1.5	Not reported
Reitsamer <i>et al.</i> , 2006 (17)	188	Electrons	0	Not reported
Present study	154	50-kV X-ray	1.5	Grade 3, 7% (3 y)
Harms <i>et al.</i> , 2002 (33)	113	Brachytherapy	5	Grade 3, 12% (5 y)
Lemanski <i>et al.</i> , 2006 (14)	50	Electrons	4 (9 y)	Grade 2, 14% (9 y)
Mussari <i>et al.</i> , 2006 (40)	47	Electrons	0 (4 y)	Grade 3, 6% (3 y)
Bartelink <i>et al.</i> , 2001 (4), 2007 (5)	2613	Electrons	4.1	Moderate/severe, 28% (10 y)

Abbreviations: IORT = intraoperative radiotherapy; EBRT = external-beam radiotherapy; EORTC = European Organization for Research and Treatment of Cancer; IBTR = in-breast tumor recurrence.

Radiobiology of high single doses

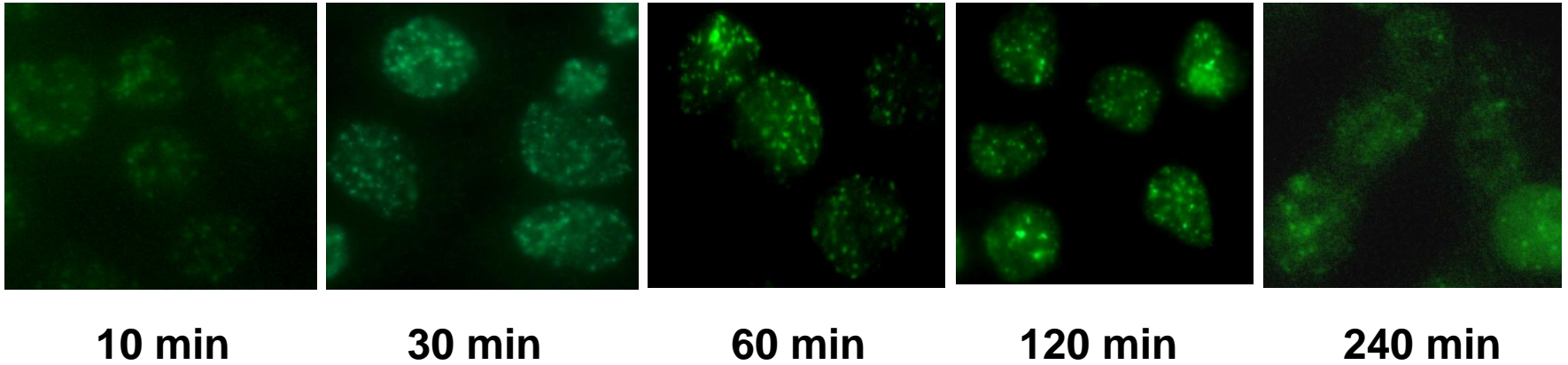


Radiobiology of high single doses

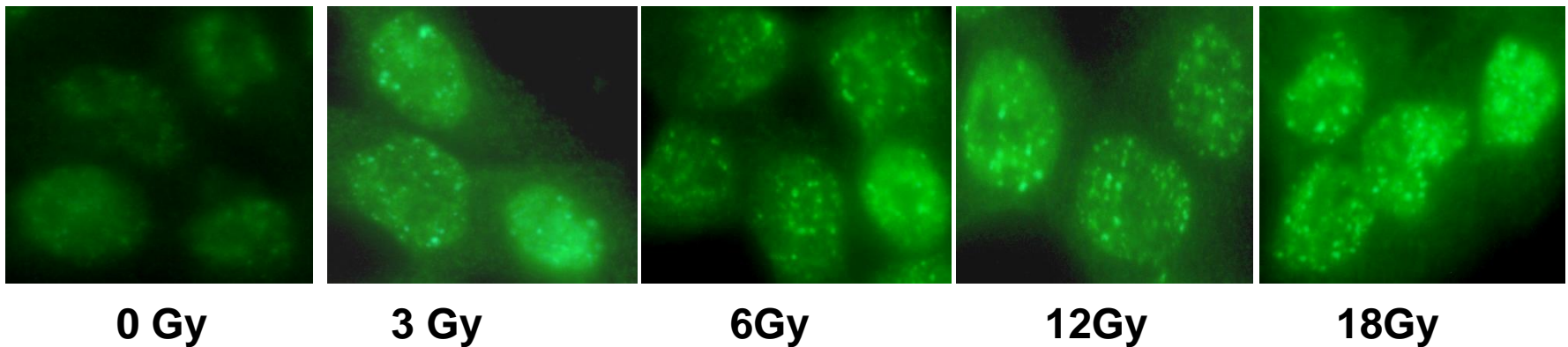


Saturation of repair

After D=2 Gy of 10 MeV electrons (V79 hamster cells)



Dose response (t=30 min)



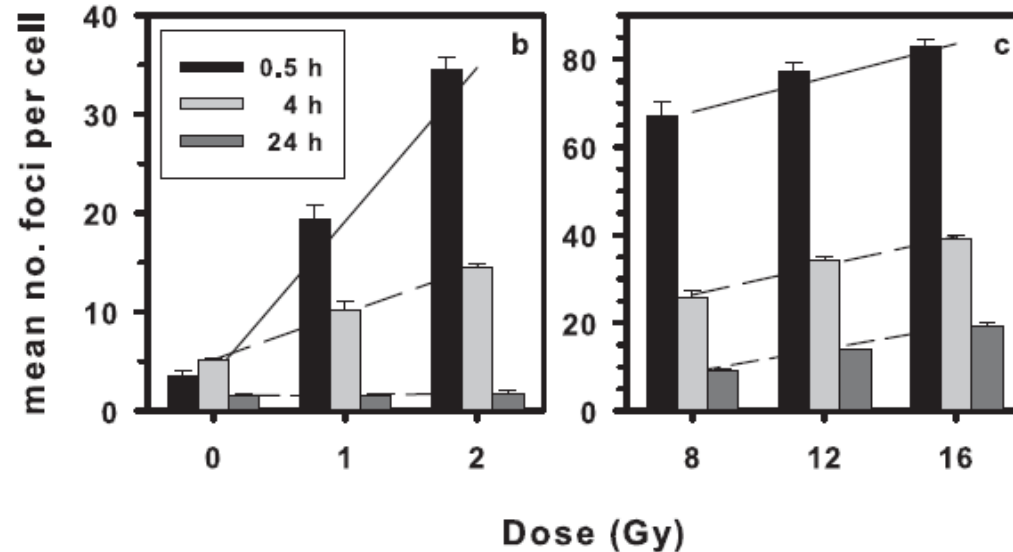
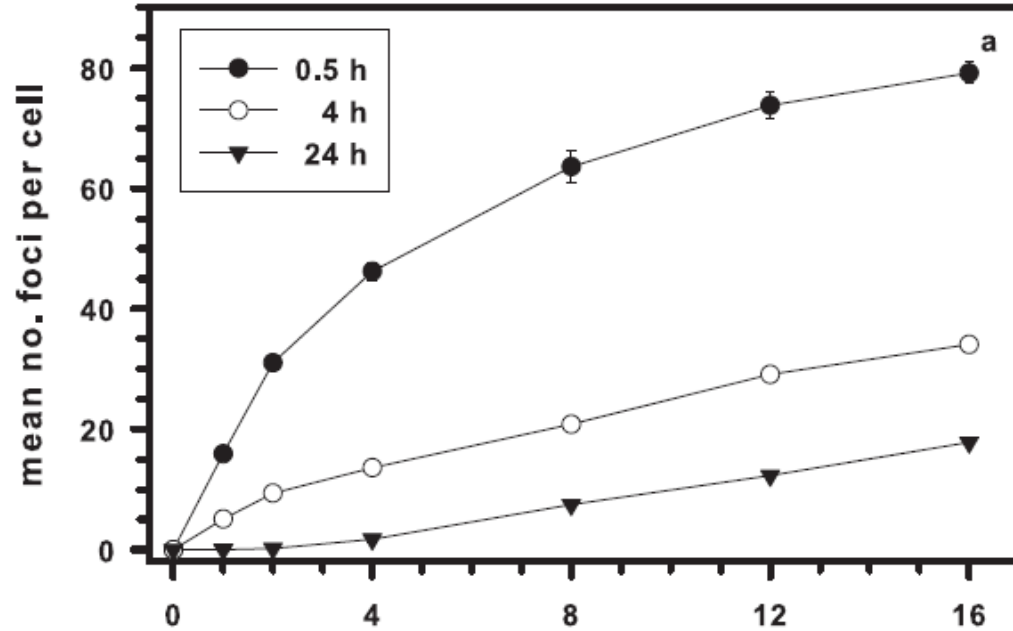
Induction and decay of DNA repair foci (γ H2AX)

L. Ma
MSc thesis

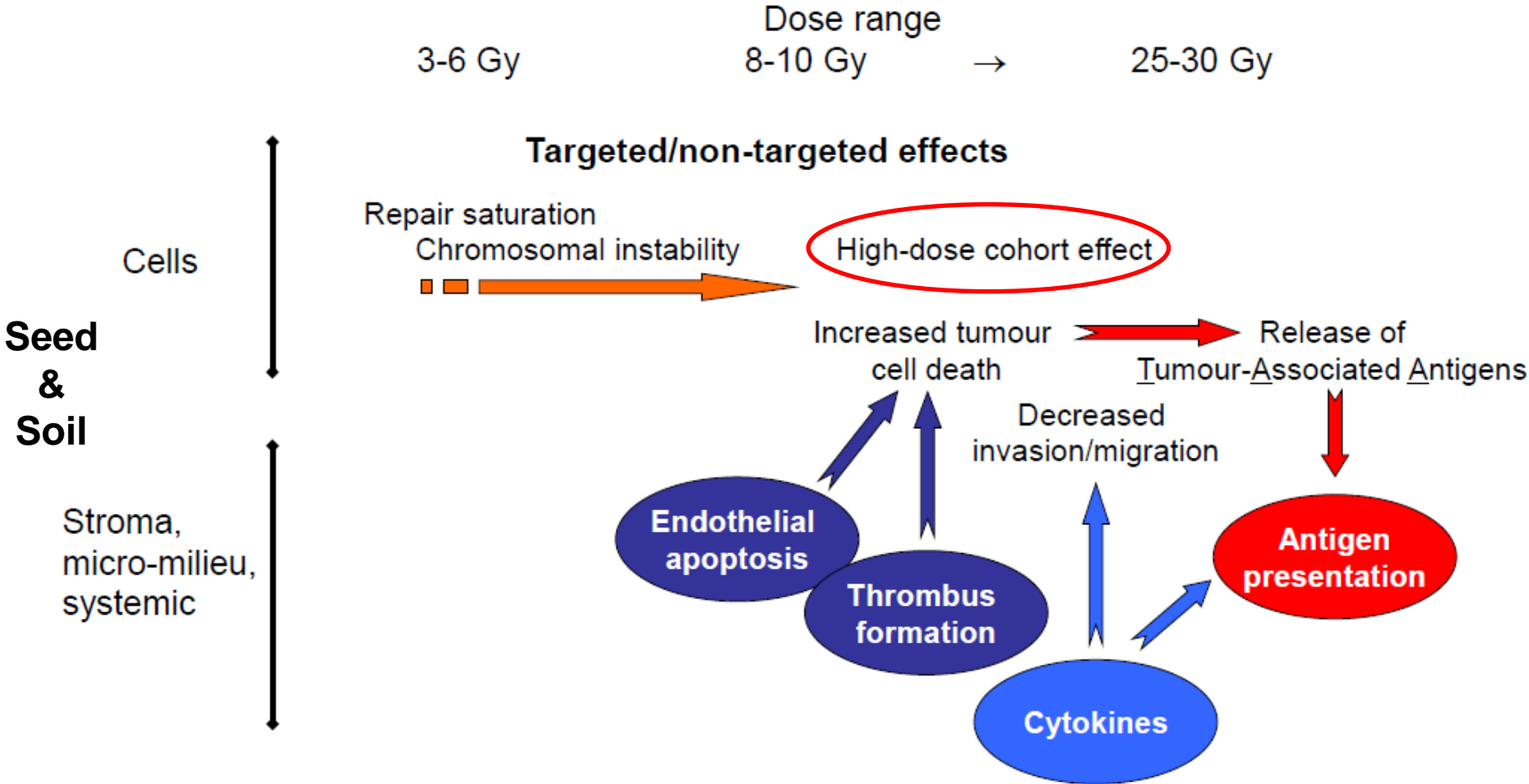
Saturation of repair

53BP1 foci
co-localize with DSB
vs dose

@low dose:
proportional to dose
@high dose:
subproportional



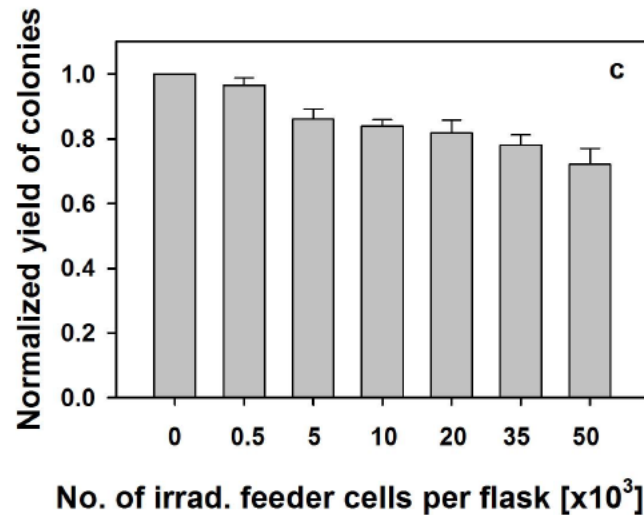
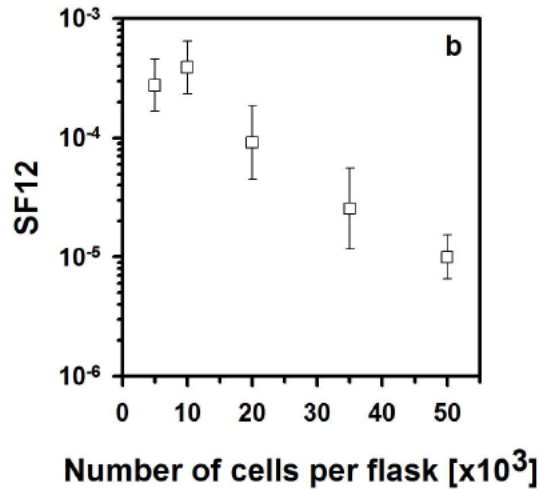
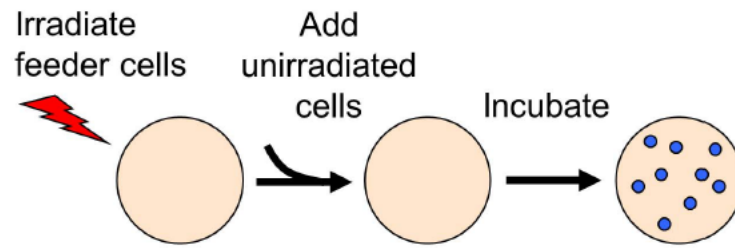
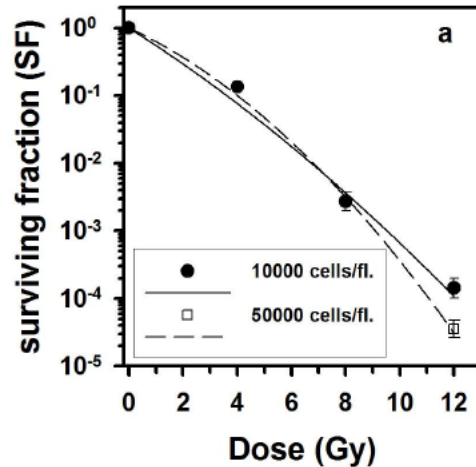
Radiobiology of high single doses



The Biological Effect of Large Single Doses: A Possible Role for Non-Targeted Effects in Cell Inactivation

Marlon R. Veldwijk¹, Bo Zhang², Frederik Wenz, Carsten Herskind*

PLoS ONE 9(1): e84991.

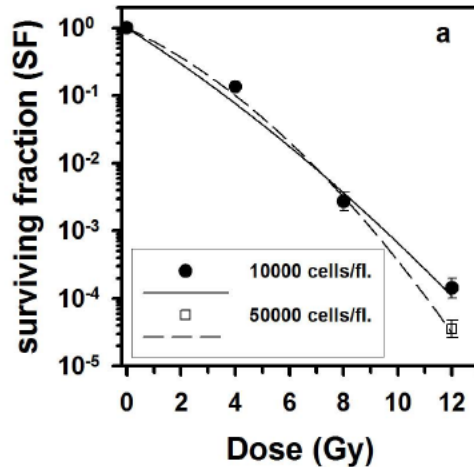


Effect of cell density on cell survival after high-dose irradiation.

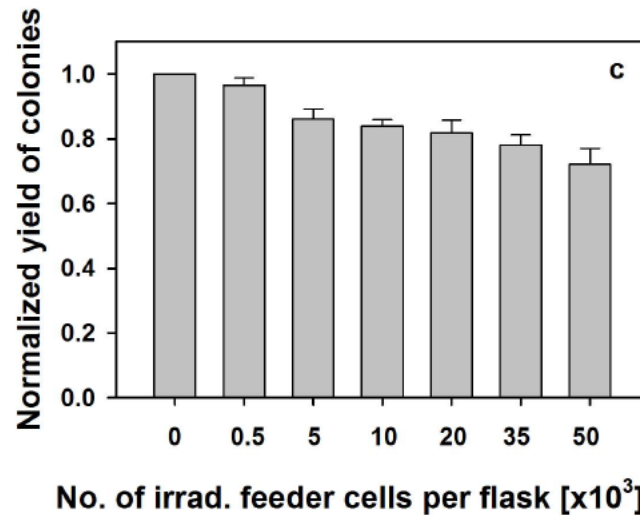
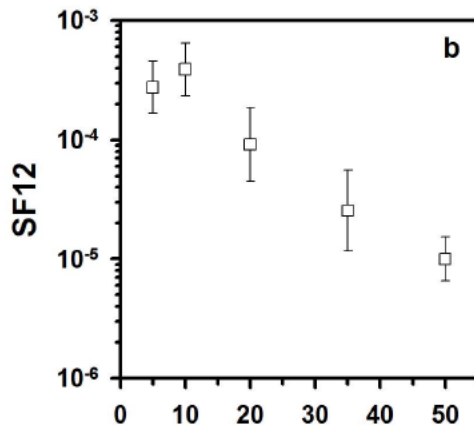
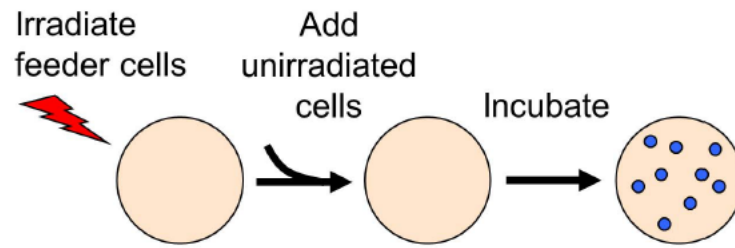
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effect depends on cell number

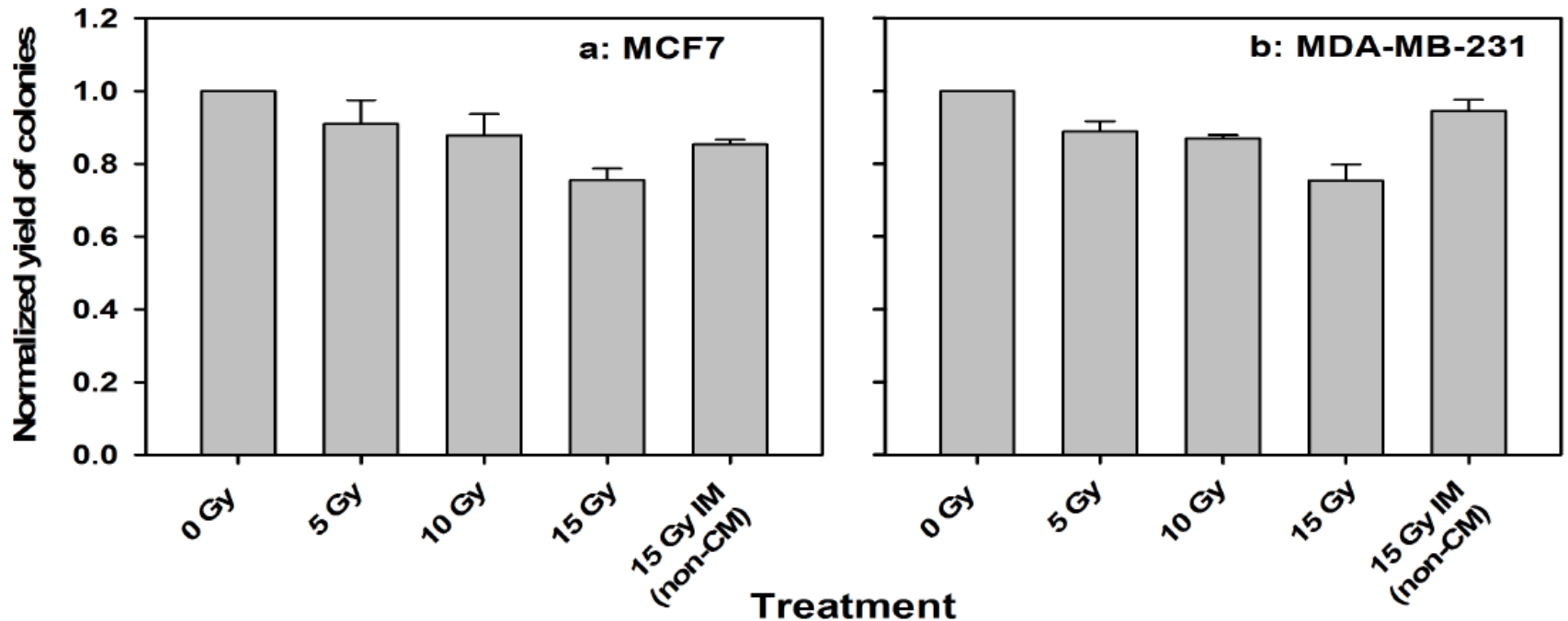
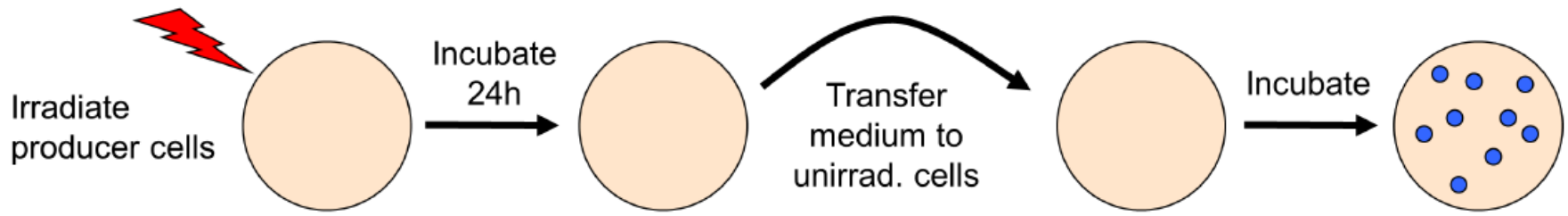


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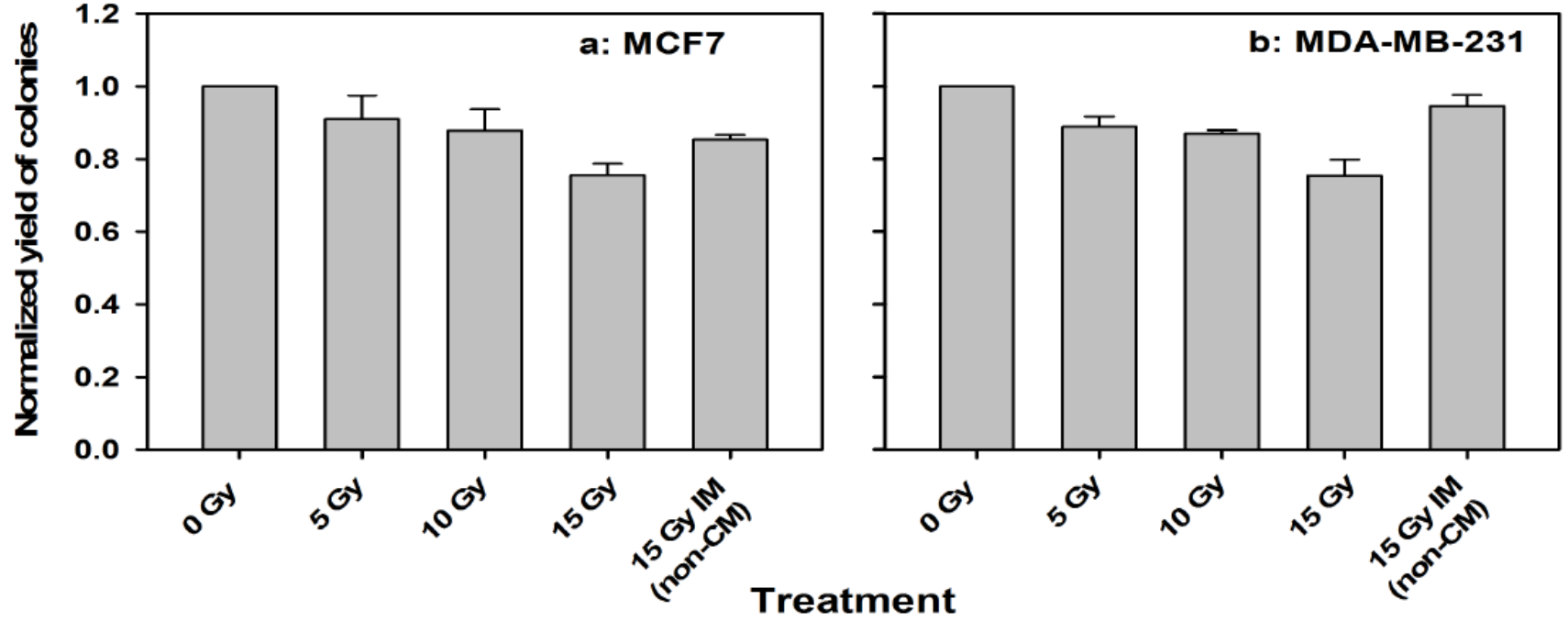
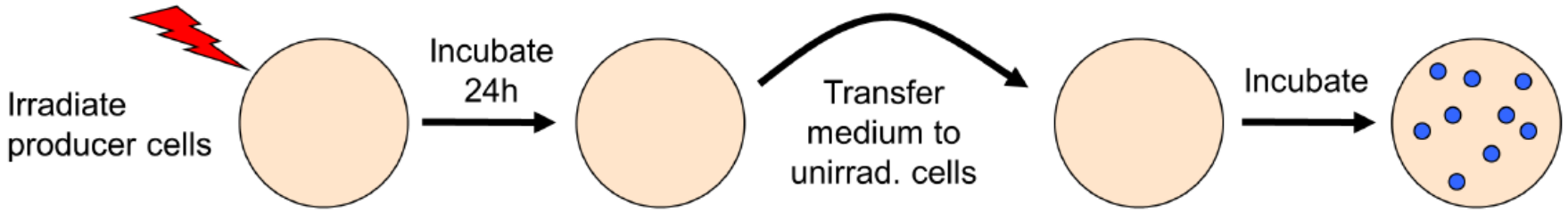
Medium transfer experiments to test effect of transferrable factor.

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PLoS ONE 9(1): e84991.

Marlon R. Veldwijk¹, Bo Zhang², Frederik Wenz, Carsten Herskind*

effect depends on cell number & dose

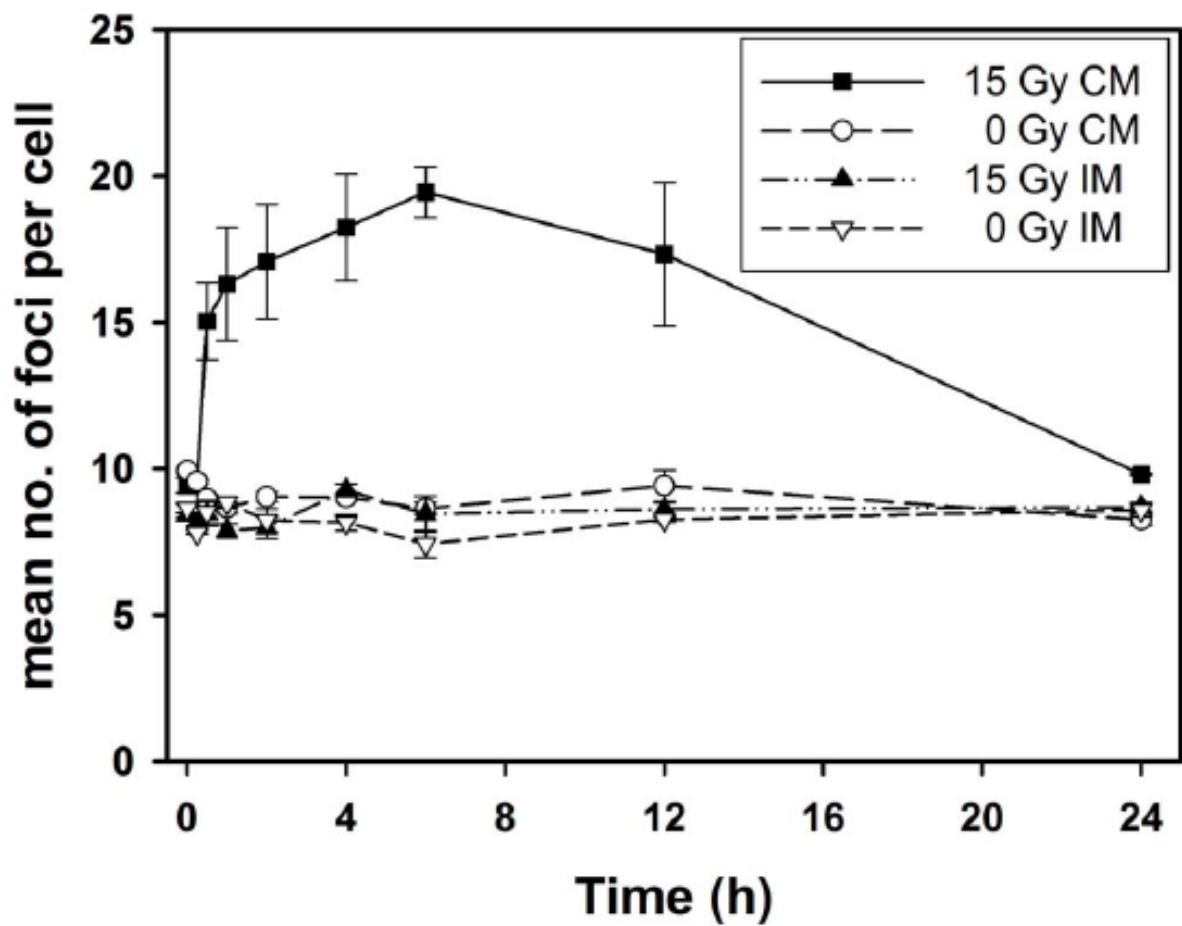


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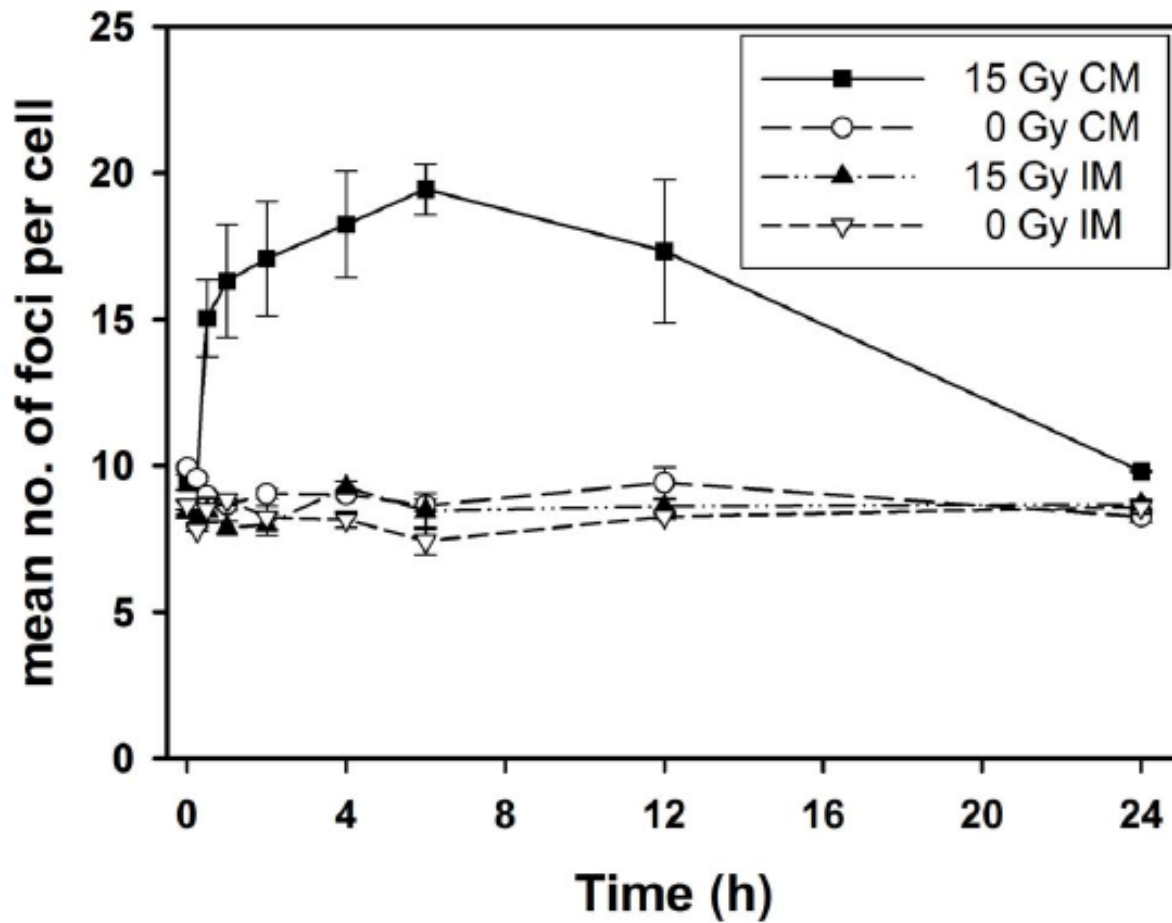
Conditioned medium (CM) induces prolonged γ -H2AX foci in MCF7 cells.

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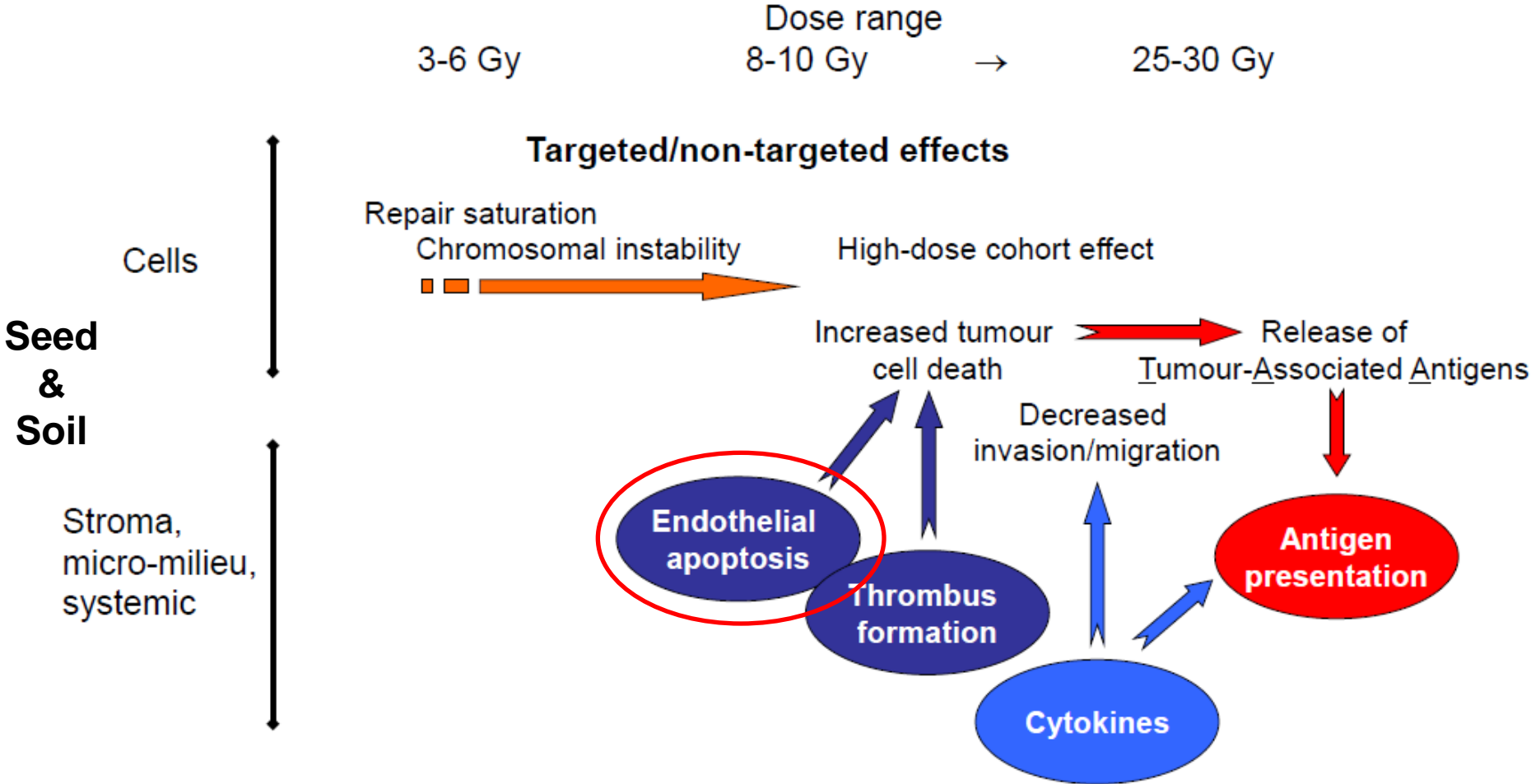
PLoS ONE 9(1): e84991.

effect depends on cell number & dose
„supposed“ dsb



Conditioned medium (CM) induces prolonged γ -H2AX foci in MCF7 cells.

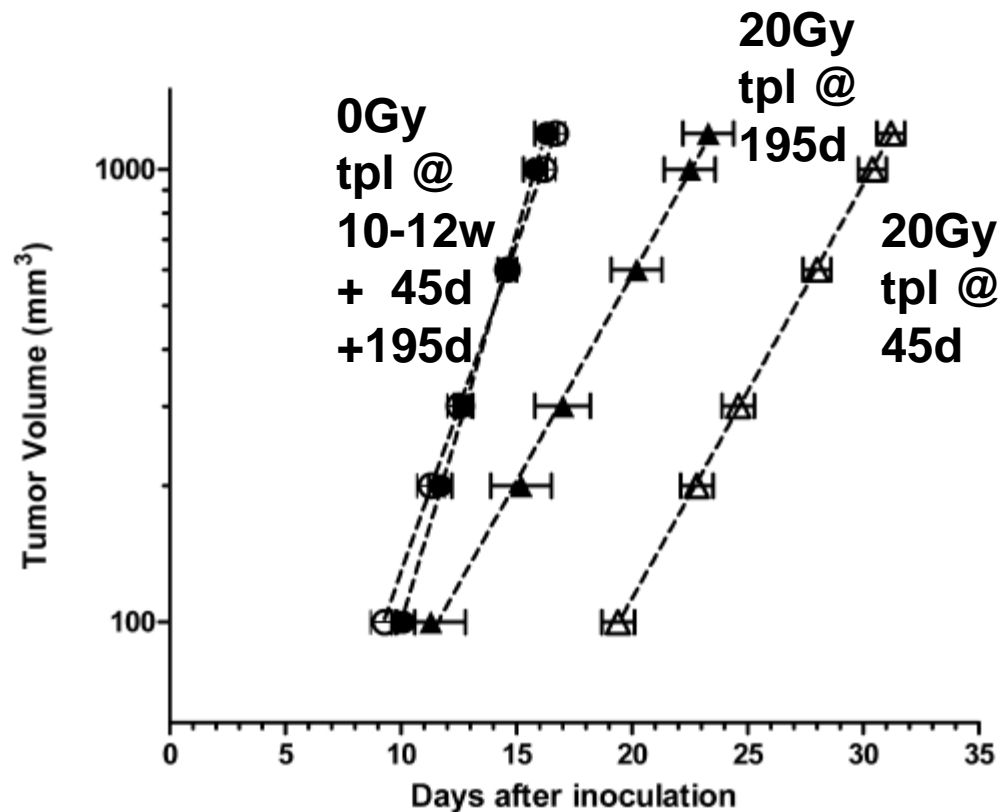
Radiobiology of high single doses



Radiobiology of high single doses – tumor bed

Residual Late Radiation Damage in Mouse Stromal Tissue Assessed by the Tumor Bed Effect

Jaap HAVEMAN*¹, Hans RODERMOND¹, Chris van BREE¹, Jan WONDERGEM²
and Nicolaas A.P. FRANKEN¹ *J. Radiat. Res.*, 48, 107–112 (2007)



M8013 tumors
mice breast ca

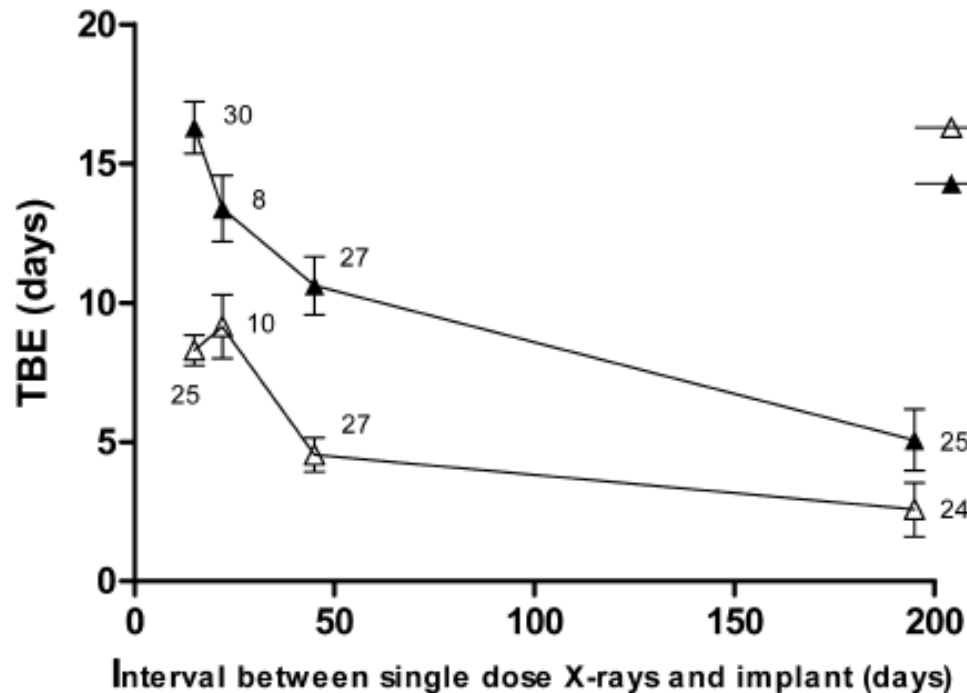
TBE
(tumor bed effect)

dose dependent
time dependent

Radiobiology of high single doses – tumor bed

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**M8013 tumors
mice breast ca**

**TBE
(tumor bed effect)**

**dose dependent
time dependent**

Radiotherapy for renal-cell carcinoma

Lancet Oncol 2014; 15: e170-77

Gert De Meerleer, Vincent Khoo, Bernard Escudier, Steven Joniau, Alberto Bossi, Piet Ost, Alberto Briganti, Valérie Fonteyne, Marco Van Vulpen, Nicolaas Lumen, Martin Spahn, Marc Mareel

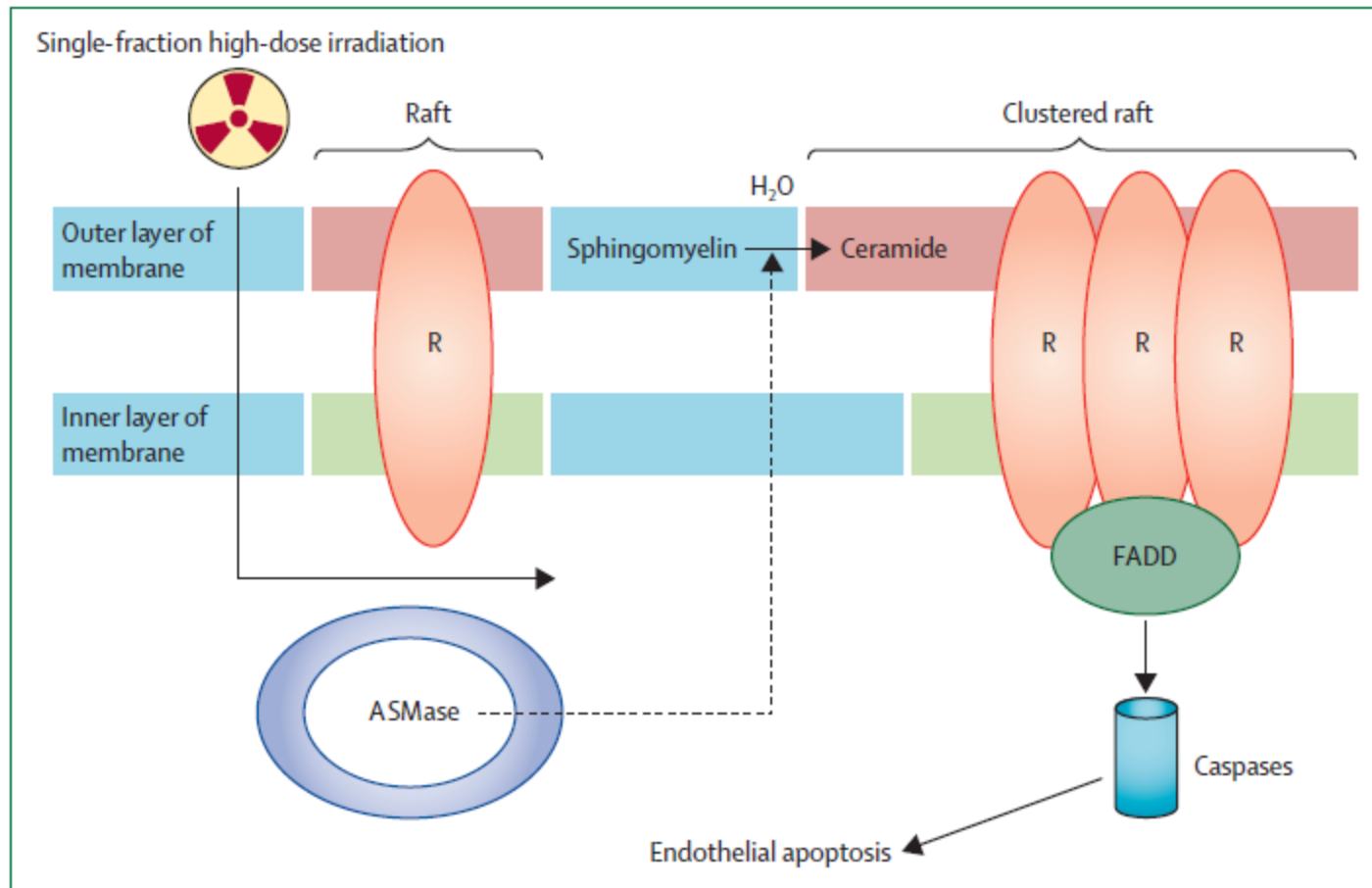


Figure 1: Activation of ceramide pathway by single-fraction high-dose irradiation

Adapted from Fuks and colleagues,⁵ Corre and colleagues,¹² and Simons and colleagues.¹³ ASMase=acid sphingomyelinase. R=receptor. FADD=FAS-associated death domains.

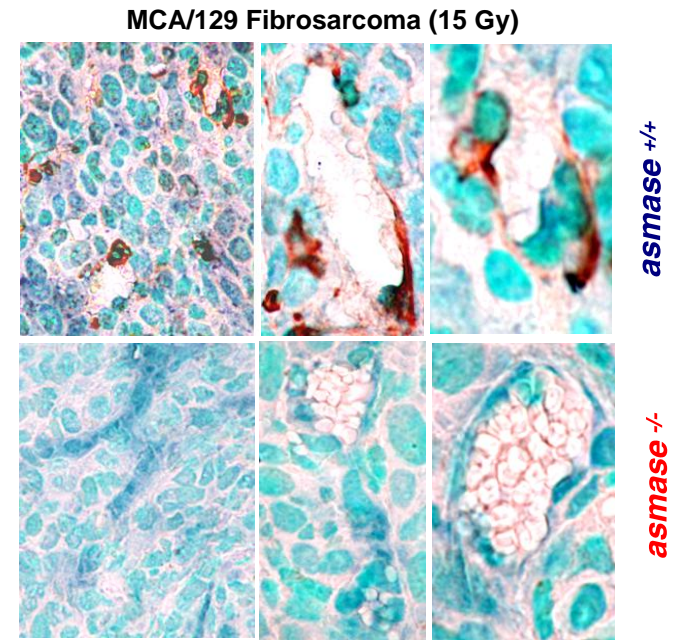
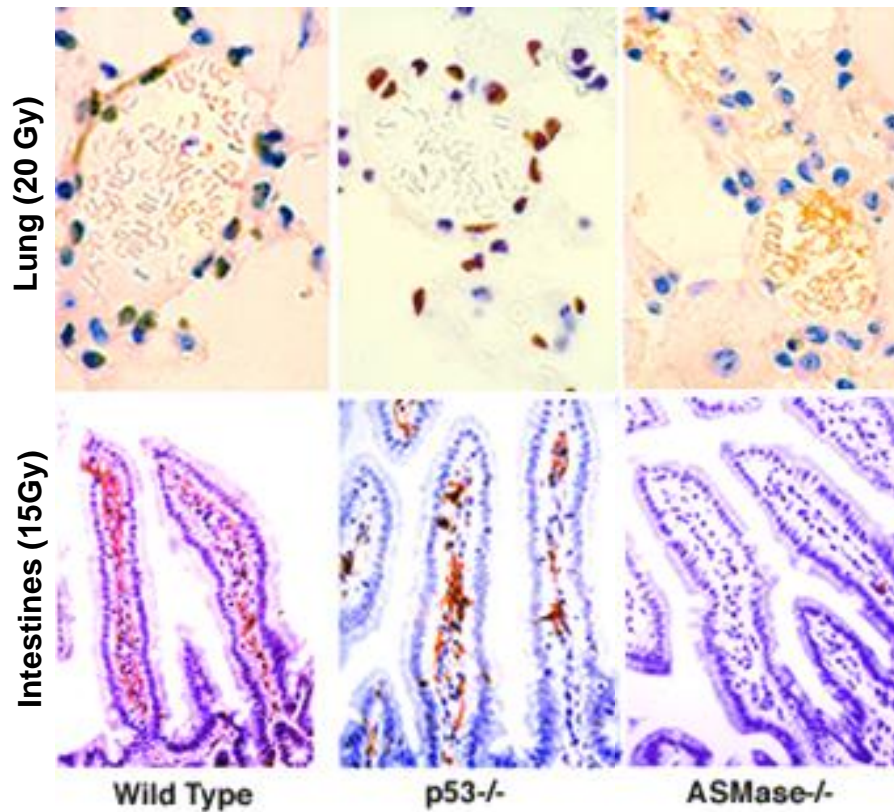


The Biological Basis of Single Dose Radiotherapy

Implications for IORT

Z. Fuks, M.D.
Madrid
June, 2008

Tissues Exhibit Acute and Transient Endothelial Apoptosis After Single-dose Exposure of ≥ 10 Gy



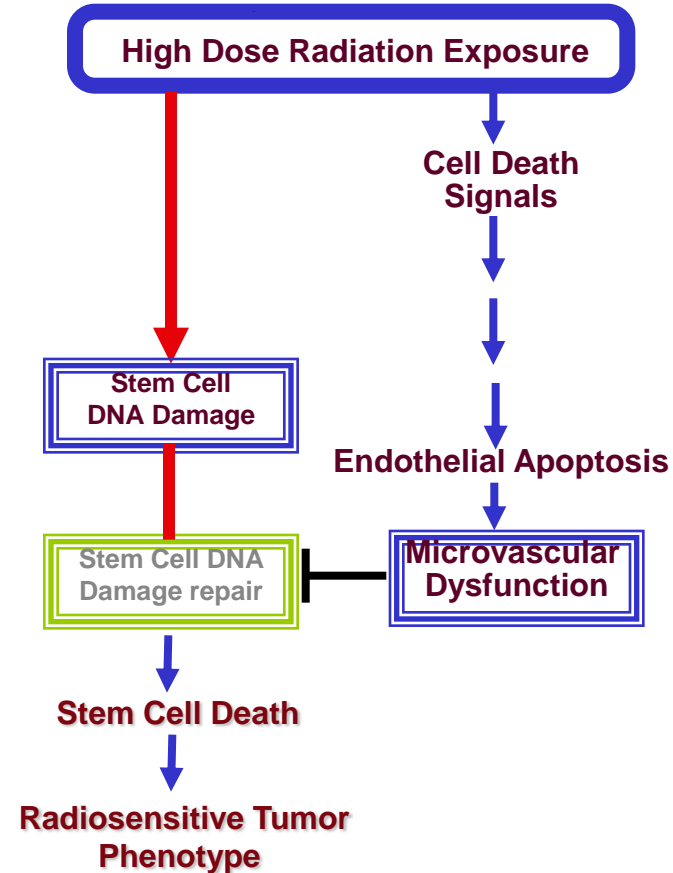


The Biological Basis of Single Dose Radiotherapy

Implications for IORT

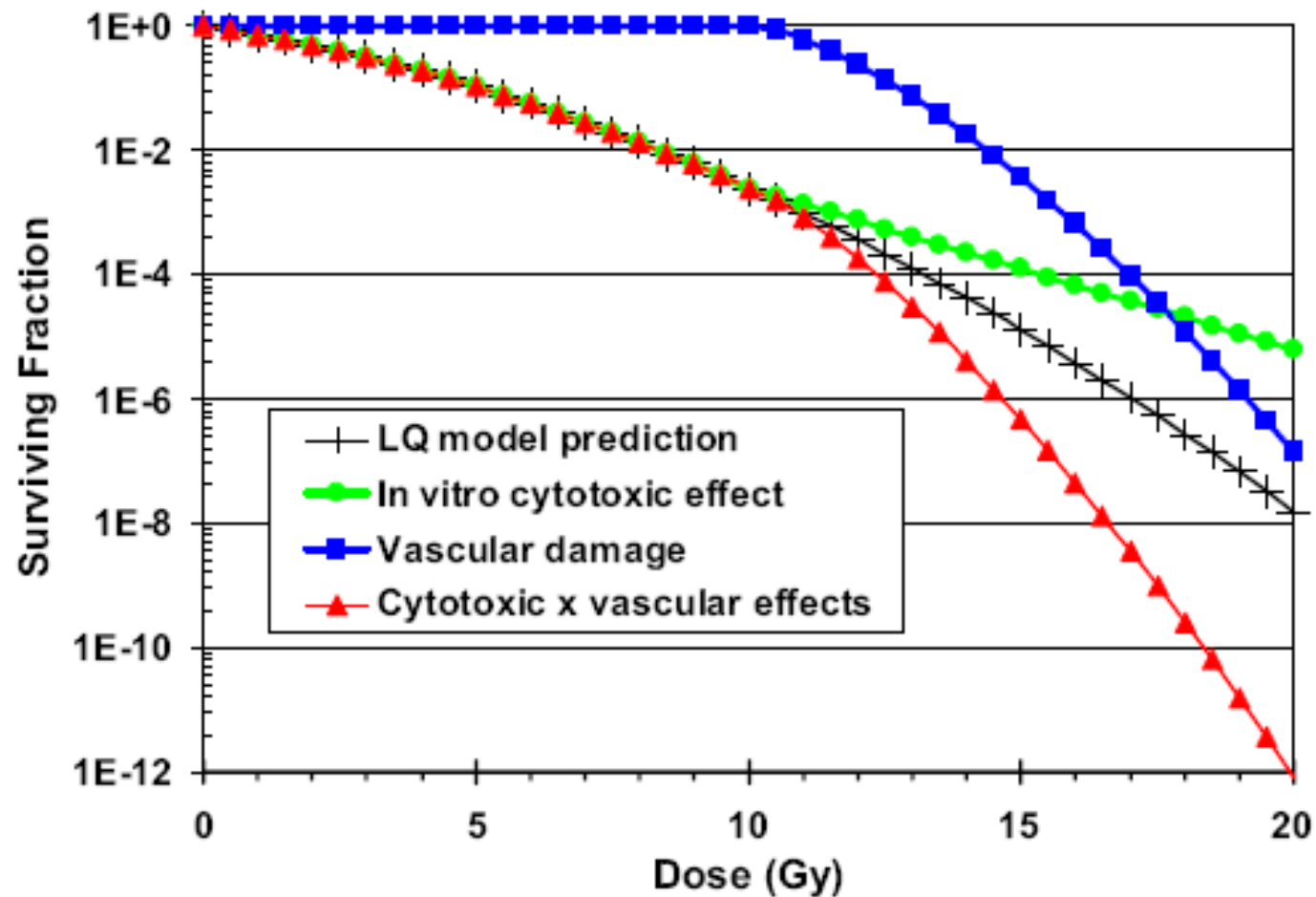
Z. Fuks, M.D.
Madrid
June, 2008

- Single-dose radiotherapy is mechanistically different from fractionated radiotherapy
- The target for high single-dose exposure is a linked system of tumor stem cells and its host-derived microvascular network
- The threshold for activating this target system is 8-10 Gy
- Transient microvascular dysfunction represses DNA dsb repair and confers a stem cell radiosensitive phenotype
- Concomitant activation of both target elements is mandatory, as inhibition of the microvascular component facilitates stem cell DNA dsb repair and confers radioresistance

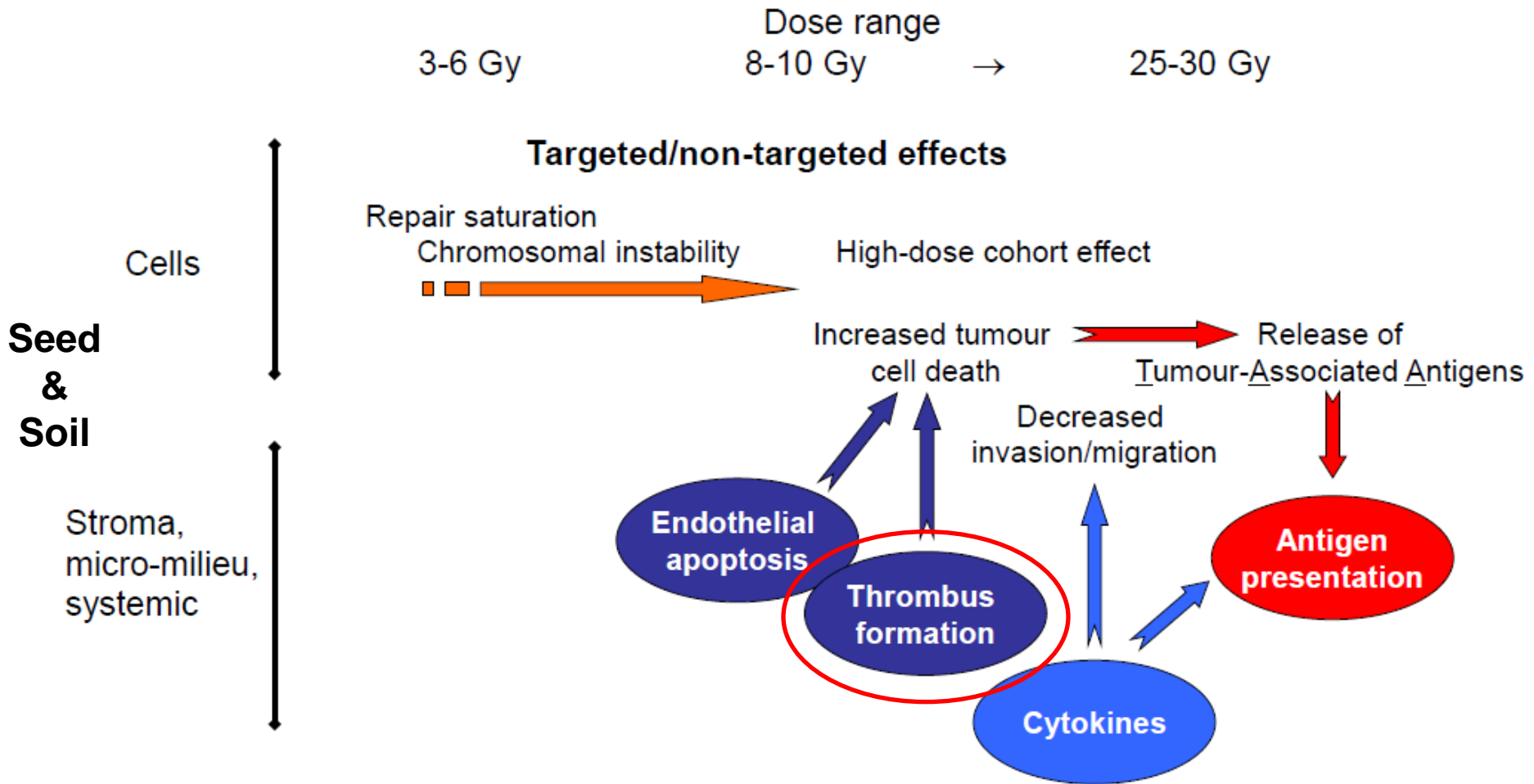


Model Is Inappropriate to Model High Dose per Fraction Effects in Radiosurgery

John P. Kirkpatrick, MD, PhD, Jeffrey J. Meyer, MD, and Lawrence B. Marks, MD



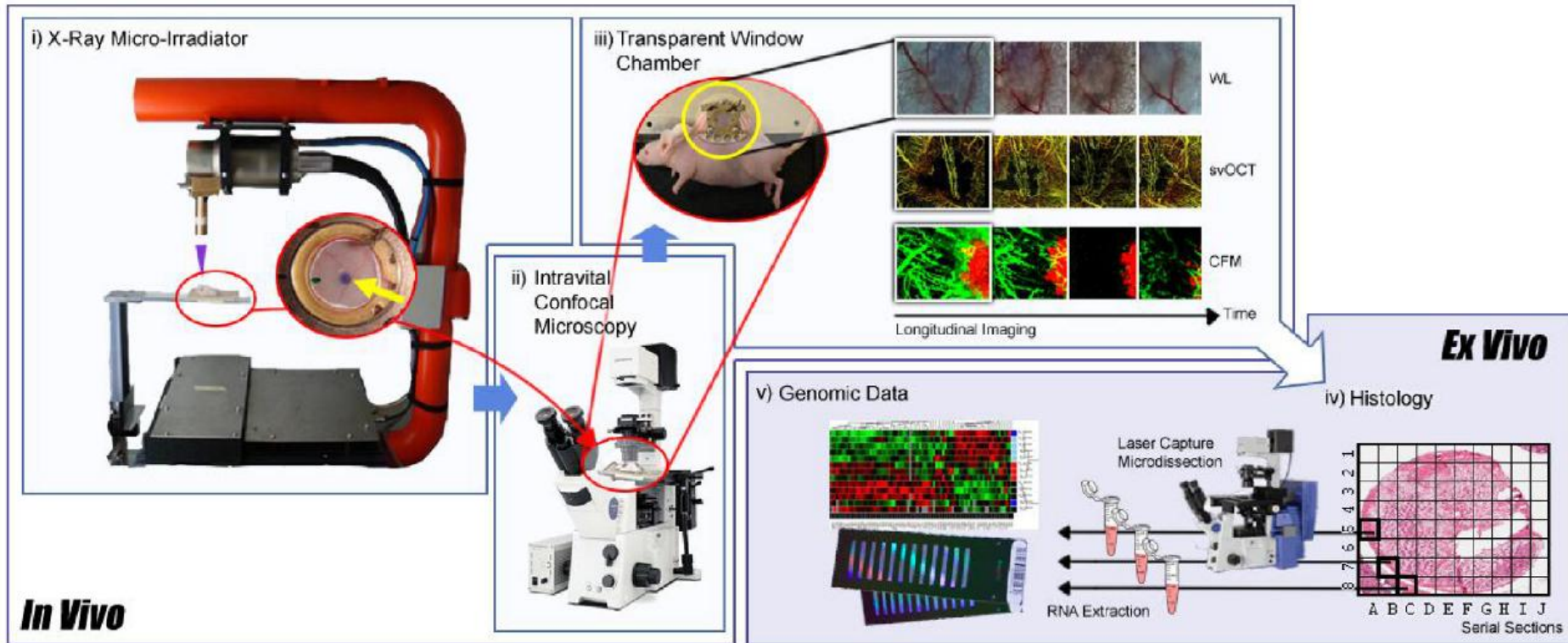
Radiobiology of high single doses



In Vivo Optical Imaging of Tumor and Microvascular Response to Ionizing Radiation

PLoS ONE 7(8): e42133. 2012

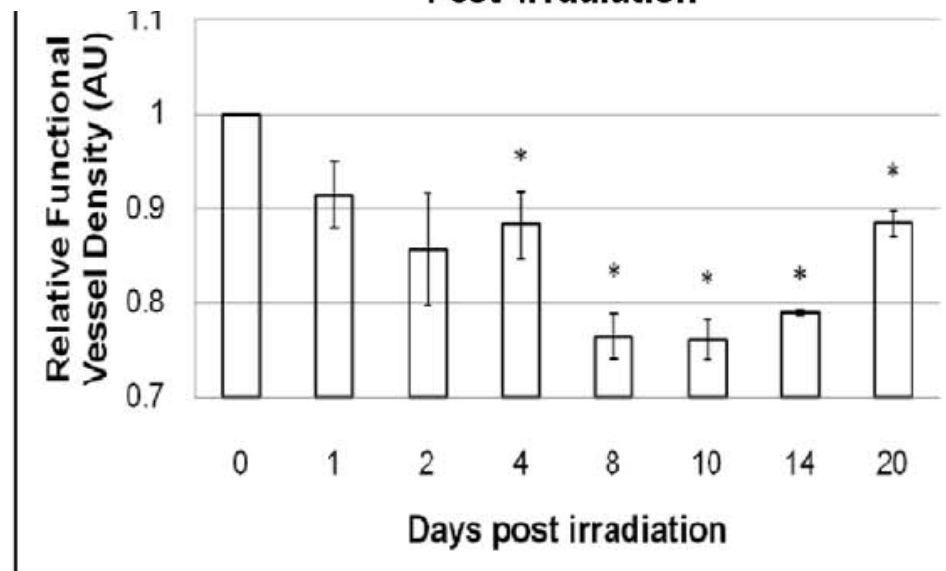
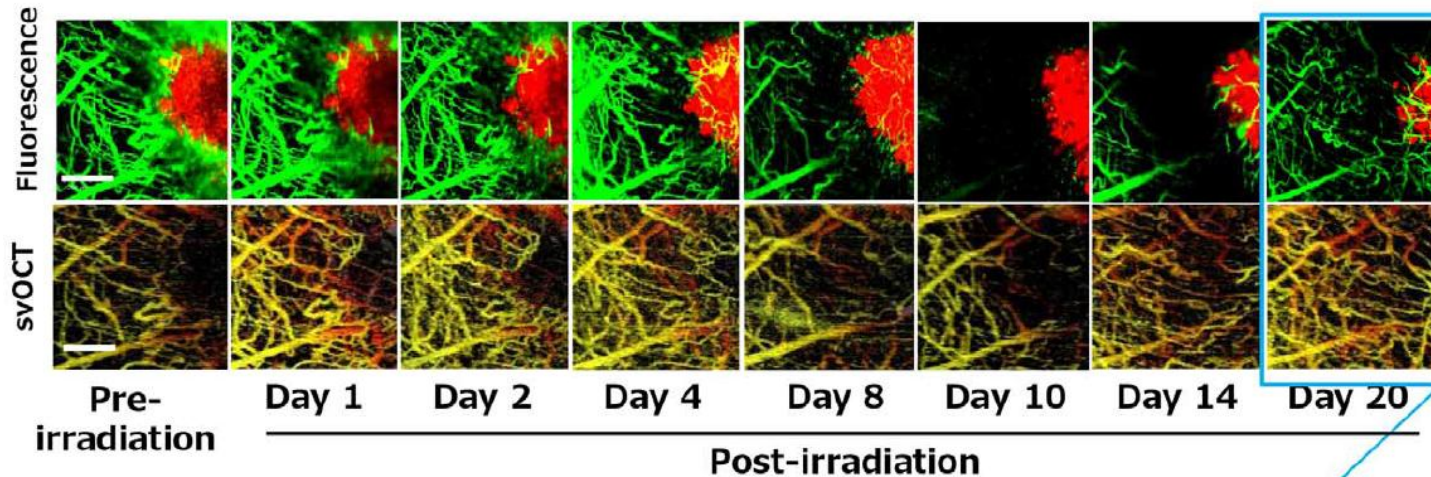
Azusa Maeda^{1,2}, Michael K. K. Leung^{1,2}, Leigh Conroy^{1,2}, Yonghong Chen¹, Jiachuan Bu¹, Patricia E. Lindsay³, Shani Mintzberg^{1,5}, Carl Virtanen^{1,5}, Julissa Tsao^{1,5}, Neil A. Winegarden^{1,5}, Yanchun Wang⁶, Lily Morikawa⁶, I. Alex Vitkin^{1,2,3}, David A. Jaffray^{1,2,3,4}, Richard P. Hill^{1,2,3}, Ralph S. DaCosta^{1,2,4*}



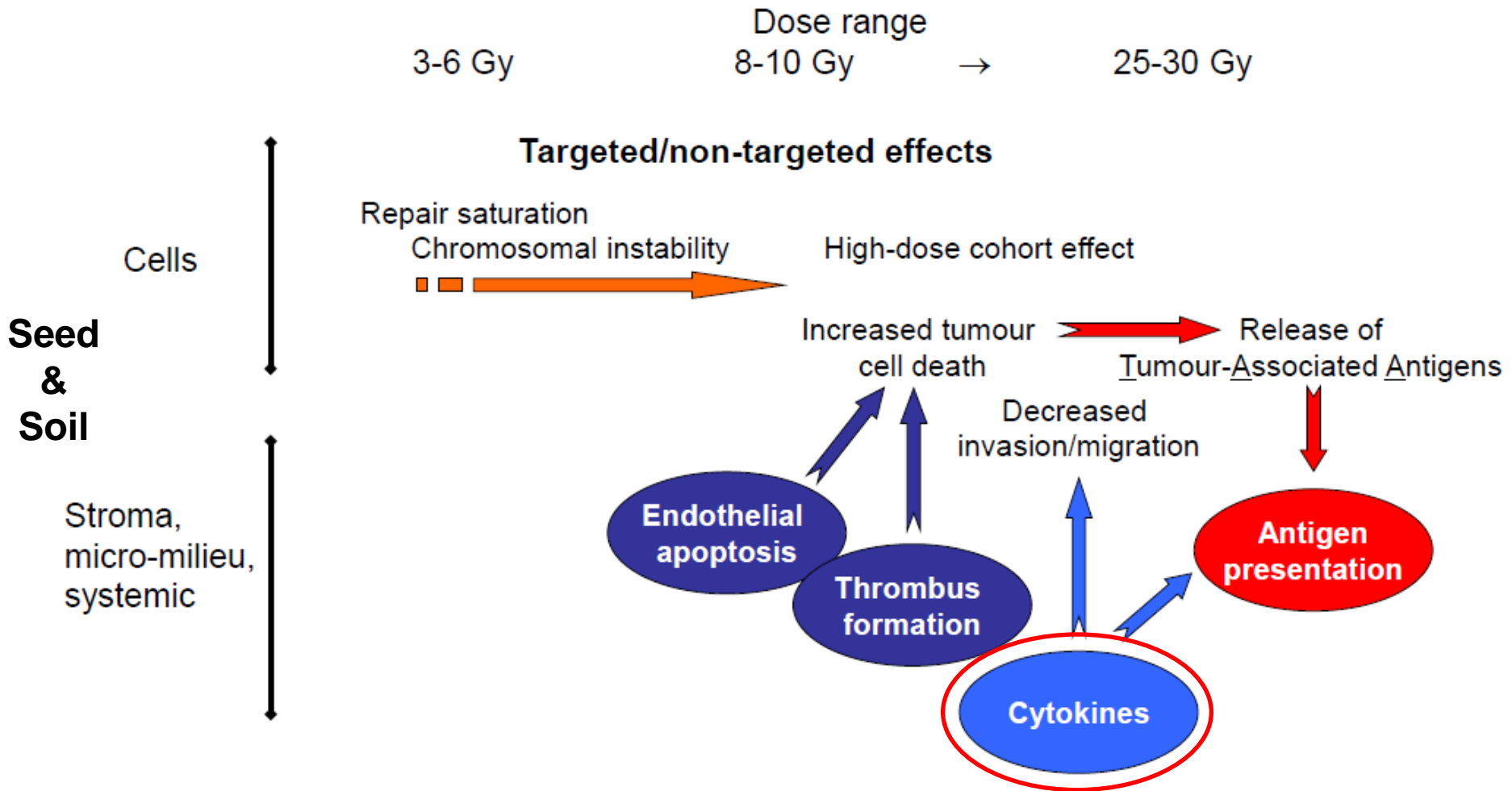
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Radiobiology of high single doses

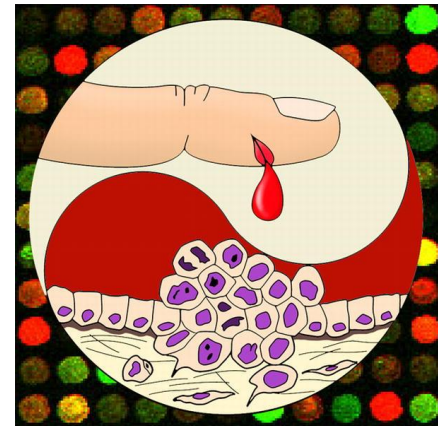
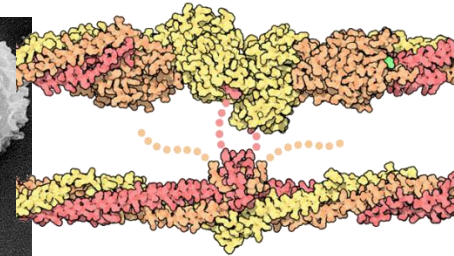
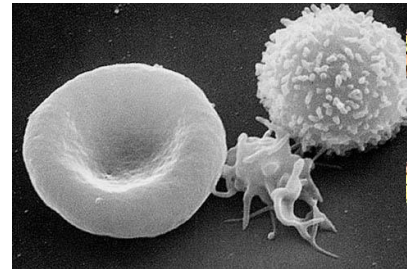


Micromilieu–Does surgery stimulate tumor cells?

Tumor stroma is “normal wound healing gone awry”

Dvorak HF (1986) Tumors: Wounds that do not heal: Similarities between tumor stroma generation and wound healing. N Engl J Med 315:1650–1659.

During normal wound healing, coagulation of extravasated blood initiates a complex cascade of signals that recruit inflammatory cells, stimulate fibroblast and epithelial cell proliferation, direct cell migration, and induce angiogenesis to restore tissue integrity. Many of these normally reparative processes may be constitutively active in the tumor milieu and critical for tumor engraftment, local invasion, and metastasis to distant organs



Micromilieu – Cytokines in Wound Fluid

Targeted Intraoperative Radiotherapy Impairs the Stimulation
of Breast Cancer Cell Proliferation and Invasion Caused
by Surgical Wounding

Clin Cancer Res 2008;14(5) March 1, 2008

Barbara Belletti,¹ Jayant S. Vaidya,⁷ Sara D'Andrea,¹ Frank Entschladen,⁸ Mario Roncadin,¹ Francesca Lovat,¹ Stefania Berton,¹ Tiziana Perin,⁴ Ezio Candiani,² Sonia Reccanello,³ Andrea Veronesi,^{5,6} Vincenzo Canzonieri,⁴ Mauro G. Trovò,³ Kurt S. Zaenker,⁸ Alfonso Colombatti,¹ Gustavo Baldassarre,^{1,6} and Samuele Massarut^{2,6}



drawing of **pre-**
surgery serum



+ n = 24
- n = 20



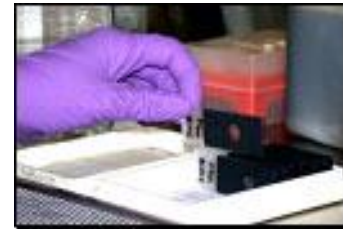
drainage of **post-**
surgery serum



processing



centrifuging



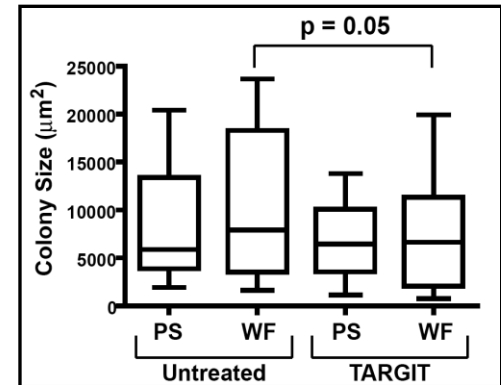
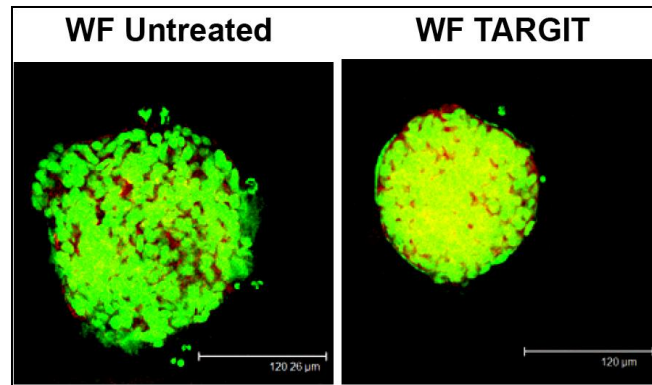
storage

Micromilieu – Cytokines in Wound Fluid

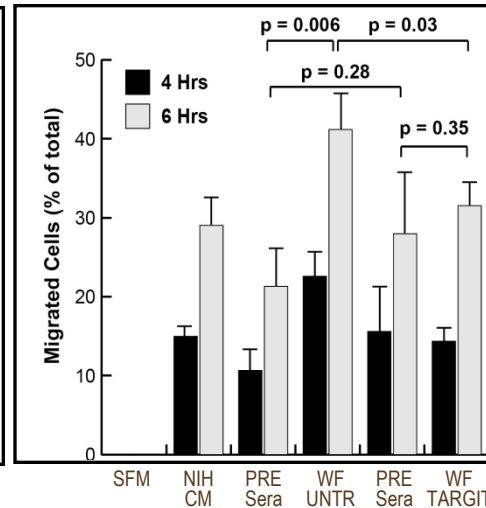
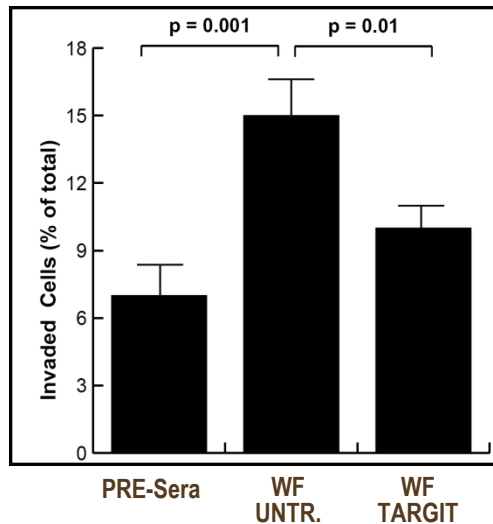
Targeted Intraoperative Radiotherapy Impairs the Stimulation
of Breast Cancer Cell Proliferation and Invasion Caused
by Surgical Wounding Clin Cancer Res 2008;14(5) March 1, 2008

Barbara Belletti,¹ Jayant S. Vaidya,⁷ Sara D'Andrea,¹ Frank Entschladen,⁸ Mario Roncadin,¹ Francesca Lovat,¹ Stefania Berton,¹ Tiziana Perin,⁴ Ezio Candiani,² Sonia Reccanello,³ Andrea Veronesi,^{5,6} Vincenzo Canzonieri,⁴ Mauro G. Trovò,³ Kurt S. Zaenker,⁸ Alfonso Colombatti,¹ Gustavo Baldassarre,^{1,6} and Samuele Massarut^{2,6}

MCF-7 in MATRIGEL



MDA-MB 231



Micromilieu – Cytokines in Wound Fluid

Targeted Intraoperative Radiotherapy Impairs the Stimulation

of Breast Cancer Cell Proliferation and Invasion Caused

by Surgical Wounding

Clin Cancer Res 2008;14(5) March 1, 2008

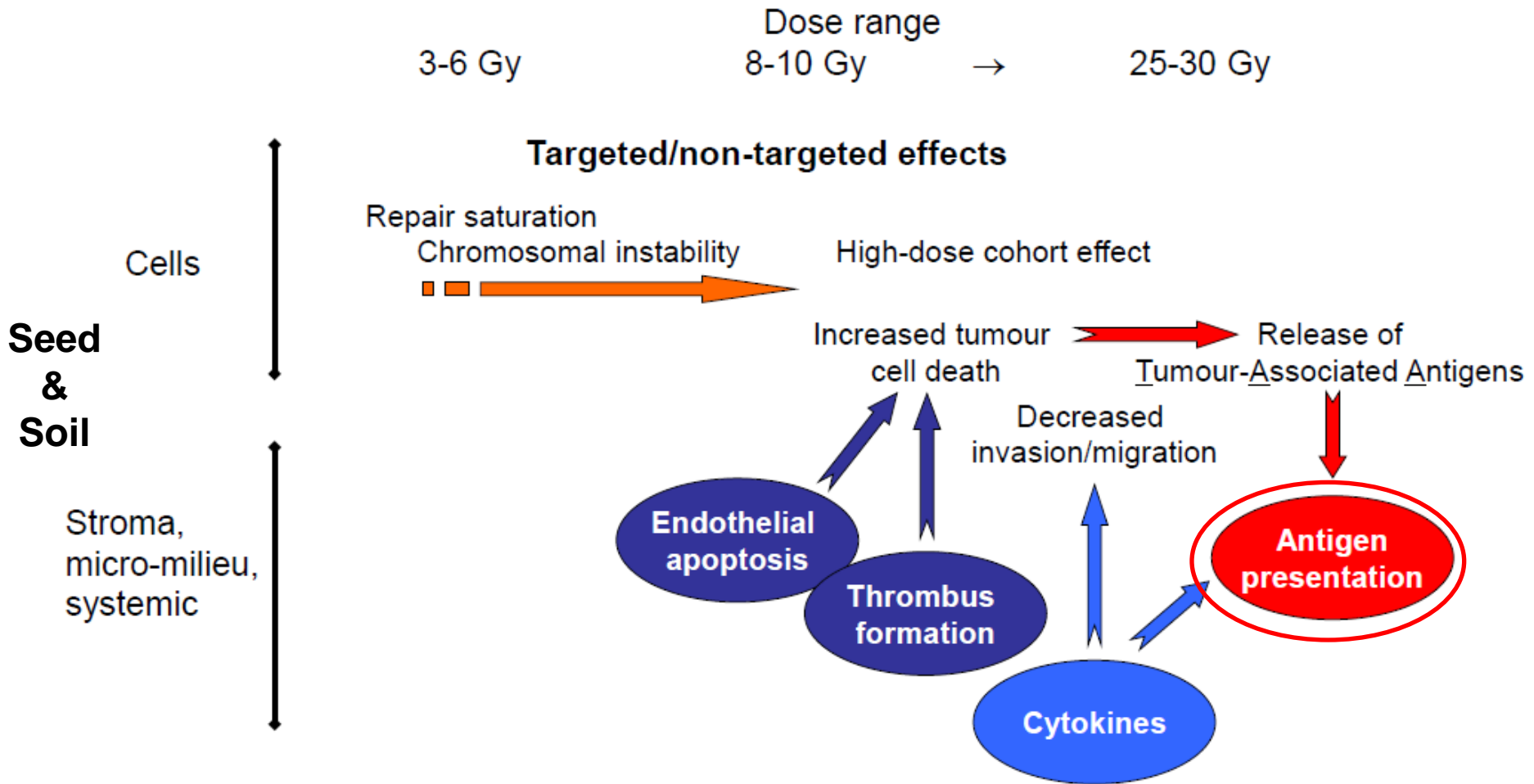
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- **Wound Fluid triggers breast cancer cell proliferation (but not that of normal cells)**
- **Wound Fluid stimulates migration and invasion of breast cancer cells (but not of normal cells)**
- **Both these effects are counteracted by IORT**
- **Wound Fluid from IORT-treated patients display a different molecular profile from other WF**

Table 1. WF proteins altered by TARGIT

Decreased by TARGIT	Increased by TARGIT
Angiogenin	AgRP
Flt-3 ligand	EGFR
IL-10	FAS/TNFRSF6
IL-6	FGF-4
IL-7	G-CSF
Leptin	IGFBP-6
MCP-1	IL-13
MCP-2	IL-4
RANTES	IL-5
PDGF-BB	Mip-1d
GRO	
HGF	
IL-8	
Mip-1a	
sTNFR-II	
sTNFR-I	
uPAR	
VEGF-R3	
Tie-1	
Tie-2	

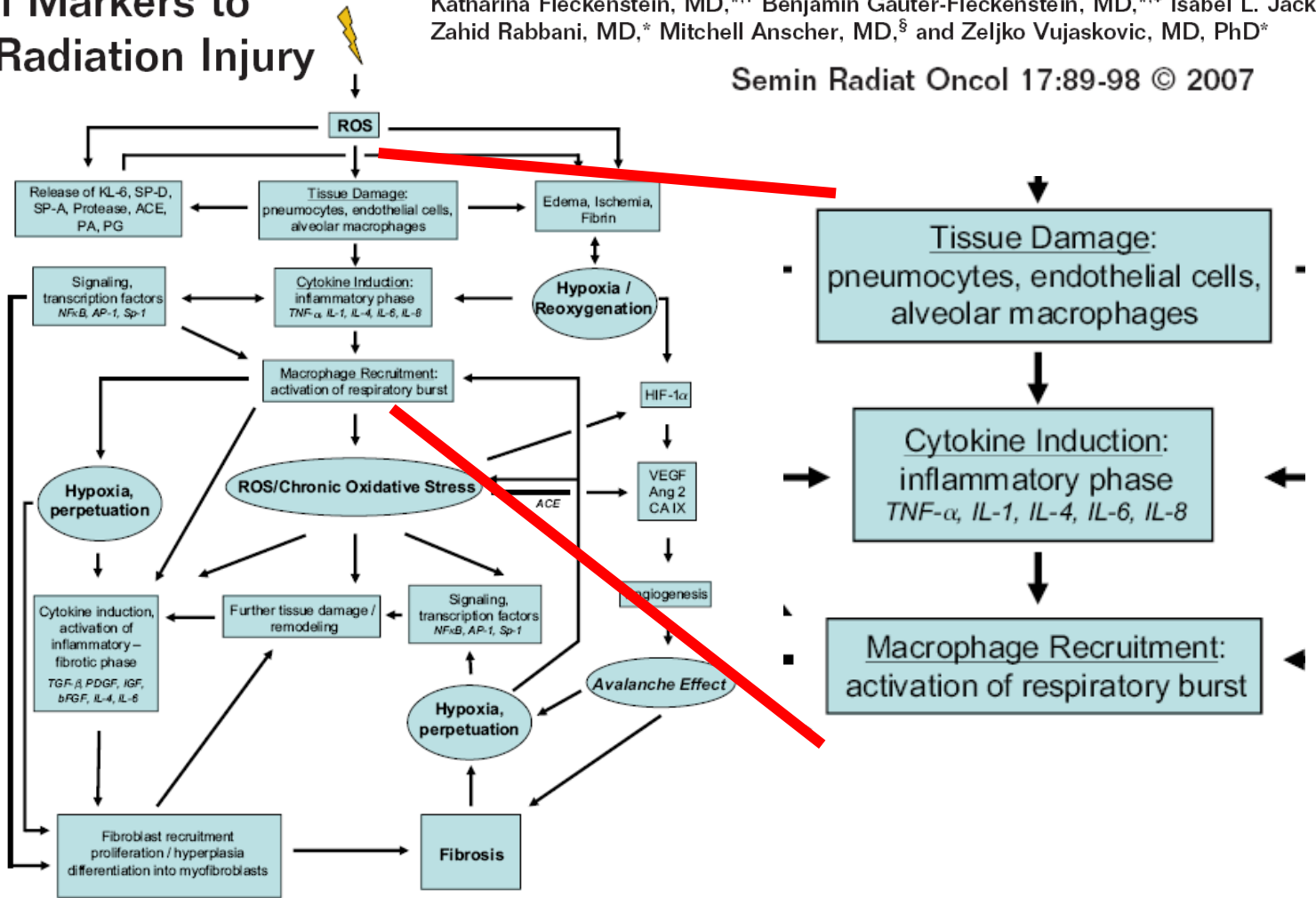
Radiobiology of high single doses



Using Biological Markers to Predict Risk of Radiation Injury

Katharina Fleckenstein, MD,^{*,†} Benjamin Gauter-Fleckenstein, MD,^{*,‡} Isabel L. Jackson, Zahid Rabbani, MD,^{*} Mitchell Anscher, MD,[§] and Zeljko Vujaskovic, MD, PhD^{*}

Semin Radiat Oncol 17:89-98 © 2007



Role of T lymphocytes in tumor response to radiotherapy

Sandra Demaria^{1*} and Silvia C. Formenti²

August 2012 | Volume 2 | Article 95 |

frontiers in
ONCOLOGY

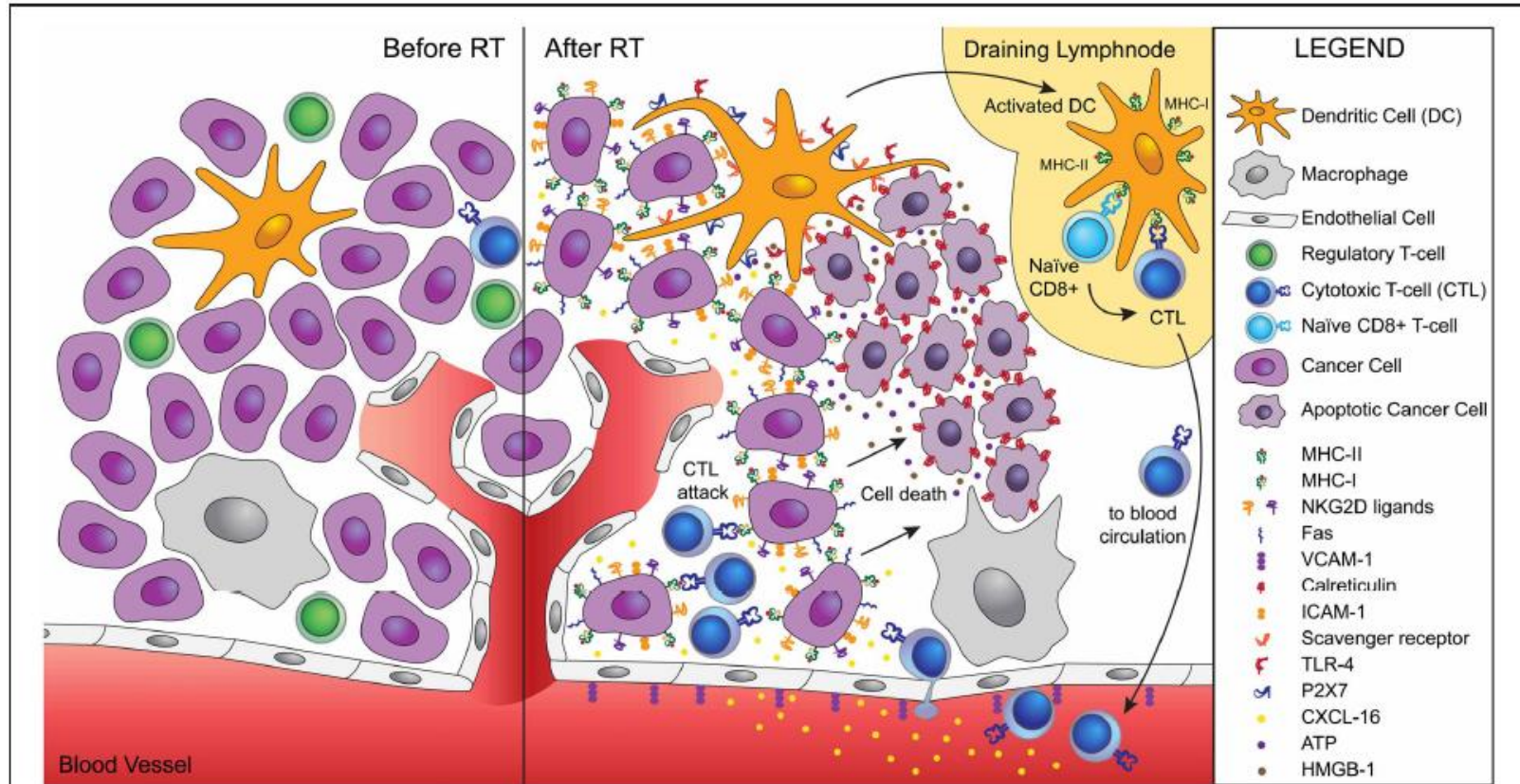


FIGURE 1 | Ionizing radiation acts as a modifier of the tumor microenvironment converting the tumor into an *in situ* vaccine.

Radiation induces an immunogenic cell death of tumor cells characterized by calreticulin translocation to the surface of dying cells, and release of HMGB-1 and ATP. Calreticulin allows uptake of dying cells by dendritic cells via scavenger receptor(s). HMGB-1 binds to TLR4 and promotes the cross-presentation of tumor antigens, while ATP binds to P2X7 and triggers the activation of the inflammasome. Activated dendritic cells migrate to the draining lymph node, where they activate naïve T cells specific for tumor

antigens. Activated CD8 T cells acquire effector functions and traffic to the tumor guided by radiation-induced chemokines. Tumor infiltration by CTLs is facilitated by radiation-induced upregulation of VCAM-1 on the vascular endothelium. Once in the tumor, CTLs interact efficiently with tumor cells expressing increased levels of MHC-I, ICAM-1, NKG2D ligands, and Fas that promote the formation of stable immunological synapses between targets and effectors and facilitate the killing of tumor cells by CTLs. Tumor cells killed by CTLs become a source of antigens for cross-presentation, thus fueling the process.

Radiotherapy for renal-cell carcinoma

Lancet Oncol 2014; 15: e170-77

Gert De Meerleer, Vincent Khoo, Bernard Escudier, Steven Joniau, Alberto Bossi, Piet Ost, Alberto Briganti, Valérie Fonteyne, Marco Van Vulpen, Nicolaas Lumen, Martin Spahn, Marc Mareel

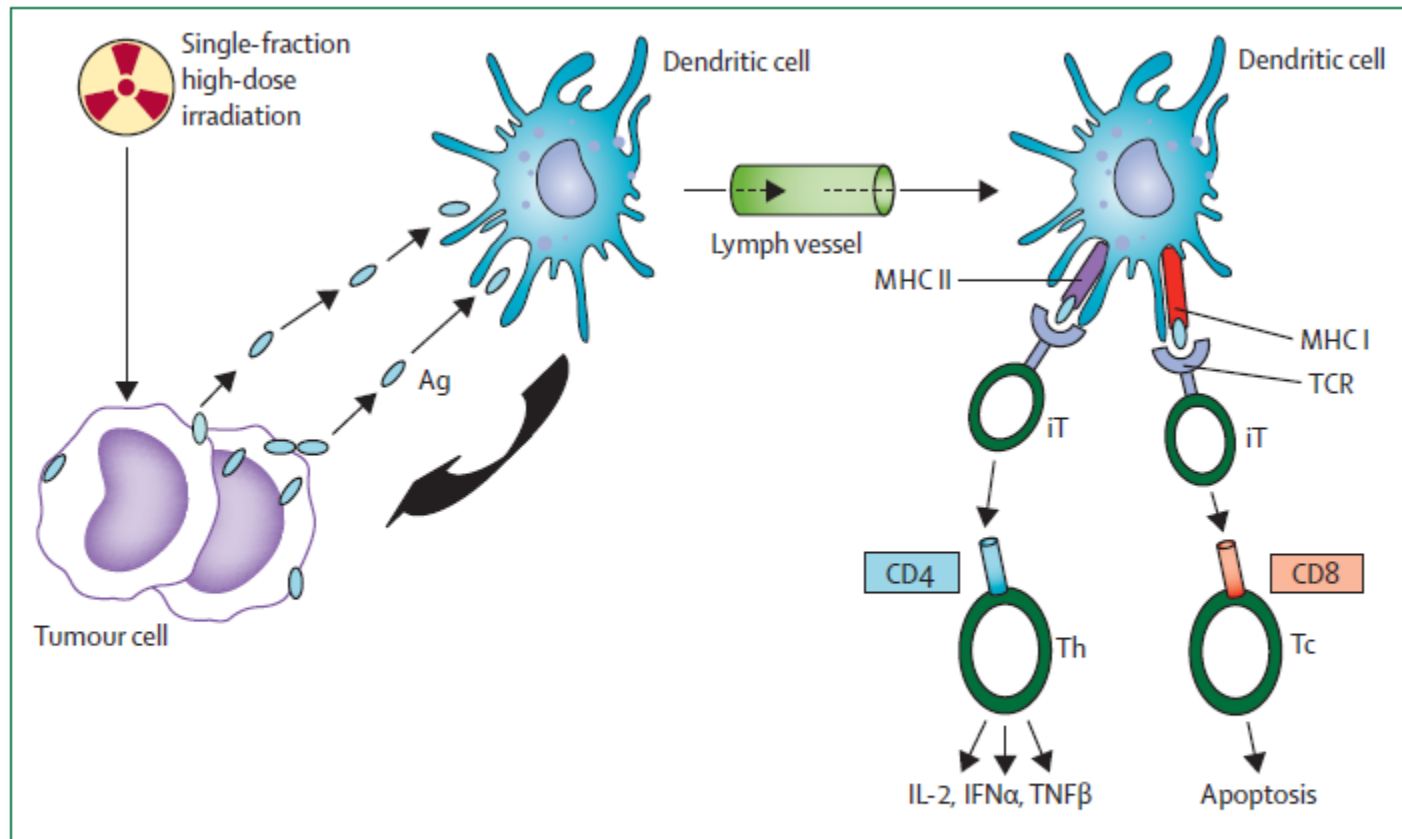


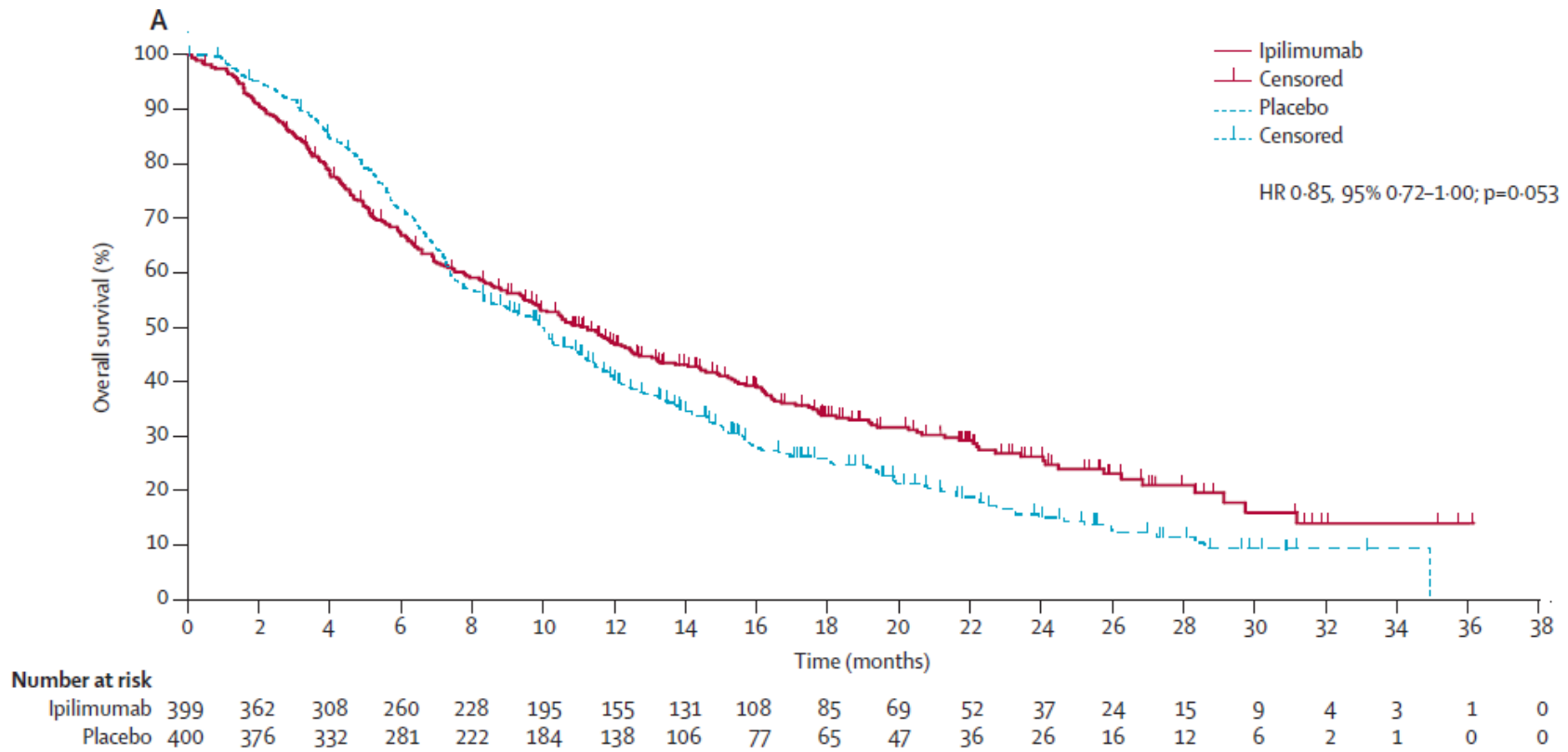
Figure 2: Immunological interpretation of the abscopal effect

Ag=antigen. TCR=T-cell receptor. iT=immature T-cell. Th=T-helper cell. Tc=cytotoxic T cell. IL2=interleukin 2. IFN α =interferon α . TNF β =tumour necrosis factor β .

Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial

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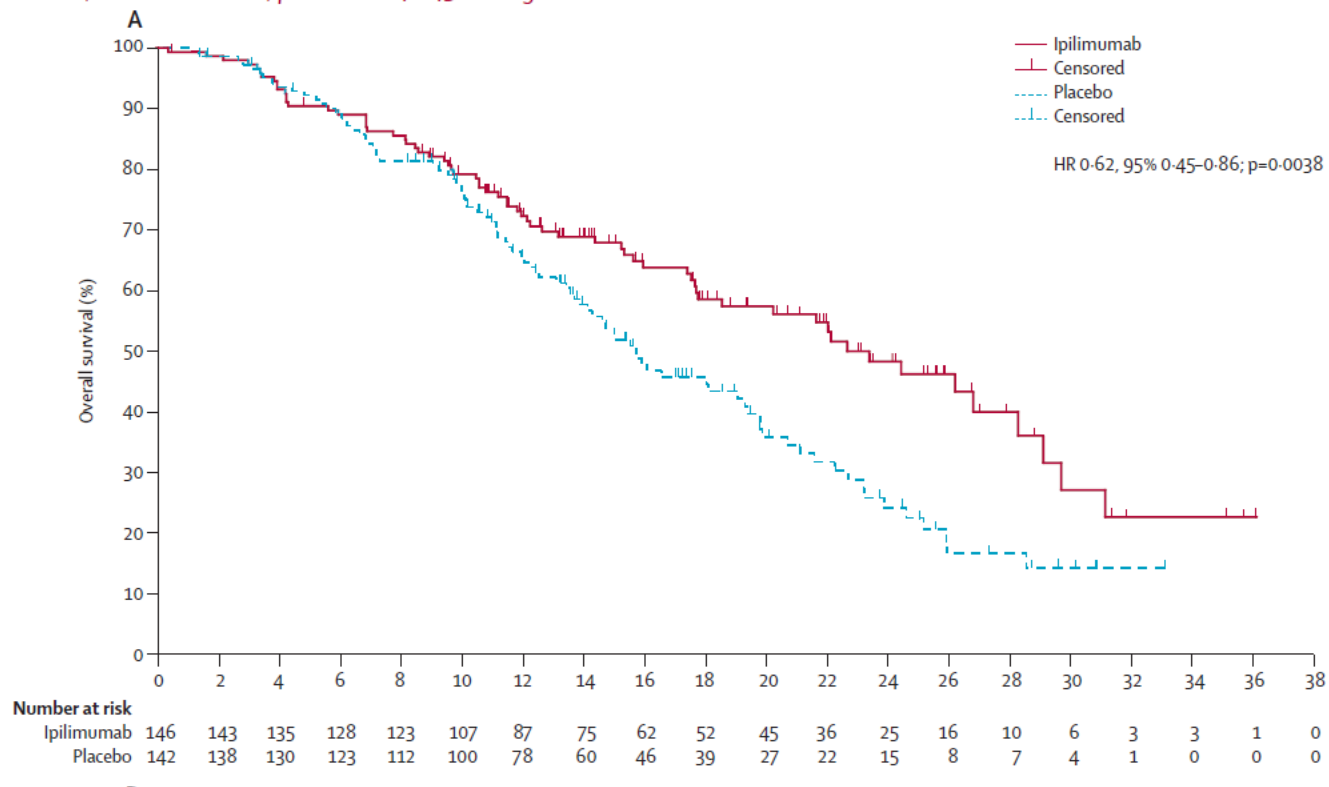
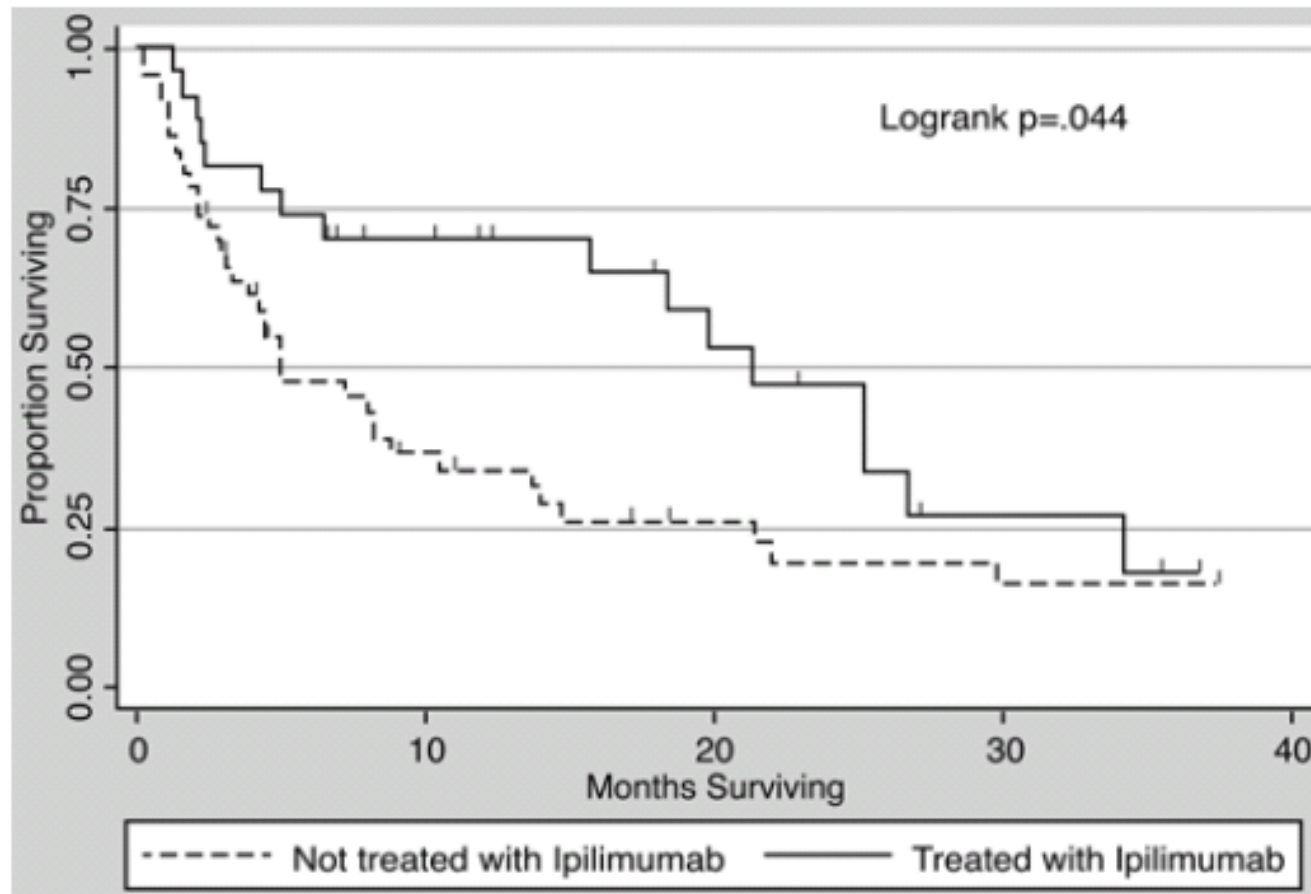


Figure 3: Post-hoc subgroup analyses of overall survival in patients with good (A) and poor prognostic features (B)
 (A) Overall survival in patients with alkaline phosphatase concentration less than 1.5 times the upper limit of normal (ULN), haemoglobin concentration of 110 g/L or more, and no visceral metastases (ipilimumab, n=146; placebo, n=142). (B) Overall survival in patients with at least one adverse prognostic feature—ie, alkaline phosphatase concentration of 1.5 times ULN or higher, haemoglobin concentration less than 110 g/L, or presence of visceral metastases (ipilimumab, n=253; placebo, n=258).

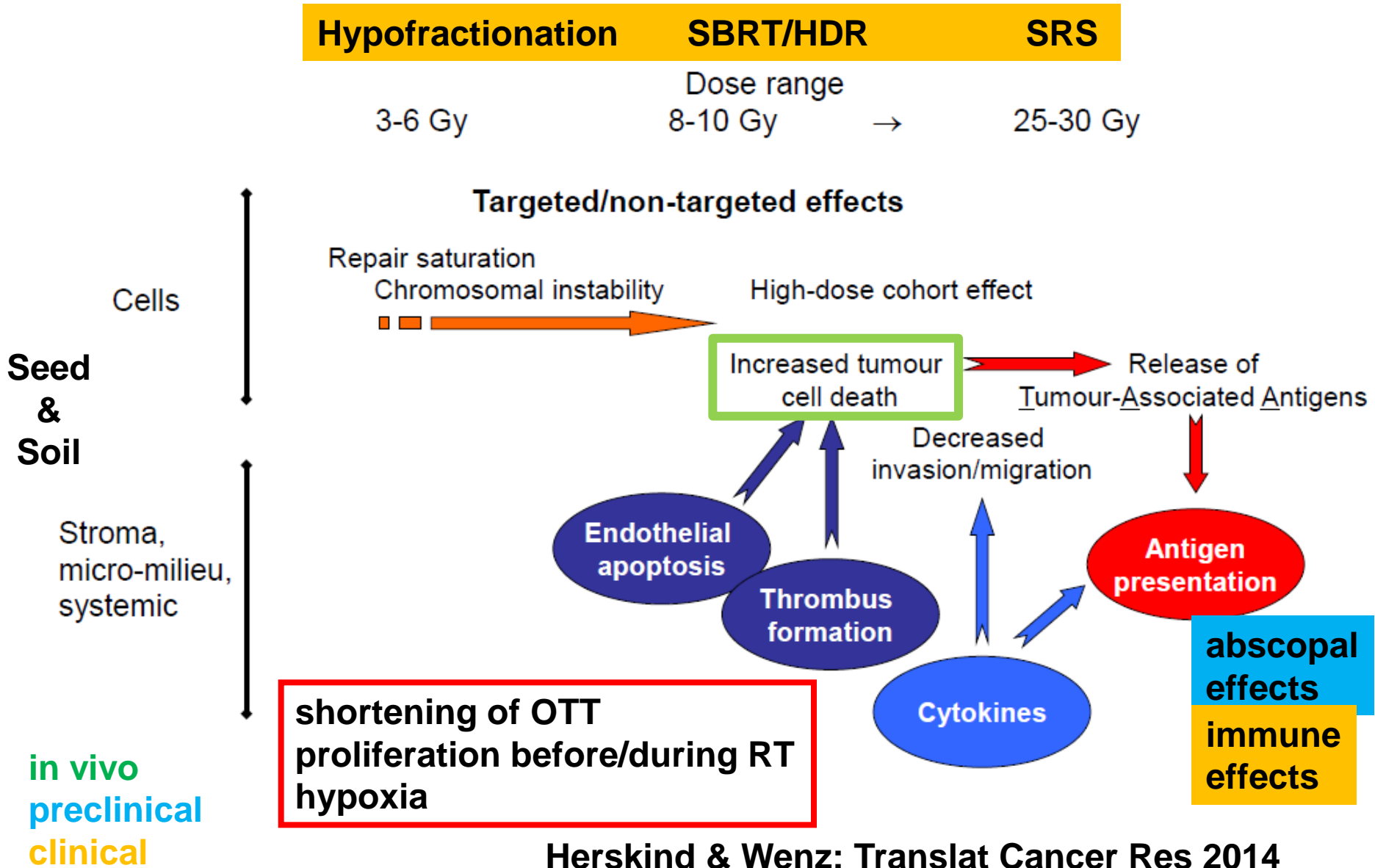
Radiosurgery for melanoma brain metastases in the ipilimumab era and the possibility of longer survival

Clinical article

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MARIO SZNOL, M.D.,^{2,3} HARRIET M. KLUGER, M.D.,^{2,3} AND VERONICA L. S. CHIANG, M.D.^{3,5}



Radiobiology of high single doses



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