Meeting in radiation oncology – Brescia 2008

Experimental models and future directions

Prof. Vincenzo Tombolini
Giovanni Luca Gravina M.D.

Department of Experimental Medicine
Division of Radiation Oncology
University of L'Aquila

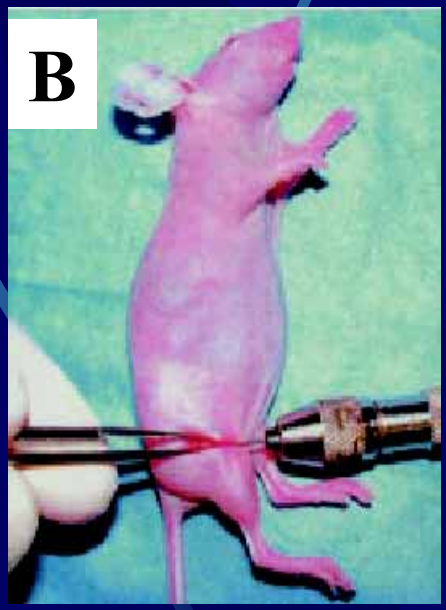
Experimental models and future directions in the study of solid tumors

Cellular continuous lines					
Prostate: PC3, DU145, LNCap, 22rv1, CWR22, CWR22R					
Brain Tumors: T98G, U373, U87MG, U251, SF539 Tumor Angiogenesis : HUVEC, UMEC					
Tumoral Cell Inoculation	**Model Aim**	**Effectiveness**	**Local growth**	**Bone Metastas.**	**Metastas. in other sites**
Subcutaneous	Local growth in physiologyc milieu	Good model to control tumor growth in vivo. Generally metastases are not observed	☺	✗	✗
Intracardiac	Extravasal Phase Diffusion on whole arterial system	Good rates of lumbar and lower limb metastases but poor rates of metastases in other sites. High technical difficulty with poor reproducibility	N.V.	☺	✗
Directly Intrabone	Local Growth in the sites of metastases	All the cases, by use of PC3, are positive. Other lines that don't metastasize bone can growth in the bone (LNCaP) with high rates.	☺	N.V.	✗

N.V. = non valuable ☺ = Good ✗ = absent ∿ = occasional

INOCULO CELLULE TUMORALI

Nave Mouse CD-1 nu/nuBR 4 week



100.000 cells are injected in 100 ml of physiologic solution
(That low cell number allows to simulate the real diffusion of tumor from his original site)

After 40 days (max), the animals are sacrificed.
Radiographies (also after 30 days) , autopsies and
Immuno-histochemical studies are performed

RADIOGRAPHIC VALUATION* OF METASTASES

Radiography whole body

Digital Magnification

Normal view

Osteolytic lesions

*Dedicate Machine: Faxitron

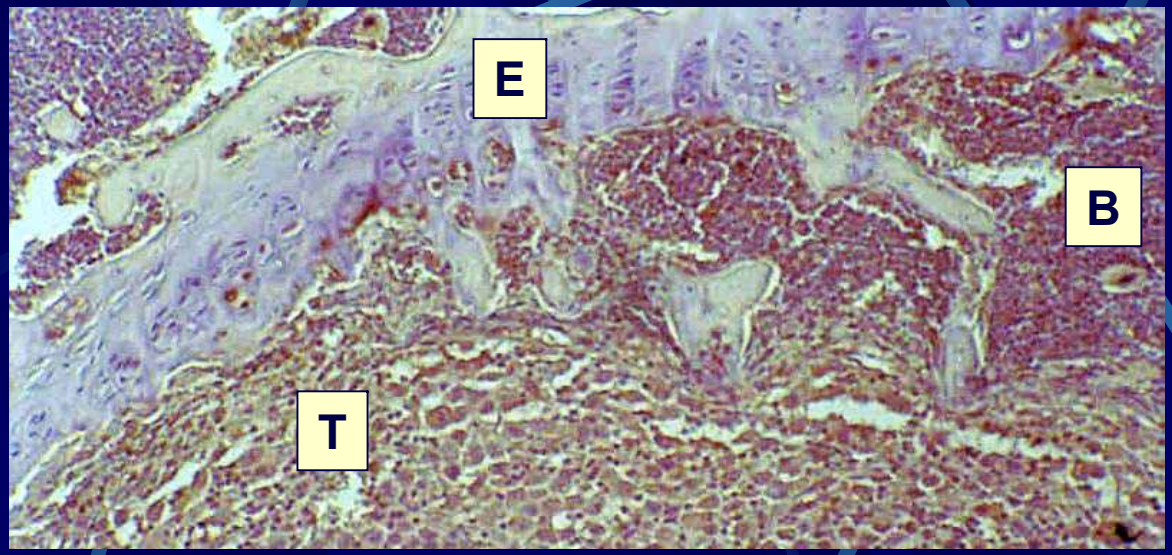
VALUTAZIONE E RACCOLTA DELLE METASTASI

Autopsy and Examination of the animals
Systematic Control of lungs, liver and adrenal glands

Evidence of metastatic lesions

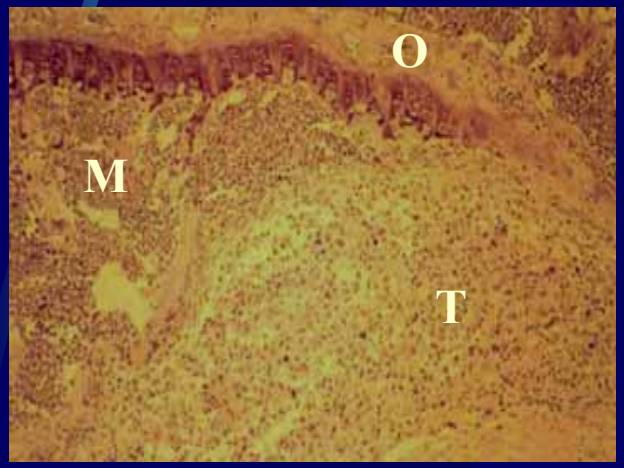
Recovery of metastatic tissues

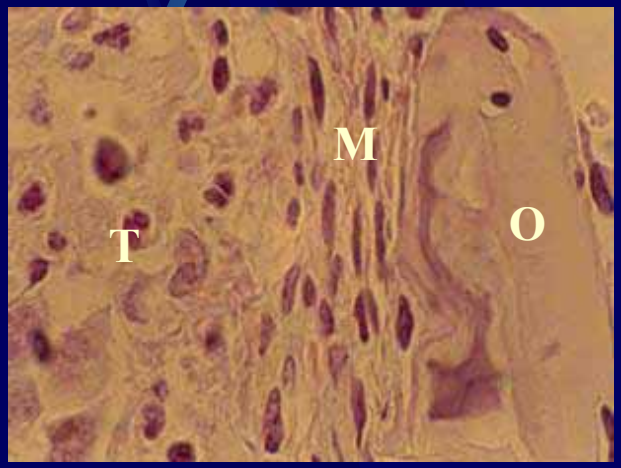
INTRACARDIAC INJECTION



MMP9 positive in bone metastases

B = bone marrow cells
T= tumor cells
E= cartilaginous epiphysis





Hematoxylin-eosin staining:
T= Tumor
O= Bone trabecula
M= Bone marrow

INTRATIBIAL INJECTION

The extension of osteolithic lesions is much variable. We may observe lesions of varied extension, from well localized and like point areas to large and destroying metastases that invade surrounding tissues. The epithelial origin confirmation of tumor sub lines is performed by immuno-histochemical array of 8 and 18 cytokeratins.

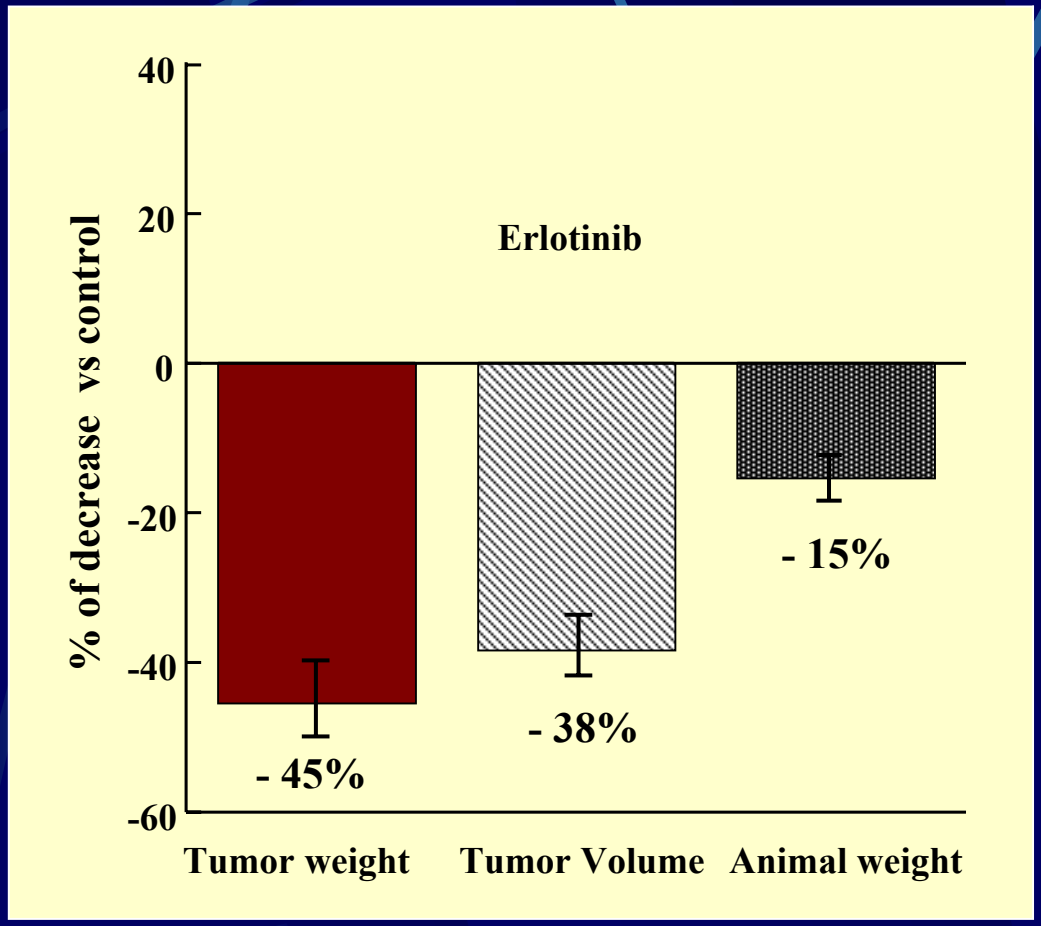
macroscopic view

radiographic view

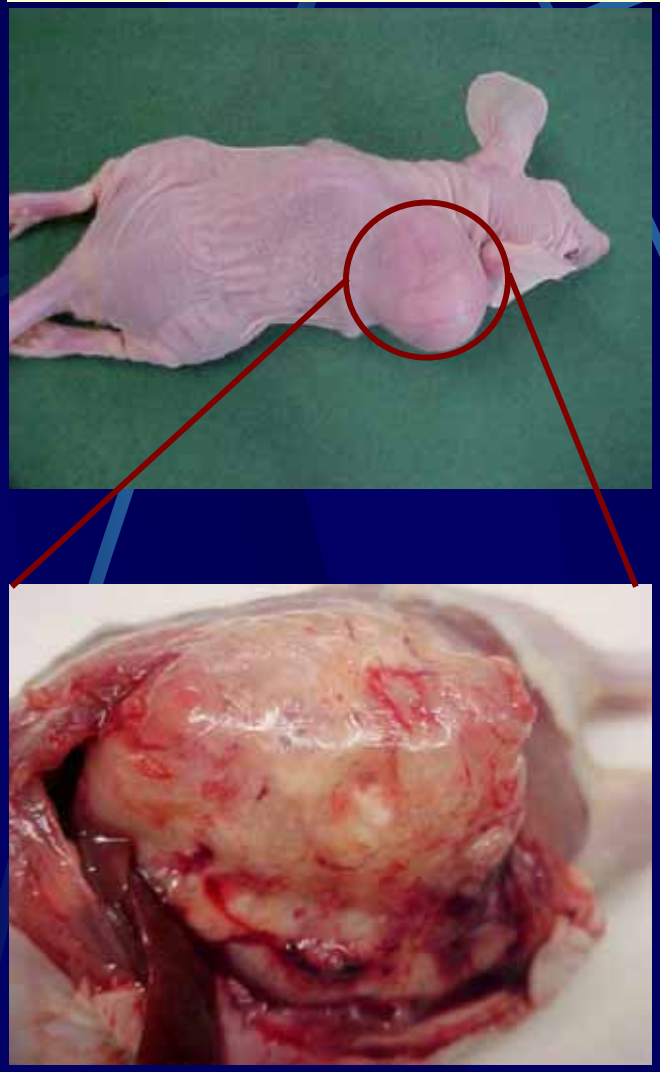
Enzymatic Digestion

ImmunoHistochemic of 18 Cytokeratin

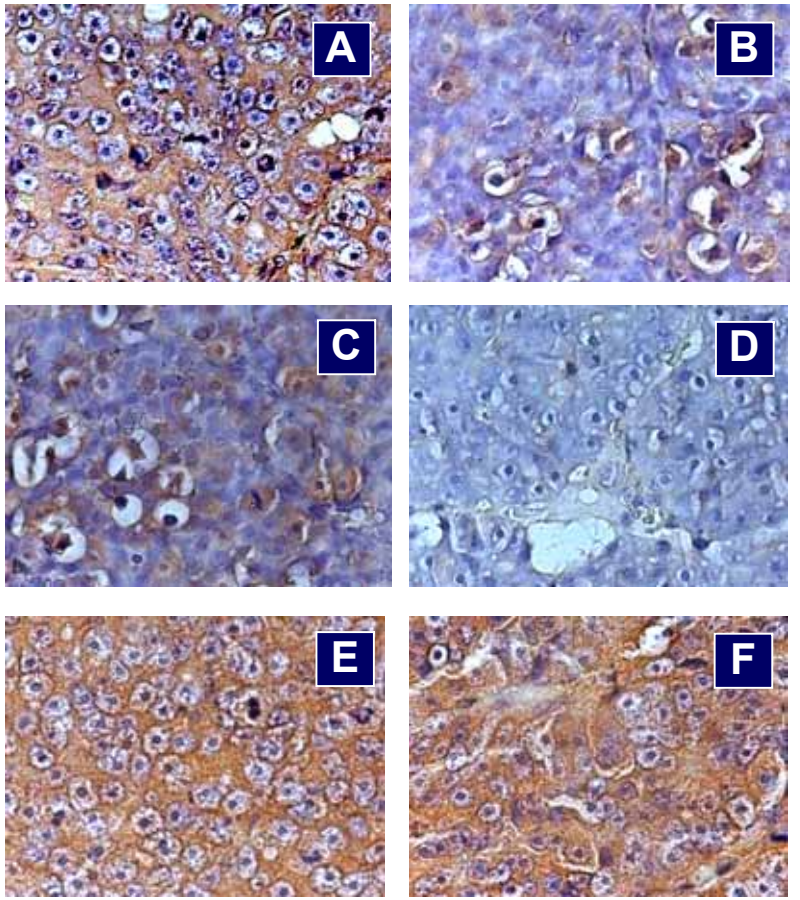
Local tumor growth in vivo: Xenograft Model



Local tumor growth in vivo: Xenograft Model



Morphologic Valuation



A: EGFR; B: HER2/neu; C: AKT; D: p-AKT; E: TRK-A; F: TRK-B

Injection Modality Tumor cells	Analysis Techniques of Tumor Tissues	
Subcutaneous	**Proteomic**: Analysis of intracellular proteins(Western Blot , Dot blot), FACS (Apoptosis, cell cycle , autophagy, expression of membrane antinuclear antigens) Immunohistochemistry	**Gene Analysis** : Analysis of messenger RNA (RT-PCR) DNA methylation
Intracardiac	**Proteomic**: Analysis of intracellular proteins (Western Blot , Dot blot) Immunohistochemistry	**Gene Analysis** : Analysis of messenger RNA (RT-PCR) DNA methylation
Intrabone Directly	**Proteomic**: Analysis of intracellular proteins(Western Blot , Dot blot) Immunohistochemistry	**Gene Analysis** : Analysis of messenger RNA (RT-PCR) DNA methylation

In progress Projects of translational researches

Prostate Tumors	Study of Epigenetic drugs (HDAC e Demethyilant) and Anti-target therapy like radio sensitizing in models of androgen dependent, independent and bone metastatic tumors
Brain Tumors	Study of Epigenetic drugs (HDAC e Demethyilant) and Anti-target like radio sensitizing in models of chemotherapy respondent and not respondent tumors
Tumor Angiogenesis	The chance of Epigenetic drugs (HDAC e Demethyilant) and Anti-target in tumor angiogenesis with and without Radiotherapy

Università Degli Studi Dell'Aquila

Facoltà di Medicina e Chirurgia

Dipartimento di Medicina Sperimentale
U.O. di Radioterapia
Presidio Ospedaliero – S. Salvatore – L'Aquila

Prof. Vincenzo Tombolini
Dott. Giovanni Luca Gravina
Dott. Pierluigi Bonfili
Dott. Mario Di Staso

Dipartimento di Medicina Sperimentale
Laboratorio di Istopatologia

Prof. Anna Teti
Dott. Nadia Rucci

Dipartimento di Medicina Sperimentale
Laboratorio di Patologia Generale

Dott. Claudio Festuccia

Dipartimento di Medicina Sperimentale
Laboratorio di Patologia Clinica

Prof. Vincenza Dolo
Dott. Danilo Millimaggi
Dott. Sandra D'Ascenzo