



Molecular mechanisms involved in organ-specific metastatic growth processes in breast cancer

LSHC-CT-2004-503049

EU FRAMEWORK 6 PROGRAMME

- Specific Targeted Research Project (STREP)
- 36 months: 2004-2006/7

Why MetaBre?

- More than 200,000 women diagnosed with breast cancer in Europe every year
- Lifetime risk of developing breast cancer is ~1 in 10
- Leading cause of death in women between ages 35 to 55
- Although survival rates are improving, prognosis is poor once distant metastases are established
- **Lymphatic, bone, brain and visceral metastases present distinct pathological and therapeutic issues**

Objectives of MetaBre

- Identification of genes and proteins involved in **organ-specific** metastasis in breast cancer
- Investigation of previously identified candidate genes
- Functional analysis and molecular mechanisms of action
- Evaluation of signatures and biomarkers for organ-specificity and development of new diagnostic tests
- Development of therapeutic agents towards clinical trials

MetaBre partners and activities

❖ UNIVAQ

- Anna Maria Teti, Bone Biopathology Group,
Dept Experimental Medicine, University of L'Aquila, ITALY
 - coordination and scientific leadership
 - collection of tissue samples
 - gene profiling related to bone metastasis
 - investigation of organ-tumour cell interactions in bone
 - in-vivo testing of therapies and clinical trials of diagnostic techniques.
 - Includes M.Baldwin (project manager) and P.Gromov proteomics advisor

MetaBre partners and activities

❖ CMNS

- Roberto Buccione, Molecular Pharmacology of Tumour Cells Unit, Dept Cell Biology and Oncology, Consorzio Mario Negri Sud, ITALY
 - characterisation of invadopodia/podosomes
 - molecular machinery of trafficking and secretion of MMPs.

❖ CRH

- Rosette Lidereau, Centre René Huguenin, Saint-Cloud, FRANCE
 - collection of clinical samples
 - gene profiling and functional analysis

MetaBre partners and activities

❖ INSERM

- Philippe Clezardin, INSERM Unit 664, Lyon, FRANCE
 - gene profiling and proteomic analysis
 - in vivo assays of bone metastases (MDA MB 231 B02 xenograft model)

❖ PSK

- Philippe Clement-Lacroix, ProStrakan, Romainville, FRANCE
 - gene profiling (Affymetrix platform)
 - development and testing of pharmacological therapies for bone metastasis

MetaBre partners and activities

❖ LUMC

- Gabri van der Pluijm, Department of Endocrinology, University of Leiden Medical Centre, NETHERLANDS
 - bioluminescent in-vivo imaging of metastases
 - in vitro and in vivo models of bone metastasis
 - gene function studies

❖ WAU

- Maciej Ugorski, Dept Biochemistry, Agricultural University of Wroclaw, POLAND
 - mechanisms of cell adhesion and invasion
 - role of carbohydrate antigens

MetaBre partners and activities

❖ ICR

- Suzanne Eccles, Tumour Biology and Metastasis Group,
Cancer Research UK Centre for Cancer Therapeutics, ICR, UK
 - deputy coordinator
 - mechanisms of metastasis to lymph nodes (*in vitro* and xenograft models)
 - ErbB oncogene signalling pathways as targets for therapy

❖ IRO

- Angels Sierra, Center of Molecular Oncology,
Institut de Recerca Oncologica, Barcelona, SPAIN
 - proteomics and protein interaction network analysis (PIANA)
 - molecular mechanisms of metastasis to brain
 - imaging of metastases with MRI/MRS

MetaBre partners and activities

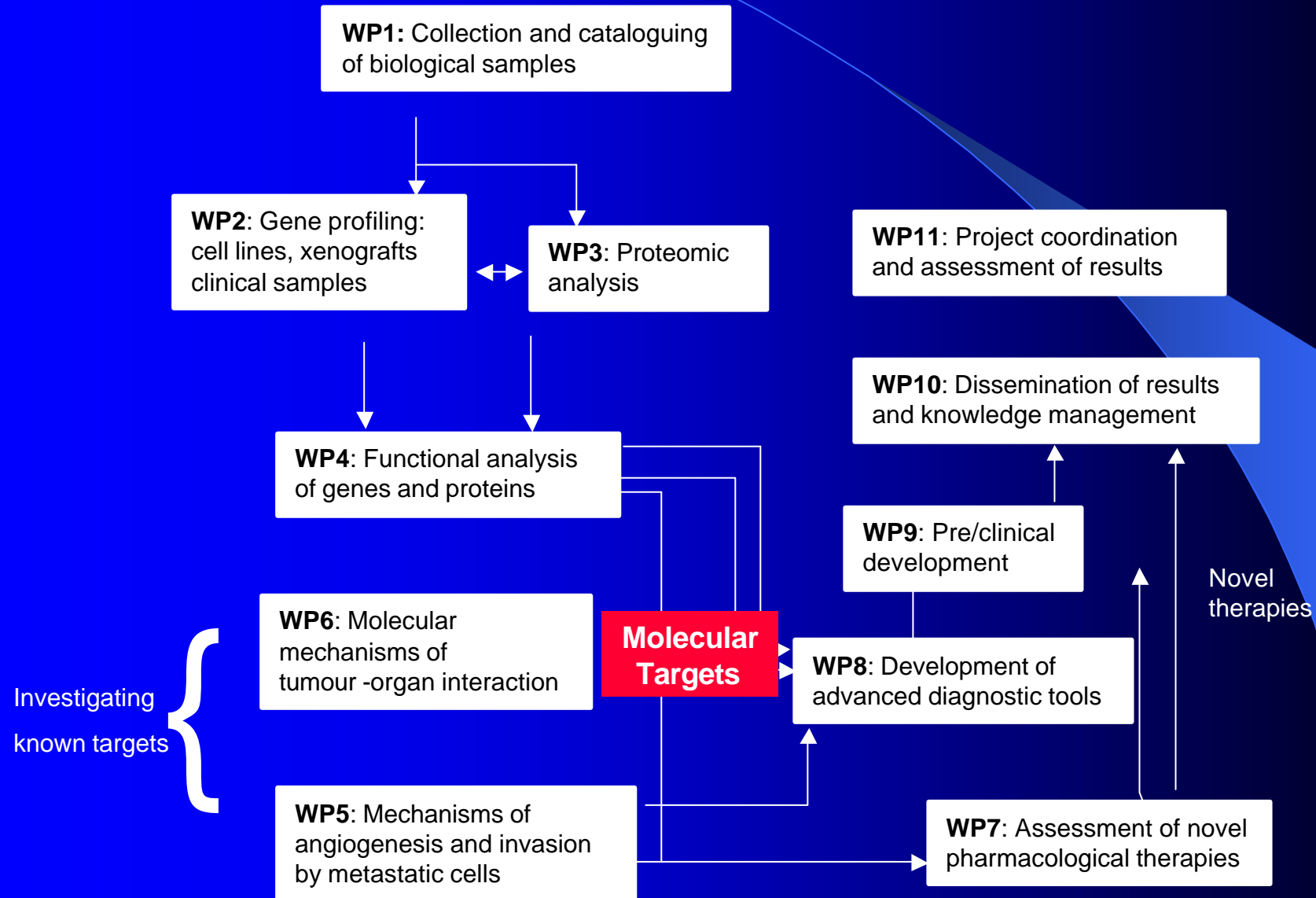
❖ UGent

- Marc Bracke, Laboratory of Experimental Cancerology, Faculty of Medicine and Health Sciences, University of Gent, BELGIUM
 - Role of cadherins in angiogenesis and invasion
 - development of diagnostic techniques

❖ ULG

- Vincent Castronovo, Metastasis Research Laboratory, University of Liege, BELGIUM
 - collection of tissue samples, gene validation
 - bone matrix protein expression in osteotropic cancer
 - angiogenesis inhibition (*in vitro* and *in vivo* models)

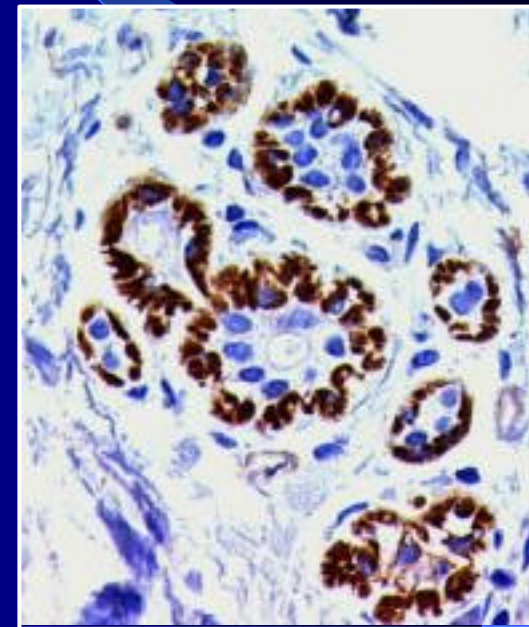
MetaBre workplan



MetaBre achievements to date

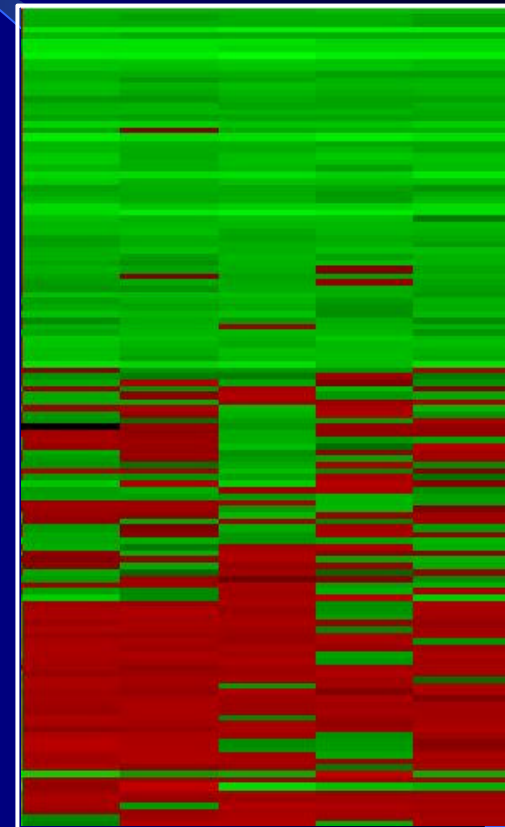
WP1: Collection and cataloguing of biological samples

- Catalogued collection of frozen and fixed clinical samples with detailed patient histories
- Collection of sera for validation of circulating biomarkers
- Characterisation of human breast cancer cell lines and metastasis models for functional studies



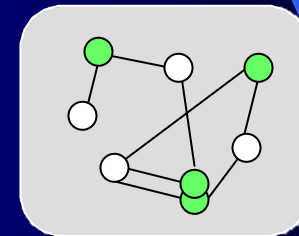
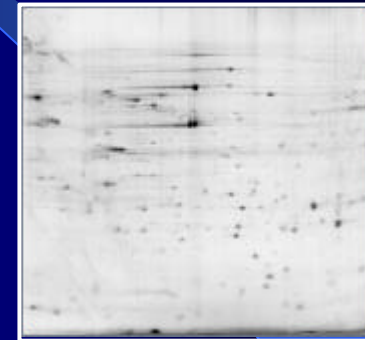
WP2: Gene profiling of tissue samples and cell lines

- Gene expression profiling of B02 cell line and other organ-specific cell line variants
- Gene expression profiling of metastases vs normal tissue and primary breast tumours
- Identification of signatures for site-specific bone, lung, liver and brain metastases
- Selection of genes for validation and functional analysis in WP4



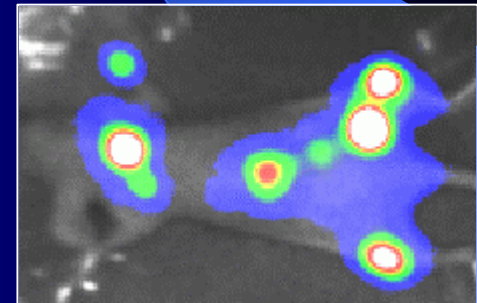
WP3: Proteomic analysis

- Identification of proteins differentially expressed in metastatic breast cancer cell lines showing tropism for lung or brain
- Comparison with Affymetrix gene profiling data identified common expression patterns
- Protein interaction maps (PIANA) used to identify potential functional pathways



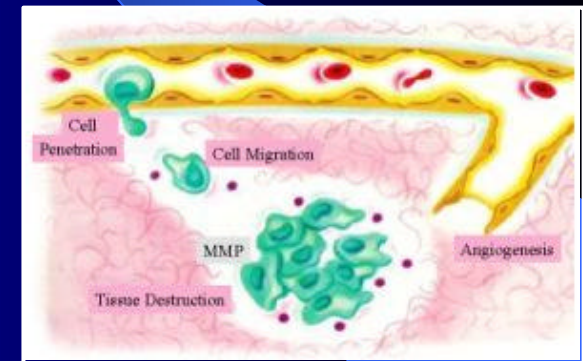
WP4: Functional analysis of key genes and proteins

- Key genes selected from B02 analysis for further investigation
- Additional genes selected from gene profiling and proteomics of bone/soft tissue metastases
- *In vivo* “proof of concept” for RNAi silencing of genes of interest in FRT cell lines with optical reporter genes
- Overexpression and silencing of genes *in vitro* and *in vivo* to be performed in year 3-4



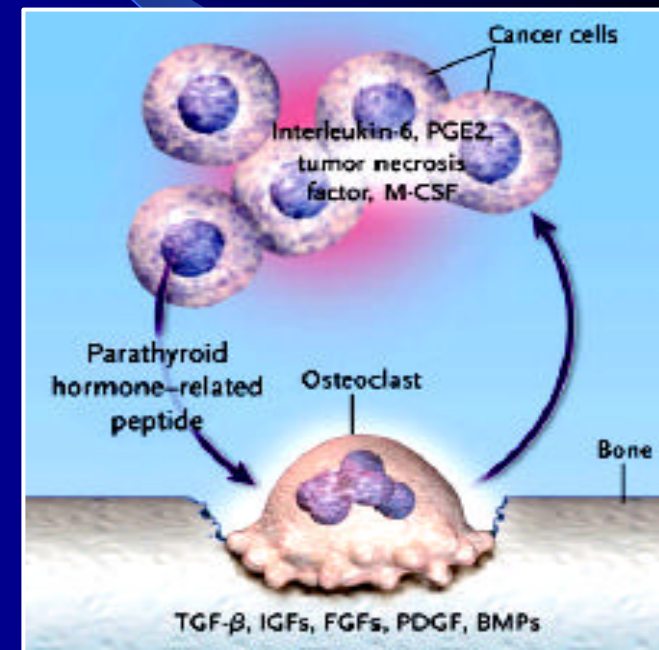
WP5: Mechanisms of angiogenesis and invasion by metastatic cells

- Elucidation of mechanisms of action of known candidate genes
- Identification of cell lines for study of sialyl LeA antigens and successful inhibition with siRNA
- Analysis of lymphatic metastases from different experimental models to seek evidence for “active” vs “passive” mechanisms of dissemination
- Studies on the intracellular transport of MT-MMPs and mapping of protein-protein interactions in invadopodia.



WP6: Molecular mechanisms of tumour-organ interactions

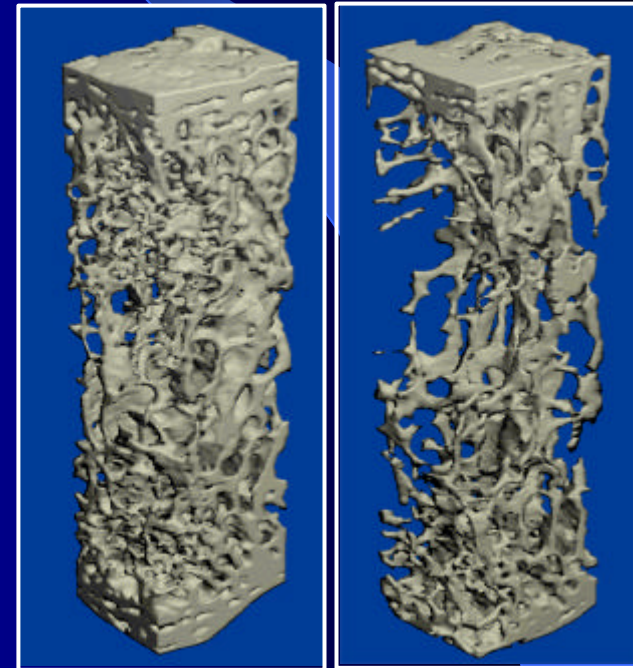
- MAPK-Src-integrin signalling pathways involved in bone metastasis
- Assays established for studies of tumour-endothelial cell interactions
- Model developed for analysis of tumour-osteoclast co-operativity



(from Roodman G.D., 2004)

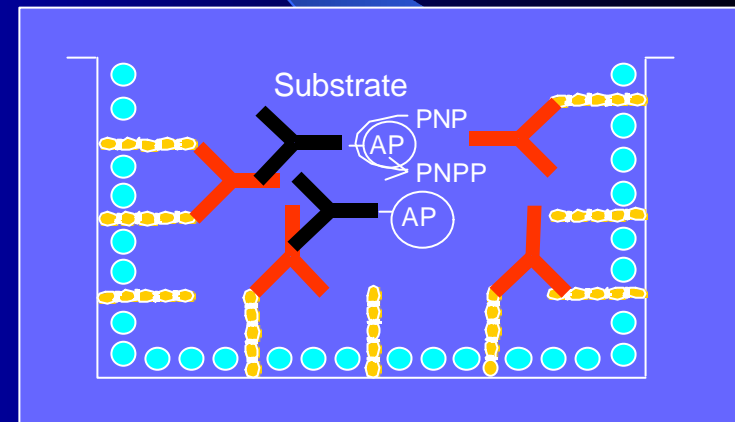
WP7: Development of novel pharmacological therapies

- Recognition that cSrc inhibitors act on metastatic cancer cells as well as inhibiting bone resorption in osteolytic lesions *in vivo*
- *In vitro* and *in vivo* testing of $\alpha v \beta 3$ -integrin antagonists for treatment of bone metastases



WP8: Development of advanced diagnostic tools

- Development of ELISA sandwich assay for serum markers
- Characterisation of experimental brain metastases by MRI and MRS
- Validation of new biomarkers in 2006-7



WP9: Clinical development

- Preclinical development by ProStrakan of non-peptide $\alpha v \beta 3$ integrin antagonist for therapy of bone metastases (PK, tox)
- Testing of diagnostic ELISA with serum samples from patients with cancer and other diseases
- Testing of additional therapies and diagnostic tests in 2006-7



MetaBre key scientific milestones for 2006

- Validation of organ-specific signatures in further clinical samples by immunohistochemistry and RT-PCR
- Functional analysis of key genes/proteins in human tumour cell lines and xenografts and validation of new therapeutic targets
- Preclinical development of new therapies and diagnostic tests



MetaBre

Molecular mechanisms involved in organ-specific
METAstatic growth processes in BREast cancer

Thank you for your attention

<http://www.metabre.org>