# The development of Radiobiology in Italy: critical points

- Controlled clinical trials are increasingly promoted by Pharmaceutical Industry (sponsored trials).
- The main investigator is often a Medical Oncologist (not interested in radiobiology).
- Few experimental studies are planned to investigate in the lab the interaction between new therapy and radiation.
- There should be a strong need for the selection of tumors which must be investigated.
- Pharmaceutical Industry should promote also basic research in the field of radiobiology.

# Potential investigations

- ✓ In vivo studies: murine tumors, human xenografts
- ✓ In vivo studies: cell kinetics
- ✓ In vitro studies: spheroids, mono-layers
- ✓ Reverse translational research (from bedside to bench)
- ✓ Mathematical models to use for the choice of fractionation and the optimization of treatment planning

# Gruppo di Ricerca in Radiobiologia-GRR

- - a livello inter-dipartimentale (intra-istituzionale)
- - a livello inter-istituzionale (Università, IRCCS, INFN, Asl, Enti privati)

# GRR

- **Laboratory-based Research:**

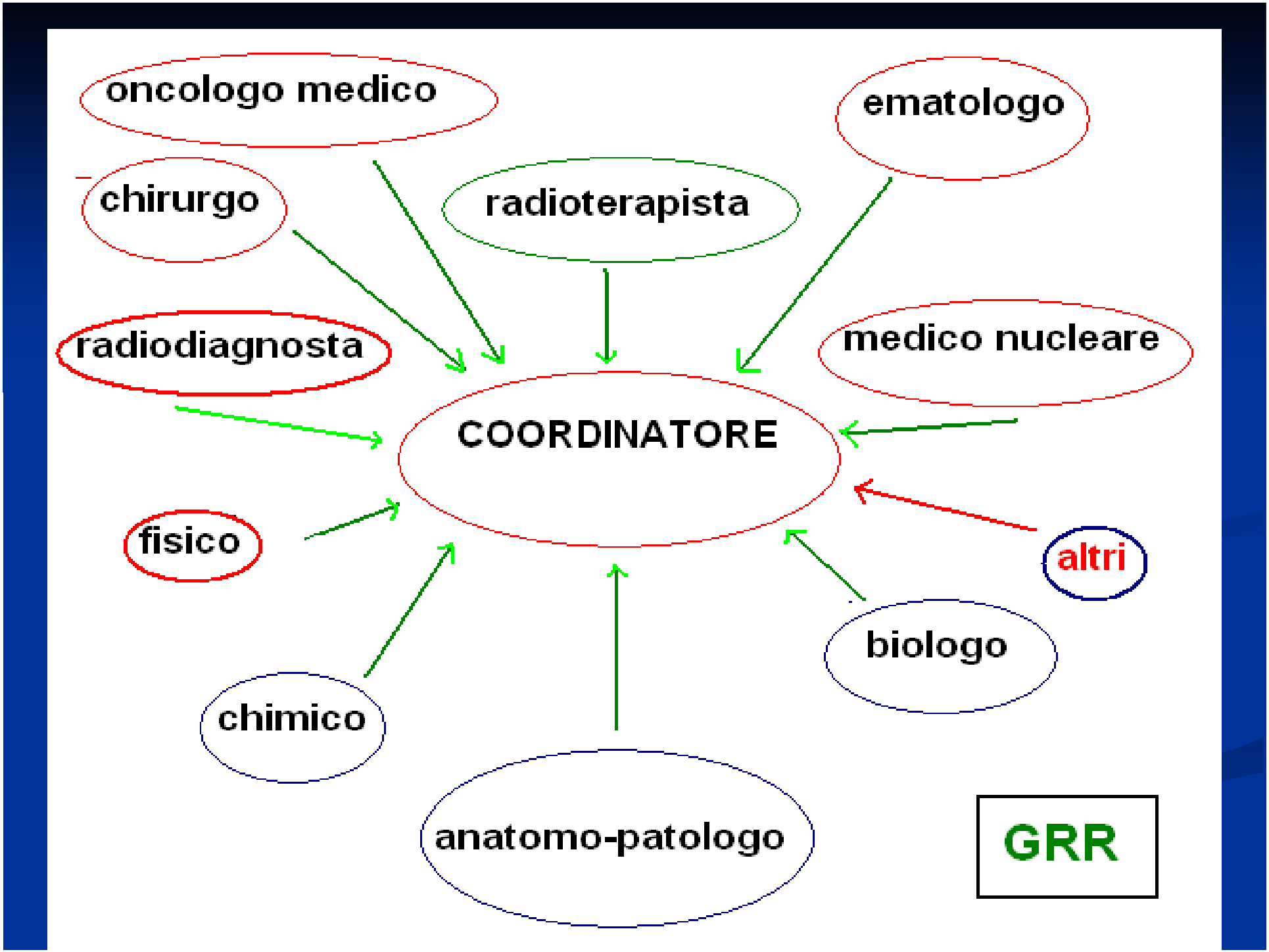
  ricercatore sperimentale (biologo,anatomo-patologo, biofisico, clinical investigator etc)

- **Image-guided Research:**

  radiodiagnosta,medico-nucleare, fisico, chimico

- **Clinical Investigation:**

  radioterapista, oncologo medico, ematologo, chirurgo, fisico


oncologo medico
ematologo
chirurgo
radioterapista
radiodiagnosta
medico nucleare
COORDINATORE
fisico
altri
biologo
chimico
anatomo-patologo
GRR

# Problematiche

- Finanziamento: progetti finalizzati nazionali (Ministero, CNR, AIRC) e internazionali (Biomed)
- Sponsorizzazione di Enti privati (evitare conflitto di interesse).

# "In vivo" investigation: constraints

- Ethical problems (studies in Humans and Animals)
- Difficulties to obtain many specimens for research after first biopsy for diagnosis
- Logistical aspects
- Need for a strong correlation between biological parameter and clinical outcome

# "In vitro" investigation: constraints

- Difficulty in the assessment of the actual role of a biological parameter;
- The pre-treatment value may change during the radiotherapy course;
- Intra and inter-tumoral heterogeneity;
- Lab analysis may be not standarized;
- The biological bank may be a solution in controlled clinical trials.

## Potential prognostic factors and predictors of response to radiotherapy in HN-SCC (spontaneous investigations)

- Intrinsic radiosensibility SF2 Gy
- Cell kinetics (LI% - Tpot, cyclin-D1, Ki 67%, others)
- Hypoxic measurement (pimonidazole index, pO2 measurement, osteopontin)
- Molecular genetics (p53, p16, p21, p 27)
- Multiple genetic aberration (LOH)
- Drug-DNA adducts and many others……
- EGF-R ?

None of the above has entered clinical practice to improve prognostic index and to select patients who may benefit from chemo-radiotherapy or altered fractionated radiotherapy

# Forward Translational Research
## (Genoa- 2000-2008)

- To investigate the in vivo Intra-Bone –Transplant (IBT) feasibility after Total Body Irradiation

  (future impact on BMT in clinics).

- To evaluate in vitro the modulation of the radiosensitivity of a radioresistant human SCC from HN embedded in collagen gels (stromal influence)

  (future impact on radiotherapy for aggressive SCC-HN).

# Total Body Irradiation for Intra-Bone Marrow -Trasplant

- Bone Marrow Transplantation → powerful strategy for the cure of congenital immunodeficiency, hematological and metabolic disorders.
- Important subject to solve: GvHD
- Goal: injection of donor bone marrow cells directly into the bone marrow cavity induces persistent donor-specific tolerance in mice even if radiation dose are reduced to sublethal levels

# Rationale

- To explore IBM-BMT in order to:
  - To shift lethal TBI dose to sublethal dose
  - To reduce GvHD by giving less donor cells
  - To avoid heavy late radiation-induced toxicity
  - To deliver BMT also in older patients
  -

# Intravenous marrow infusion

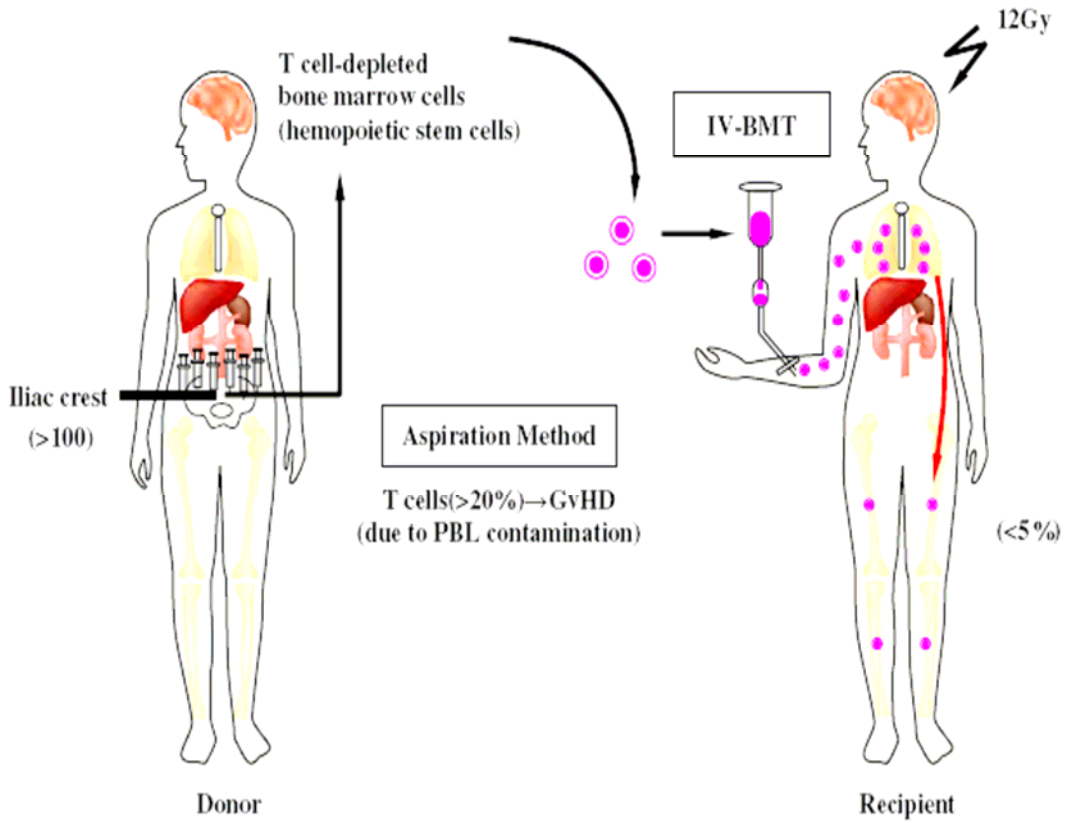



Fig. 5. Conventional BMT for allogeneic BMT. Conventional BMT is carried out using an aspiration method, followed by the intravenous injection of BMCs (IV-BMT).

STEM CELLS
TRANSLATIONAL AND CLINICAL RESEARCH

# Prevention of Graft-Versus-Host Disease by Intra-Bone Marrow Injection of Donor T Cells

JUNICHI FUKUI,[a,b] MUNEO INABA,[a,c] YUSUKE UEDA,[a,d] TAKASHI MIYAKE,[a,b] NAOKI HOSAKA,[a,c] A-HON KWON,[b] YUTAKU SAKAGUCHI,[a,e] MASANOBU TSUDA,[a,f] MARIKO OMAE,[a,g] YASUO KAMIYAMA,[b] SUSUMU IKEHARA[a,c]

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EXPERIMENTAL HEMATOLOGY

Experimental Hematology 31 (2003) 1142–1146

# A novel strategy for allogeneic stem cell transplantation: perfusion method plus intra–bone marrow injection of stem cells

Susumu Ikehara

*Kansai Medical University, Osaka, Japan*

# Intra-Bone Marrow-BMT



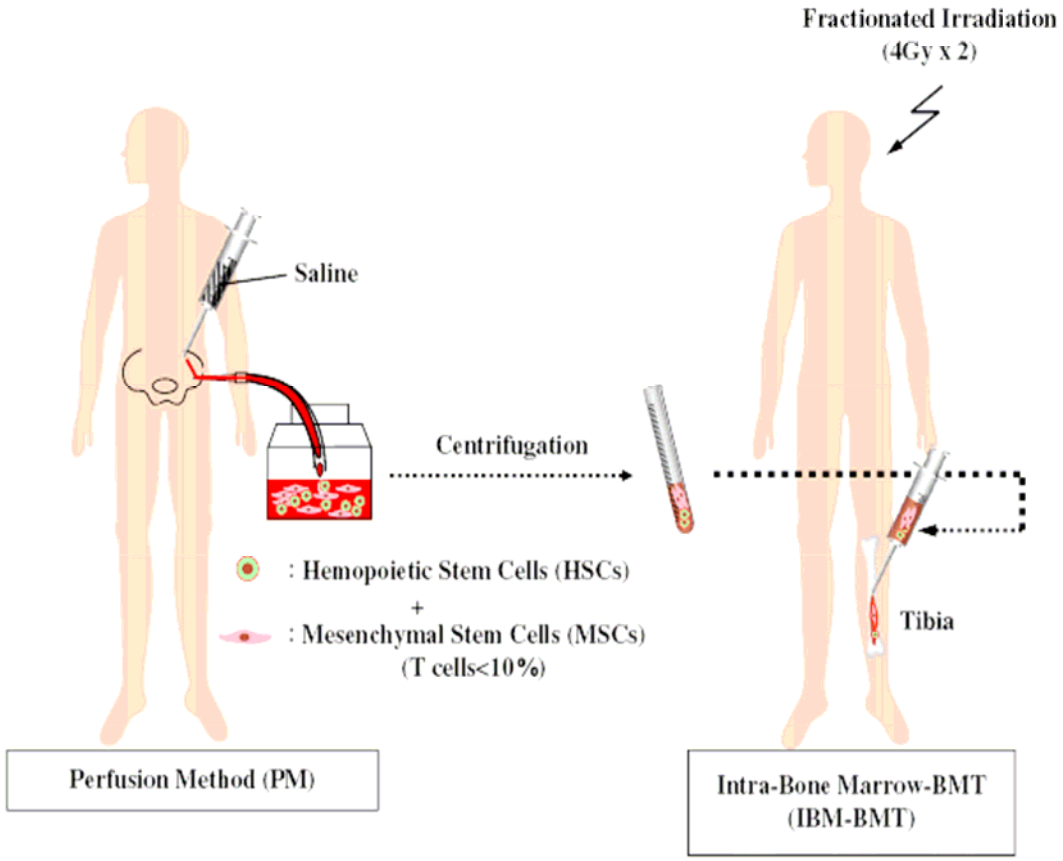


Fig. 6. A new BMT method for allogeneic BMT. The new method consists of PM + IBM-BMT.

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EXPERIMENTAL HEMATOLOGY

Experimental Hematology 32 (2004) 782–787

# Intra–bone marrow injection of bone marrow and cord blood cells: An alternative way of transplantation associated with a higher seeding efficiency

Sandra Castello[a], Marina Podestà[a], Vincenzo G. Menditto[a], Adalberto Ibatici[a], Anna Pitto[a], Osvaldo Figari[a], Daniele Scarpati[b], Lorenzo Magrassi[c], Andrea Bacigalupo[a], Giovanna Piaggio[a], and Francesco Frassoni[a]

[a]*Centro Cellule Staminali e Terapia Cellulare e* [b]*Servizio di Radioterapia, Ospedale San Martino, Genova, Italy;* [c]*Neurochirurgia, Dipartimento di Chirurgia, Università di Pavia, IRCCS Policlinico S., Pavia, Italy*

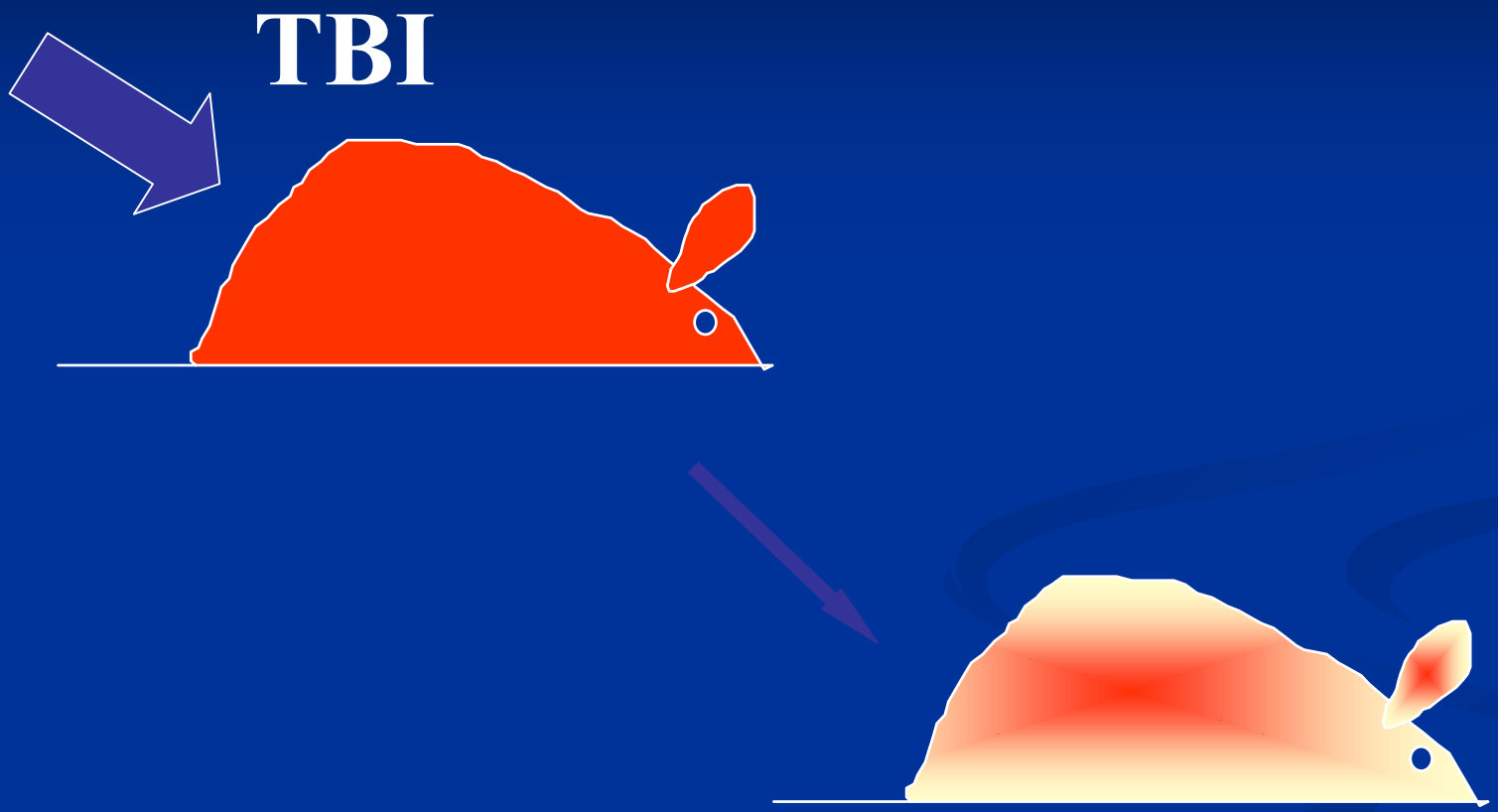
(Received 13 June 2003; revised 17 May 2004; accepted 18 May 2004)

# Results

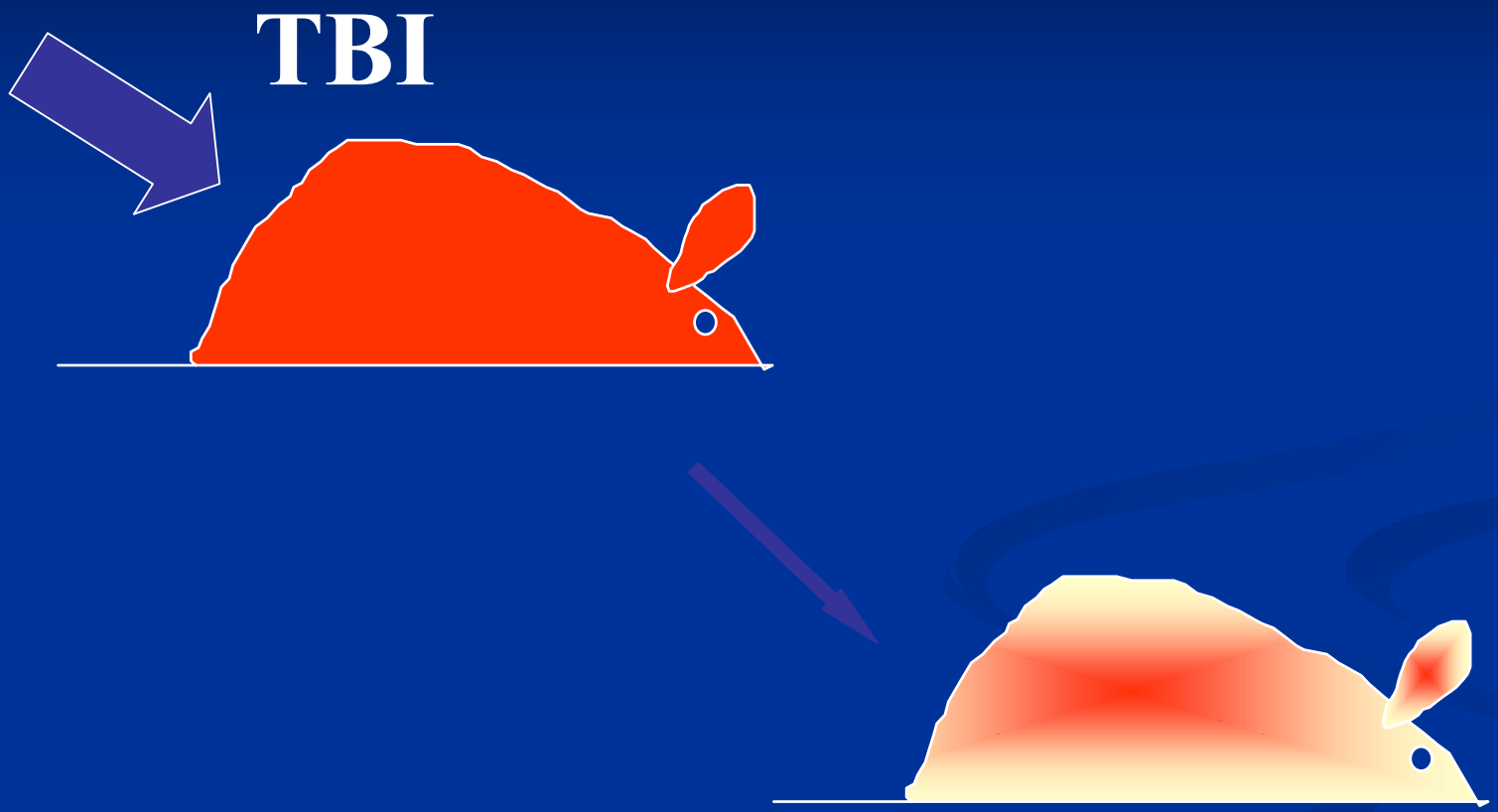
- → Intra-bone transplantation (IBT) is associated with a seeding efficiency 15 times greater than intra-venous transplantation.

- IBT may be useful when a limited number of donor progenitors are available such as cord blood cells
- With IBT the dose of TBI may be reduced.

# Conditioning regimen for bone marrow transplant



# Conditioning regimens for stem cell transplantation



# Ongoing investigation

- To evaluate the distribution of in vivo hematopoietic cells injected either i.v. or intra-bone in Lewis mice submitted to irradiation
- Day – 2: Total Mouse Irradiation (range 6-9 Gy)
- Day 0: Transplant (i.v. or intra-bone) with radiotracer Tc99.
- Analysis of distribution by gamma-camera.

# The “in vitro collagen gel” model

- Collagen gels are found wide applications in biology as a in vitro scaffold for growing a viariety of cell types  reproducing the 3-D enviroment found in vivo.

- As compared to monolayer (collagen fibers)  in the 3-D spatial volume (spheroid) cell metabolic activity is limited. This model is similar to in vivo model with respect to radiosensitivity.

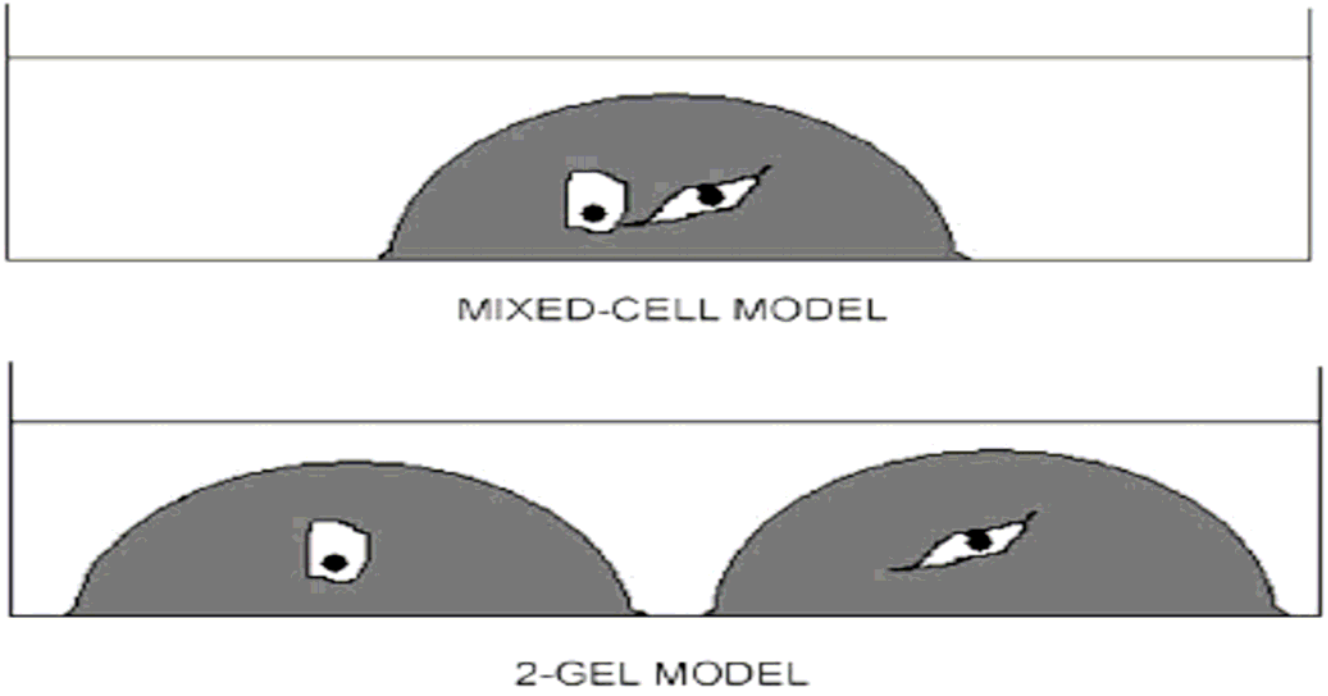



**Figure 1** Schematic representation of the cell culture models designed to study the interaction of different cell types in collagen gel. The aim of the mixed-cell model is to evaluate the effects of direct cell-cell contact, including physical contact and autocrine stimuli. That of the two-gel model is to evaluate the effects of paracrine factors, given that each cell type is cultured in separate gels nurtured by the same culture medium.

*Int. J. Cancer:* **85,** 667–673 (2000)


Publication of the International Union Against Cancer

# CO-CULTURE WITH HUMAN FIBROBLASTS INCREASES THE RADIOSENSITIVITY OF MCF-7 MAMMARY CARCINOMA CELLS IN COLLAGEN GELS

Lorenzo Rossi[1]*, Daniele Reverberi[2], Giorgia Podestà[2], Sonia Lastraioli[2] and Renzo Corvó[3]

[1]*Department of Oncology, Genetics and Biology, University of Genoa, Genoa, Italy*
[2]*Laboratory of Comparative Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy*
[3]*Department of Radiology, Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy*

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Int. J. Radiation Oncology Biol. Phys., Vol. 53, No. 5, pp. 1319–1327, 2002

0360-3016/02/$–see front matter

**PII S0360-3016(02)02865-1**

**BIOLOGY CONTRIBUTION**

# RETINOIC ACID MODULATES THE RADIOSENSITIVITY OF HEAD-AND-NECK SQUAMOUS CARCINOMA CELLS GROWN IN COLLAGEN GEL

Lorenzo Rossi, Ph.D.,*† and Renzo Corvò, M.D.‡

*Laboratory of Comparative Oncology and ‡Division of Radiotherapy–Istituto Nazionale per la Ricerca sul Cancro; and †Department of Oncology, Genetics, and Biology—University of Genoa, Genoa, Italy

Oral Oncology (2004) 40 214–222

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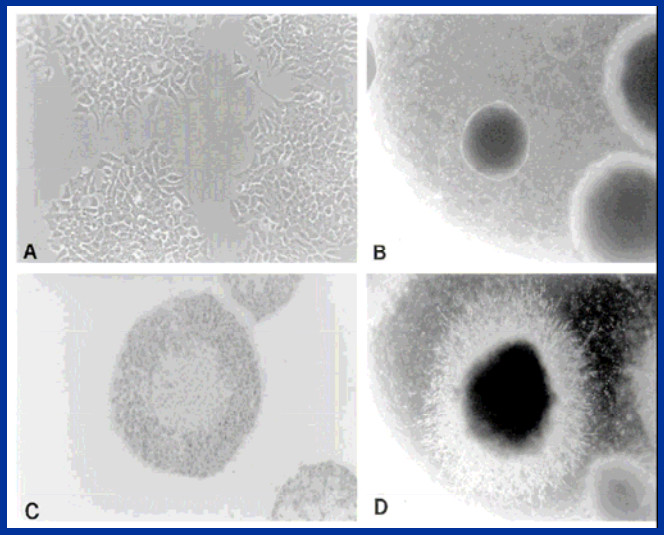
www.elsevier.com/locate/oraloncology

# Endothelial cells increase the radiosensitivity of oropharyngeal squamous carcinoma cells in collagen gel

Lorenzo Rossi[a,b,*], Francesco Boccardo[b,c], Renzo Corvò[d]



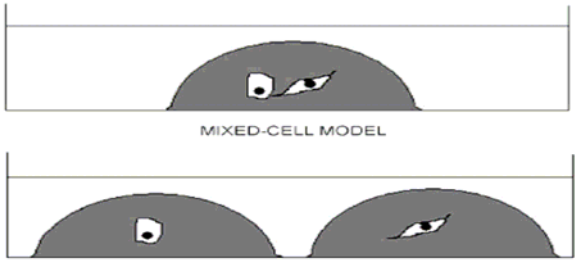
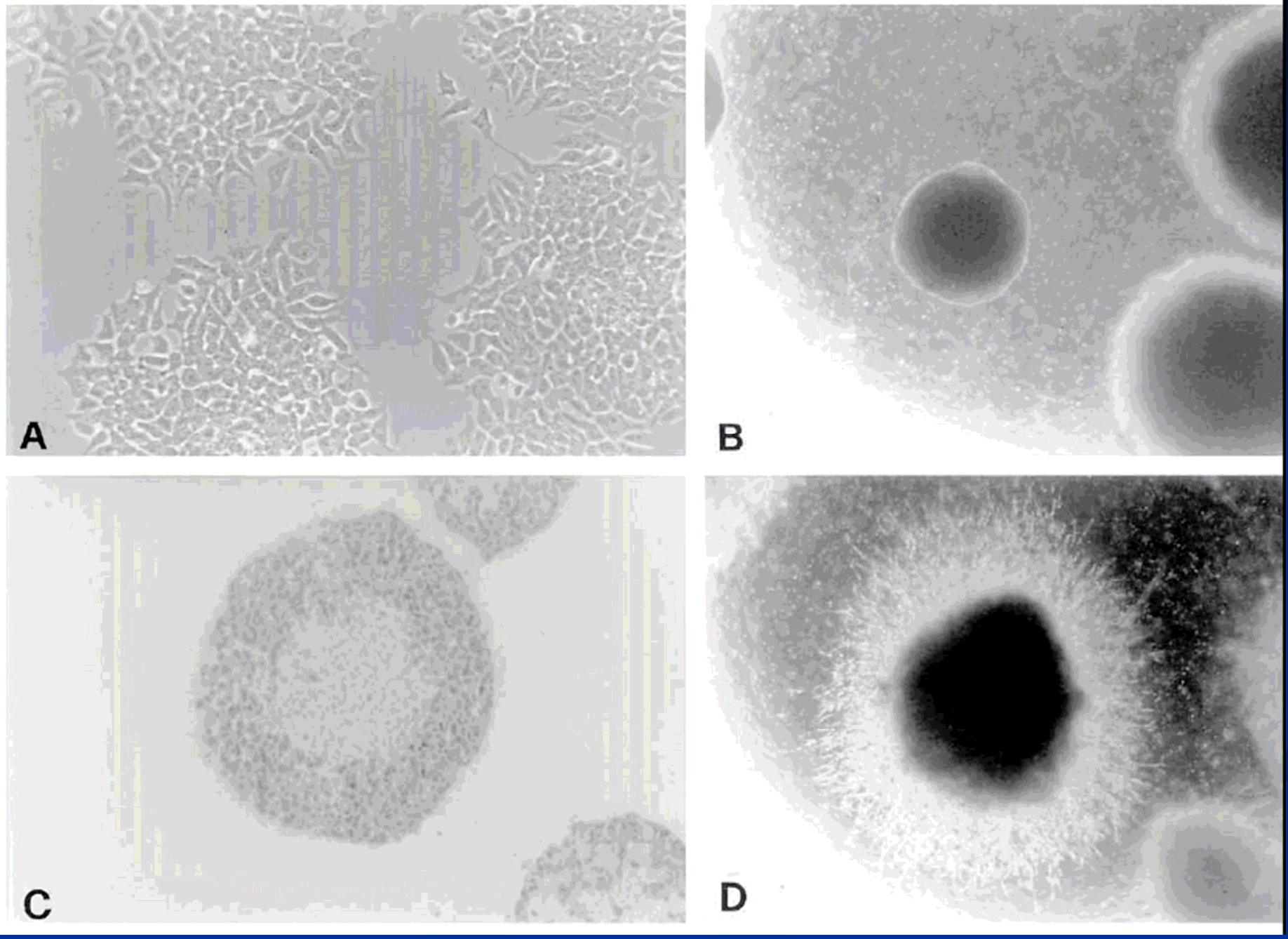




**Figure 1** Schematic representation of the cell culture models designed to study the interaction of different cell types in collagen gel. The aim of the mixed-cell model is to evaluate the effects of direct cell-cell contact, including physical contact and autocrine stimuli. That of the two-gel model is to evaluate the effects of paracrine factors, given that each cell type is cultured in separate gels nurtured by the same culture medium.


A
B
C
D

# Influence of stromal factors on radiosensitivity of SCC

- Human fibroblasts
- Contracted collagen gels
- Retinoid acid and other paracrine factors
- Stromal endothelial cells

may

increase (or modulate) radiosensitivity