Stereotactic Body Radiation and Immunotherapy—Does it make sense?

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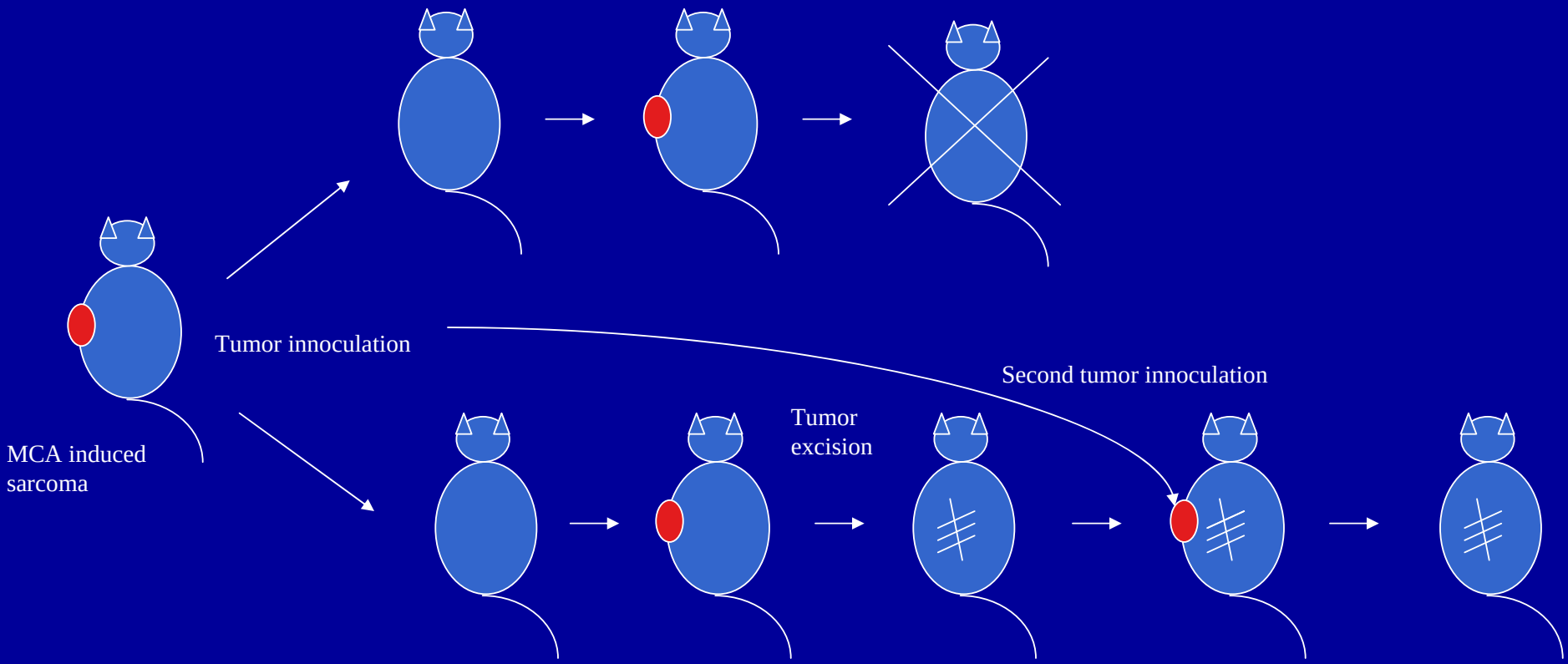
Topics

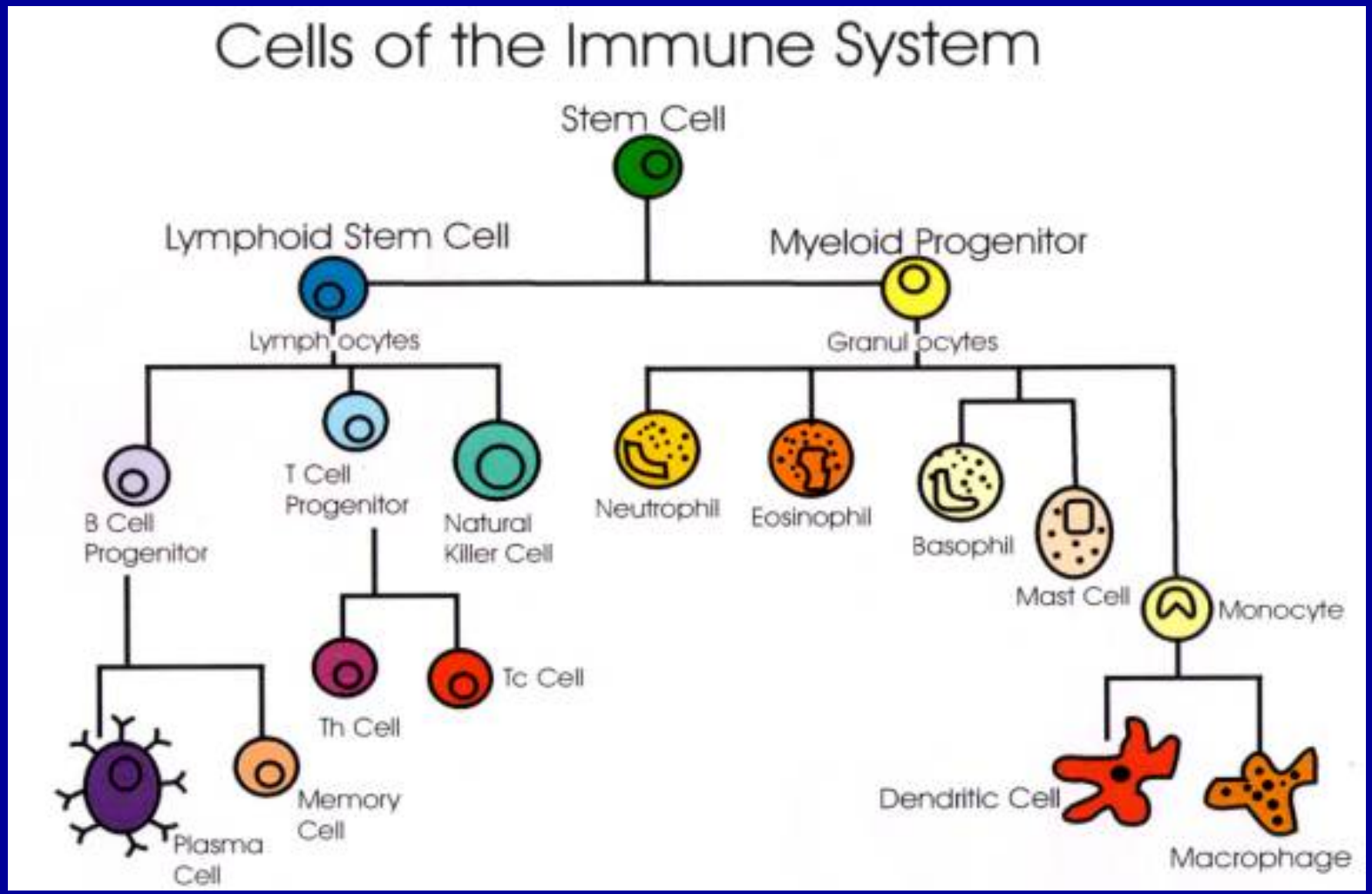
- Tumor immunity
- The problem with tumor-bearing hosts
- Effect of local high dose RT
- New tools for delivering high radiation doses to humans--IGRT and SBRT
- Effect of low dose RT
- RT and immunotherapy for oligometastases?

Tumor immunity demonstrated 50 years ago. Tumor immunity is induced IF the primary tumor is first removed.

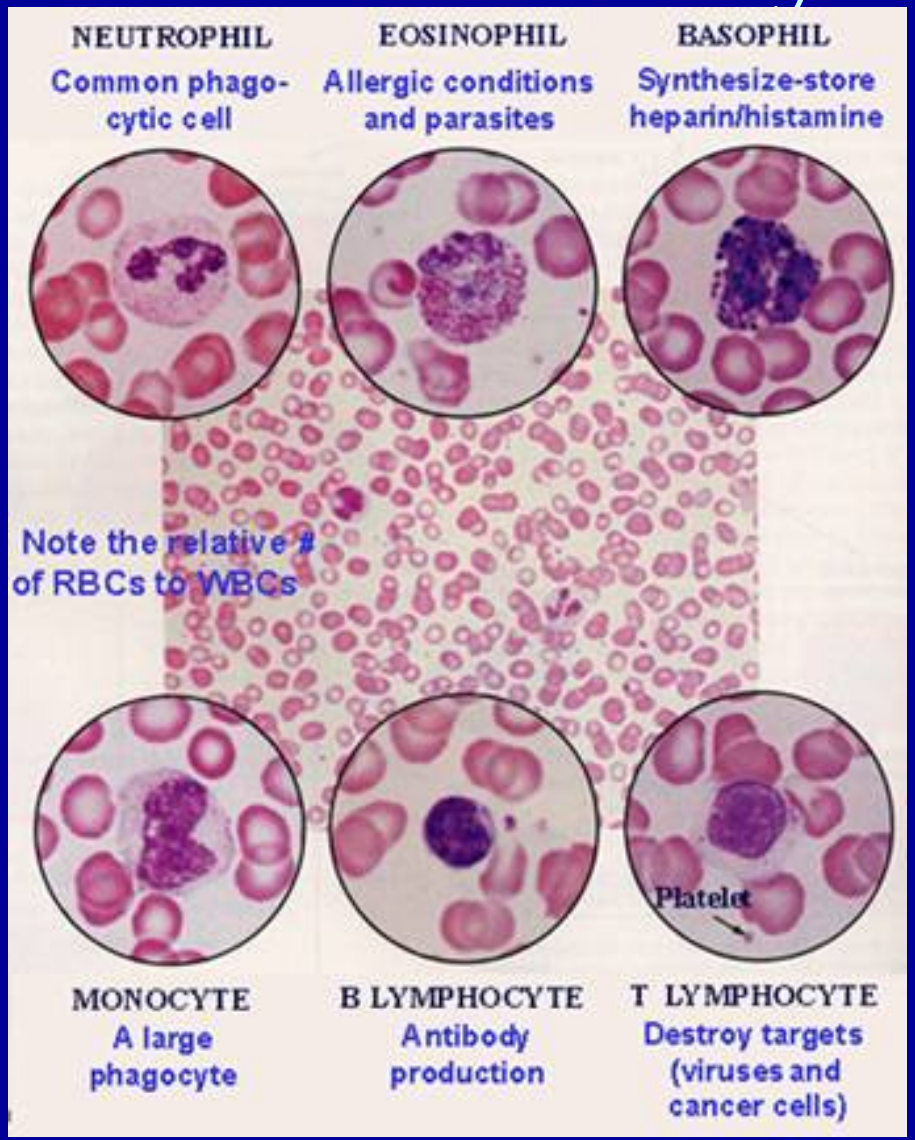


Prehn and Main, J. Natl. Cancer Inst, 1957, 18:769

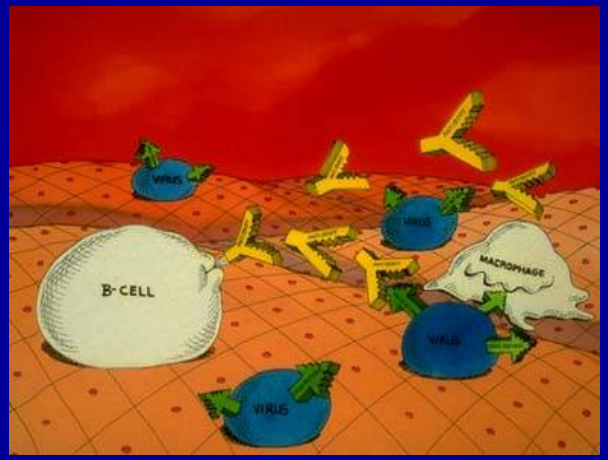
The Immune Players



The Immune Players



Antigen Presentation



Humoral response

Cell mediated response

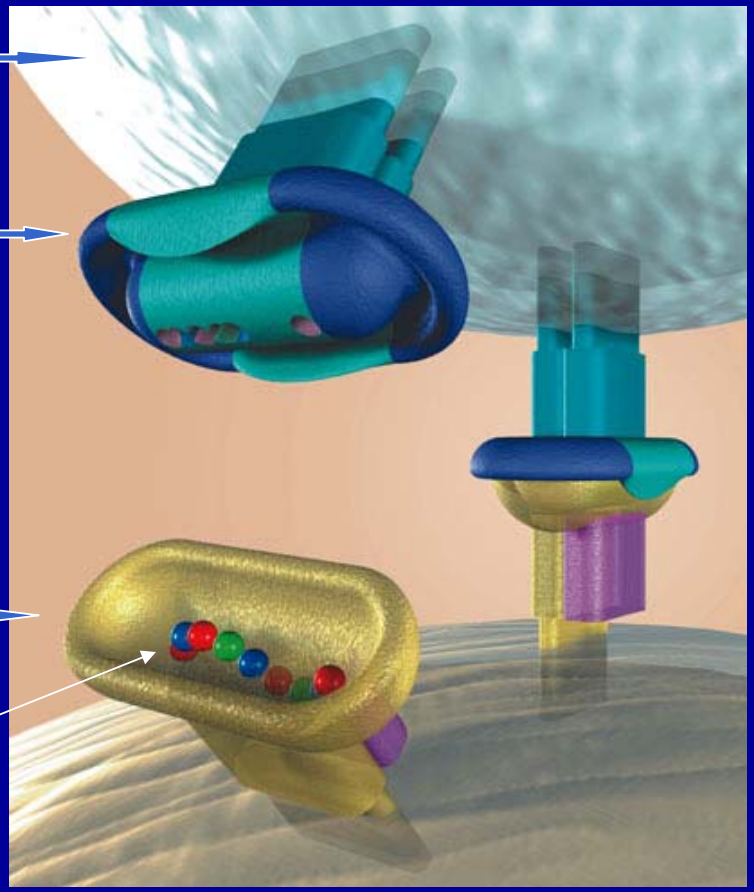
Antigen presentation

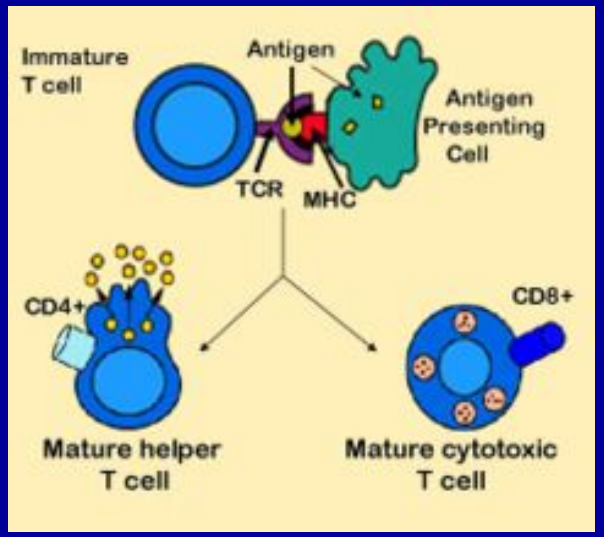
T cell

T cell receptor

MHC

Peptide





Mechanisms of immune escape by tumor cells

- Antigen loss or decreased expression
- MHC loss
- Eliciting anti-Ag T cell response to Ag not presented on tumor cell surface
- Attracting inflammatory cells that act as autocrine loop

Problem with “established” tumors in animal models

- Tumor cell inoculations are artifacts and induce local necrosis and inflammation that may not reflect the natural outgrowth of primary solid tumors
- Such models do not reflect sporadic solid tumors that arise from a single mutant within normal stroma

Problem with “established” tumors in animal models

- Most “established tumors” in literature are treated within 7-14 days post inoculation
- Tumors older than 14 days or > 1cm in diameter are rarely successfully treated in animal models.

The problem with a tumor burden

- Tumor cells themselves can express immunity suppressing cytokines
- Tumor stroma cells can be tumor promoting
  - B cells may secrete tumor-enhancing Ab
  - $CD4^{+}CD25^{+}$ T reg cells suppress maturation of cytolytic T cells
  - Gr-1^{+} tumor-associated macrophages can suppress anti-tumor activity in T cell-deficient mice

The problem with tumor burden in humans

- Not all primary tumors resectable
  - Tumor bulk may be immunosuppressive
- Regional metastases to LN rarely resected radically due to morbidity
  - Residual LN involvement may impair anti-tumor response
- Distant metastases usually not resected
  - Compounds problem with tumor burden

The problem with tumor burden in humans

- How can we cyto-reduce tumor burden to augment immune response in humans if tumor burden truly impairs anti-tumor immunity?
  - Radiation therapy
- In which patient groups should we experiment to prove this concept?
  - Oligometastases

Rationale for using radiation to reduce tumor burden

- Radiation is directly cytotoxic--the single most effective anti-cancer agent
- Radiation can potentiate anti-tumor immunity

Effects of Radiation Depends on Dose

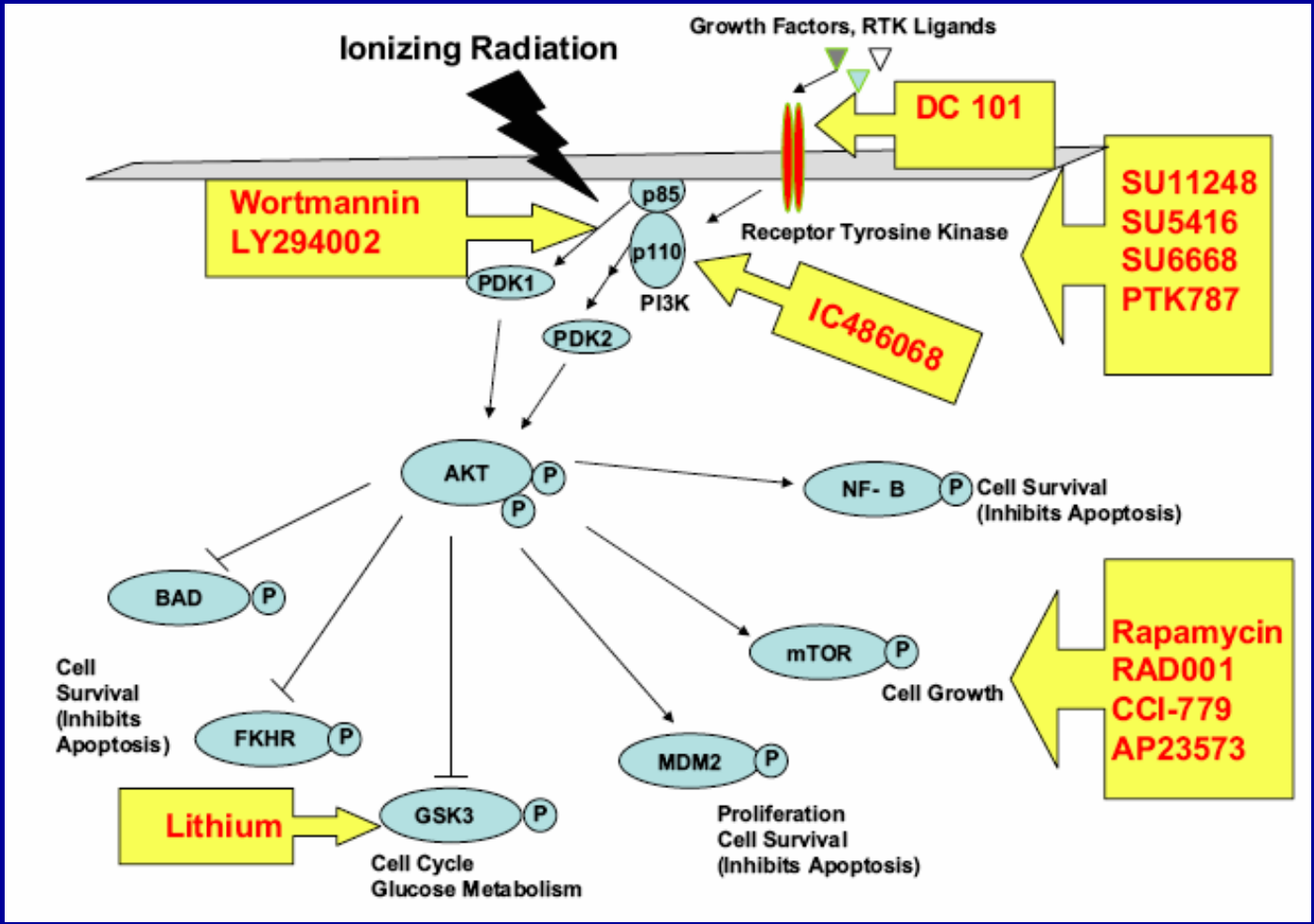
TBI absorbed dose	Effect
<0.5 Gy	No symptoms
1 Gy	N/V in 10% within 24 hrs
2Gy	N/V in 50% with drop in WBC and plts
4Gy	N/V in 90% w/in 12hrs; diarrhea 10% w/in 8hrs; 50% mortality in absence of medical care
6 Gy	100% mortality in absence of medical care
10-30 Gy	N/V in 5min; death in 2-3 wks
> 30 Gy	CNS collapse; death in 24-72 hrs

Effects of Radiation Depends on Dose

Skin single dose	Effect
3-4 Gy	Epilation in 2-3 wks
10-15 Gy	Erythema in hours to days
20 Gy	Moist desquamation; possible ulceration
25 Gy	Ulceration with slow healing
30-50	Blistering, necrosis in 3wks
100 Gy	Necrosis in 1wk

What Effects of Radiation at Cellular Level?

- Radical formation due to H_20 in cells
  - DS DNA break
    - Repair
    - Apoptosis
  - Protein cross-linking
  - Break in di-sulfide bonds
  - AA side chain oxidation

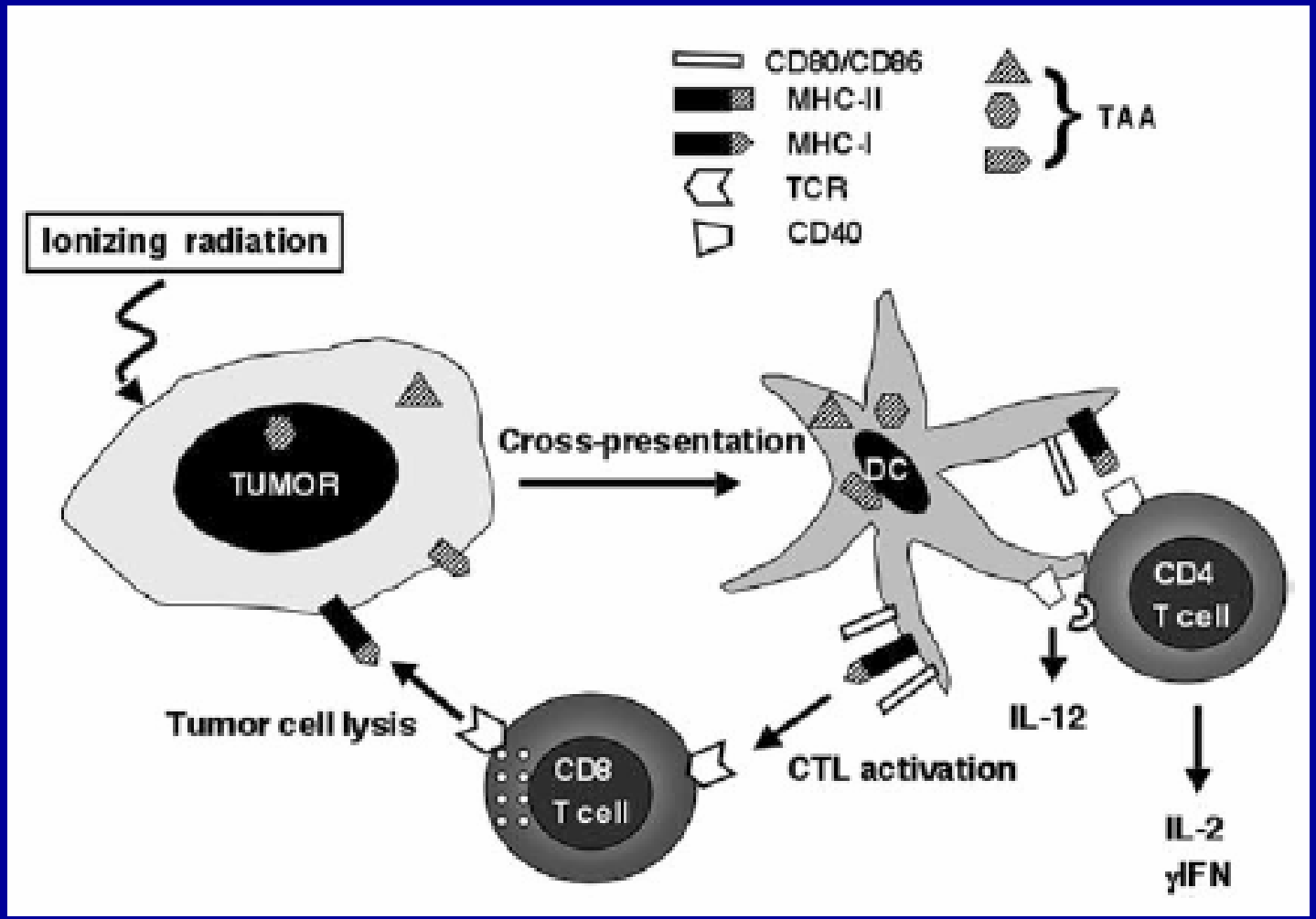


Endothelial cell response to moderate dose of RT (2-3Gy)

Kim et al, IJROBP, 2006, 64:38

Local High Dose RT on Immune System

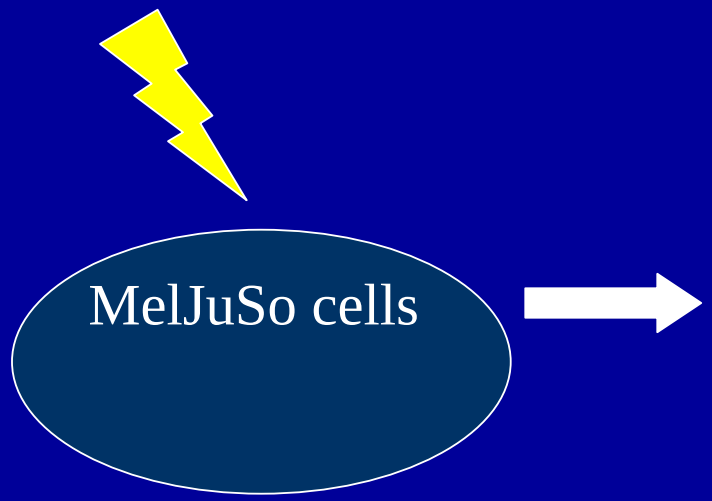
- RT induces “danger” signals
  - IL-1β (Ishihara, Rad Res, 1993, 133:321)
  - TNF-α (Hallahan, PNAS, 1989, 86:10104)
  - Upregulates MHC class I (Reits, JEM, 2006, 203:1259)
  - Upregulates Fas/CD95 (Reap, PNAS, 1997 94:50)
  - Facilitates homing of APC and T-cells to the tumor (Ganss, Cancer Res, 2002, 62: 1462)
  - Upregulates B7.1 (Vereeque, Br J Haematology, 2000, 108:825)


CD80/CD86
MHC-II
MHC-I
TCR
CD40
TAA
Ionizing radiation
TUMOR
Cross-presentation
DC
CD4
T cell
Tumor cell lysis
CD8
T cell
CTL activation
IL-12
IL-2
γIFN

Radiation can increase MHC expression and peptide presentation

Reits, JEM, 2006, 203:1259

0, 1, 7, 25 Gy



MHC staining

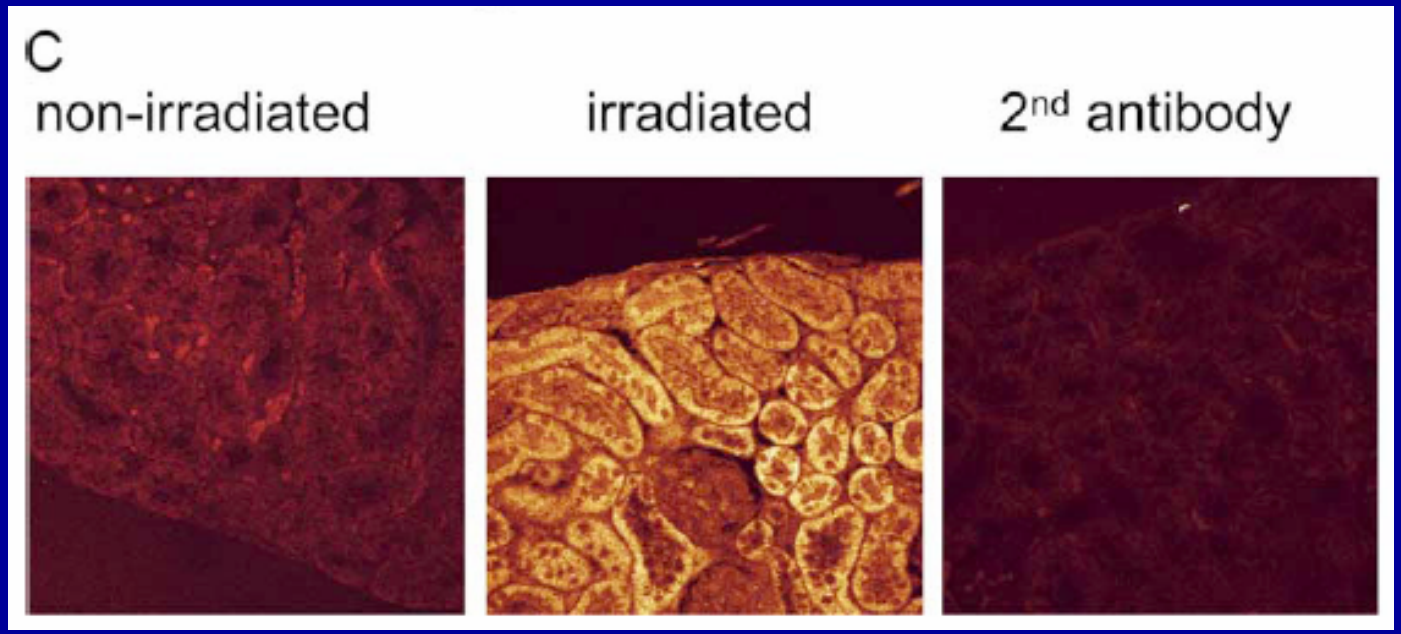
FRAP (fluorescence recovery after photobleaching)

mTOR inhibition

Peptide profiling

Reits, JEM, 2006, 203:1259

Upregulation of MHC I seen *in vivo* in normal tissues.

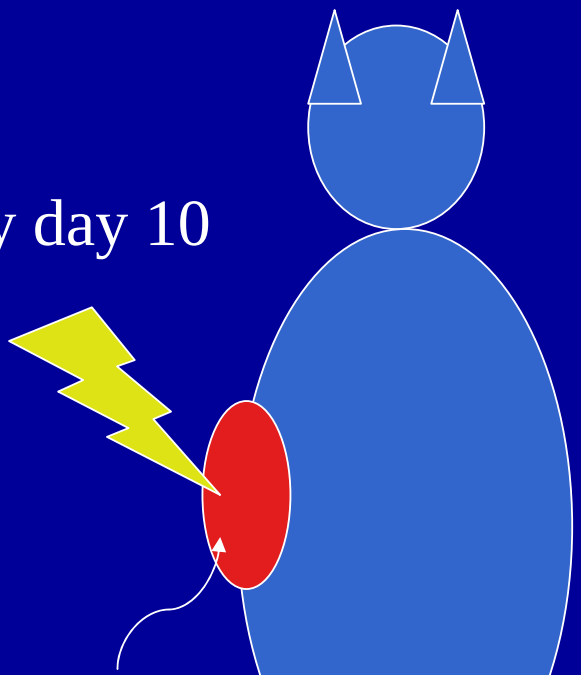


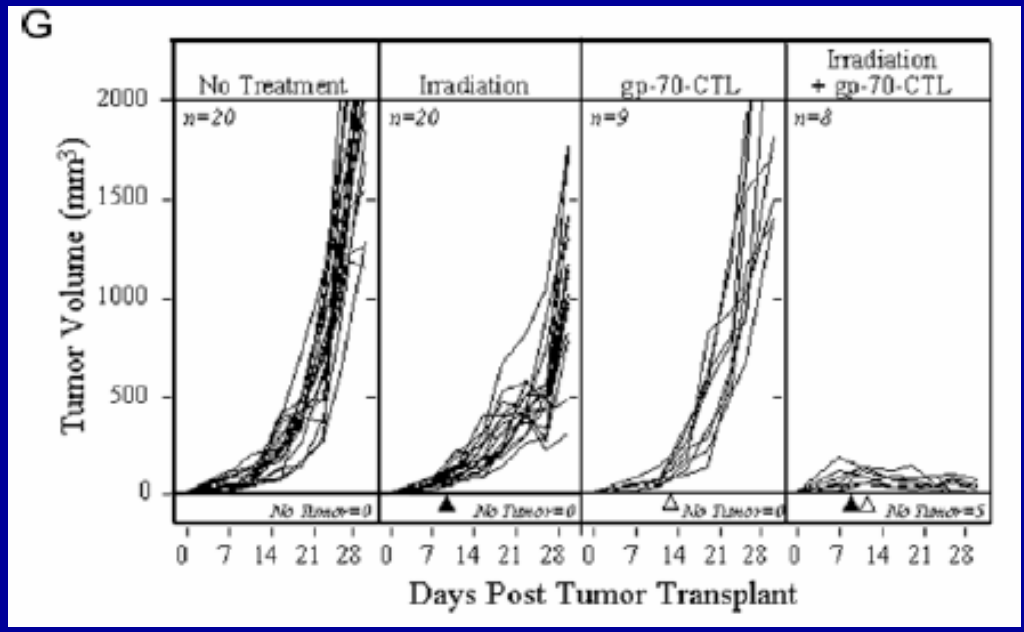
HLA-A2 mouse given 25 Gy locally to one kidney. Both kidneys stained with rabbit anti-MHC class I H chain serum followed by second Ab coupled to Cy5.

C57BL/6 mice

RT to tumor makes it a better target to CTLs

10 Gy day 10

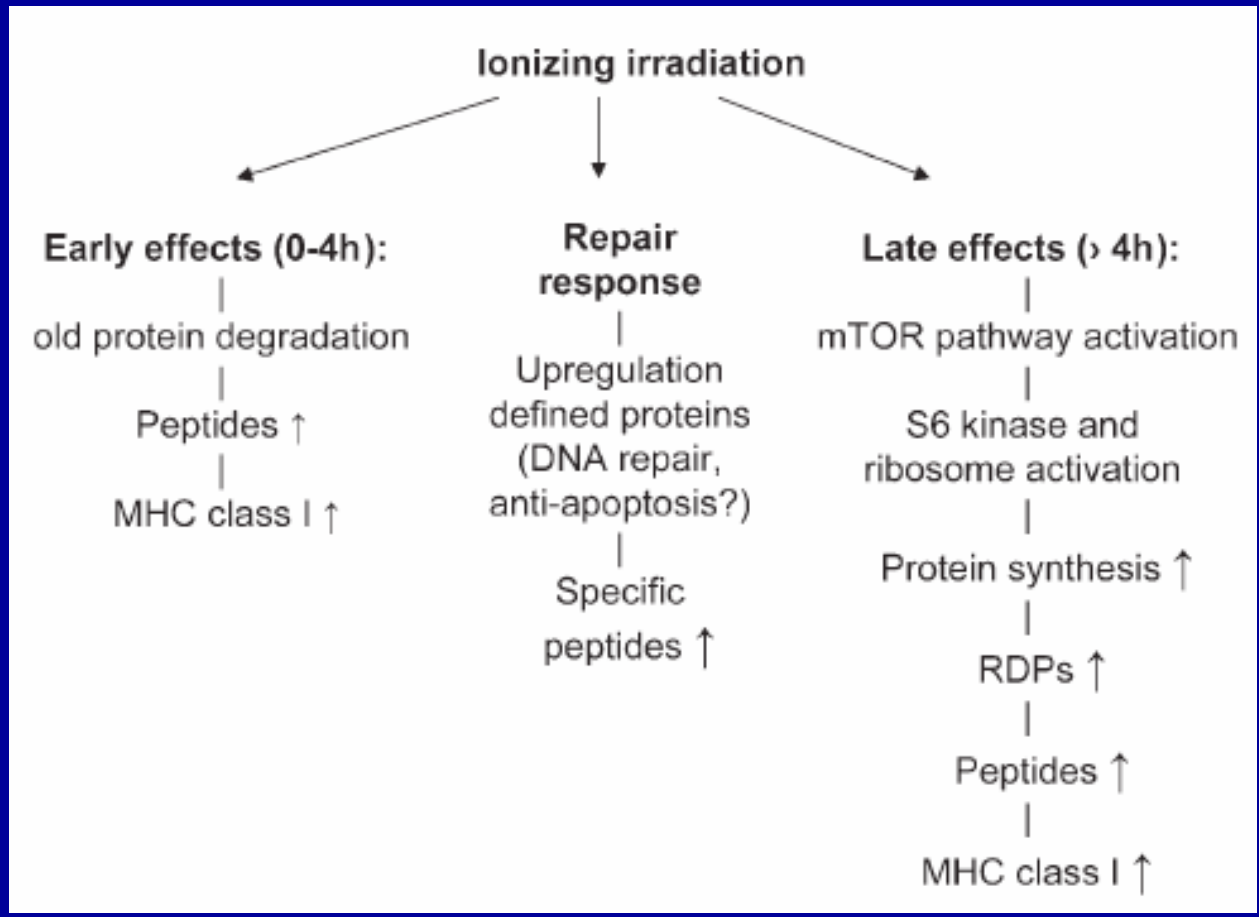




MC38 mouse colon ca
3 x 10^5 s.c.

Adoptive transfer of gp70-specific CTL at day 10

Reits, JEM, 2006, 203:1259



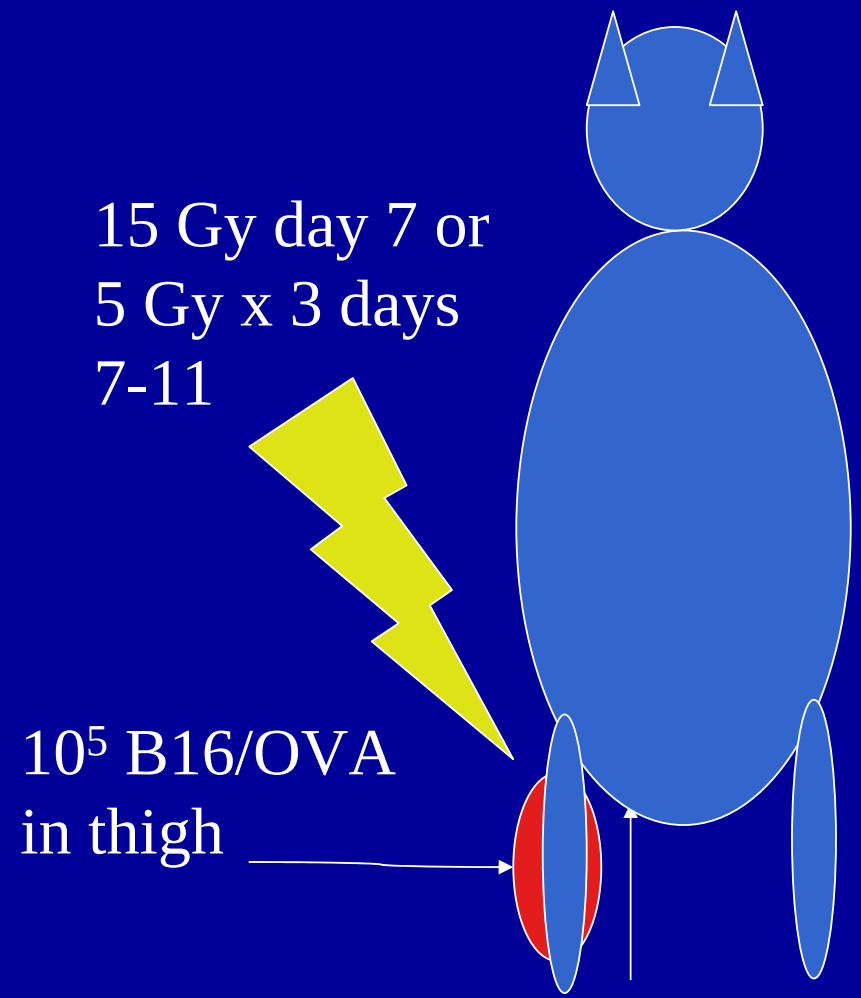
High dose local RT can make a tumor more antigenic/immunogenic

Radiation can increase trafficking of CTLs into the tumor

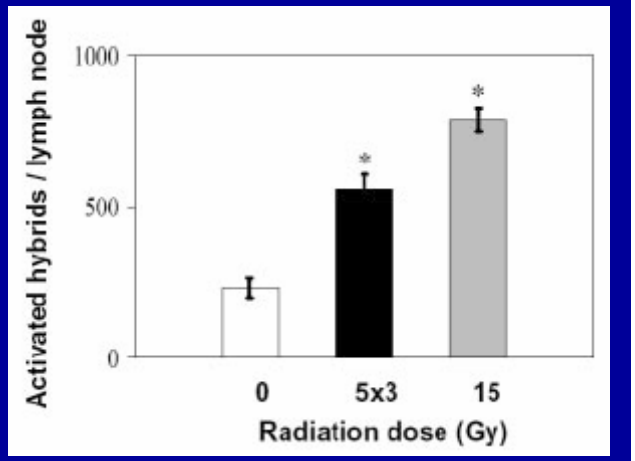
C57BL/6J mice

15 Gy day 7 or
5 Gy x 3 days
7-11

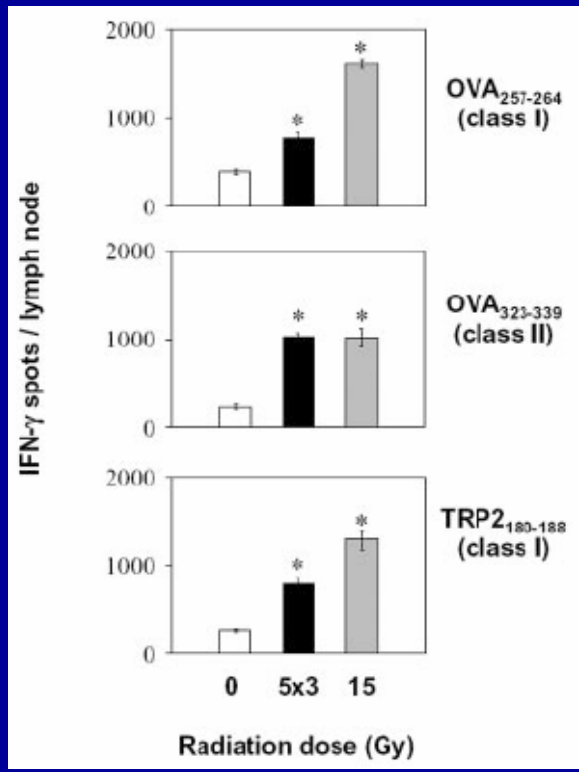
10^5 B16/OVA
in thigh



Harvest draining LN



B21 hybridoma against OVA257-264 incubated with TDLN and number of activated hybridoma measured



TDLN cultured in vitro with various peptides and IFN-γ ELISPOT performed

Lugade, J Immunol, 2005, 174:7516

OT-1 α-OVA $_{257-264}$

P14 α- LCM virus Gp51 $_{33-41}$

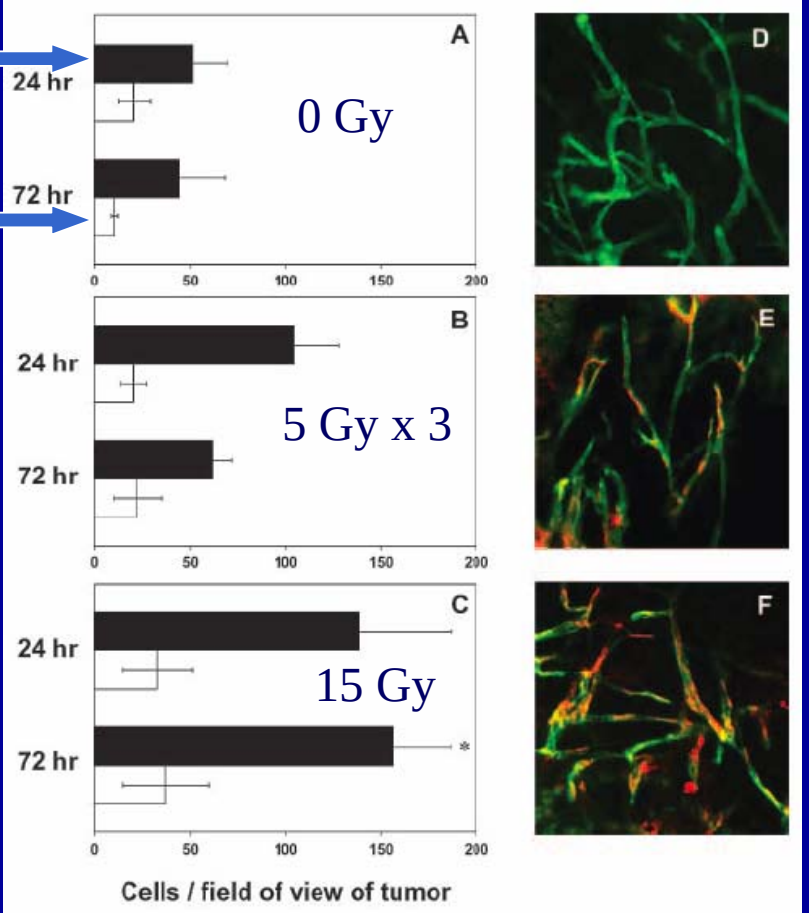


FIGURE 7. Radiation enhances the ability of tumor Ag-specific T cells to traffic to and infiltrate tumors. A total of 3×10^6 OT-1 (■) and 3×10^6 P14 (□) $CD8^+$ T cells, labeled with CFSE and CMTMR, respectively, were transferred into C57BL/6 mice bearing 7-day B16/OVA tumors and either untreated (*A*) or treated with single doses of 3 Gy (*B*) or 15 Gy (*C*). Tumors were removed either 24 or 72 h posttransfer and analyzed by whole mount fluorescent microscopy to locate migrated T cells. Tumors analyzed 72 h posttransfer in *B* reflect mice that had received three consecutive daily doses of 3 Gy (a total of 9 Gy). Results are averages ± SEM of three independent experiments. Whole mount histology on tumor samples either untreated (*D*) or treated with 3 Gy (*E*) or 15 Gy (*F*) 24 h after treatment. Overlaid images on corresponding fields are pseudocolored green for CD31 and red for VCAM-1. *, $p < 0.01$ (Holm-Sidak method) for number of OT-1 cells/field of view in 15 Gy-treated tumors compared with 0 Gy-treated tumors 72 h posttreatment.

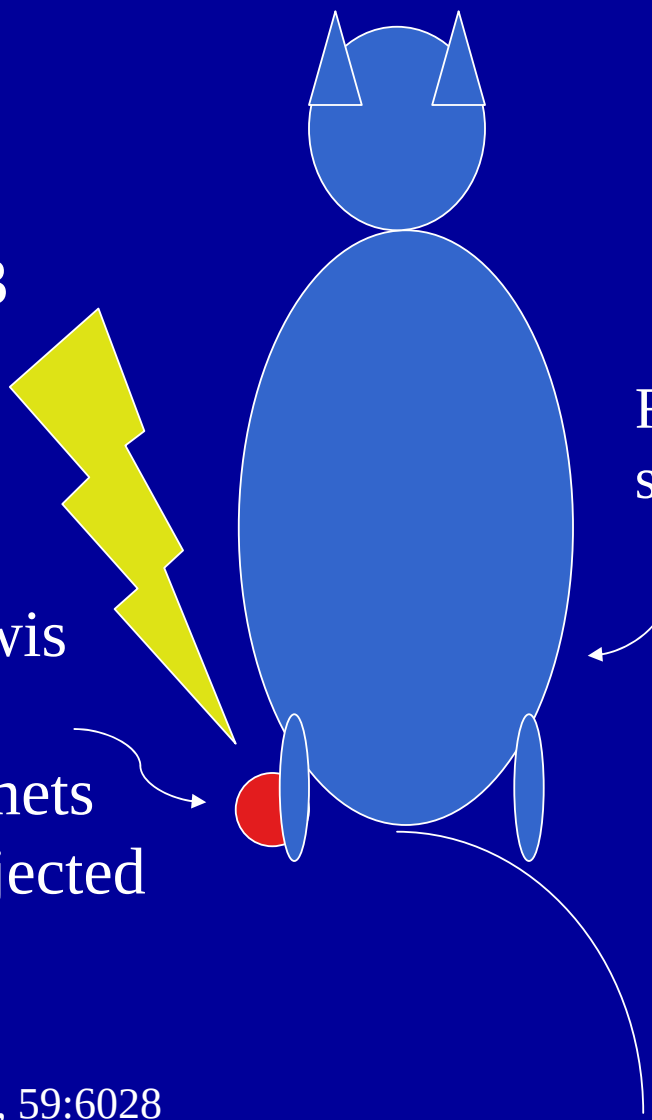
Radiation increases trafficking of CTL into the tumor as seen in whole mount staining

Lugade, J Immunol, 2005, 174:7516

Is there evidence that local RT induces immune response to distant sites *in vivo*?

- Reduction of metastases-indirect proof
  - Chakravarty et al, Cancer Res, 1999, 59:6028
- Abscopal effect of RT
  - Demaria et al, IJROBP, 2004, 58:862

C57BL/6 mice



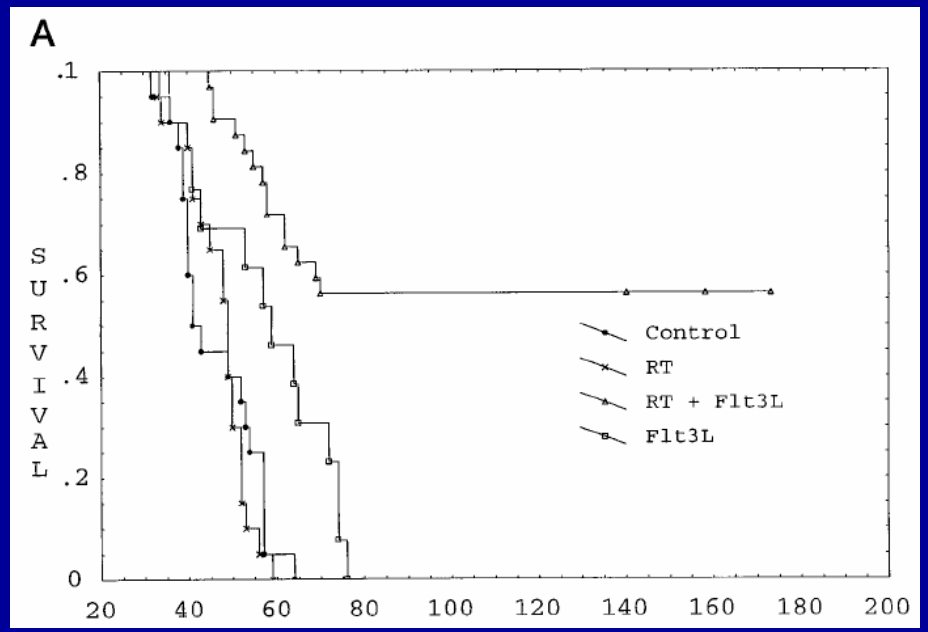
60 Gy wk 3

Flt3L 0.5 mg/kg/d i.p. x 10d
starting with RT

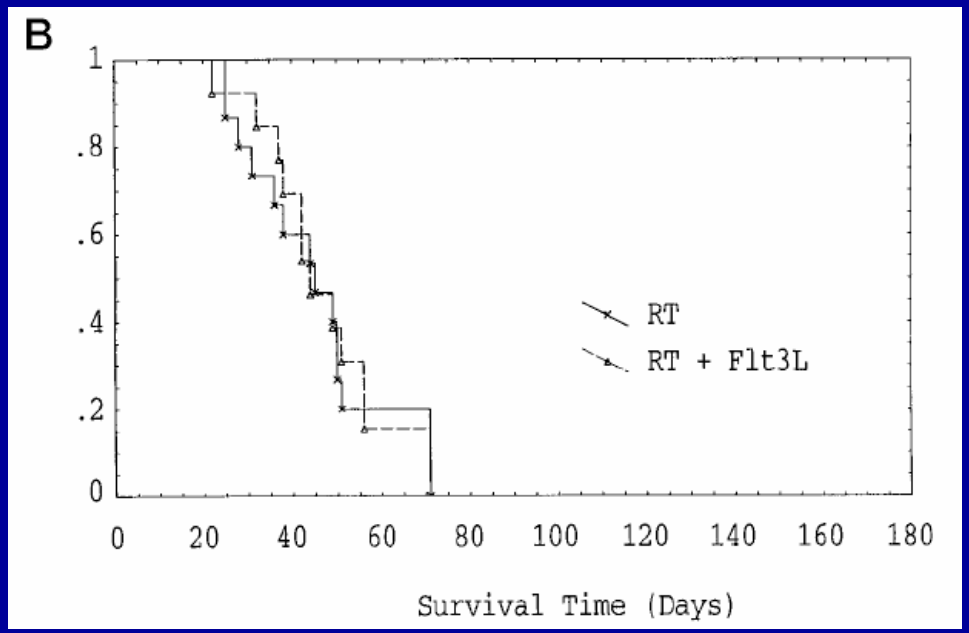
3LL/D122 Lewis
lung ca clone.
Low MHC I, mets
to lung. 10^5 injected
In foot pad.

Chakravarty et al, Cancer Res, 1999, 59:6028

Survival



Normal mice



Nude mice

Chakravarty et al, Cancer Res, 1999, 59:6028

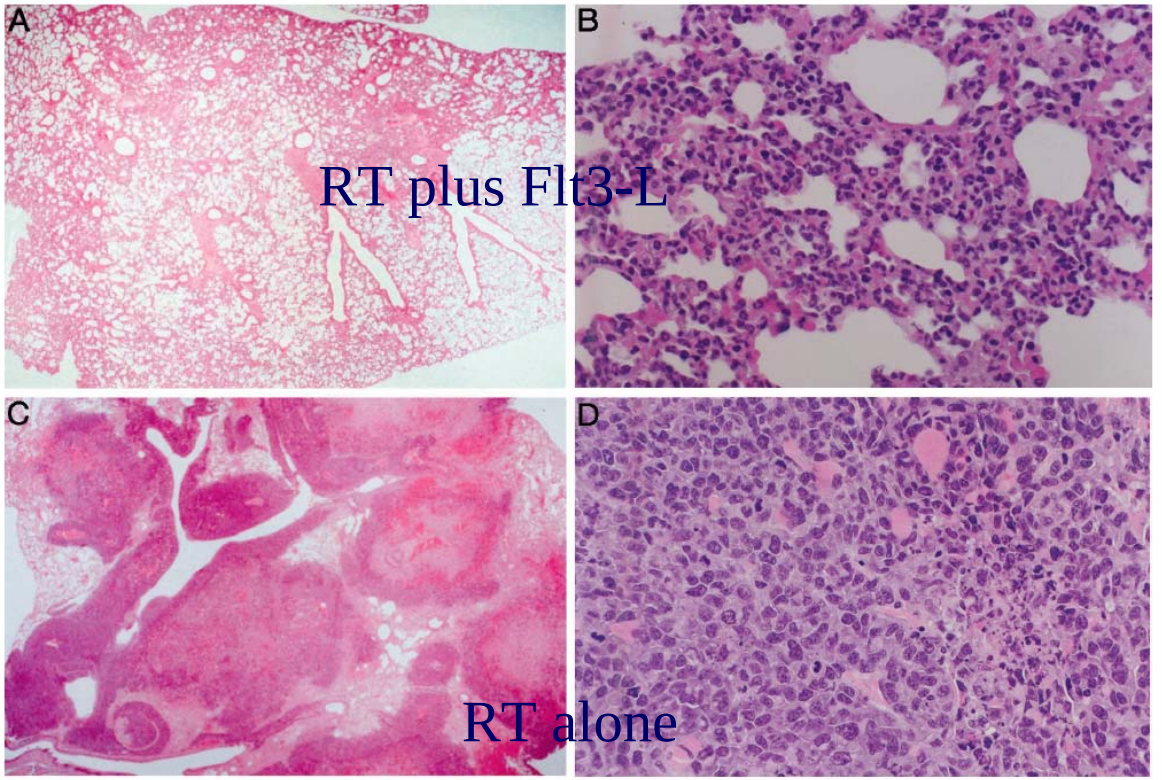


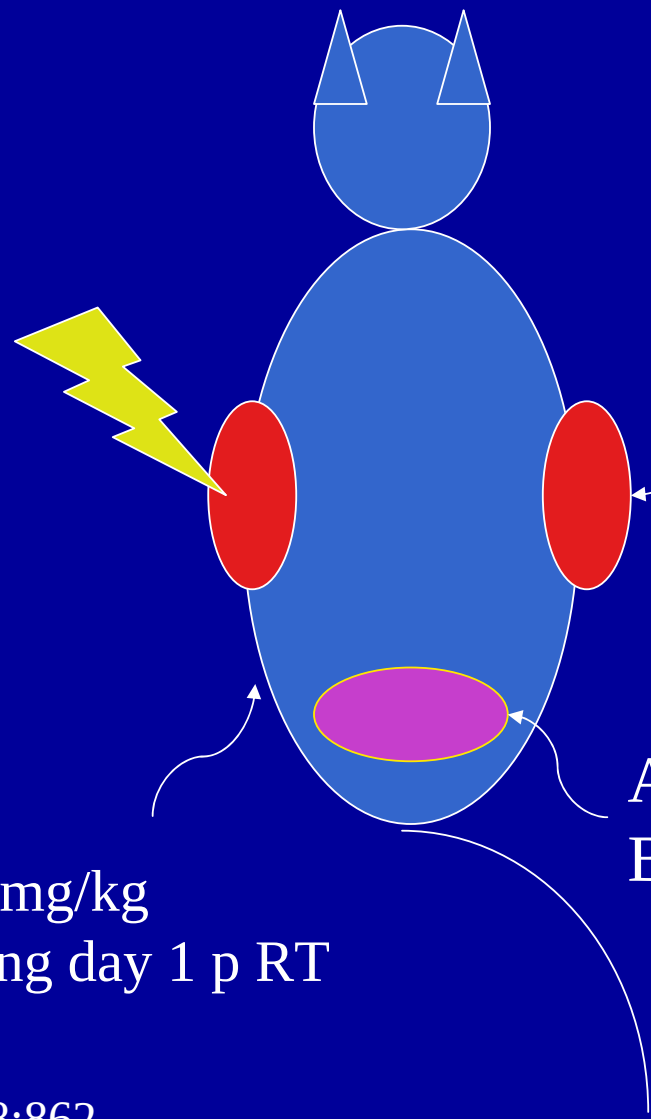
Fig. 3. Tumor-bearing animals, subjected to RT or RT + Flt3L, were sacrificed 6 weeks after 3LL tumor cell transplantation (3 weeks after initiation of treatment, either RT o Flt3L). The lung tissues were collected from different animals and were cryopreserved or formalin fixed for histological sections and H&E staining. *A*, the lungs of surviving RT + Flt3 cohorts showed no carcinoma cells but had infiltrates of neutrophils, lymphocytes, and mononuclear leukocytes, as evidenced by the H&E staining of the formalin-fixed paraffi embedded lung tissue. *B*, higher magnification (×20) of *A*. *C*, the lung sections of the RT cohort show massive tumor cell infiltration. *D*, higher magnification (×20) of *C*. Animal of the RT + Flt3L cohort that died early also showed metastatic infiltration in lungs.

Groups	*n*	Lung weight (mean ± SE)	*t* test
A. RT	12	463.2 ± 41.8	
B. RT + Flt3L	11	293.5 ± 27.1	B *vs.* A, $P < 0.003$
C. Flt3L	9	338.8 ± 44.0	C *vs.* A, $P = 0.057$

Lung wt 6wks post innoculation, 3wks post RT/Flt3L

Chakravarty et al, Cancer Res, 1999, 59:6028

Balb/c mice



Rad
2 or 6 Gy
once tumor size
100-150 mg

67NR 5 x 10^5
Mammary tumor

A20 10^6 cells
B cell lymphoma

Flt3-L i.p. 0.5 mg/kg
qd x 10d starting day 1 p RT

Demaria et al, IJROBP, 2004, 58:862

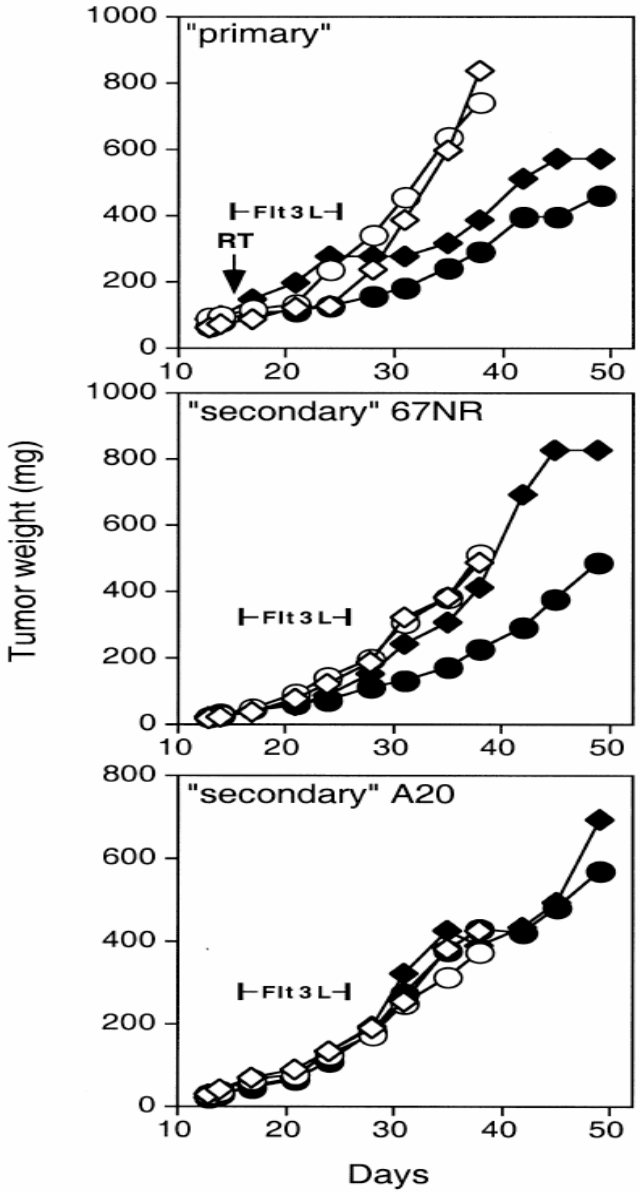


Fig. 3. The abscopal effect triggered by local RT and Flt3-L is tumor specific. Mice were injected s.c. in the left flank with 10^5 67NR cells at Day 0 ("primary" tumor). Five days later, 10^5 67NR cells were injected in the right flank ("secondary" 67NR), and 10^6 A20 cells were injected in the back ("secondary" A20). Mice were either left untreated (empty diamonds), were treated with RT (filled diamonds) at Day 14 (arrow) at a single dose of 2 Gy exclusively to primary tumors, or were given Flt3-L alone i.p. (empty circles) for 10 days starting at Day 15 or Flt3-L in com-

Summary points

- High dose local RT can make local environment potentially more immunogenic
- Local RT by itself still may not be good enough to activate APC’s
- Local RT and APC maturation signals may improve anti-tumor immunity systemically

Local High Dose RT on Immune System

- RT induces “danger” signals
  - IL-1β (Ishihara, Rad Res, 1993, 133:321) **20Gy**
  - TNF-α (Hallahan, PNAS, 1989, 86:10104) **5Gy**
  - Upregulates MHC class I (Reits, JEM, 2006, 203:1259) **7-25Gy**
  - Facilitates homing of APC and T-cells to the tumor (Ganss, Cancer Res, 2002, 62: 1462) **10 Gy**
  - Upregulates B7.1 (Vereeque, Br J Haematology, 2000, 108:825) **25 Gy**

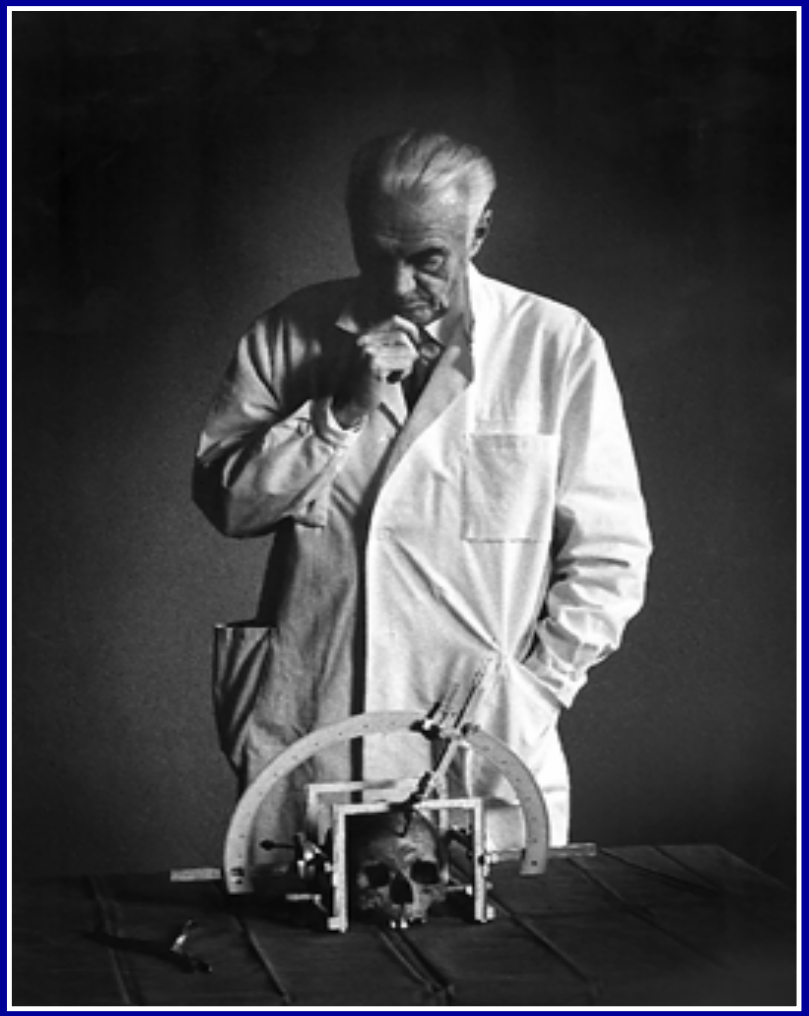
Summary points

- Most pre-clinical models suggest high radiation fractional doses are required
- These dose ranges are not used in clinical practice due to risk of normal tissue injury
  - Except for stereotactic radiosurgery/radiotherapy

Stereotactic Radiosurgery/radiotherapy

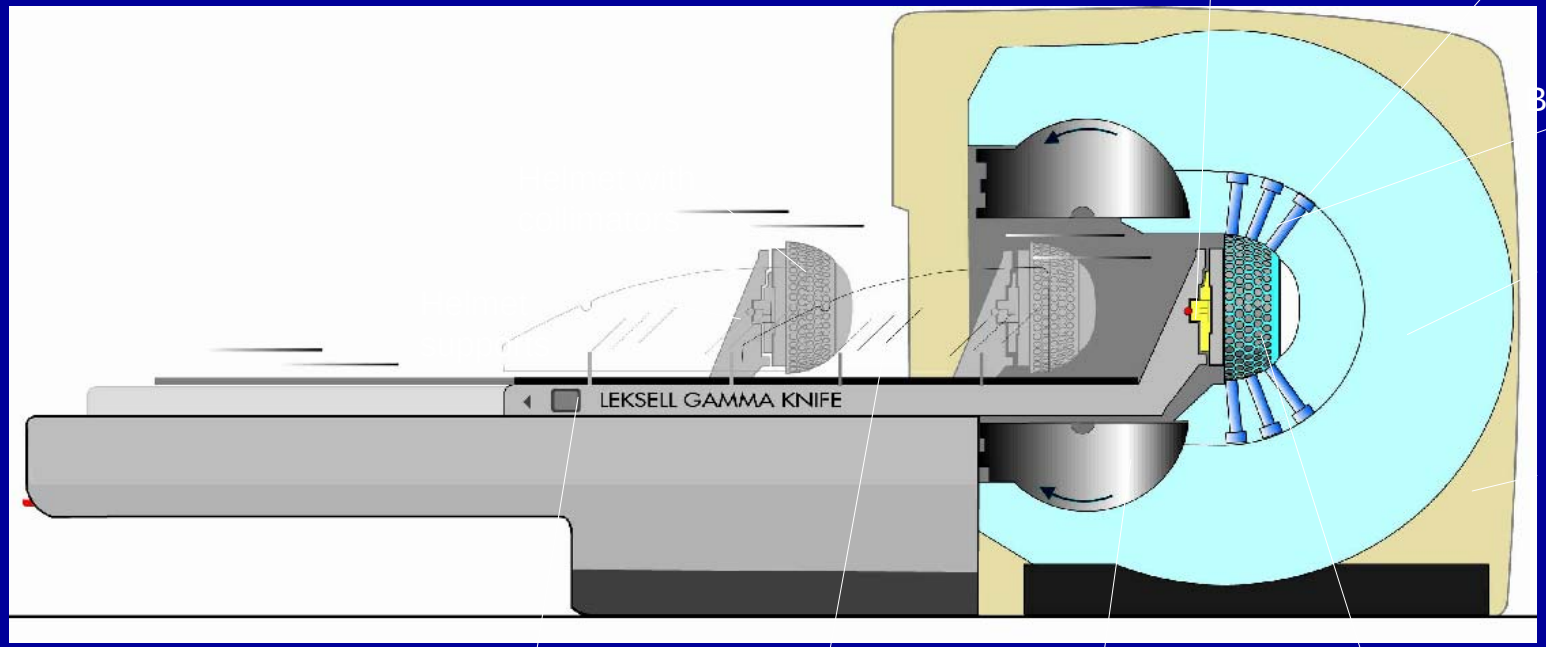
- Historically referred to the management of intracranial tumors in single treatment
- Gamma Knife the prototype
- When treatment is in few fractionated therapy, it is called stereotactic radiotherapy

RADIOSURGERY
Definition



“The delivery of a single, high dose of radiation to a small and critically located intracranial volume through the intact skull.” -
Lars Leksell, M.D. Ph.D., 1951

Leksell Gamma Knife C



Automatic positioning system

Cobalt-60 sources

Beam channel

Shielding

Plastic cover

Treatment couch with mattress

Protection panels

Shielding doors

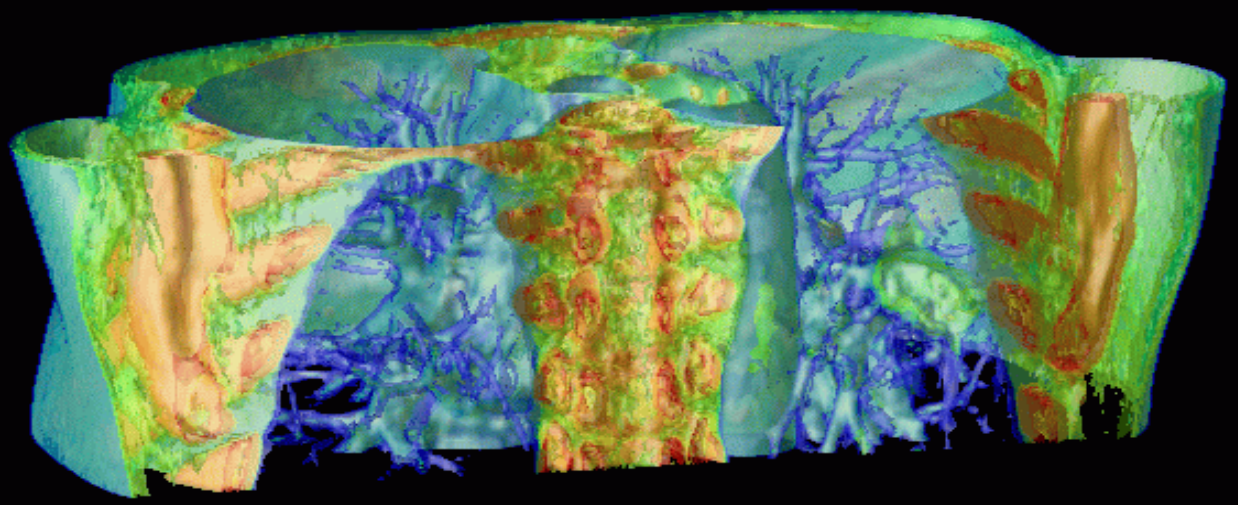
Helmet in treatment position

GAMMA KNIFE® SURGERY

Can we do radiosurgery outside the head?

- Unlike the brain, the rest of the body moves because of respiration, heart beat, urine and stool excretion, and even patient restlessness
- Volume of tumor tends to be bigger extracranially. Single large dose may be insufficient. May need to do fractionated stereotactic radiotherapy.
- Fractionated radiotherapy requires reproducible positioning

Breathing is conducive to life



- posterior view
- posterior cut

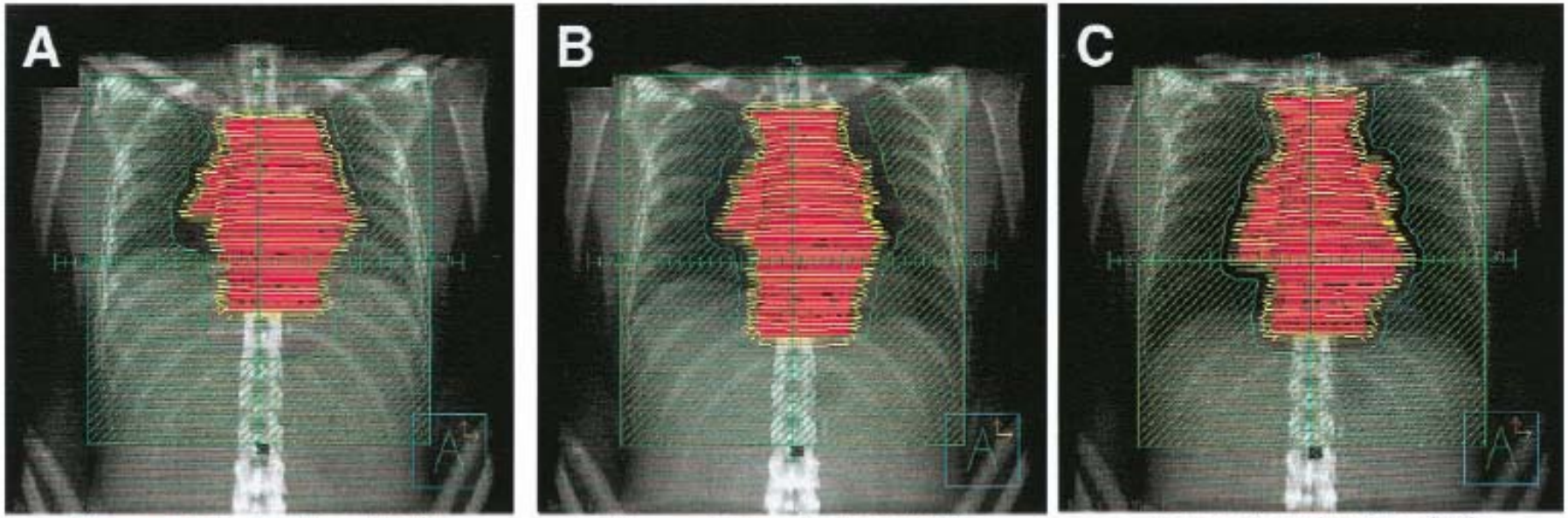
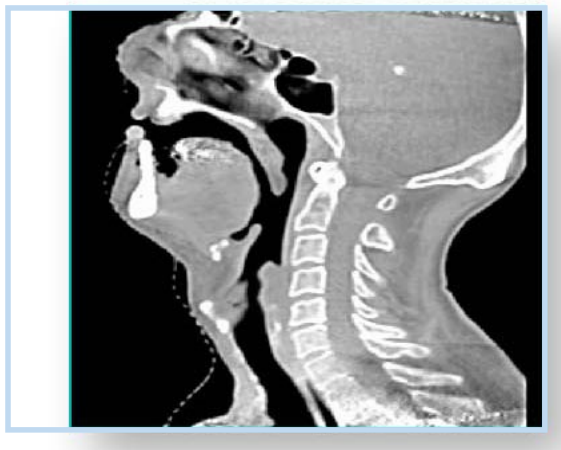

A
B
C
Normal Expiration (NE)
Normal Inspiration (NI)
Deep Inspiration (DI)

Image Guided Radiation Therapy

Imaging the patient at the time of treatment and with the patient in the treatment position



Elekta Synergy® - treatment and imaging

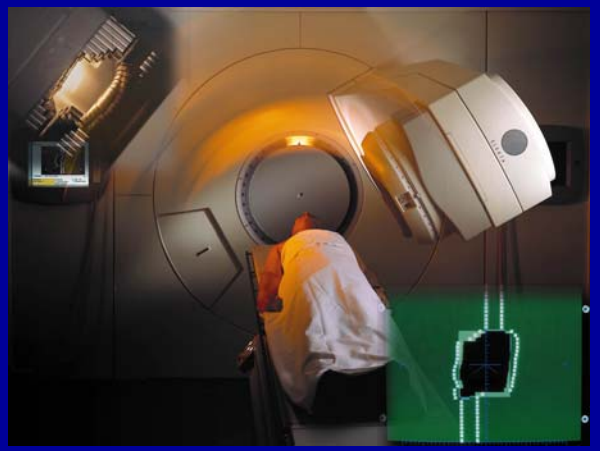
Megavoltage radiation source

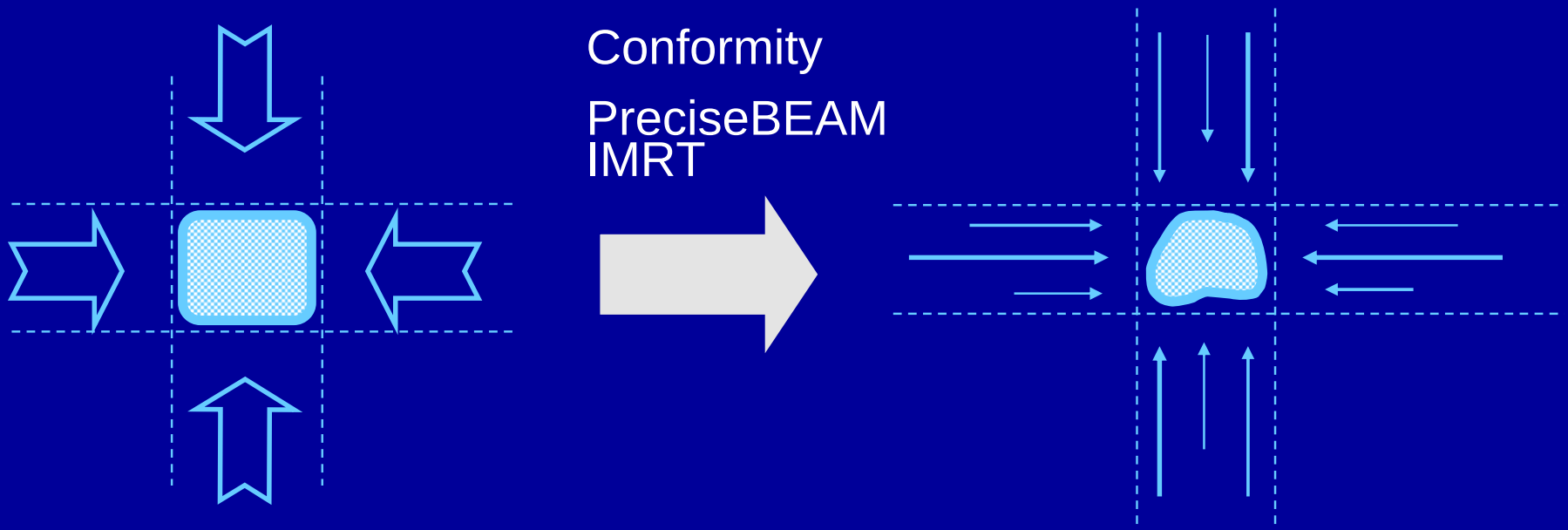
Kilovoltage radiation source

Kilovoltage imager

Megavoltage imager

IMRT gives us precise conformity – ”the right shape”

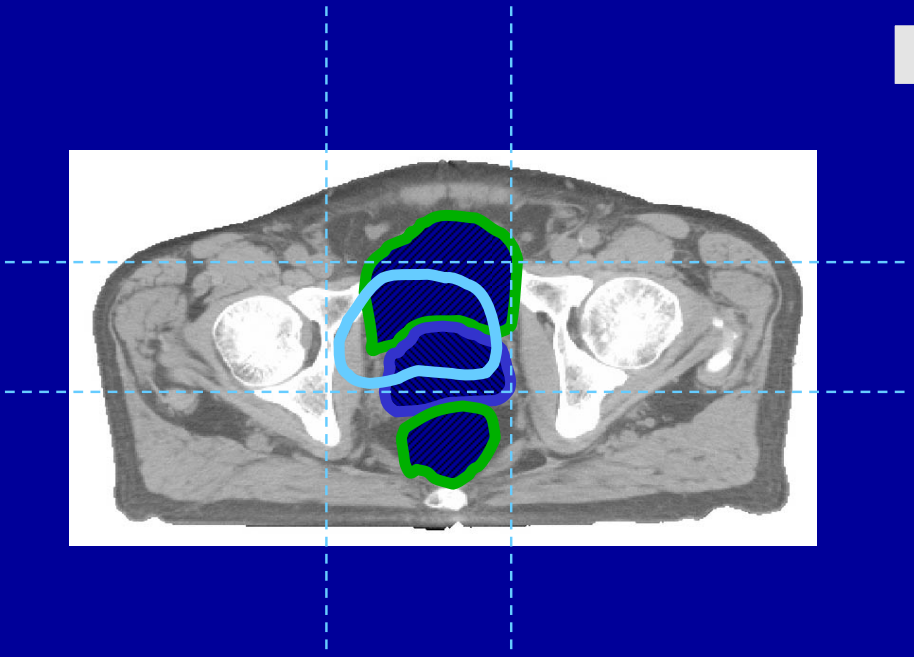


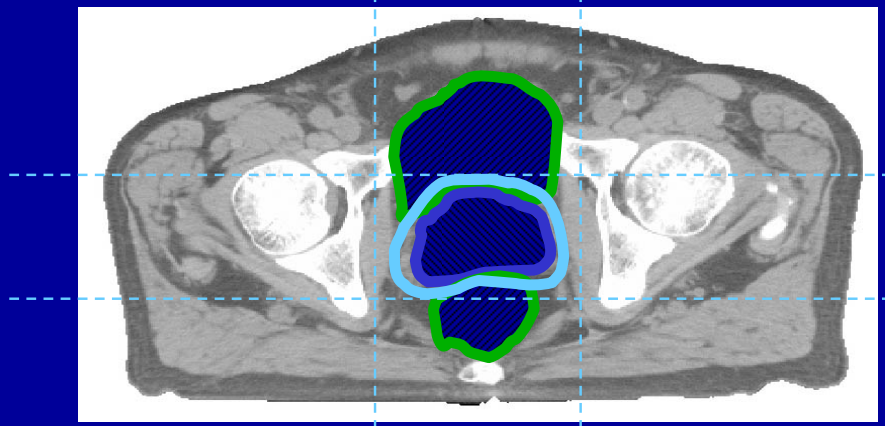


IGRT gives us Accuracy – "the right place"

IGRT

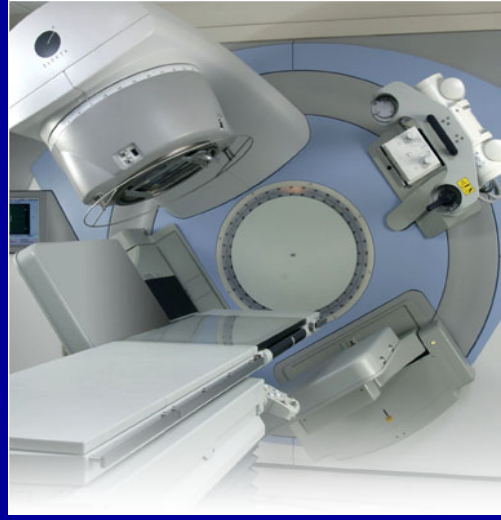
Accuracy





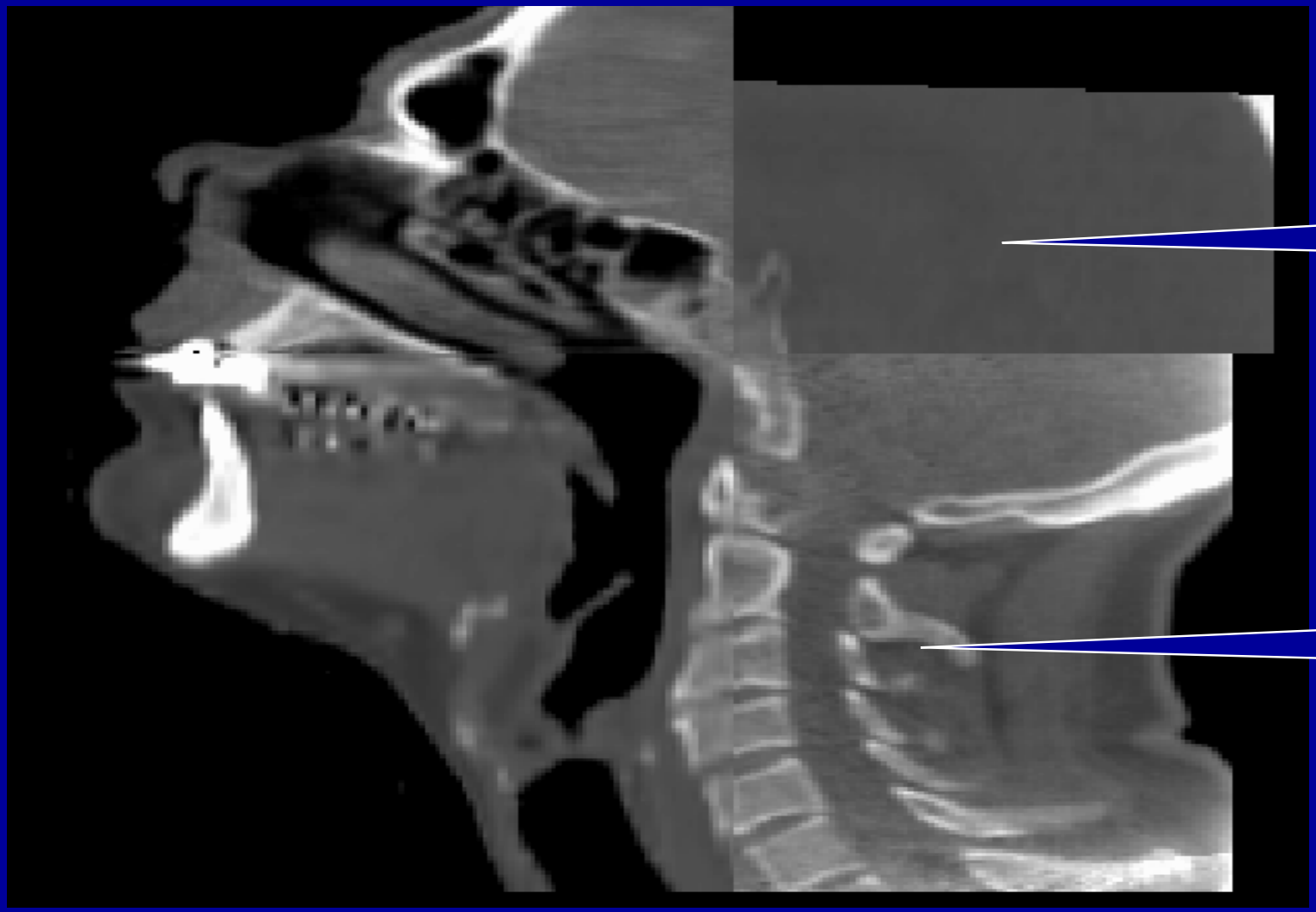
Purpose Engineered Flexibility:

MotionView™ imaging software



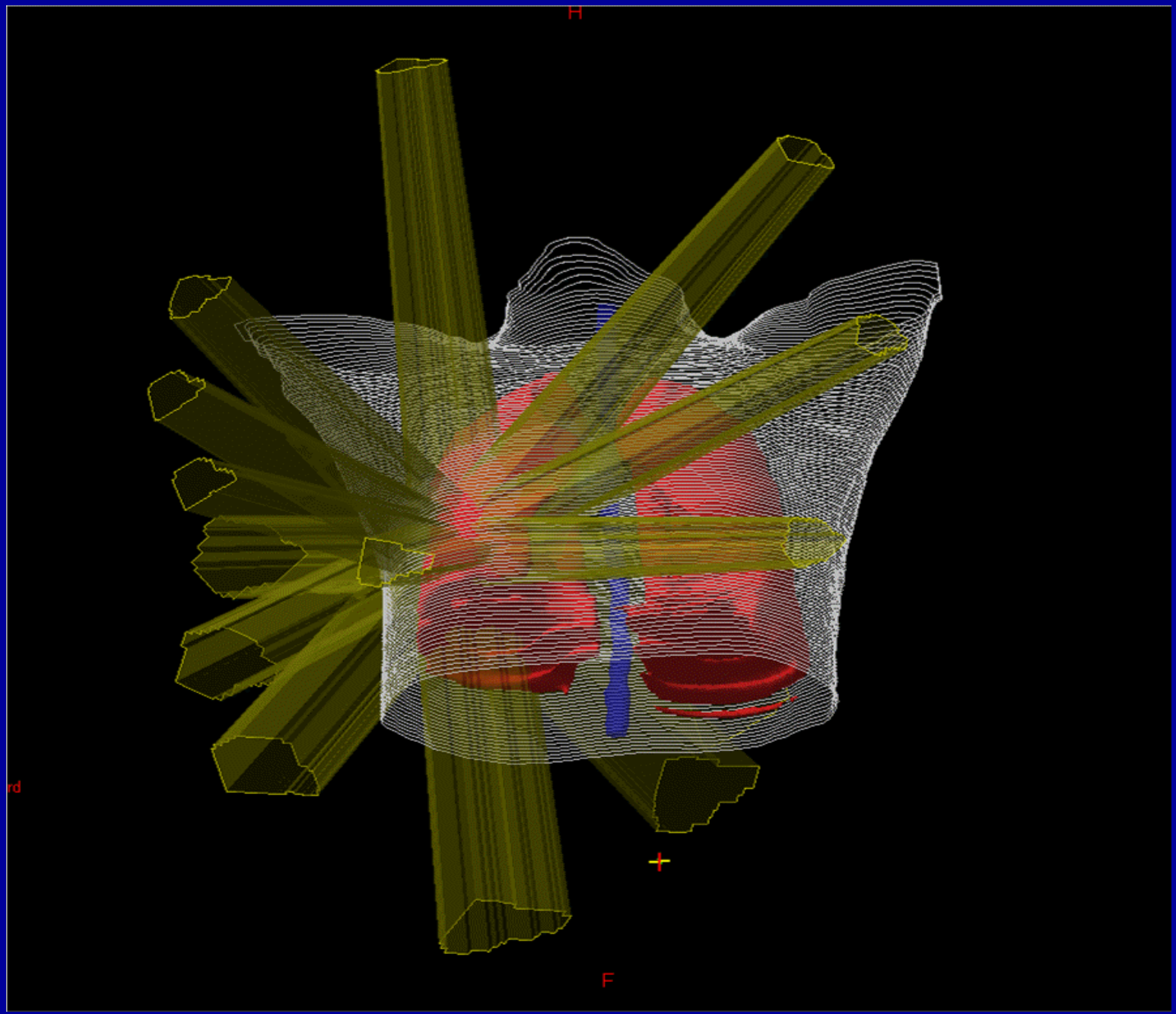
- 2D planar sequence imaging (fluoroscopy)
- Shows real-time movement of dense features:
  - lung tumors (high contrast to air)
  - bony landmarks (that don't overlie other bony features)
  - implanted (surrogate) markers in soft tissue targets

Analyze the image and make corrections



IGRT Potential

- Improved accuracy
- Reduce field margins around tumors to decrease normal tissue volume
- Potential extracranial body stereotactic radiosurgery/radiotherapy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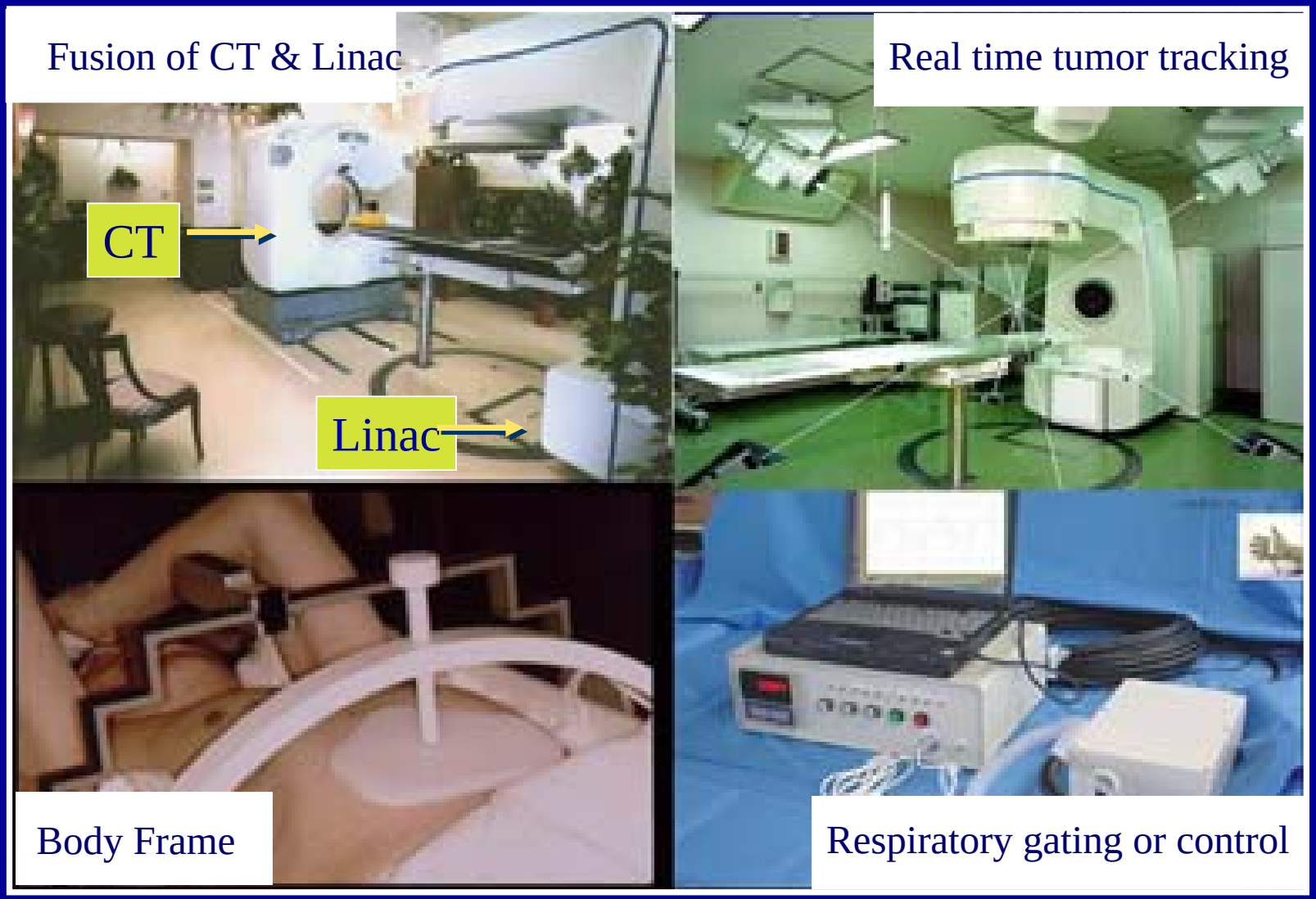
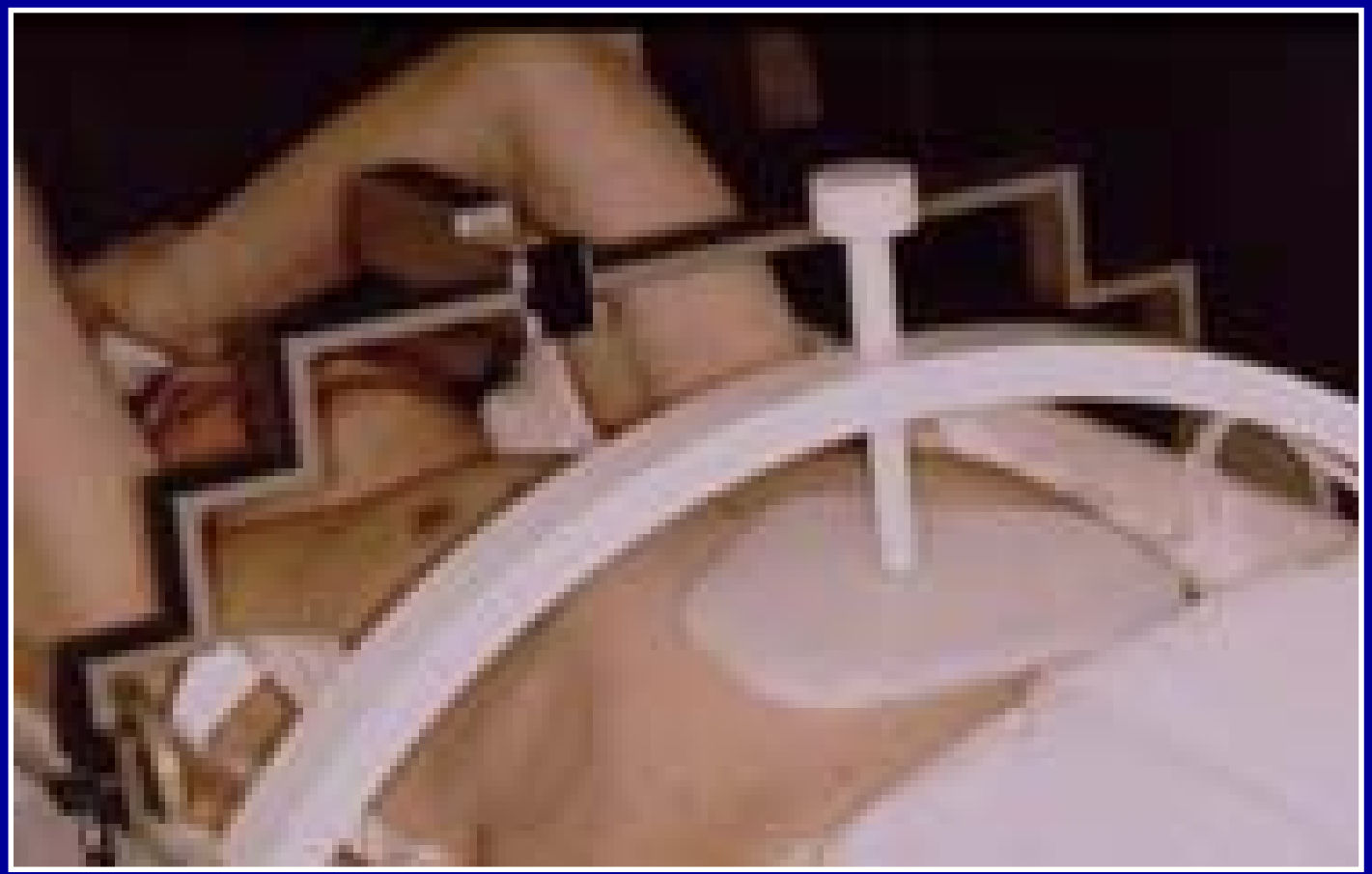
Techniques & Devices for STI to permit small margin around tumor



Adapted Onishi et al, ASCO 2004

Stereotactic Body Frame

First Introduced in 1994

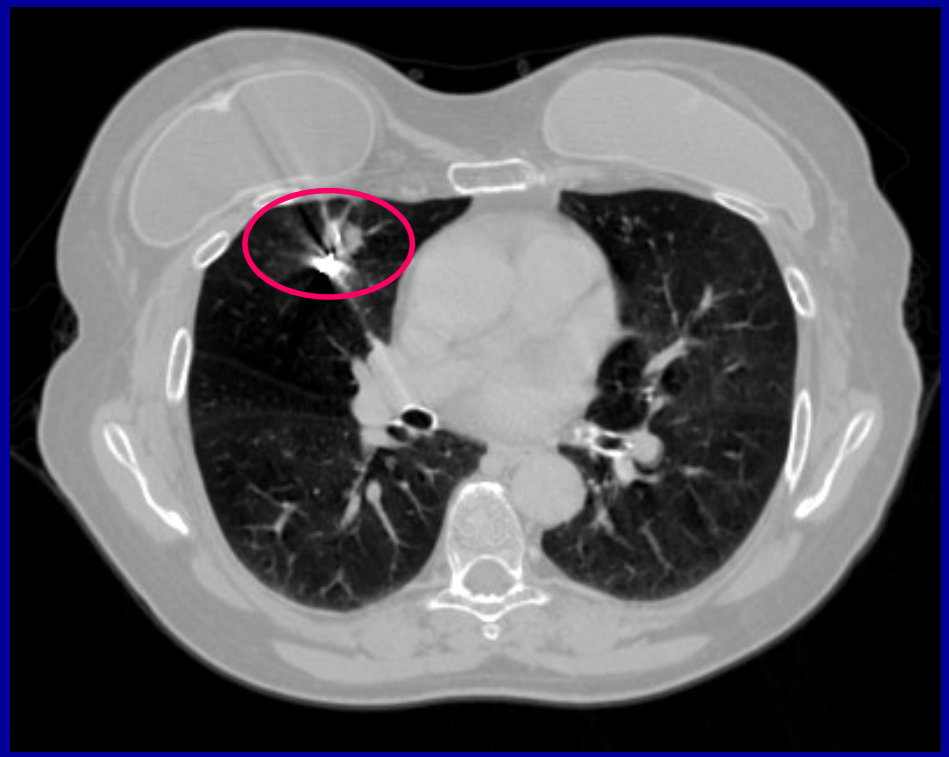


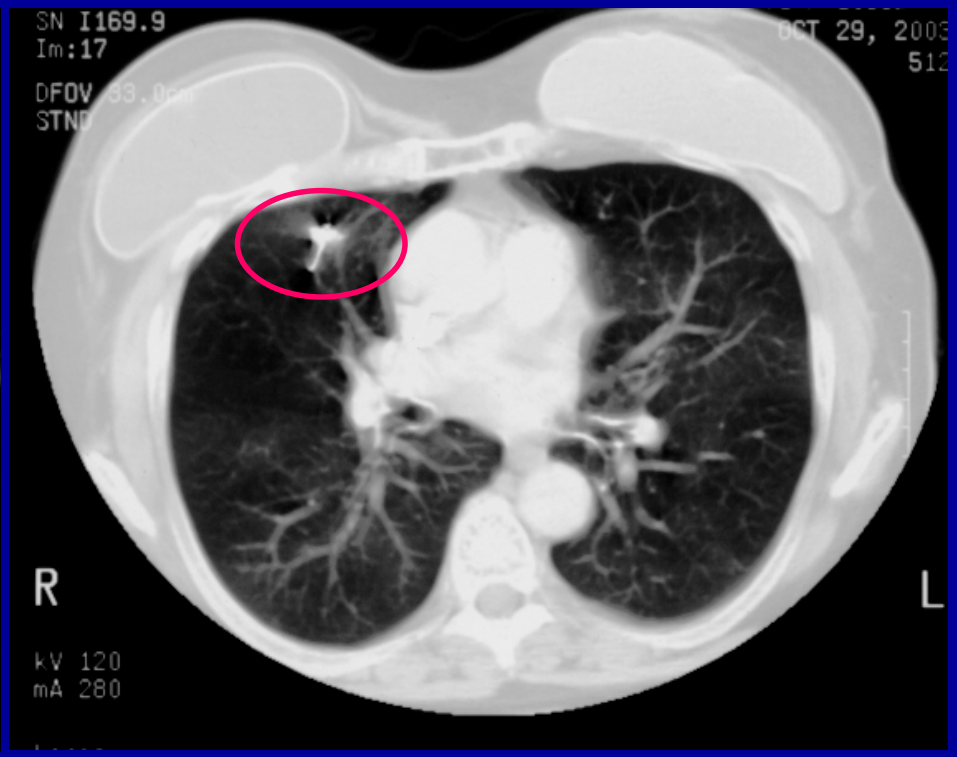
Lax et al, Acta Oncol 33:677, 1994

Results of Hypofractionated Stereotactic Irradiation for Lung Tumors

Author	# Pts	Dose/#FX	Ref Point	BED	Local control
Uematsu*	50	50-60/5-12	Margin	90-100	94%
Nagata	33	48/4	Isocenter	106	94%
Hof	10	19-26/1	Isocenter	55-94	80%
Timmerman	26 11	24-48/3 54-60/3	Margin	43-125 151-180	77% 100%
Le	9 14	15/1 25/1	Margin	38 (52-64) 169 (110-215)	56% 86%

Response in a T1N0 NSCLC after 25 Gy

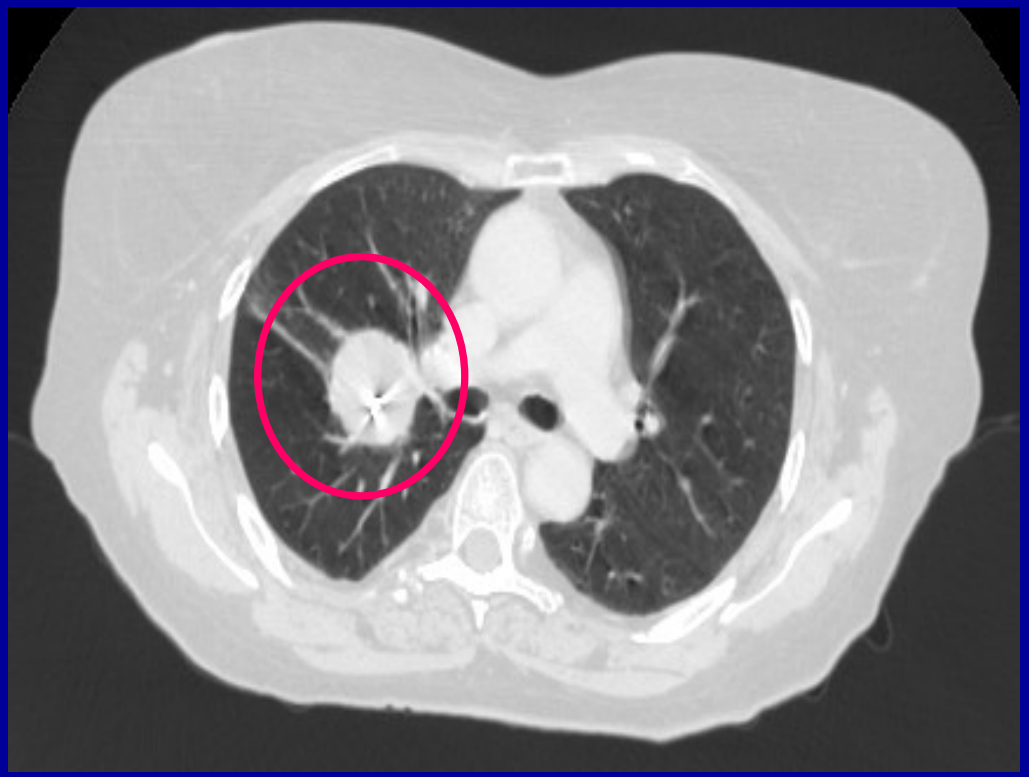




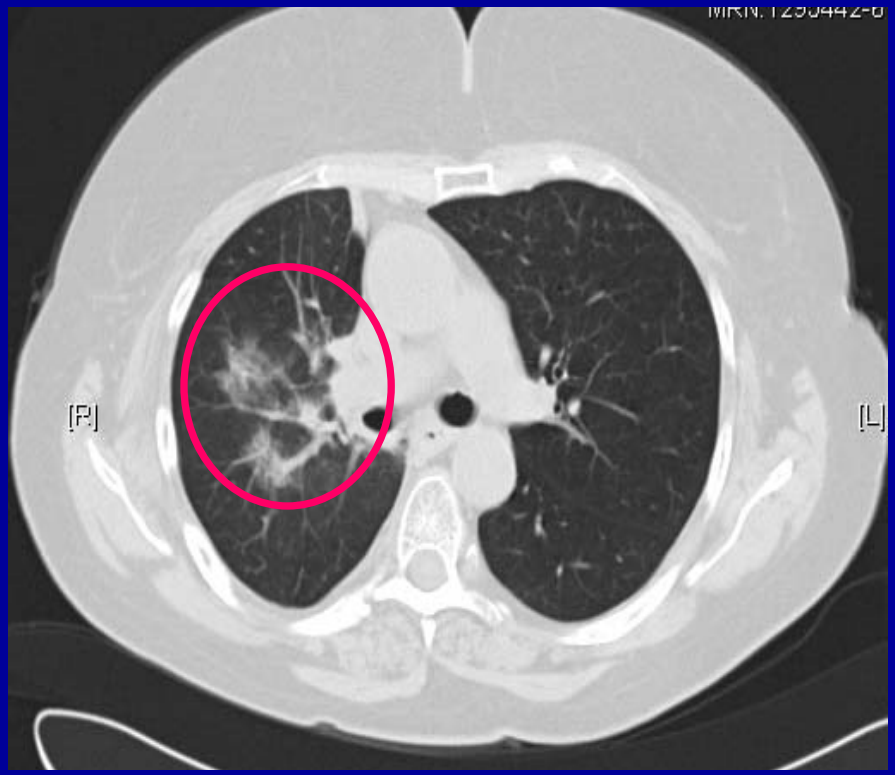
Pretreatment

Post-treatment

Post-Treatment Lung Reaction



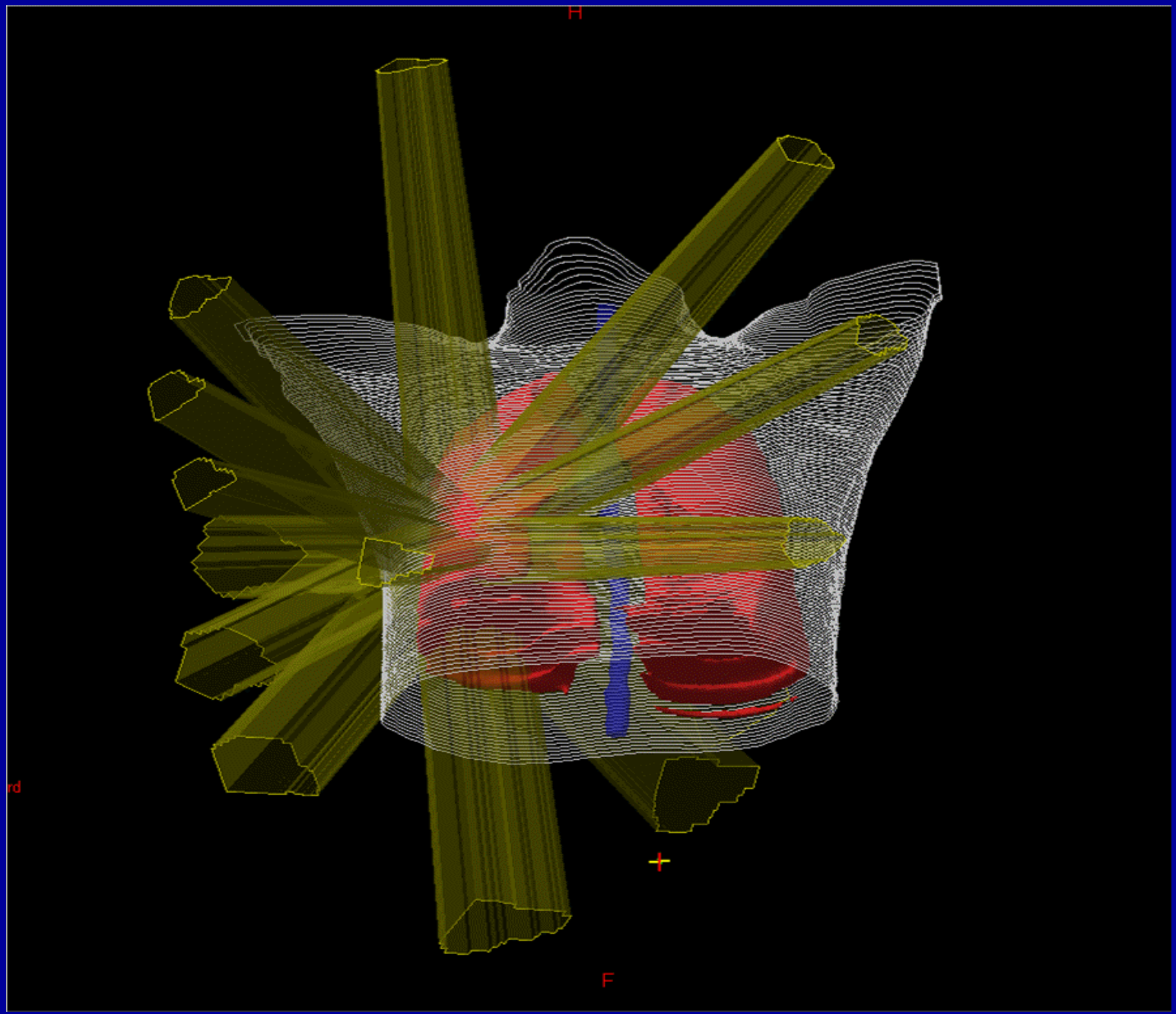
Pretreatment



Post-treatment

IGRT/SBRT summary

- Preliminary results with IGRT/SBRT stereotactic irradiation are promising. Longer follow up is needed.
- Issues to be addressed:
  Best method for tumor immobilization and targeting
  Dose & fractionation
  Long term normal tissue toxicity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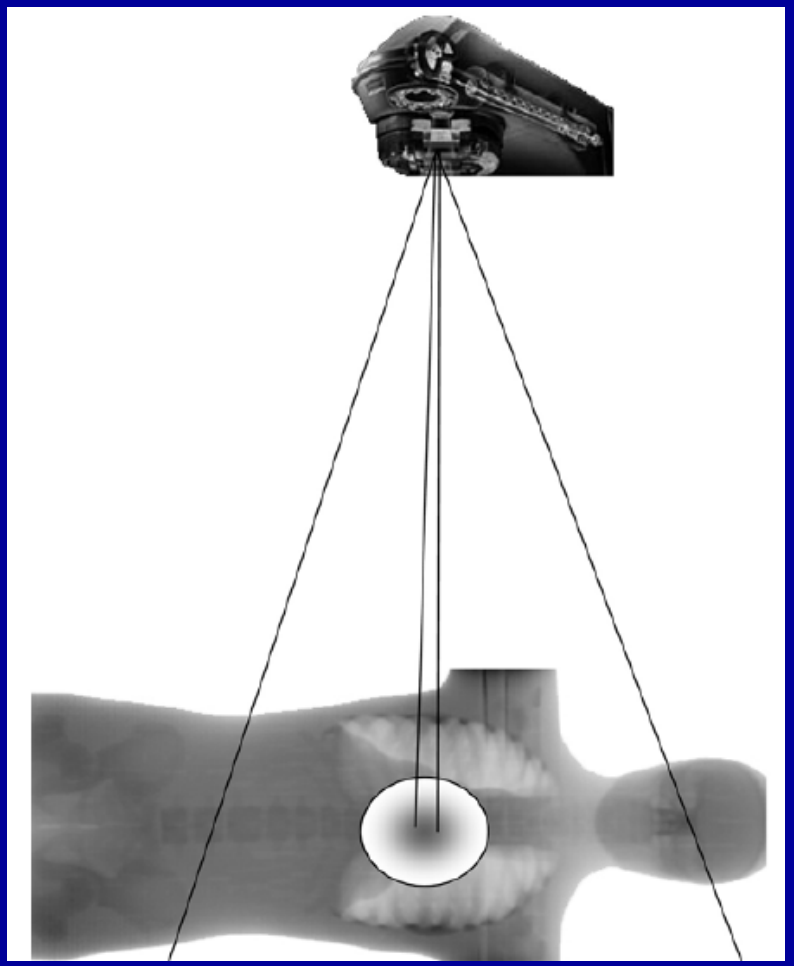
Concerns with IGRT

- Better targeting of tumor but
  - More MU needed to deliver a given dose (I.e., more leakage dose from LINAC possible
  - More beams mean more normal tissue volume receiving low doses of radiation unnecessarily

Concerns of leakage



Risk of second cancers from IMRT/IGRT

Table 3. Estimated risk of fatal radiation-induced malignancies after RT for prostate cancer (%/Sv)

Hall and Wuu (4)	
Conventional 6 MV	1.5
IMRT 6 MV	3.0
Kry *et al.* (5)	
Conventional 18-MV Varian	1.7
IMRT 6-MV Varian	2.9
Siemens	3.7
IMRT 10-MV Varian	2.1
IMRT 15-MV Varian	3.4
Siemens	4.0
IMRT 18-MV Varian	5.1

Abbreviations: IMRT = intensity-modulated radiation therapy; MV = megavoltage; RT = radiation therapy.

Is there a benefit from low dose total body exposure?

- Animal data
  - 0.1 Gy TBI in mice give 6-15 hr pre tumor transplantation increases TD50 (Sakamoto, Gan To Kagak Ryolo, 1987,14:1545)
  - 0.2 Gy TBI in rats transplanted with KDH-8 hepatoma cell lines decreases mets to lung (Hashimoto, Nippon Igaku Hoshasen Gakkai Zasshi, 1997, 57:717)

Is there a benefit from low dose total body exposure?

- Animal data
  - Subset of Lyt-1,2^{+} CTL can be killed in vitro with 0.1-0.25Gy (Spellman, JEM, 1982, 155:1858)
  - Low dose TBI decreased relative number of B cells but increased T and NK cells in spleens (Fourquet, Radiother. Oncol, 1993, 26:219)

Is there a benefit from low dose total body exposure?

- Animal data
  - Tumor bearing rats given 0.2Gy TBI (but not to tumor) increased IFN-γ and TNF-α expression in spleenocytes, but did not increase IL4, IL6 or IL10 (Hashimoto, Radiat. Res, 1999, 151:717)

Is there a benefit from low dose total body exposure?

- Human data
  - Chemo-refractory NHL pts tx'd c 1.2-1.8 Gy TBI enhanced in vitro immune response; clinical improvement correlated with increased mitogen-induced proliferative response (Yonkosky, Cancer, 1978, 42:1204)
  - Helper and cytolytic T cells increase while suppressor T cells decrease in NHL pts getting TBI (Takai, Nippon Gam Chiryo Gakkai Shi, 1989, 24:1288)

Summary of topics thus far

- High local RT can make tumors more immunogenic/antigenic
- Local RT and APC maturation signals may induce abscopal effect
- IMRT/IGRT permits high local RT in humans
- Low dose leakage may increase risk of second malignancies
- Low dose leakage may theoretically augment immune response

What can we do with these tools?

- Oligometastases
  - State of limited number of metastases before widespread dissemination

Oligometastases

- Tumors early in the chain of progression may have metastases limited in number and location because the facility for metastatic growth has not been fully developed and the site for such growth is restricted (this is in contrast to micrometastases, which, although small in size, are extensive in number).
- With further progression, the tumor seeding efficiency increases and becomes less fastidious with regard to the location of metastatic growth.
- The increasing primary tumor size and therefore cell number should also be correlated with the increasing number of cells seeding.
- Tumor size is the principal basis of tumor staging and, with histologic grade, correlates with the likelihood of metastases.

Oligometastases

- If this state exists, it is possible, by attempts at local eradication of these metastases (and the primary), to cure these patients.
- By adding adjunctive immunotherapy in this setting of cytoreduction (decreasing tumor burden), we may increase the likelihood of eradicating subclinical disease
  - Assuming that metastatic tumor cells retain antigenicity

Radiation SBRT for lung oligomets

Table I. Patient and treatment characteristics.

Number of patients	49
Curative/palliative	30/19
Number of treated targets total	125
Number of targets per patients mean (range 1–5)	2.6
Gender	
Male	22
Female	27
Age (years)	
Range	37–86
Median/mean	60/60
Histology type	
Adenocarcinoma	30
Squamous Cell	6
Others	14
Tumor size (longest diameter (cm))	
Range	0.3–7.7
Median/mean	2.1/2.5
Treatment volume (ml)	
GTV (min/max; median/mean)	0.1/125; 4.7/11.8
V_{20} and V_{10}	
V_{10} (%) (median/mean)	22.8/23.8
V_{20} (%) (median/mean)	10/12.6
Treatment dose (Gy)	
GTV min dose (median/mean)	4.8/4.4
GTV max dose (median/mean)	5.4/5.3
Follow-up time (months)	
Median/mean	14.9/18.7
Range	3.7–60.9

V_{20} = percentage of total lung volume that received ≥ 20 Gy.
V_{10} = percentage of total lung volume that received ≥ 10 Gy.

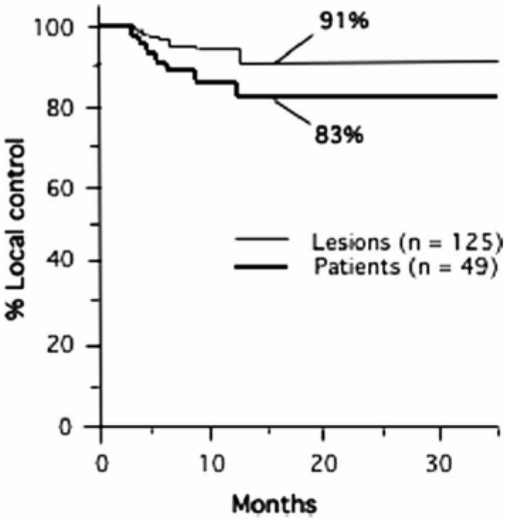


Figure 4. Actuarial local control rate for lesions (n = 125) and for patients (n = 49) is shown. Local failures were all seen during the first 15 months after treatment. At 15 months the local control rate for all lesions and patients was 91% and 83%, respectively. There were 20 patients at risk at 15 months. Crude local control was achieved in 117 of 125 lesions (94%). One patient had two local failures leading to local control in 42 of 49 patients.

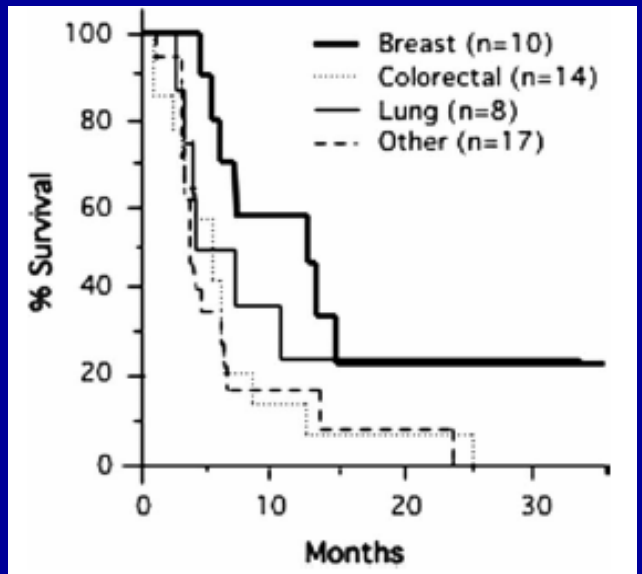


Figure 3. Actuarial progression-free survival by primary tumor types (breast, colorectal, lung, and others) among all patients is shown. Long-term progression-free status was most obvious for breast and lung cancer.

Okunieff, Acta Oncologia, 2006, 45:808

Conclusions

- Tumor burden may be too much burden for effective immunotherapy
- SBRT allows effective local cytoreduction while increasing antigenicity of tumors
- Secondary low dose TBI from SBRT may augment immune response

Melanoma stage IV undergoing systemic/immunotherapy

- PS 0-2
- 1-4 metastatic lesions, none larger than 4cm maximum diameter
- No new lesions within the last two months
- Treatment: SBRT during systemic therapy (IL2, GMCSF, Flt3L?). Continue with systemic care.
- Endpoints: survival, local control, time to new mets