TUMOR RESPONSE TO RADIOTHERAPY

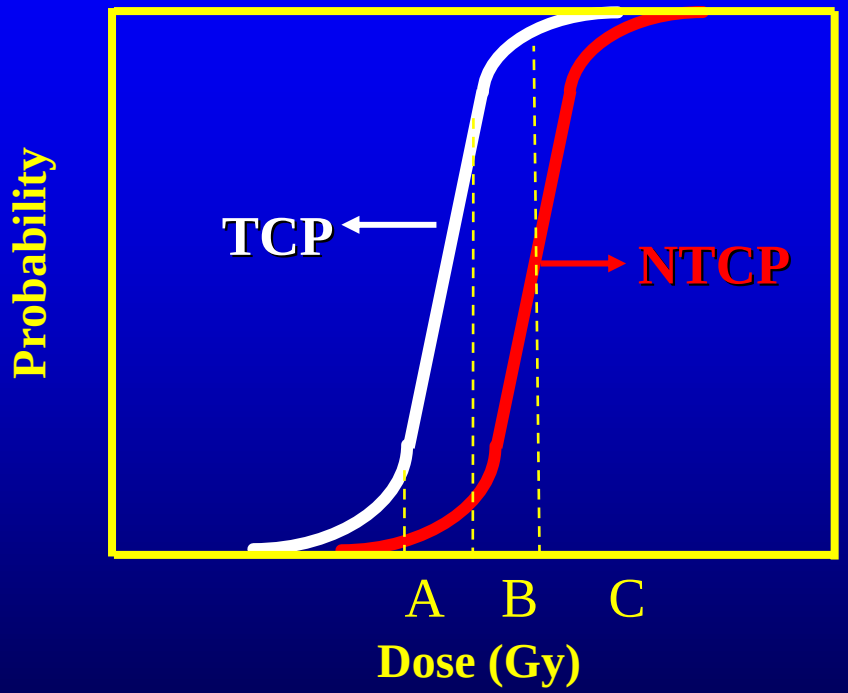
|

TARGETS OTHER THAN TUMOR CELLS

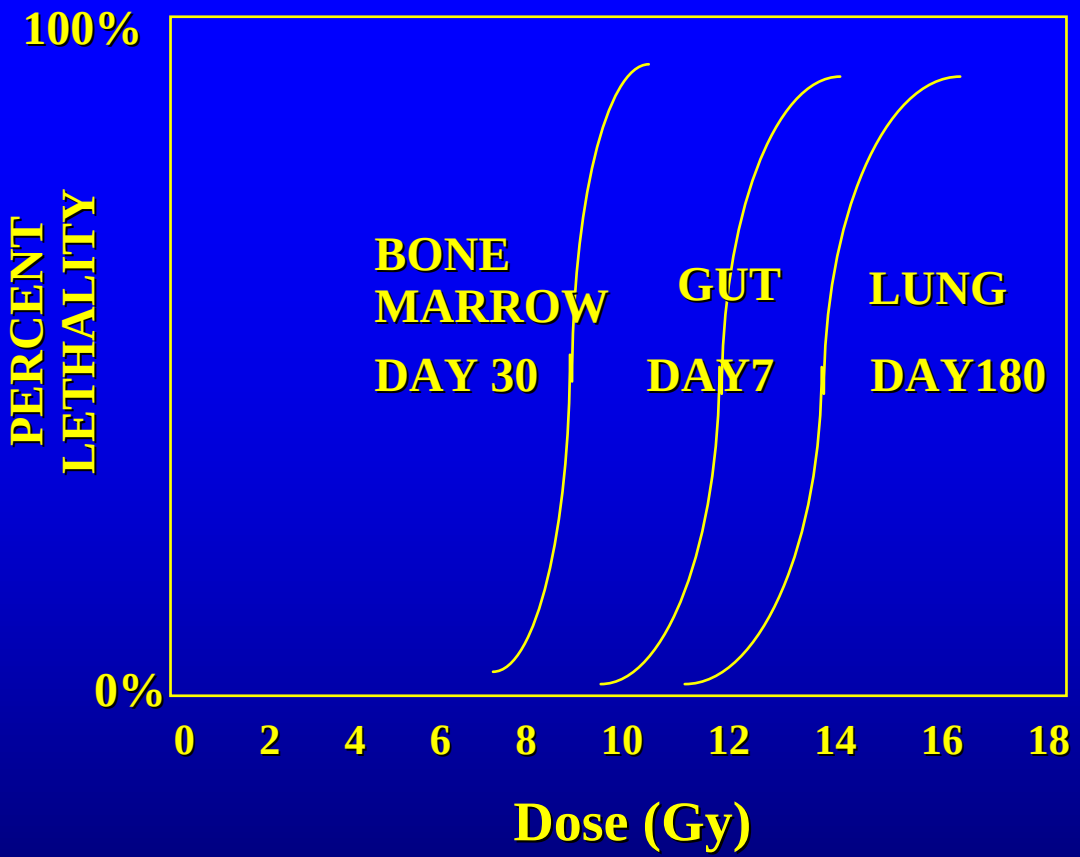
2006/02/25

The Aim of Radiobiology (The Art of Radiotherapy)

- To separate the curves of TCP and NTCP
  - Sensitizers
  - Protectors
  - Dose fractionation
  - etc….



NORMAL TISSUE RADIOBIOLOGY



Different tissues have different tolerances to irradiation and fail at different times after irradiation. In this case, mice were given whole body irradiation, abdominal irradiation, or thoracic irradiation to determine the response of bone marrow, gut, and lung, respectively.

Tolerance

‘ Tolerance' depends not only on the intrinsic radiosensitivity of the target cells but also on the number of clonogens required to make an FSU. Survival of an FSU requires survival of at least one clonogen.

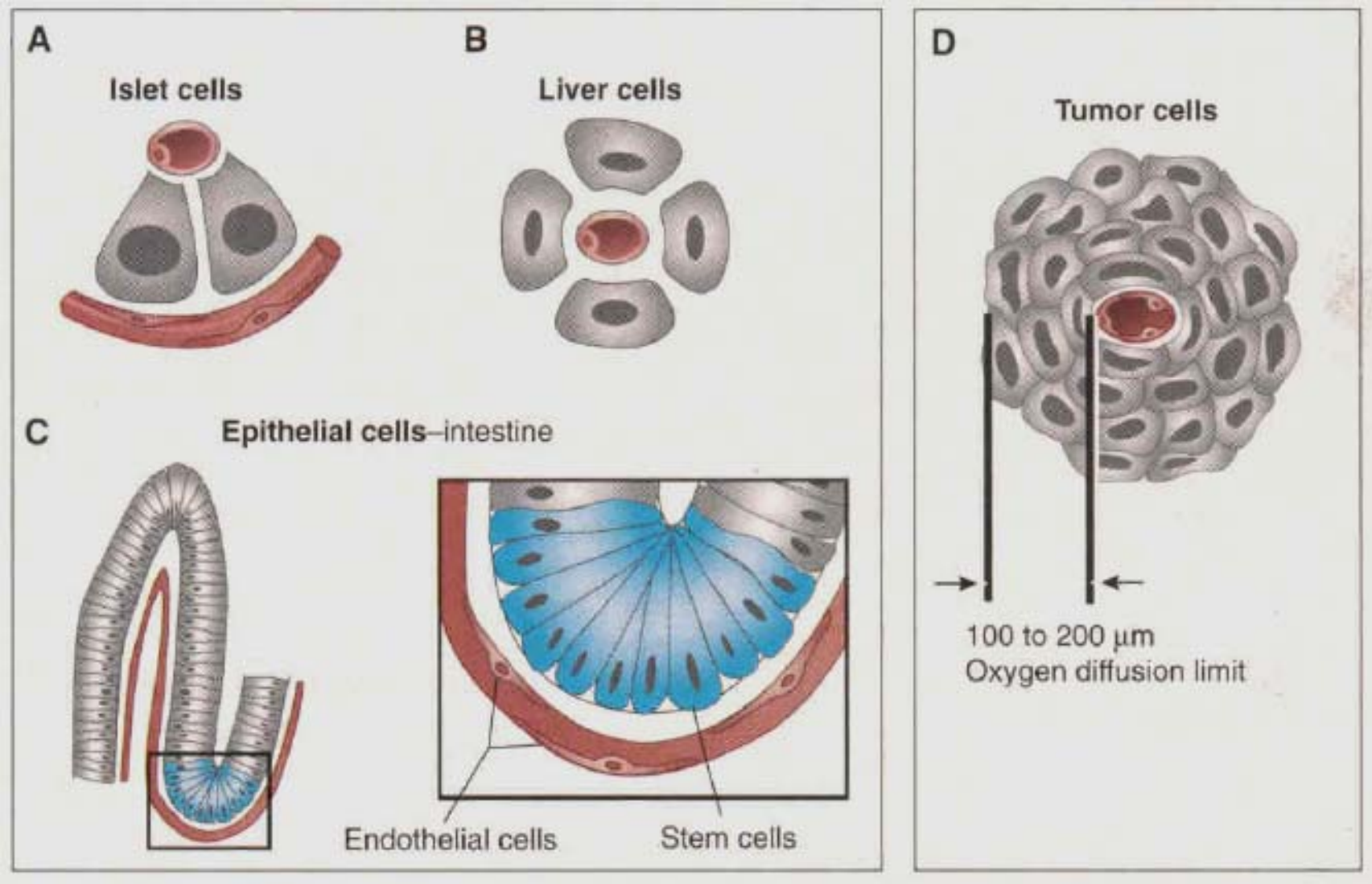
eg.

- Hair loss occurs at a lower dose than skin damage because hair follicles have less clonogenic cells.
- Hair depigmentation occurs at a lower dose than skin because follicles contain less melanocytes.

Latency

- Determined by **STEM CELL** turnover kinetics in a tissue.
- Not an indicator of radiosensitivity. For example,
  - after WBI leukocyte and platelet numbers drop rapidly, anemia is slow to develop because red cells have a long life span.
  - In testis, it takes 60 days to produce 1000 sperm per spermatogenic stem cell. This is why sperm counts remain normal for many weeks after irradiation and then fall precipitously
  - In gut, symptoms take 1 ~ 2 weeks to appear as this is the time taken for the epithelial cells to traverse up the villus and be shed into the lumen

Target cells responsible for radiation damage



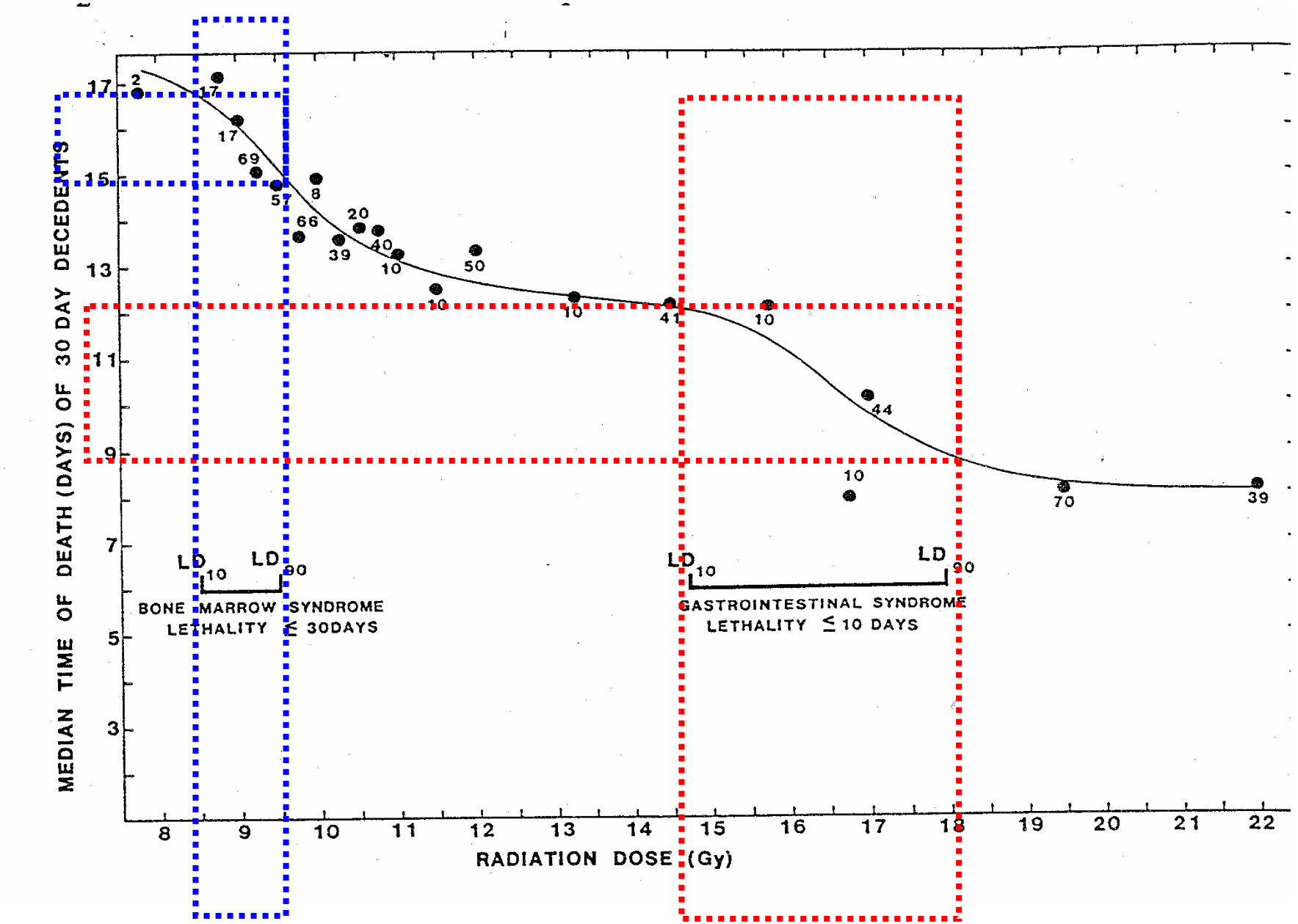
Science, 293:227, 2001 **Judah Folkman and Kevin Camphausen**

Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice

Paris, F., Z. Fuks, A. Kang, P. Capodieci, G. Juan, D. Ehleiter, A. Haimovitz-Friedman, C. Cordon-Cardo, and R. Kolesnick

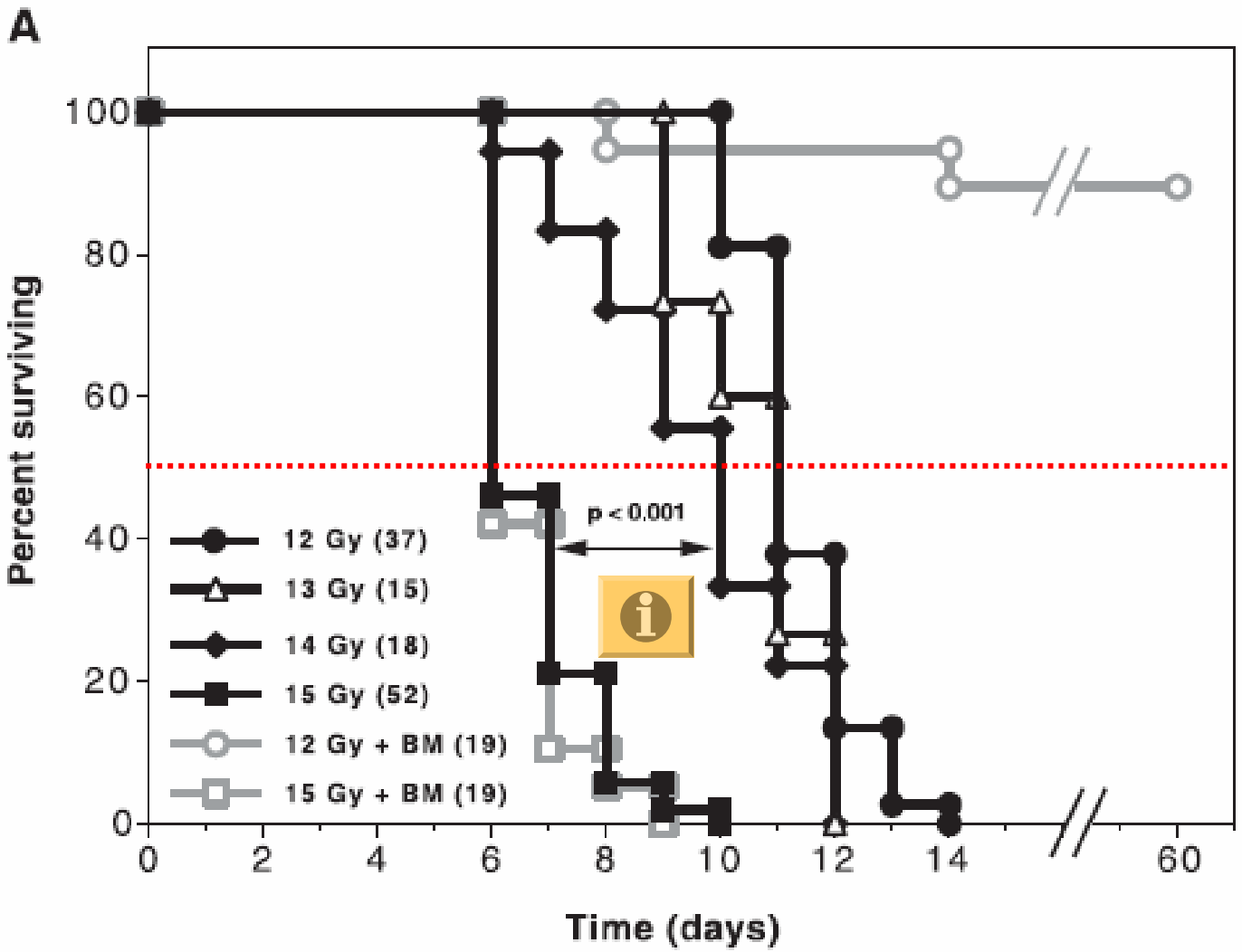
Science, 2001, 293:293

Typical Radiation Response in C3H/HeN/Kam mice to TBI



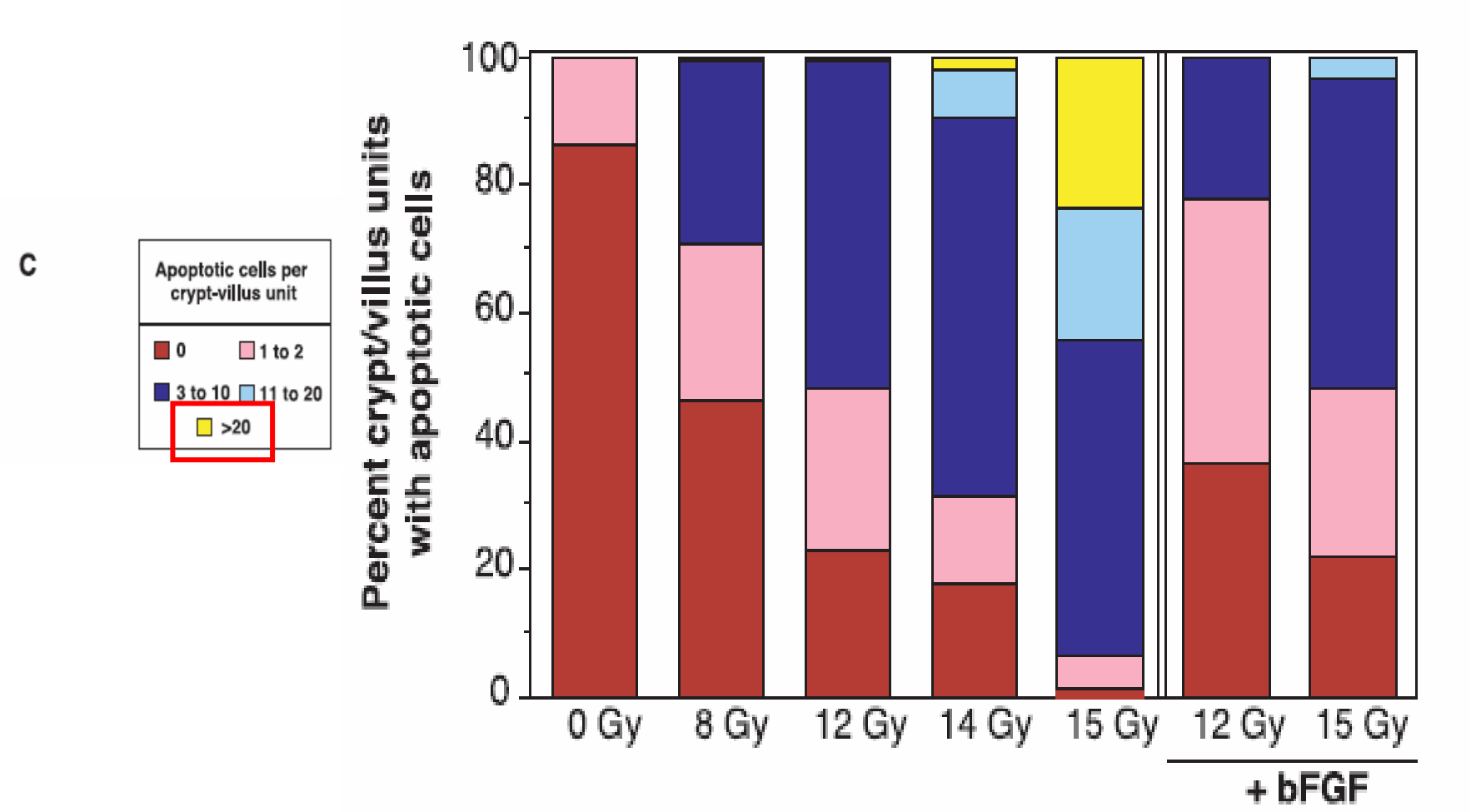
Mason, K. (1989) Int J Radiat Bio, 55:1-5

Survival curve following 12 ~ 15 Gy TBI



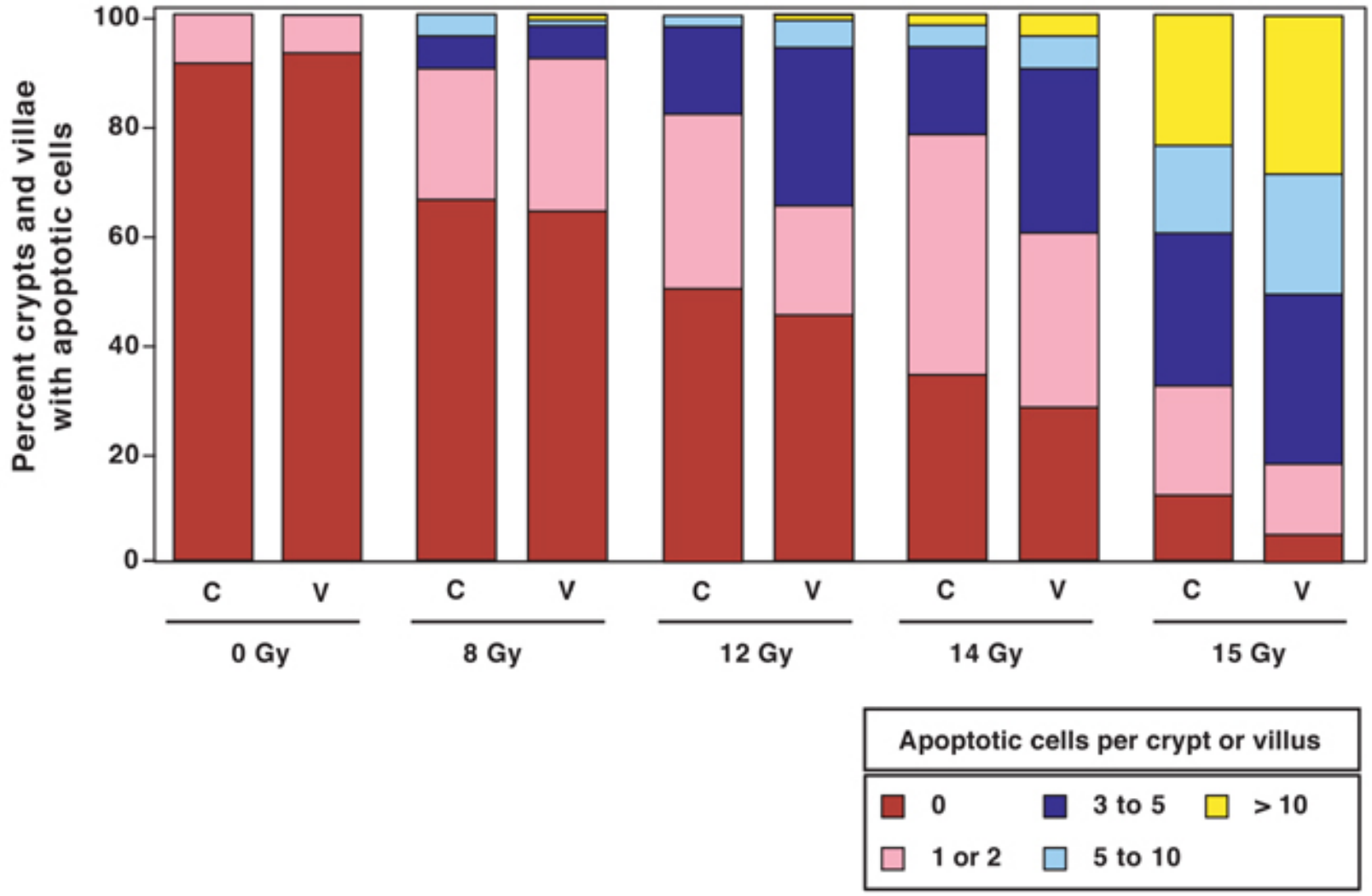
"14 Gy is a threshold for the switch from death caused by marrow failure to that from the GI syndrome"

Frequency histograms of apoptotic cells in the lamina propria at 4 hr after various doses of WBI



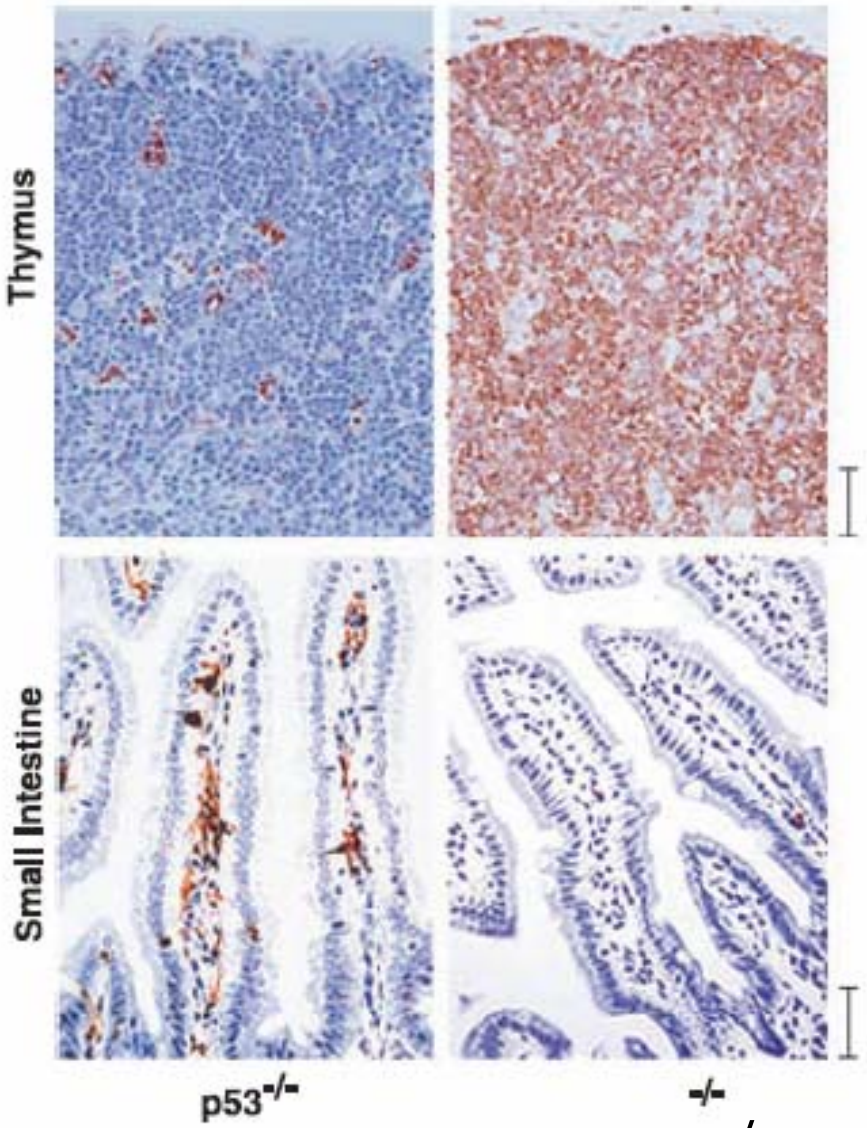
"14 Gy is a threshold for massive endothelial apoptosis, which correlates with the switch from death caused marrow failure to that from the GI syndrome"

Frequency histograms of apoptotic cells in the crypt and villus at 4 hr after various doses of WBI



Further identification of the role of endothelial apoptosis on RT-induced GI syndrome

15 Gy



ASMase deficiency protects against the GI syndrome

Supplemental Table 1. ASMase deficiency protects against the GI syndrome. The median survival was derived from product-limit Kaplan-Meier survival curves. *P* values were calculated by the Mantel log-rank test. Quantification of endothelial cell (EC) apoptosis per crypt-villus unit was as described in Fig. 2C. Student's *t* test and chi-square test for small populations were performed for statistical comparisons.

	Genotype		
Measurement	**Wild type**	***asmase*$^{-/-}$**	**Significance**
Median survival (days)	6	9	*P* < 0.001
Units with ≥ 3 apoptotic cells	77.44%	32.90%	*P* < 0.001
Units with ≥ 11 apoptotic cells	42.07%	3.95%	*P* < 0.001
Units with >20 apoptotic cells	12.81%	0%	*P* < 0.001
Mice with GI death	4/5	1/6	*P* < 0.006

ASMase deficiency → ↓ apoptosis in EC → ↑ crypt survival → protect RT-induced GI death

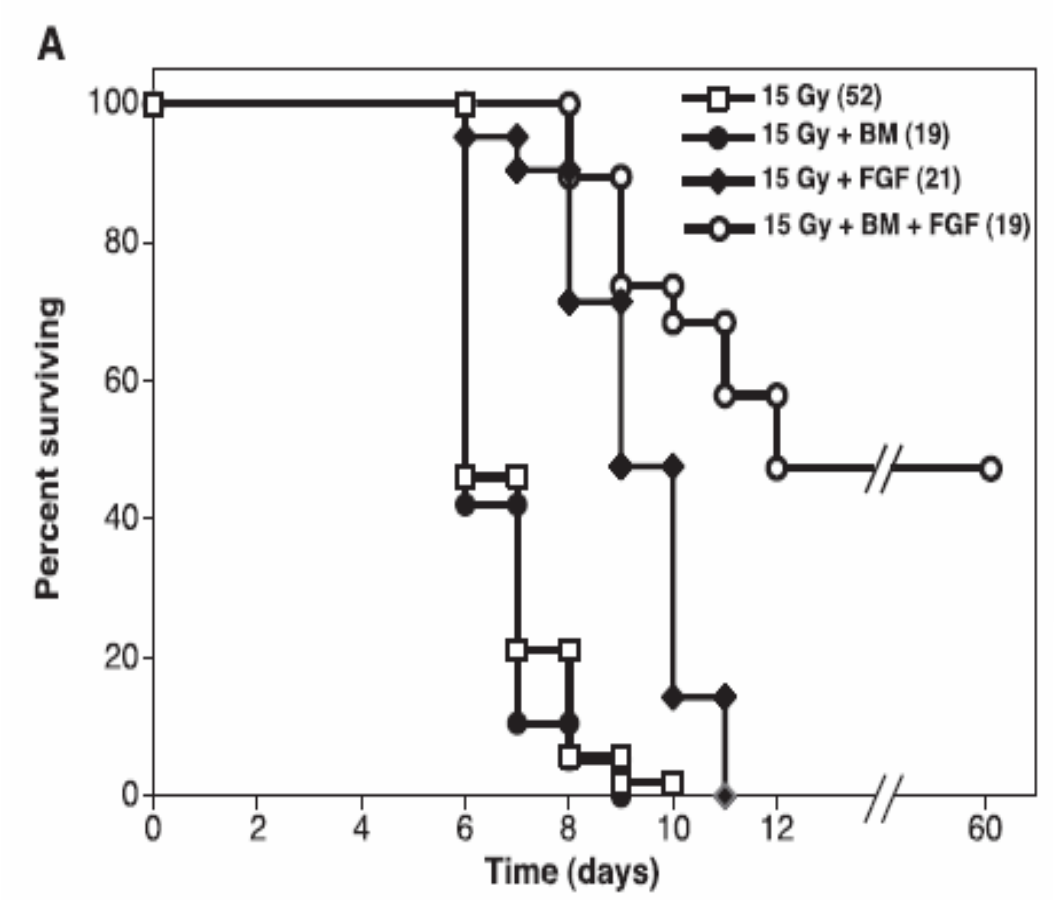
p53 deficiency does not protect against the GI syndrome

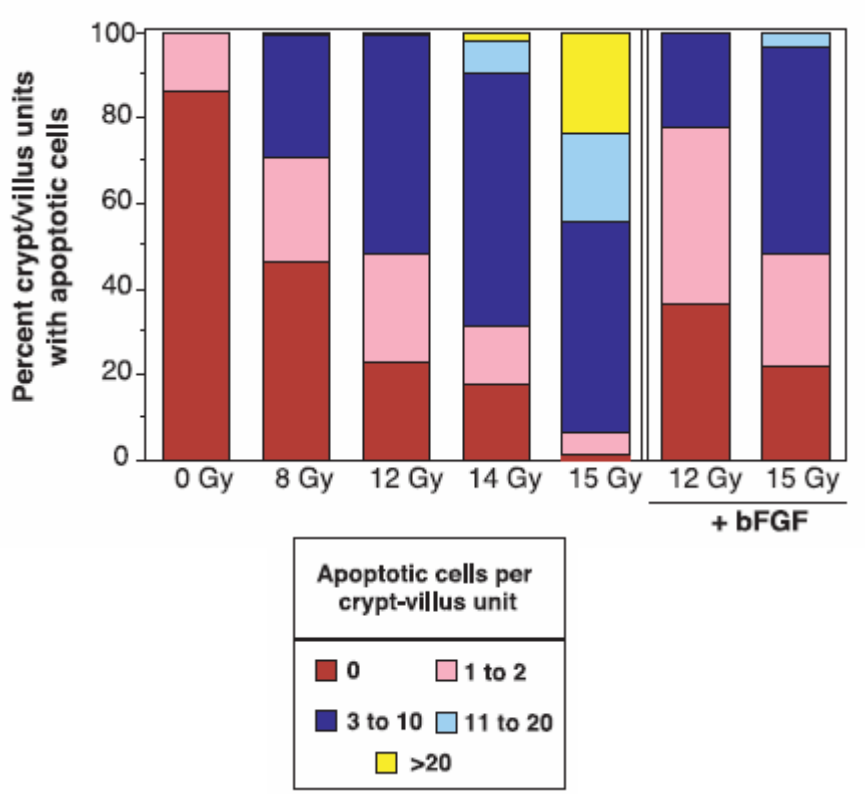
Supplemental Table 2. p53 deficiency does not protect against the GI syndrome. The median survival was derived from product-limit Kaplan-Meier survival curves. *P* values were calculated by the Mantel log-rank test. Quantification of endothelial cell (EC) apoptosis in villus-crypt units was as described in Fig. 2C. Student's *t* test and chi-square test for small populations were performed for statistical comparisons.

	Genotype		
Measurement	**Wild type**	**p53$^{-/-}$**	**Significance**
Median survival (days)	6	6	
Units with ≥ 3 apoptotic cells	93.81	87.28	*P* = 0.06
Units with ≥ 11 apoptotic cells	44.09	57.44	*P* = 0.09
Units with >20 apoptotic cells	23.61	28.72	*P* = 0.12
Mice with GI death	5/5	5/5	

p53 deficiency → ↓ apoptosis, but not in EC → no effect on crypt survival → Do not protect RT-induced GI death

Role of microvasculature on RT-induced GI syndrome

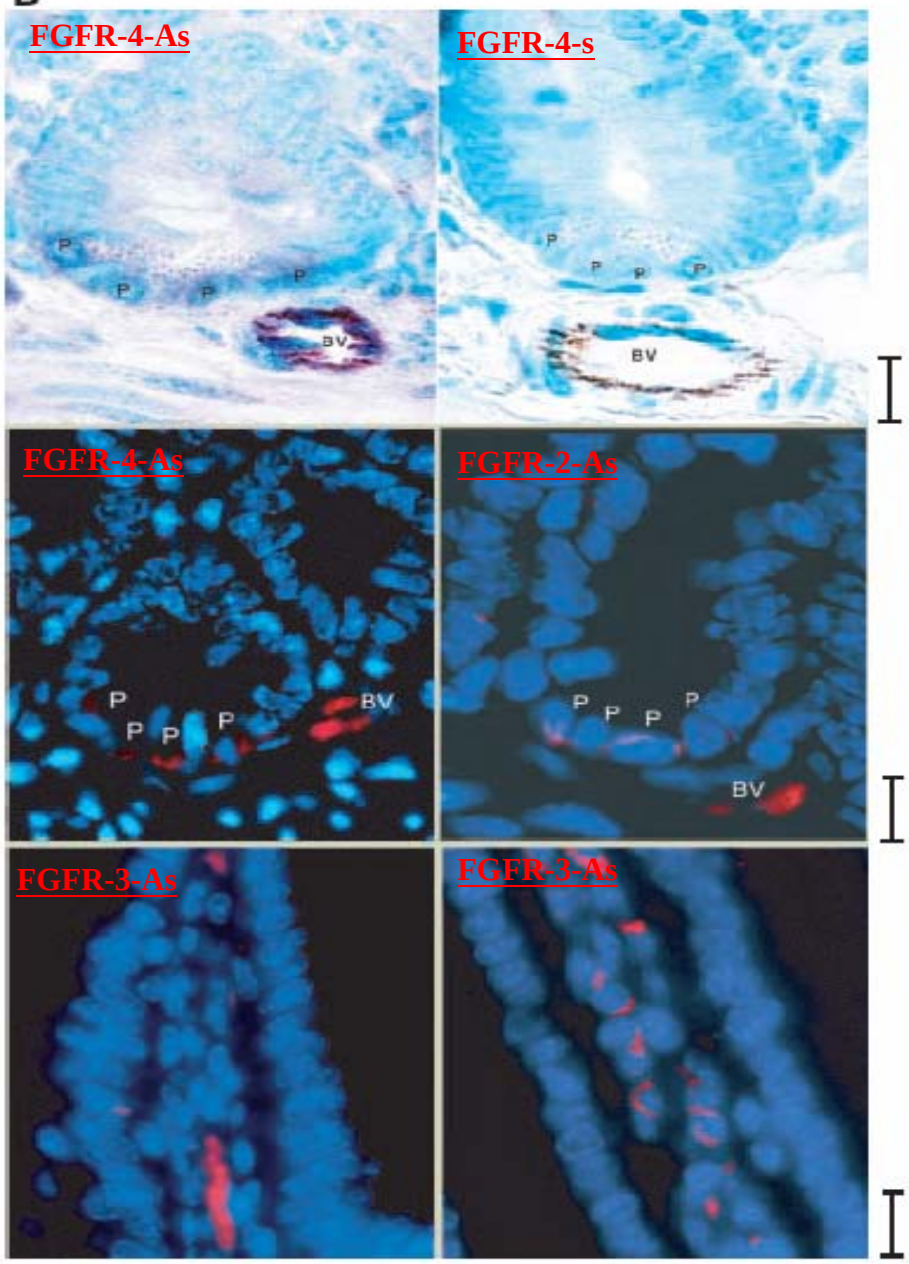


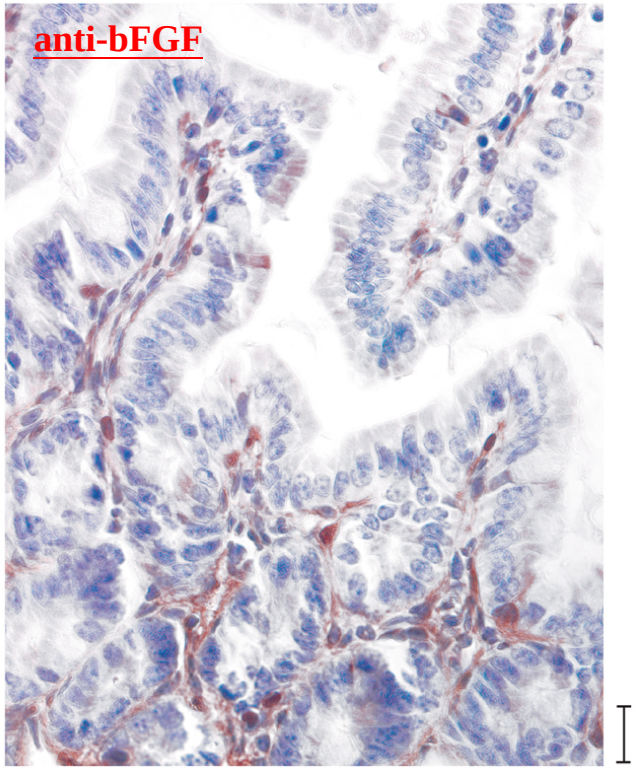


- bFGF could protect RT-induced GI syndrome.
- Is this due to the protection of endothelial or crypt stem cells ?

Expression of FGFR in Crypt

In situ hybridization / CD31 immunostaining





FGFRs were only expressed in microvasculature

The apoptosis of endothelial cells is responsible for RT-induced GI syndrome

Supplemental Table 3. Incidence of stem cell apoptosis at position 4 to 5 from the crypt base. Irradiated animals were exposed to 15 Gy WBR. Small bowel specimens were stained with TUNEL 4 hours after irradiation. Three animals were scored at each point, and 200 crypts were scored for each animal. Student's *t* test and chi-square test for small populations were performed for statistical comparisons.

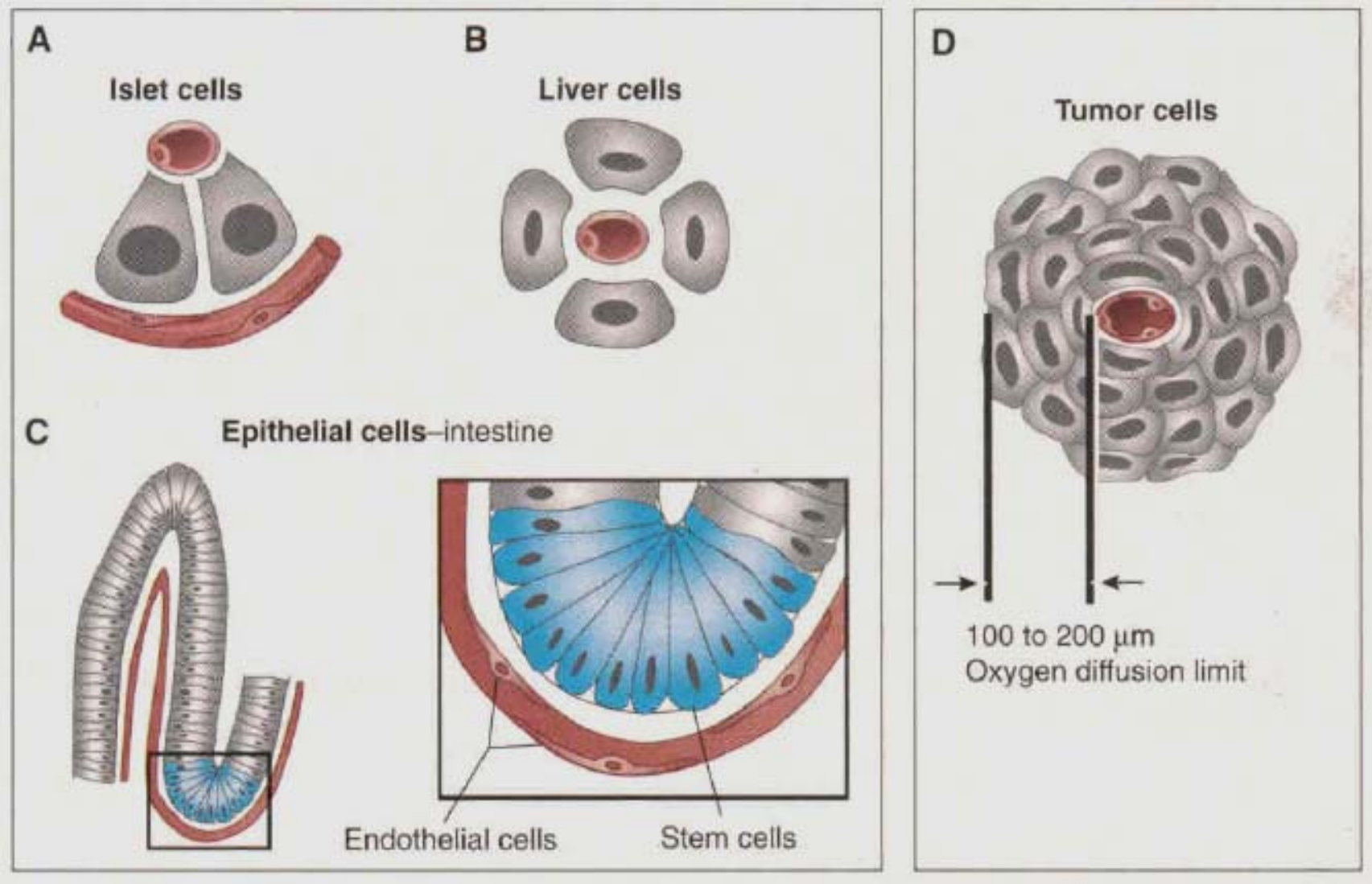
	Apoptosis for radiation dose of		
	0 Gy	15 Gy	**Significance**
Wild type	6 ± 1%	64 ± 8%	$P < 0.001$
p53$^{-/-}$	6 ± 8%	8 ± 2%	$P = 0.12$
asmase$^{-/-}$	7 ± 3%	57 ± 3%	$P < 0.001$
Wild type + bFGF	8 ± 1%	57 ± 6%	$P < 0.001$

The apoptosis at crypt base stem cell was not responsible for RT (15Gy)-induced GI syndrome. The endothelial stem cells did.

Microvascular damage is a key mechanism in GI response to radiation.

Does this also apply in tumor response to RT ?

Target cells responsible for radiation damage

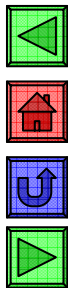


Science, 293:227, 2001 **Judah Folkman and Kevin Camphausen**

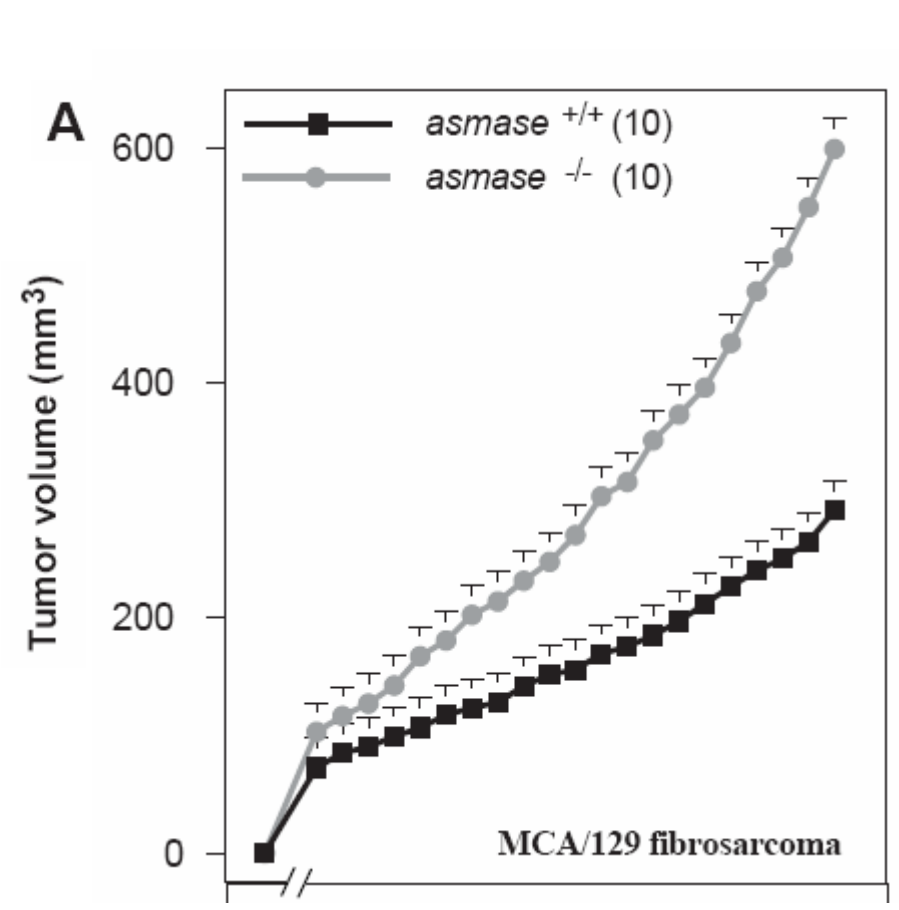
Tumor Response to Radiotherapy Regulated by Endothelial Cell Apoptosis

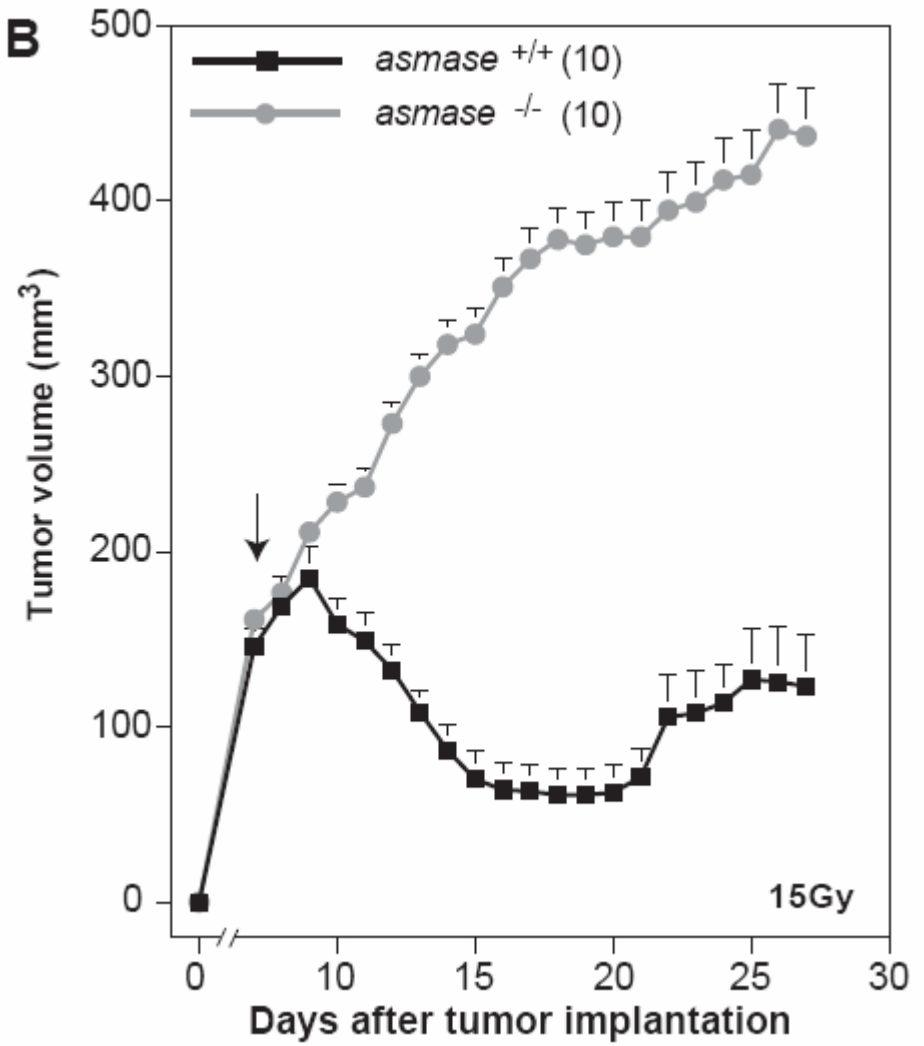
Monica Garcia-Barros, Francois Paris,
Carlos Cordon-Cardo, David Lyden,
Shahin Rafii, Adriana Haimovitz-Friedman,
Zvi Fuks, Richard Kolesnick

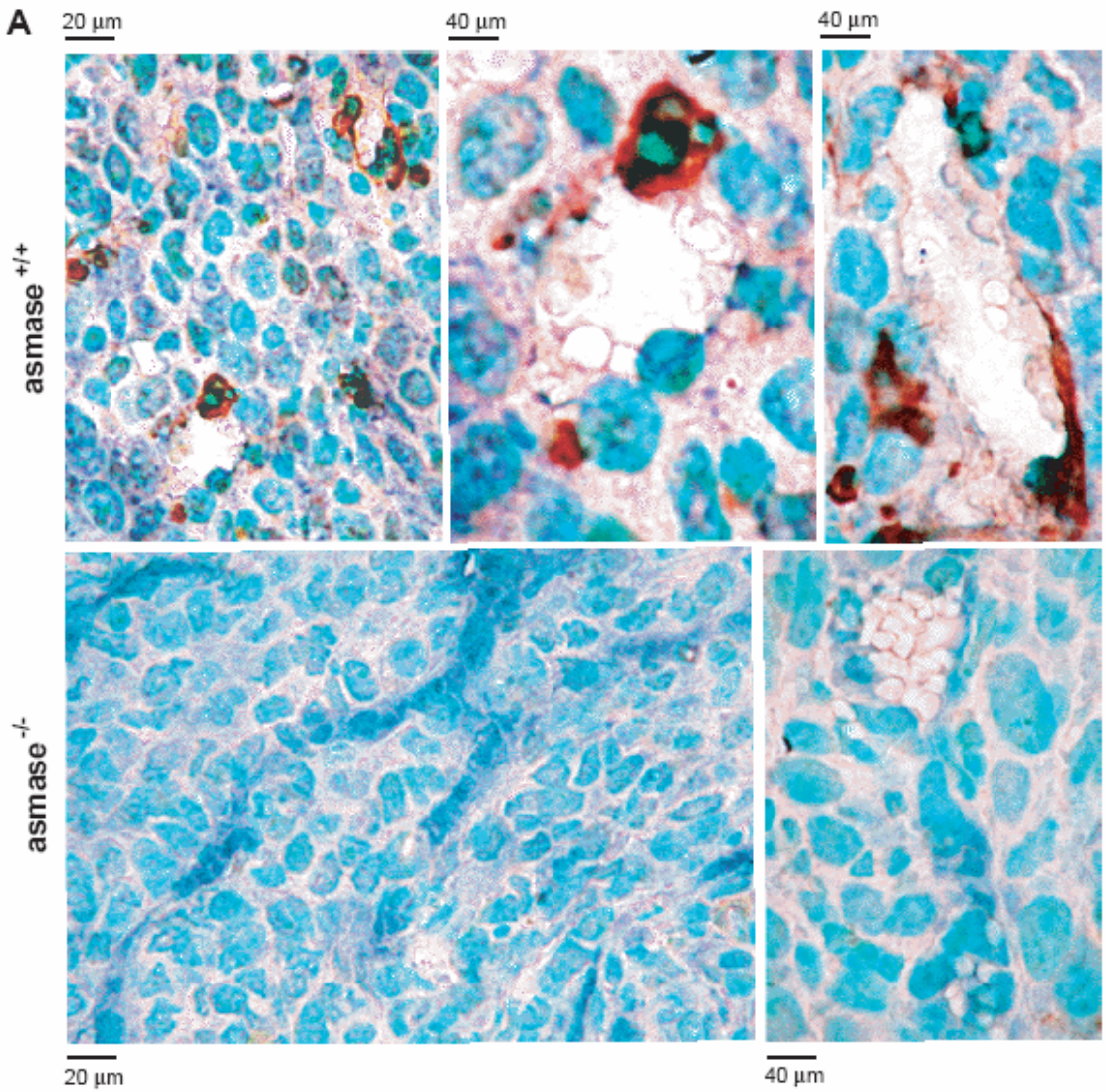
Science, 2003, 300:1155

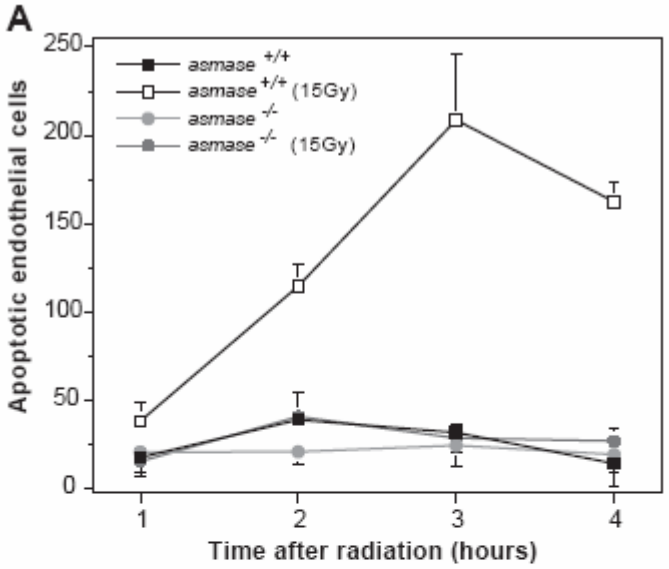


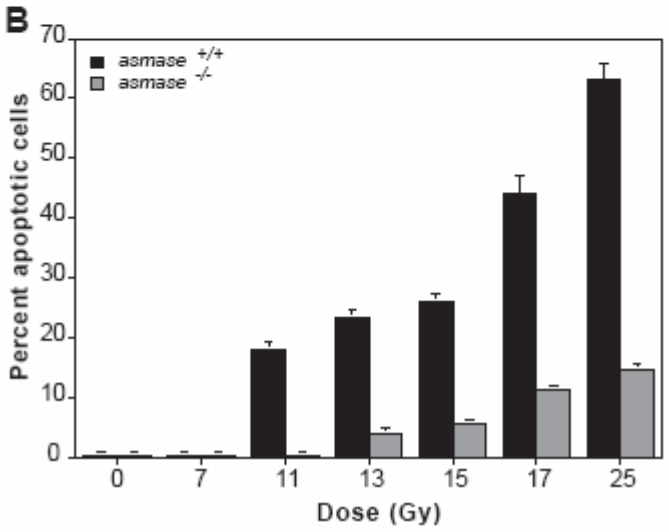
The *asmase* genotype of the host angiogenic compartment affects tumor growth and its response to RT



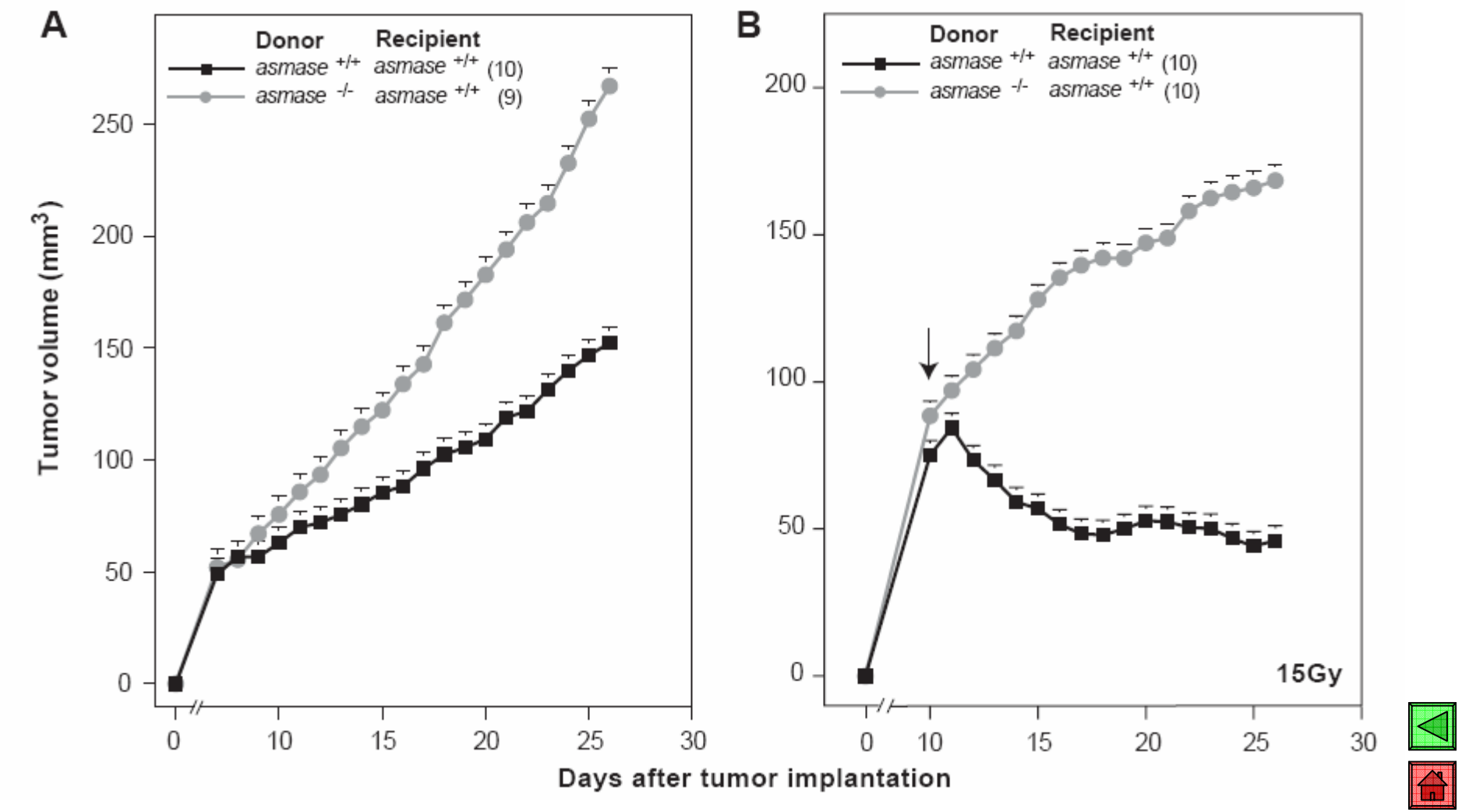



A
20 µm
40 µm
40 µm
asmase +/+
asmase -/-
20 µm
40 µm


A
asmase +/+
asmase +/+ (15Gy)
asmase -/-
asmase -/- (15Gy)
Apoptotic endothelial cells
250
200
150
100
50
0
1
2
3
4
Time after radiation (hours)


B
asmase +/+
asmase -/-
Percent apoptotic cells
70
60
50
40
30
20
10
0
0
7
11
13
15
17
25
Dose (Gy)

Effects of BM transplantation on growth of MCA/129 fibrosarcomas implanted into *asmase*$^{+/+}$ and *asmase*$^{-/-}$ mice



Summary of Zvi Fuks and Richard Kolesnick's studies

- Microvascular damage is a key mechanism in GI and tumor response to radiation.
- The sphingomyelin pathway is a possible target for improving cancer treatment, perhaps by manipulating ASMase levels in tumor endothelial cells or in endothelial precursors.
- Does this occur in clinical fractionation scheme ?

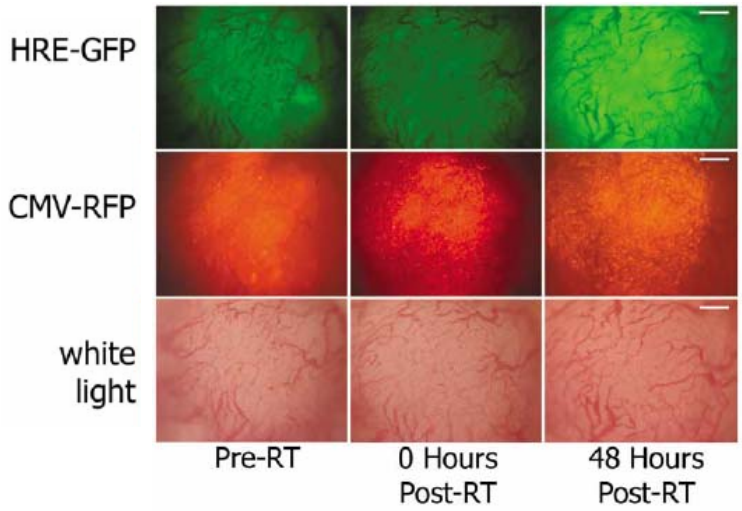
Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: Role of reoxygenation, free radicals, and stress granules

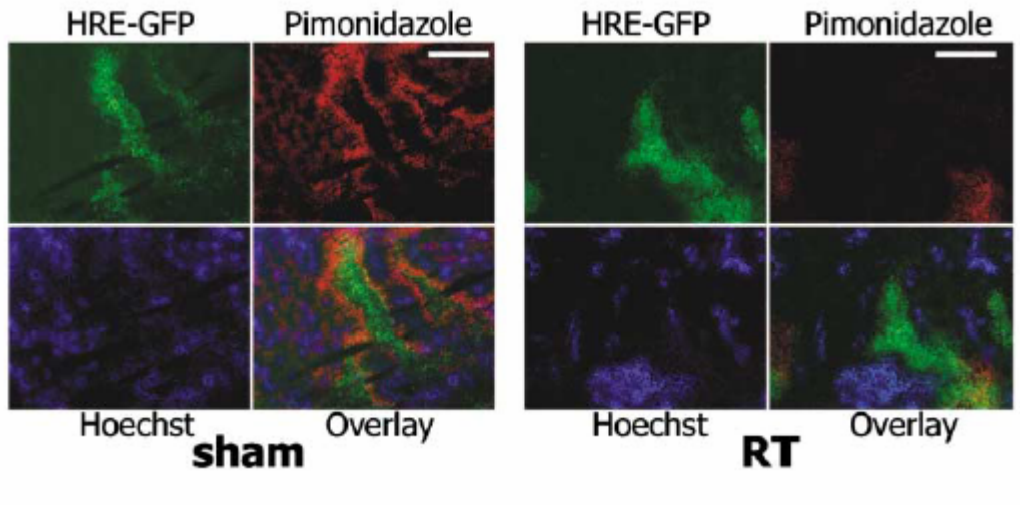
Benjamin J. Moeller, Yiting Cao, Chuan Y. Li, and Mark W. Dewhirst

Cancer Cell, 2004, 5:429

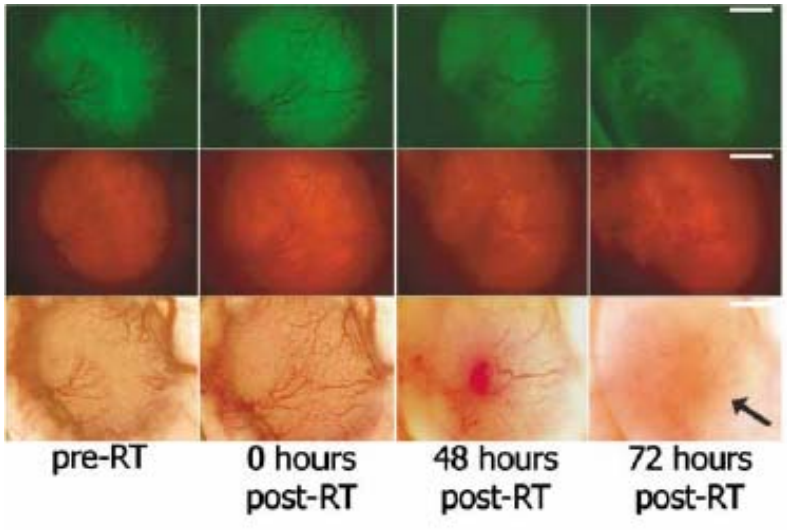
Relationship among RT, HIF1, and Free Radical

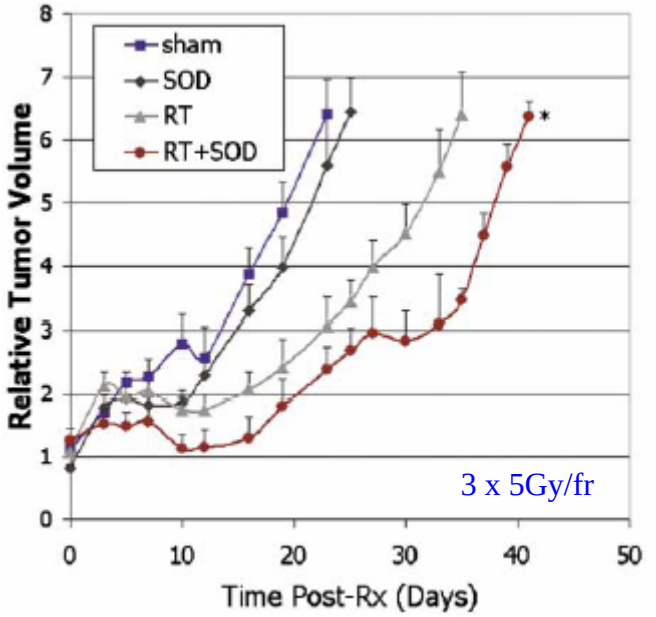
CON





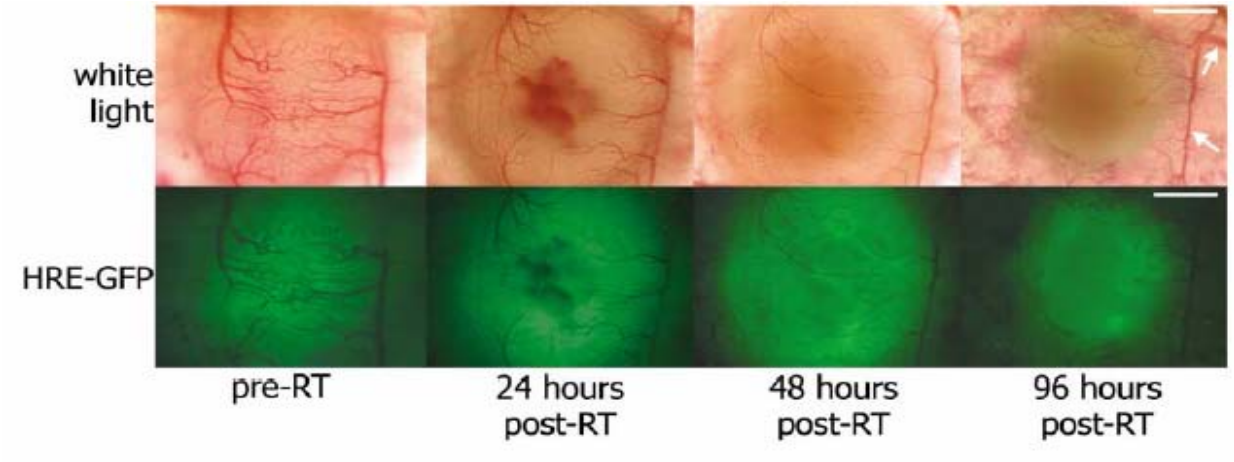
SOD

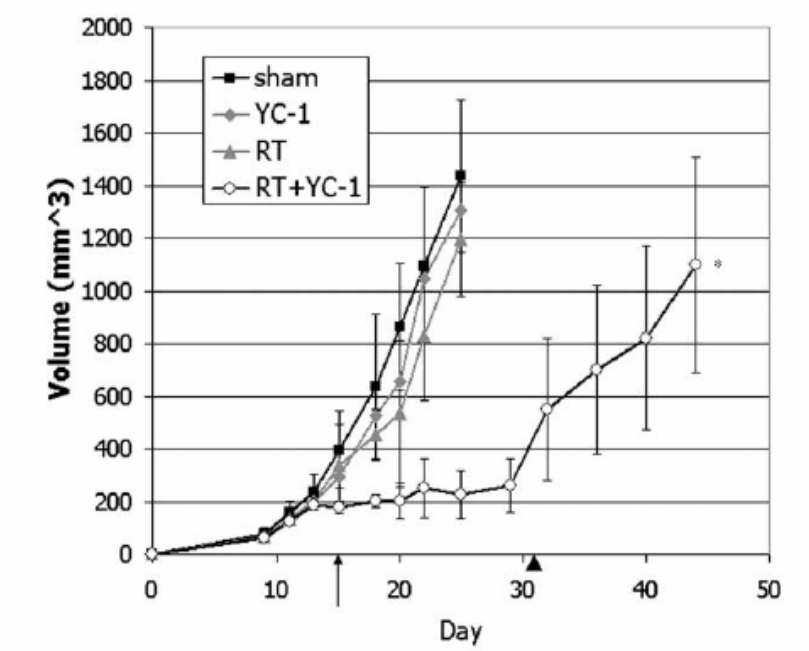




HIF-1 blockade enhances RT efficacy

YC-1 treatment





Conclusions:

Following radiotherapy, tumor reoxygenation leads to:

(1) Nuclear accumulation of HIF-1 enhanced translation of HIF-1-regulated transcripts in response to reactive oxygen.

(2) The resulting increase in HIF-1 enhances endothelial cell radioresistance.

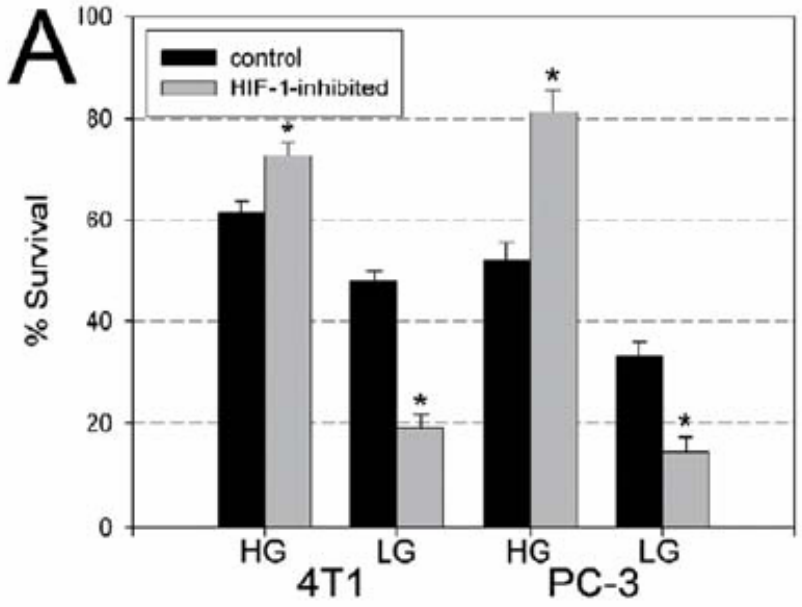
(3) Inhibiting postradiation HIF-1 activation significantly increases tumor radiosensitivity as a result of enhanced vascular destruction.

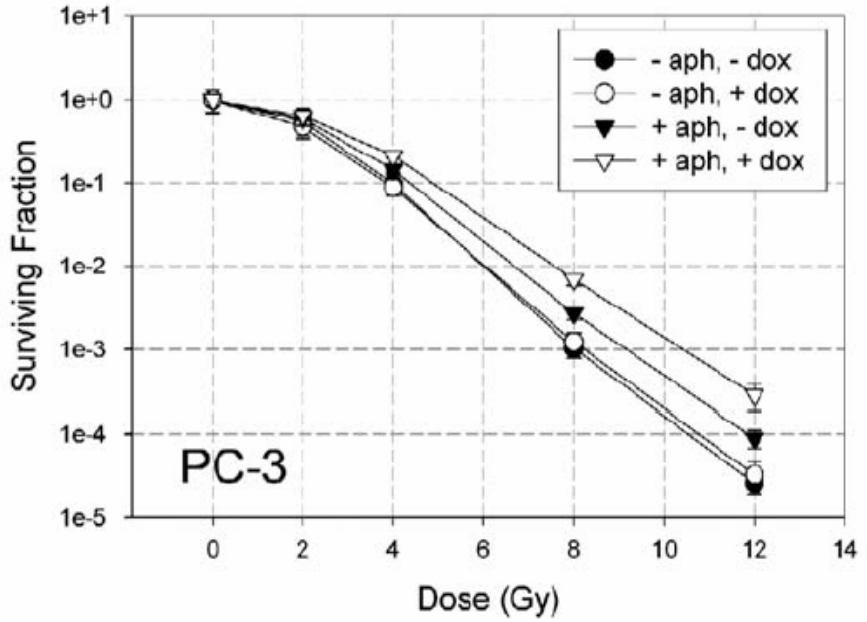
Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity

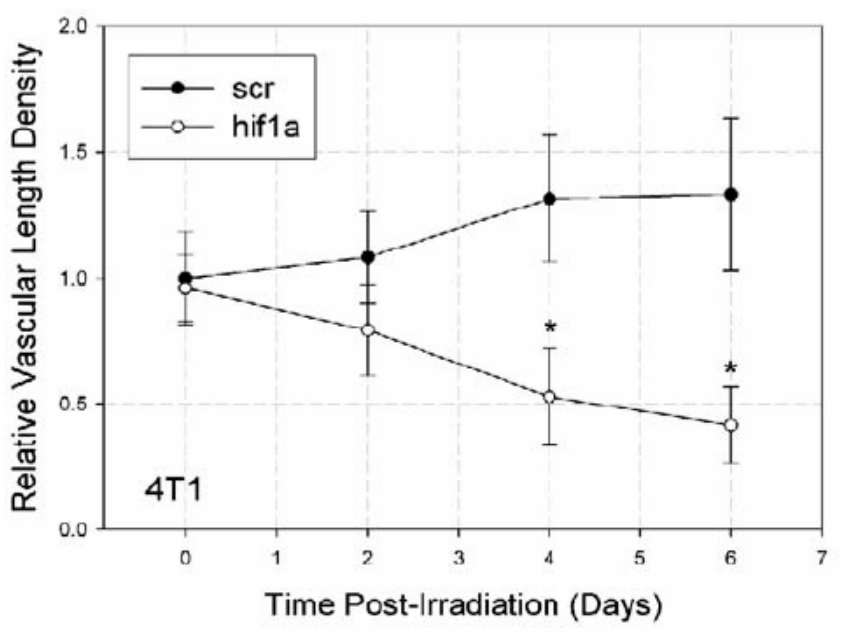
Benjamin J. Moeller, Matthew R. Dreher, Zahid N. Rabbani, Thies Schroeder, Yiting Cao, Chuan Y. Li, and Mark W. Dewhirst

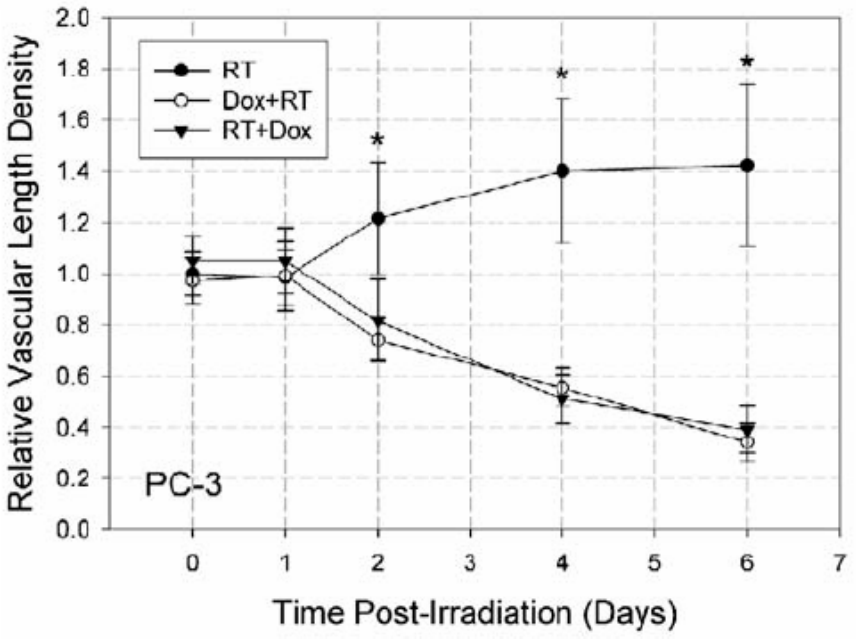
Cancer Cell, 2005, 8:99

Pleiotropic effects of HIF-1 blockade

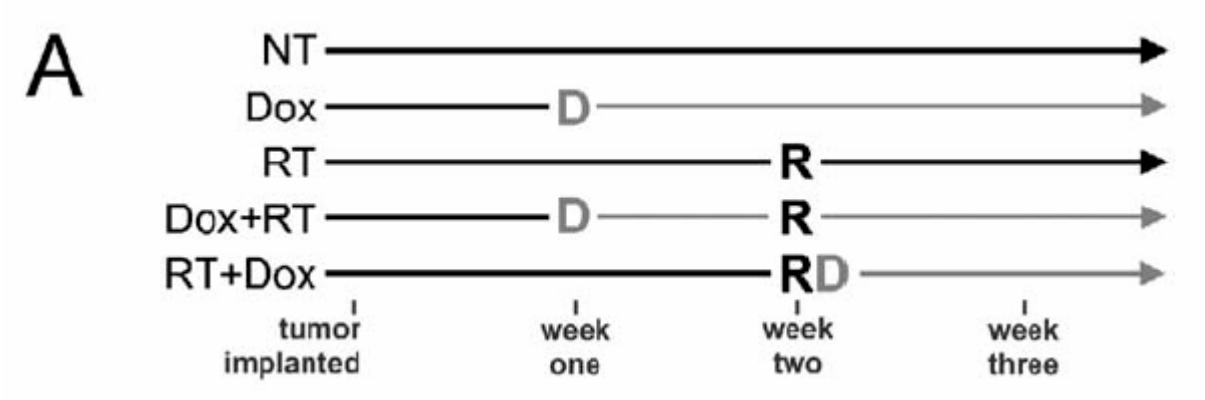


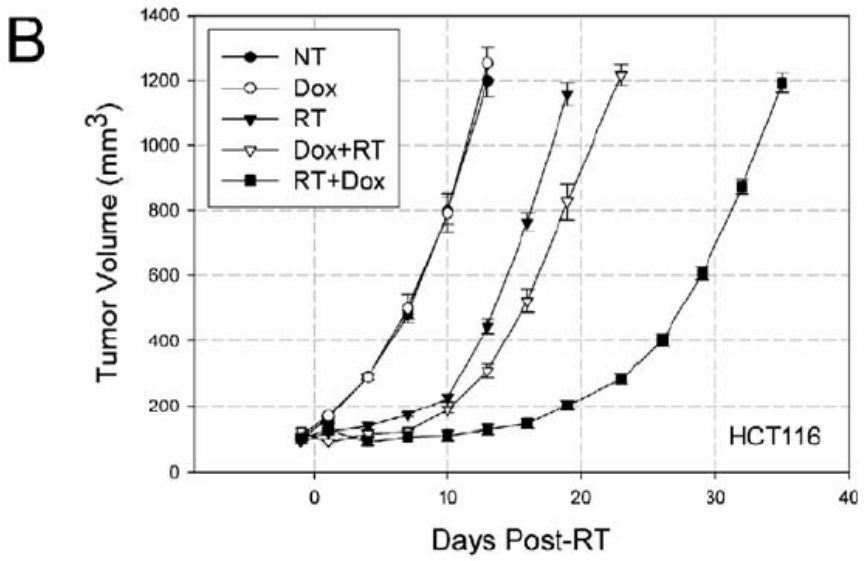


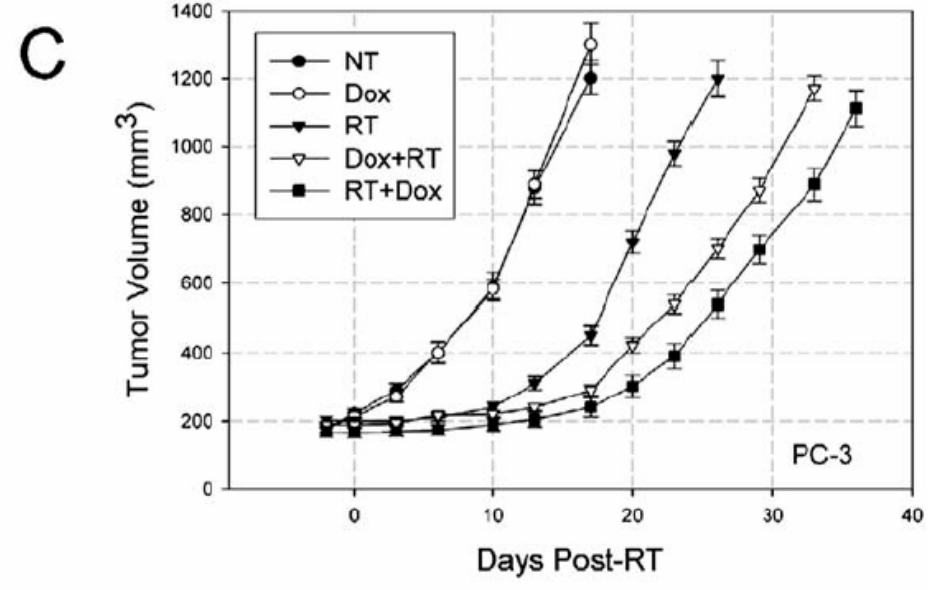




HIF-1 blockade is maximally effective following radiation



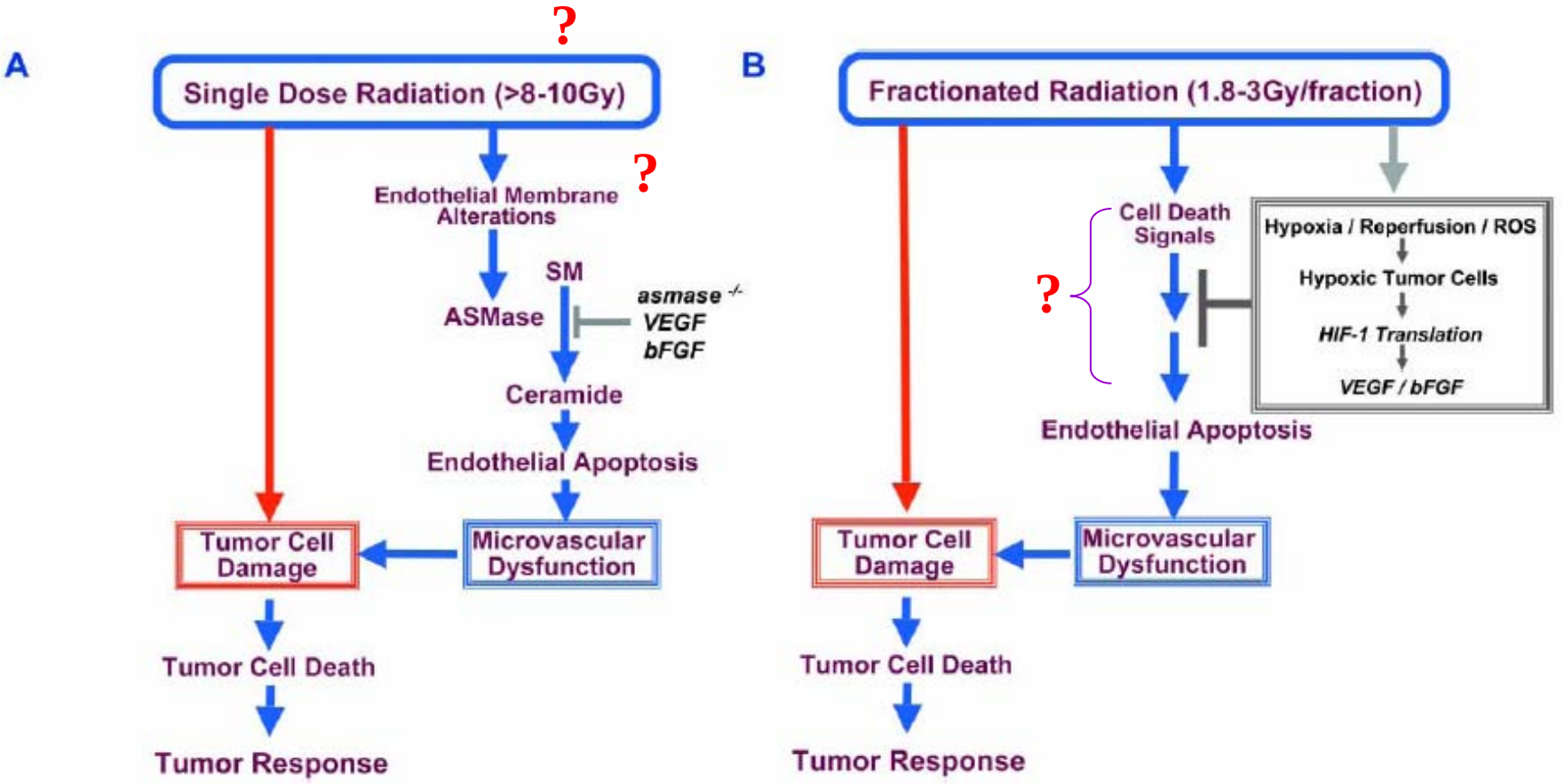




Summary

- **Fractionation radiation increases HIF-1 activity in tumors.**
- **HIF-1 causes significant radioprotection of the tumor vasculature.**
- **HIF-1 has a radiosensitizing effect on tumors.**
- **The net effect of HIF-1 blockade on tumor radioresponsiveness is highly dependent on treatment sequencing.**

Models of microvascular endothelial engagement in tumor response to single-dose or fractionated radiotherapy



Fuks, Z., and R. Kolesnick. 2005. Cancer Cell 8:89

Mechanisms for RT effects

Conventional Model

- Radiation therapy controls tumor growth is via cellular DNA damage, primarily double-strand breaks, resulting in reduced TUMOR CELL proliferative potential and clonogenic survival.

New Model

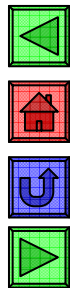
- Tumor growth control is influenced by radiation-induced ENDOTHELIAL CELL damage.

Implication

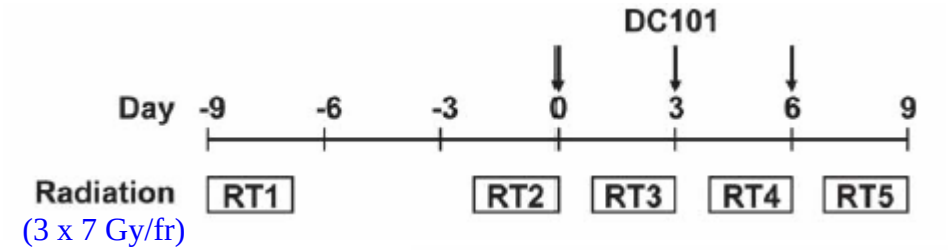
- EC seems an important target responsible for RT effects.
- Angiogenesis inhibition has generated considerable excitement in cancer therapy and led to the discovery of numerous inhibitors.
- Can it be combined with radiation therapy to improve cancer cure ?
- If yes, how can it be effective ?

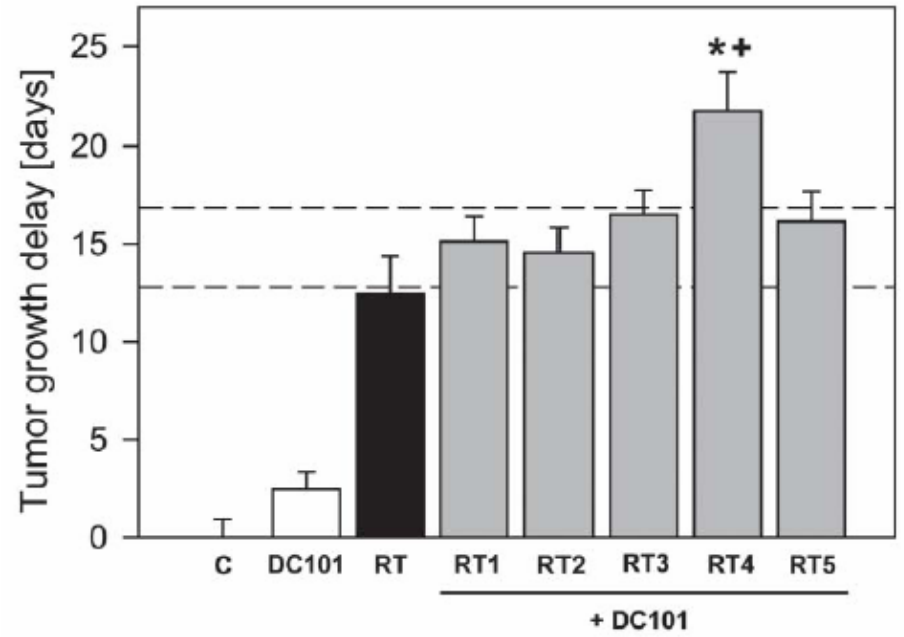
"Attempts to combine antiangiogenic therapy with radiation therapy have produced INCONSISTENT FINDINGS: some experimental studies have demonstrated an additive tumor growth delay, others have shown a stronger, synergistic effect (Teicher et al., 1995; Mauceri et al., 1998; Lee et al., 2000; Kozin et al., 2001; Wachsberger et al., 2003), and one study showed a compromised therapeutic response (Murata et al., 1997)."

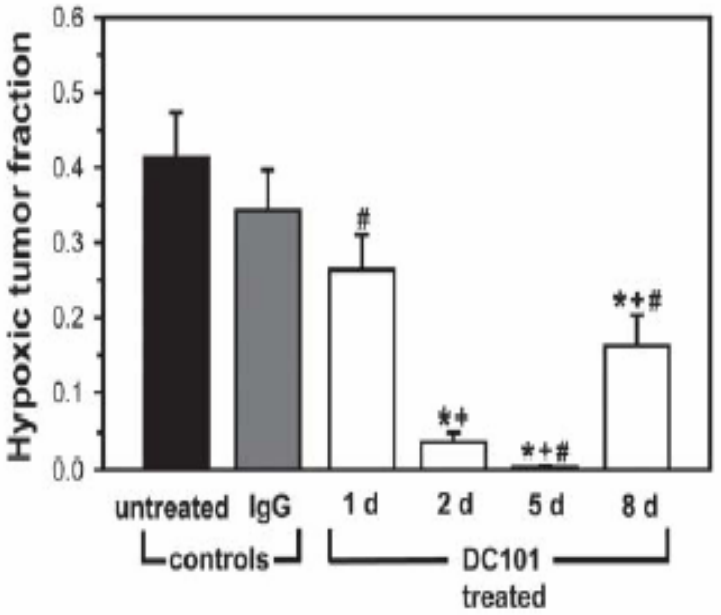
_ Frank Winkler, et al. CANCER CELL : DECEMBER 2004 ,6:553



A combination of radiation and antiangiogenic therapies is only synergistic during a "normalization window" when tumor hypoxia is greatly diminished

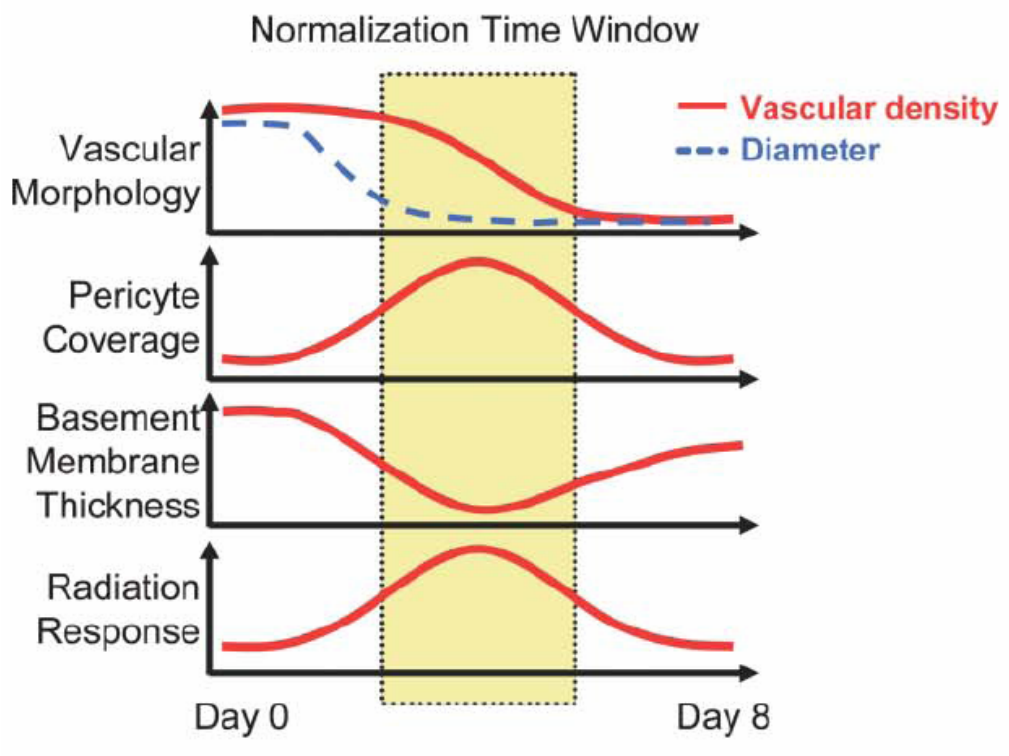


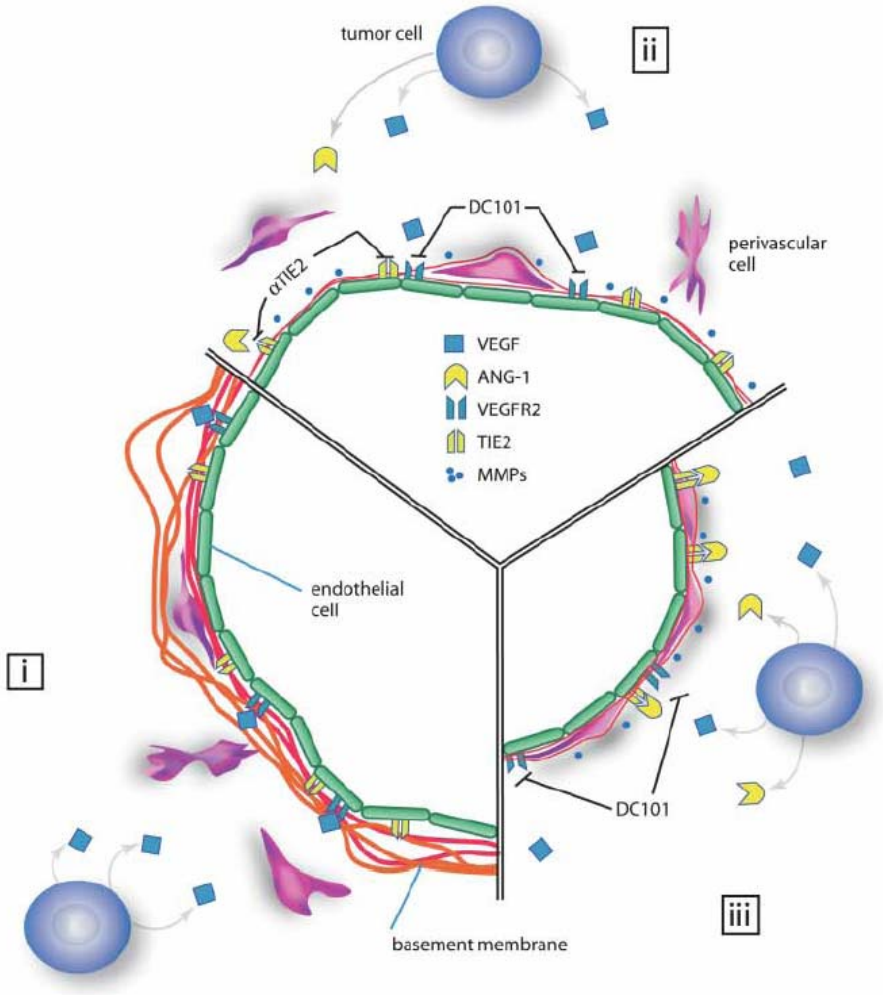




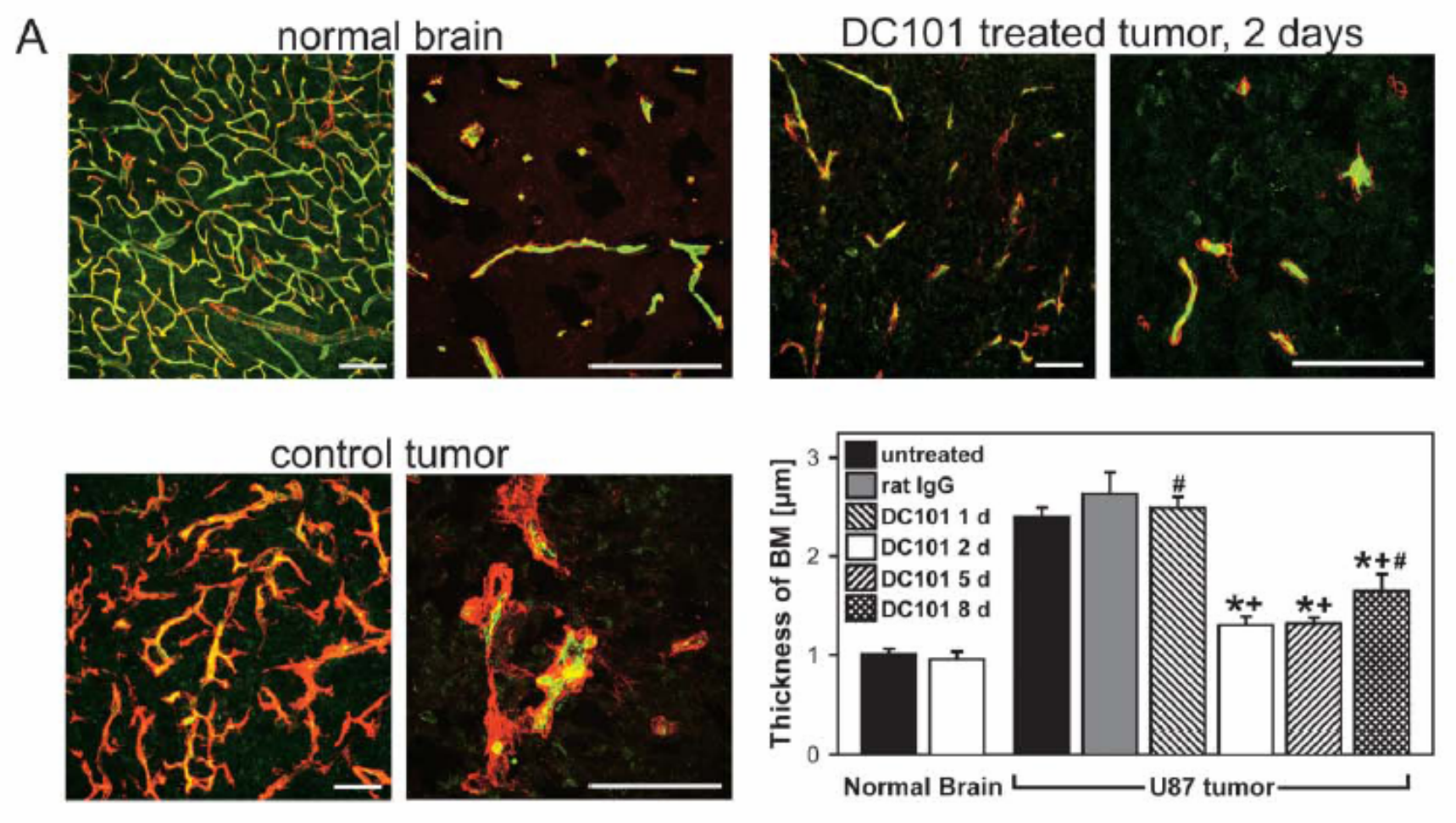
CANCER CELL : DECEMBER 2004 ,6:553

Time course and mechanisms of vascular normalization



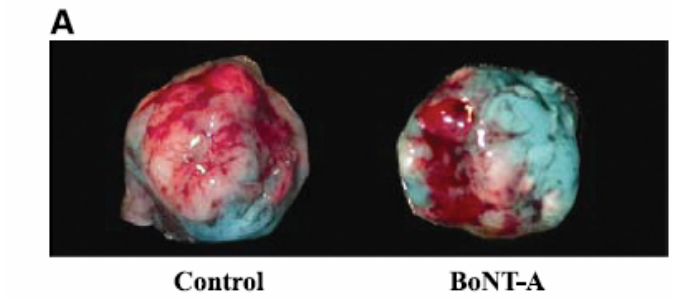


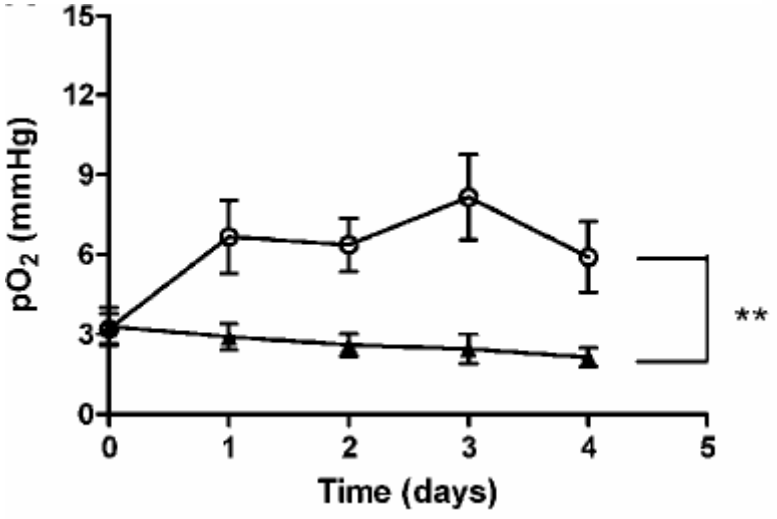
VEGFR2 blockade thins the abnormally thick basement membrane (BM) of tumor vessels

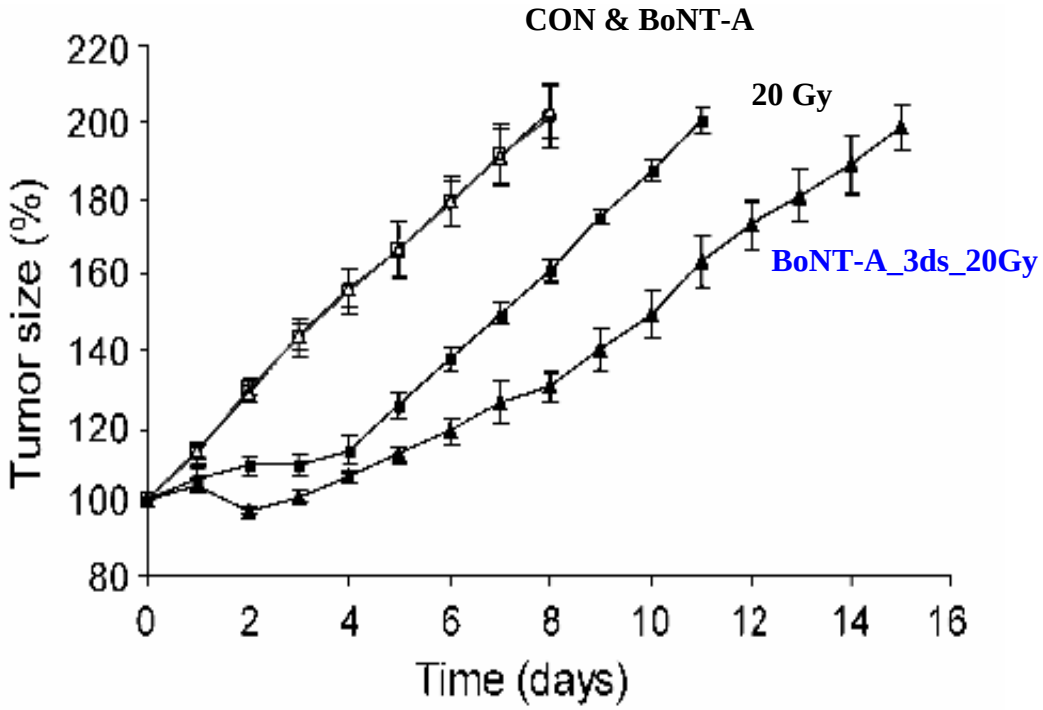


CANCER CELL : DECEMBER 2004 ,6:553

Botulinum Toxin Potentiates Cancer Radiotherapy and Chemotherapy

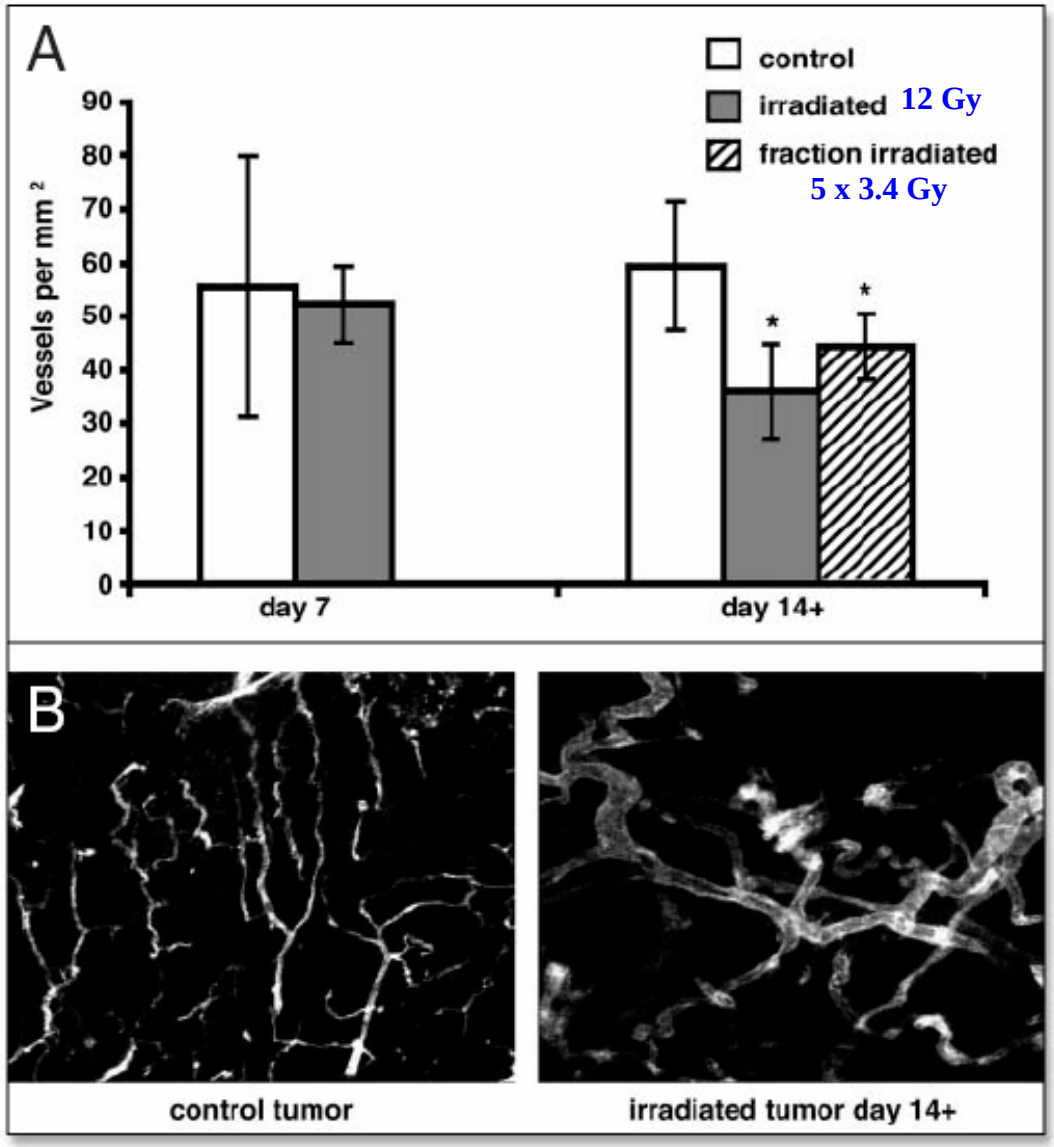


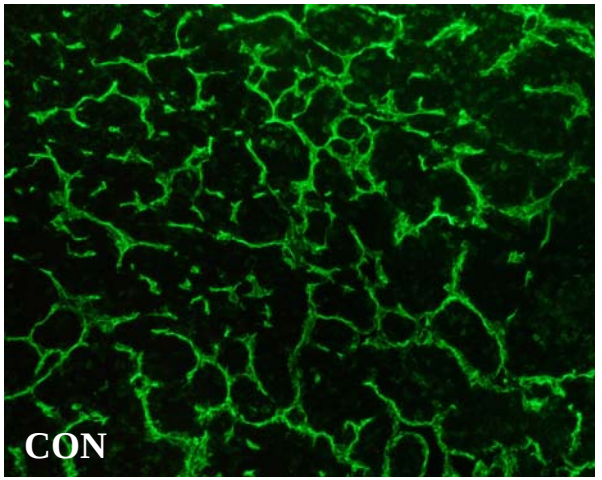


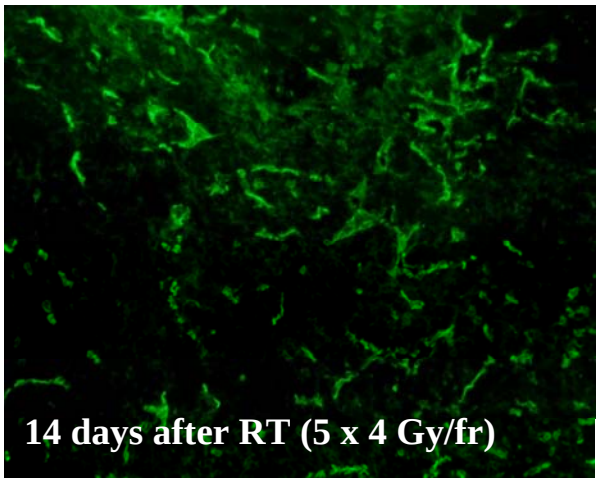


Clin Cancer Res 2006;12(4) February 15, 2006, p.1276

Radiation Induces Ineffective Tumor Angiogenesis



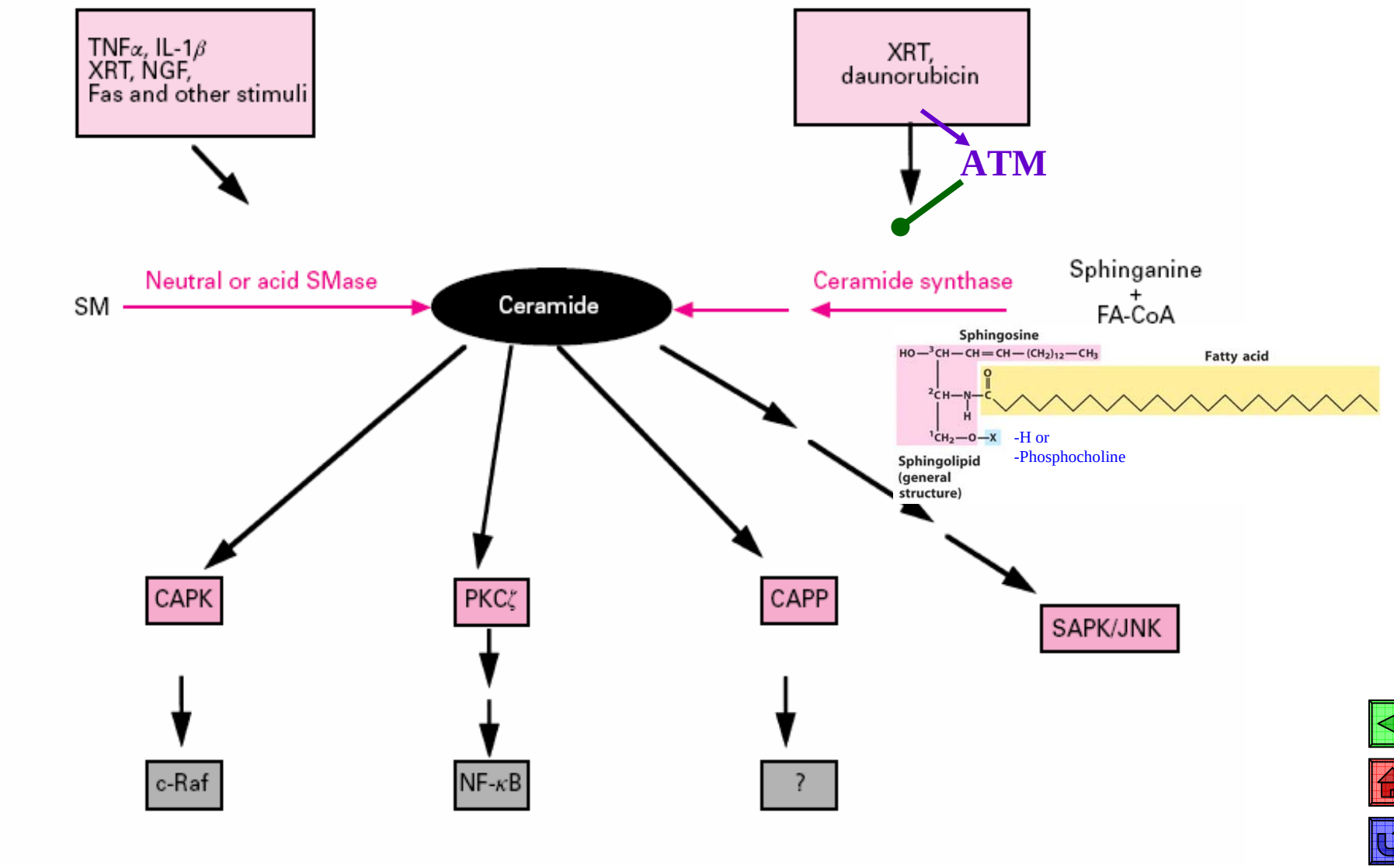




Cancer Biology & Therapy 4:12, e1-e6, EPUB Ahead of Print,


http://mx.nthu.edu.tw/~cschiang

Role of ATM and ceramide synthase in RT-induced cell death



Biochem. J. (1998) *335*, 465-480

Combined effects of angiostatin and ionizing radiation in antitumour therapy

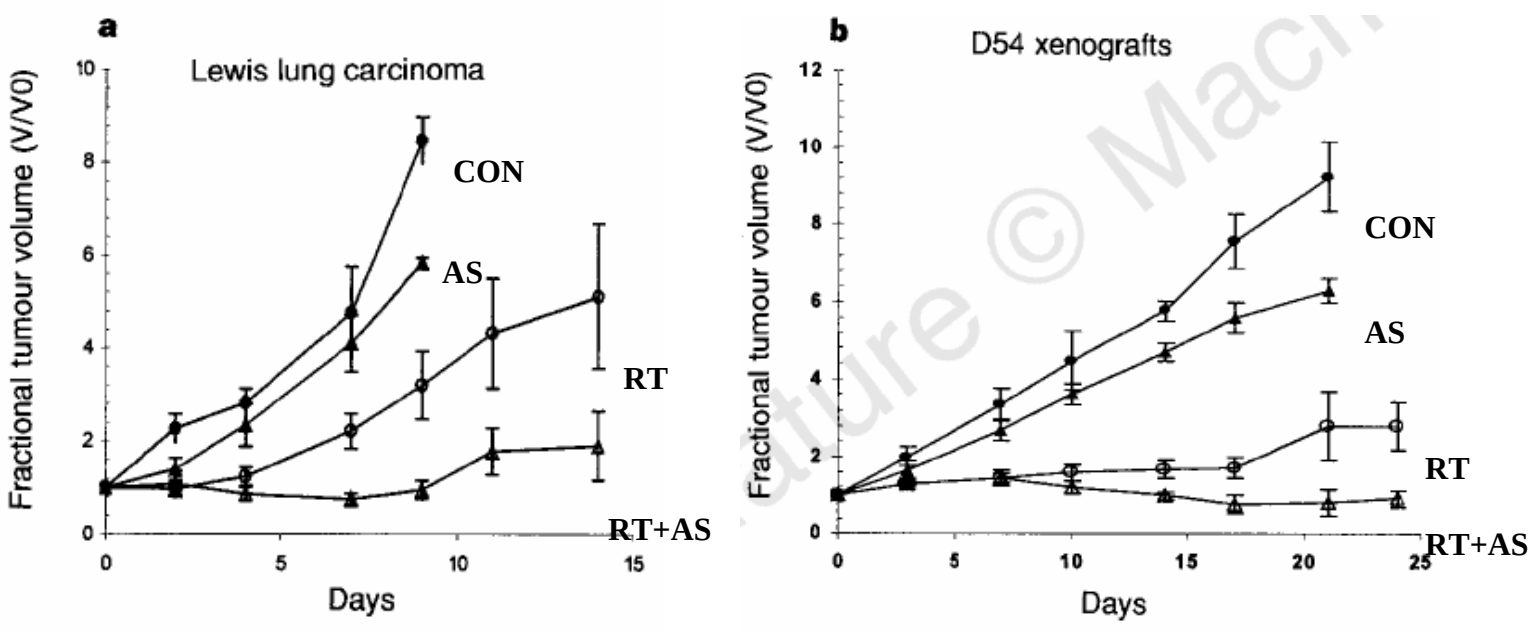
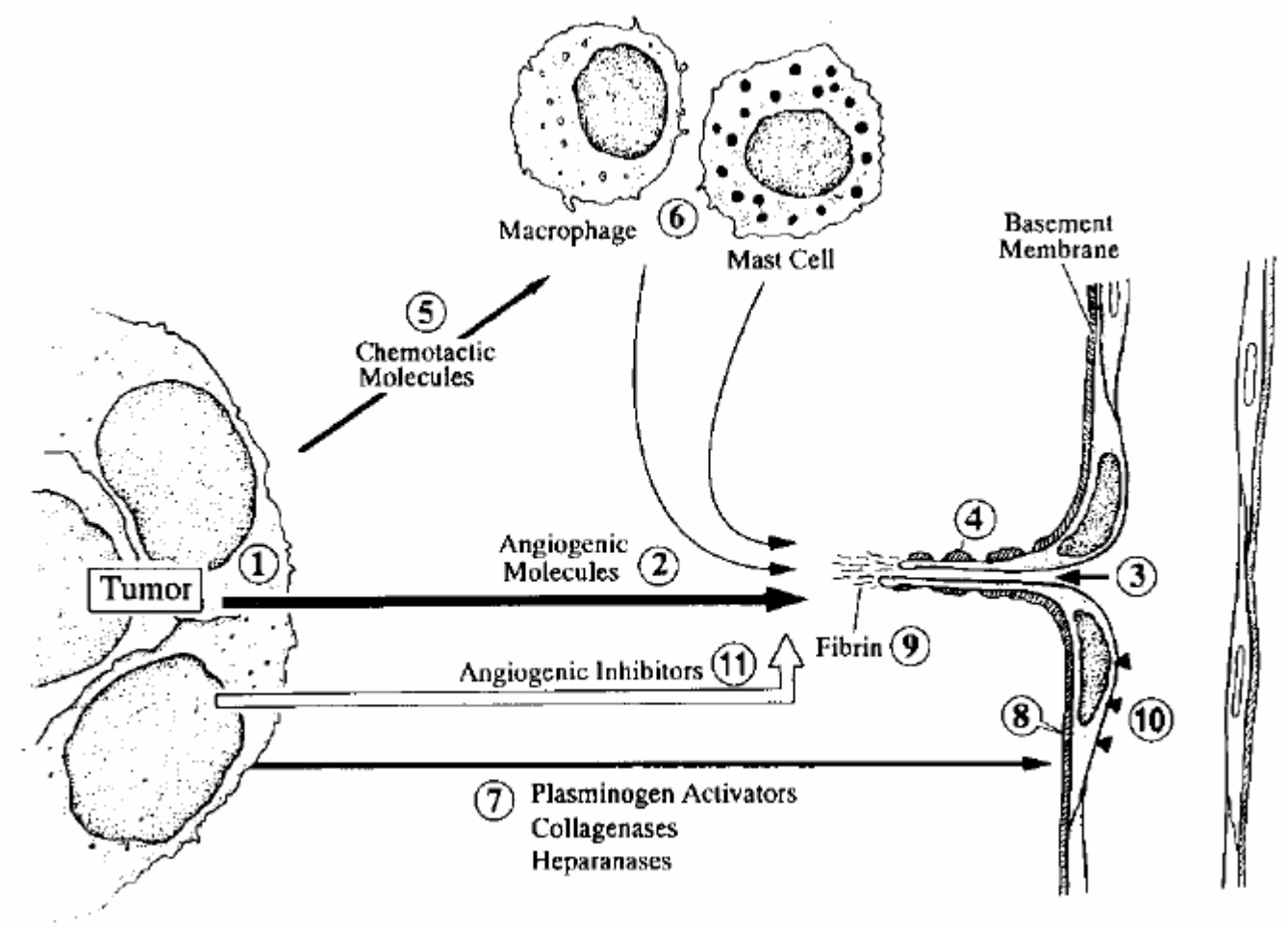


Table 1 Summary of fractional tumour volume as a function of treatment

Tumour designation	Control	Radiation	Human angiostatin	Human angiostatin + radiation	
LLC day 9 ($n = 28$)	8.49 ±0.51	3.21 ±0.73 (40 Gyr)	5.85 ±0.12	0.96 ±0.20	$P < 0.05$
D54 day 21 ($n = 20$)	9.25 ±0.91	2.78 ±0.89 (30 Gy)	2.94 ±0.07	0.38 ±0.16	$P < 0.005$
SQ-20B day 21 ($n = 31$)	2.17 ±0.50	1.05 ±0.31 (50 Gy)	2.94 ±0.07	0.38 ±0.16	$P < 0.005$
PC3 day 42 ($n = 38$)	*2.55 ±0.05	0.75 ±0.35 (40 Gy)	*1.98 ±0.55	0.09 ±0.05	$P < 0.001$

Angiogenesis



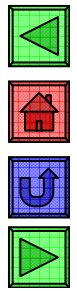
Judah Folkman and Yuen Shing,
THE JOURNAL OF BIOLOGICAL CHEMISTRY, 1992, Vol. 267, pp. 10931-10934

Summary of Normal Tissue Response to RT

Tissue	Do (Gy)	Dq (Gy)	n	Latency (days)
Jejunum	1.3	4 – 4.5	27	7
Skin	1.35	3.5	12	12-24
Bone Marrow	0.95	0.38	1.5	30
Testes	1.68	2.7	5	60
Mammary gland	1.27	2.04	5	
Thyroids	2			
Kidney	1.53	2.7	1.7	300
Liver	2.45	2.33	1.57	
Lung				120/180
Spinal cord				150
Brain				180

References

1. Paris, F., Z. Fuks, A. Kang, P. Capodieci, G. Juan, D. Ehleiter, A. Haimovitz-Friedman, C. Cordon-Cardo, and R. Kolesnick. 2001. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. Science 293:293.
2. Maj, F, Paris, F et al. 2003 Microvascular function regulates intestinal crypt response to radiation. Cancer Res. 63:4338-4341,
3. Garcia-Barros, M., F. Paris, C. Cordon-Cardo, D. Lyden, S. Rafii, A. Haimovitz-Friedman, Z. Fuks, and R. Kolesnick. 2003. Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 300:1155.
4. Garcia-Barros, M., D. Lacorazza, H. Petrie, A. Haimovitz-Friedman, C. Cardon-Cardo, S. Nimer, Z. Fuks, and R. Kolesnick. 2004. Host acid sphingomyelinase regulates microvascular function not tumor immunity. Cancer Res 64:8285.
5. Moeller, B. J., Y. Cao, C. Y. Li, and M. W. Dewhirst. 2004. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. Cancer Cell 5:429.
6. Moeller, B. J., M. R. Dreher, Z. N. Rabbani, T. Schroeder, Y. Cao, C. Y. Li, and M. W. Dewhirst. 2005. Pleiotropic effects of HIF-1 blockade on tumor radiosensitivity. Cancer Cell 8:99.
7. Fuks, Z., and R. Kolesnick. 2005. Engaging the vascular component of the tumor response. Cancer Cell 8:89.
8. Ch'ang, H. J.(常慧如）, J. G. Maj, F. Paris, H. R. Xing, J. Zhang, J. P. Truman, C. Cardon-Cardo, A. Haimovitz-Friedman, R. Kolesnick, and Z. Fuks. 2005. ATM regulates target switching to escalating doses of radiation in the intestines. Nat Med 11:484.
9. Truman, J. P., N. Gueven, M. Lavin, S. Leibel, R. Kolesnick, Z. Fuks, and A. Haimovitz-Friedman. 2005. Down-regulation of ATM protein sensitizes human prostate cancer cells to radiation-induced apoptosis. J Biol Chem 280:23262.



ATM regulates target switching to escalating doses of radiation in the intestines

Ch’ang, Hui-Ju et al.

Nature Medicine, 11:484, 2005

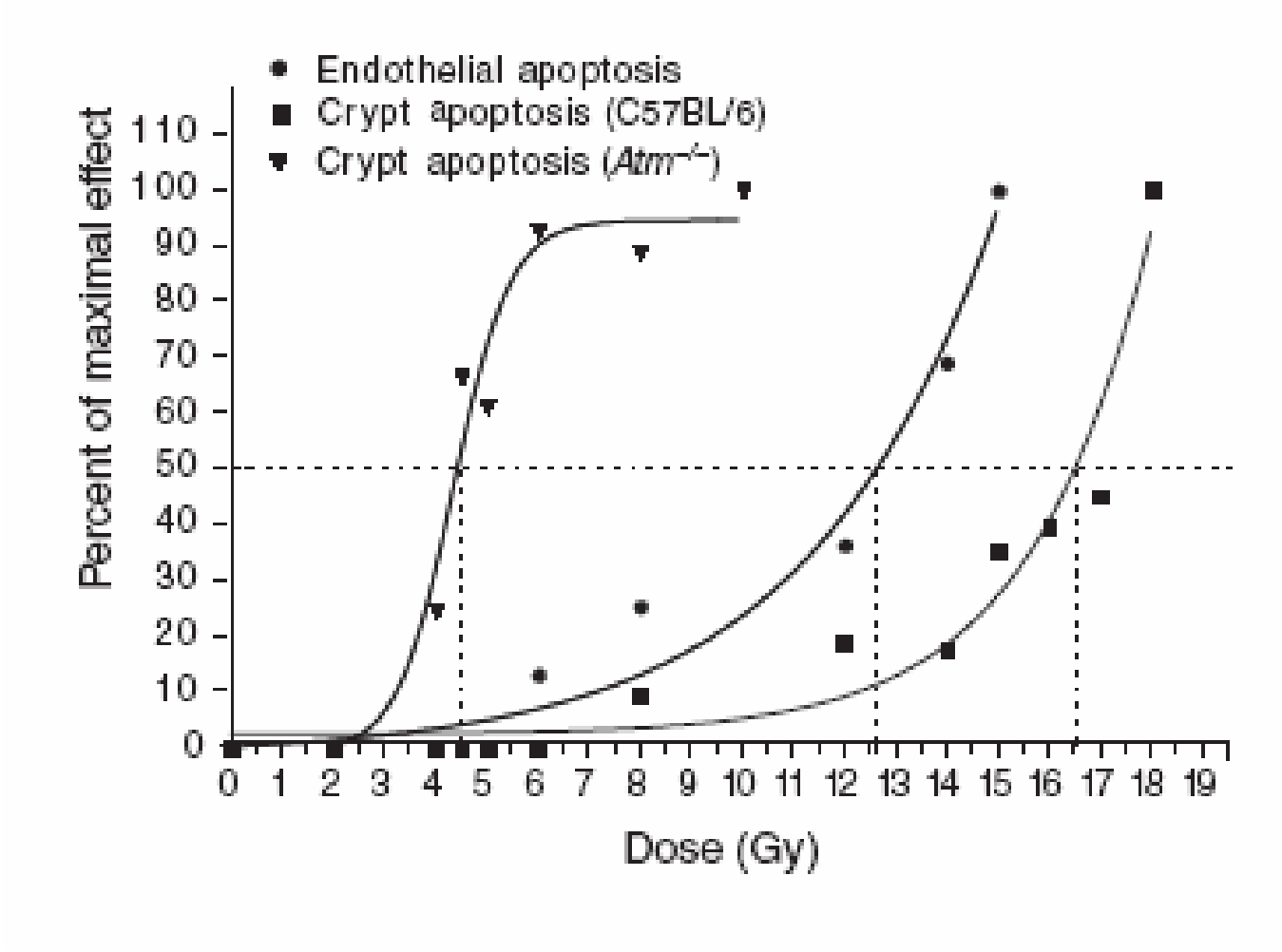
Dose escalation shifts the mechanism of intestinal damage

Table 1 Survival and autopsy results of mice exposed to WBR

Treatment	Survival (d ± s.d.)	*P* value	GI damage	BM damage
15 Gy (52)	6.8 ± 0.9		+++	±
15Gy+bFGF (21)	9.2 ± 1.3	<0.05	–	+++
16 Gy (22)	6.6 ± 1.1		+++	±
16Gy+bFGF (5)	7.6 ± 0.9	<0.05	–	+++
17 Gy (12)	5.4 ± 0.5		+++	++
17Gy+bFGF (10)	5.5 ± 0.5	NS	+++	++
18 Gy (10)	5.3 ± 0.5		+++	++
18Gy+bFGF (11)	5.5 ± 0.5	NS	+++	++
Smpd1$^{-/-}$ 16 Gy (18)	7.0 ± 1.0		–	+++
Smpd1$^{-/-}$ 18–19 Gy (7)	5.1 ± 0.1	<0.05	+++	++
Atm$^{-/-}$ 6 Gy (11)	7.1 ± 1.4		+++	+++
Atm$^{-/-}$ 9 Gy (5)	8.6 ± 2.0	NS	+++	+++

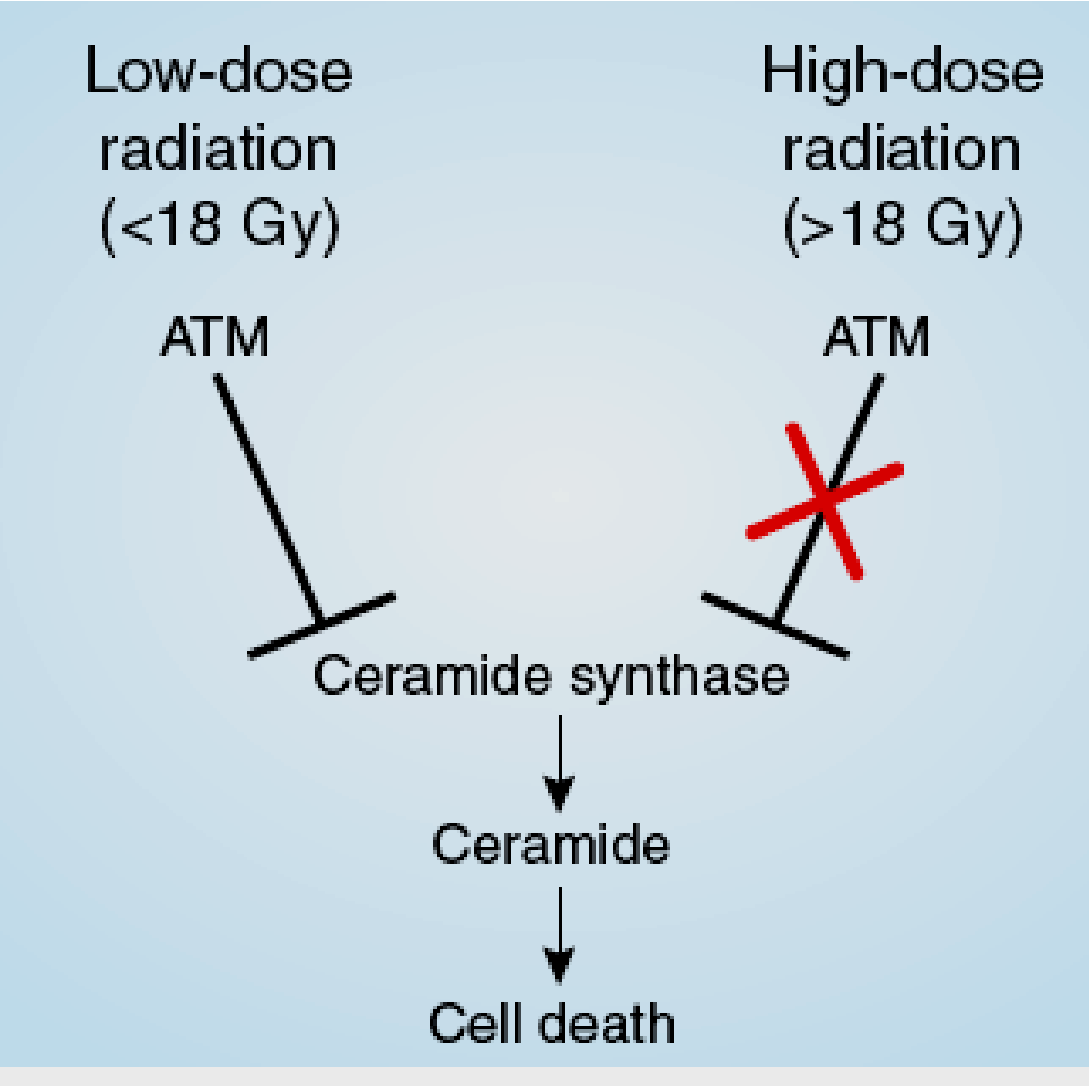
Nature Medicine, 11:477, 2005

Dose-dependent targets for RT-induced GI syndrome



Nature Medicine, 11:477, 2005

"Target-Switching" model (dose-dependent "target")

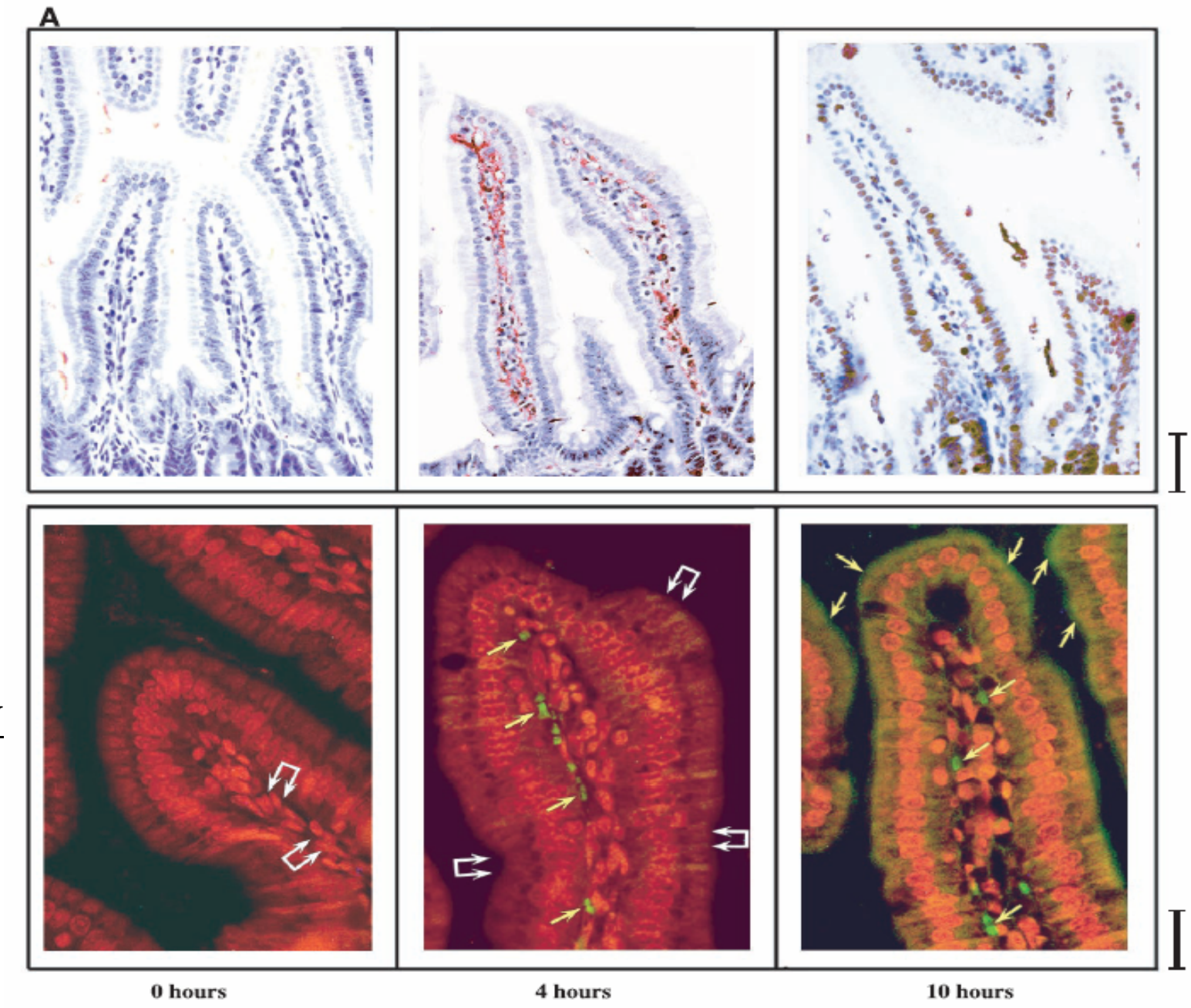


Nature Medicine, 11:477, 2005

Radiation-induced apoptosis after 15 Gy TBI

TUNEL

Annexin V



bFGF rescues C57BL/6 mice receiving 17 Gy whole-abdominal irradiation