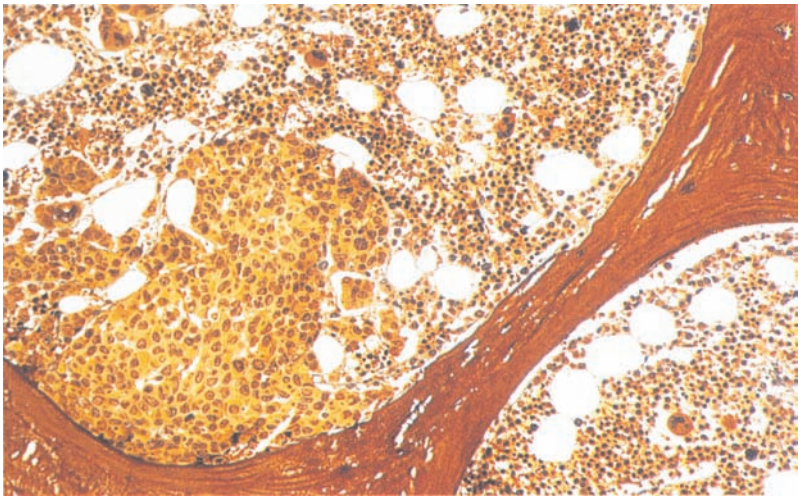
promet

PROstate cancer Molecular-oriented detEction
and Treatment of minimal residual disease



# prostate cancer molecular-oriented detection and treatment of minimal residual disease

Specific Targeted Research Project
funded by the European Commission under
the Sixth Framework Program
Contract no: LSHC-CT-2006-018858

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**pictures**
**cover:** Micrometastatic cancer deposit in the bone marrow (Univ. of Bern, Dep. of Urology)
**page 10:** Bone marrow micrometastases in bone (left) and magnification (right) (Univ. of Bern, Dep. of Urology)
**page 13** left: Magnified colony from clonogenic assay with PrEGM serum-free medium (Univ. of Bern, Dep. of Urology), right: The new emCCD camera and the fast filter changer (Berthold Technologies)
**page 15** left: Fluorescent imaging of quantum-dot labelled cancer cells (705 nm nanoparticles) (Medical Center Leiden), center and right: Small blood vessels, imaged by the optoacoustic technique. (Univ. of Bern, Inst. of Applied Physics)
**page 16** left: Sphere formation on ultra low adherent plates with PrEGM serum-free medium. (Univ. of Bern, Dep. of Urology), right: X ray of the axial skeleton with osteosclerotic bone metastases. Note the dense whitish structure of the vertebral body in the upper part of the image (Univ. of Bern, Dep. of Urology)

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In the European Union ~200.000 men are diagnosed with **prostate cancer** every year and this number is likely to increase due to the growth of the population at risk because of ageing. Through the progress made in the treatment of the primary tumour, mortality in cancer patients is increasingly linked to metastatic disease; often occult (= micrometastasis or "minimal residual disease" MRD) at the time of diagnosis/therapy of the primary tumor.

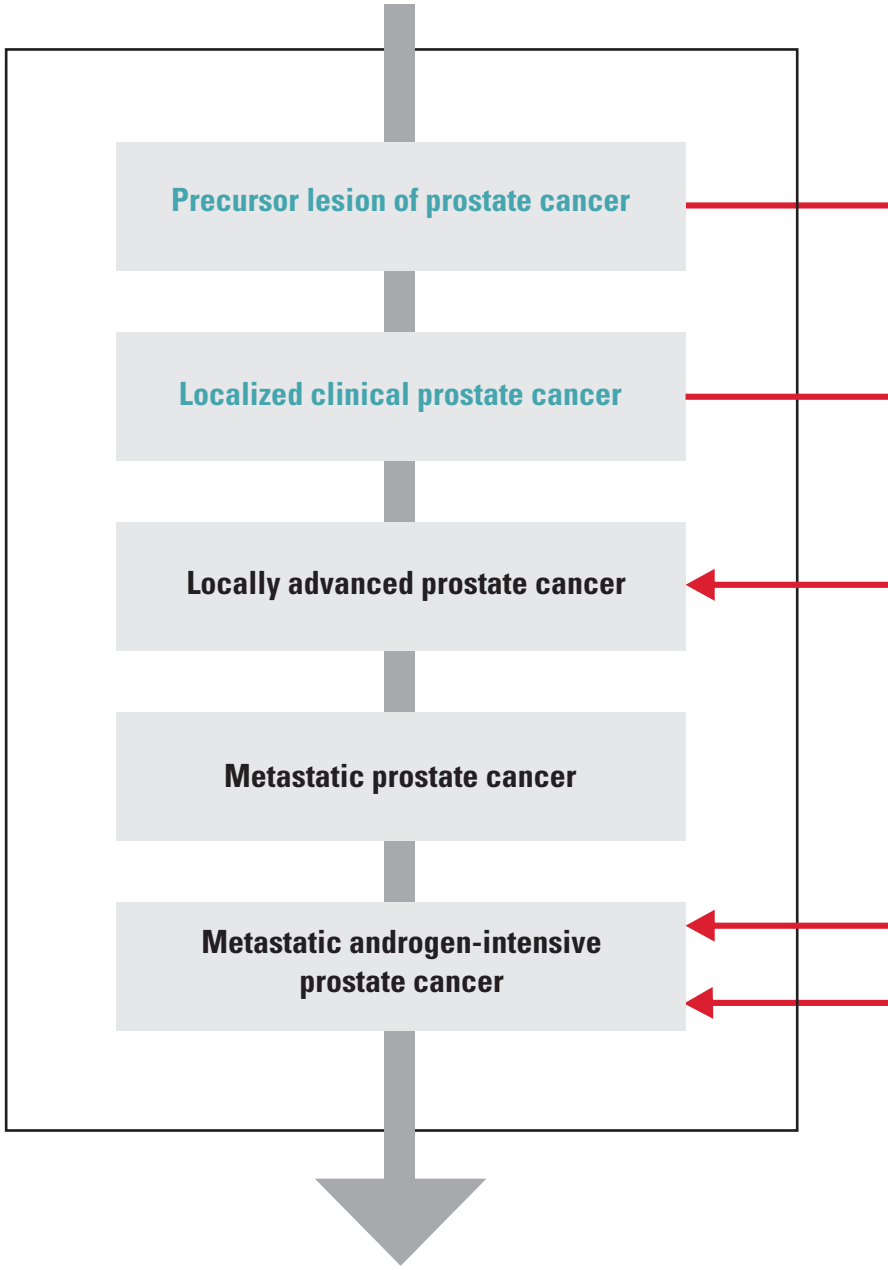
Understanding the complex mechanisms of metastasis formation from circulating tumour cells into micrometastases into metastases at the molecular and physiological level is crucial for successful detection of minimal residual disease and for evolving possible strategies for the prevention of their development into overt metastases.

In this project, called **promet**, we intend to elucidate the mechanisms and the signature of minimal residual disease in prostate cancer for improving diagnostics and developing novel therapeutic approaches to prevent the formation of minimal residual disease to overt metastases.

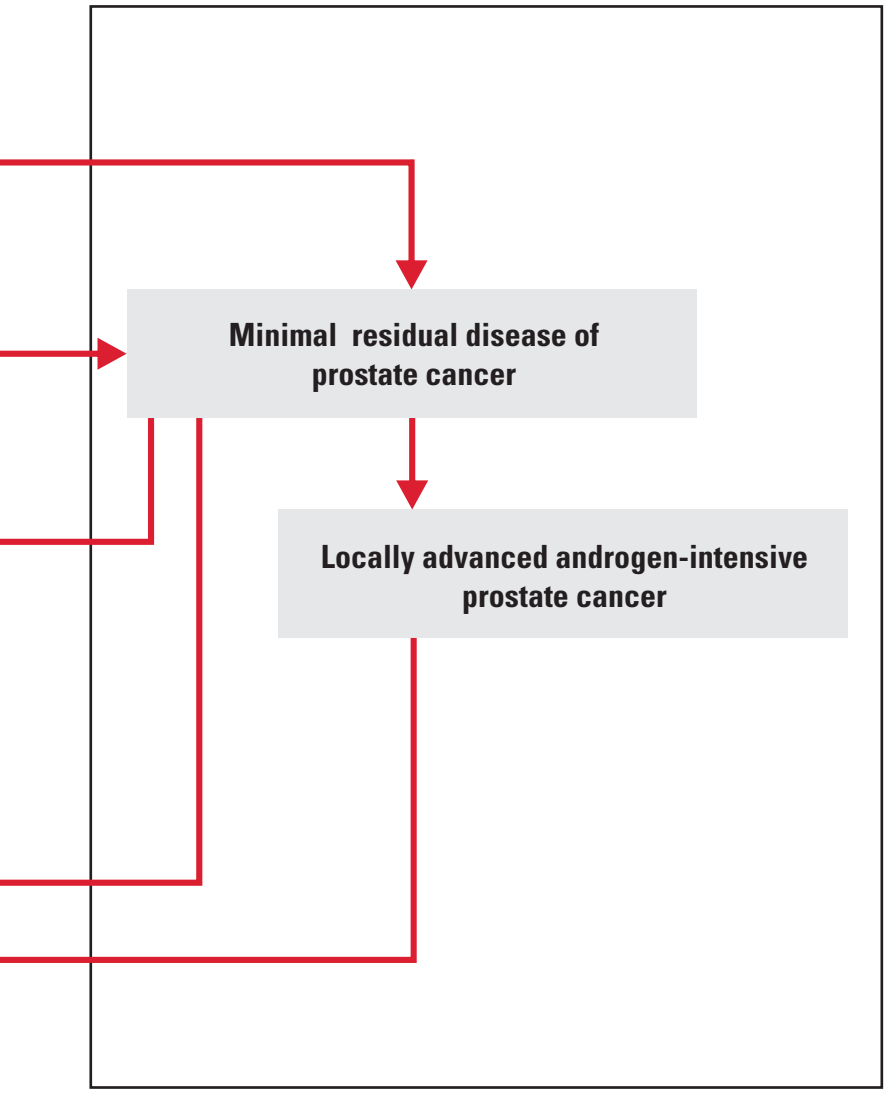
**The goal** is to identify at least 2 signal transduction targets, to develop a diagnostic test for the detection of the presence of minimal residual disease and to define a novel therapeutic strategy for the treatment of this disease in prostate cancer.

Thus, earlier detection and disease-specific treatment may decrease morbidity and mortality and ultimately have an impact on socio-economical costs.

In this targeted approach to combat minimal residual disease in prostate cancer we will pursue different approaches at the various levels at which to attack the malignant process and validate them at a phenotypic and functional level.



# promet concept




The clinical stages of prostate cancer progression
- green: curative intervention available
- black: no curative options, i.e. unmet needs!

Meddiscovery

Development of therapeutic proteins for the treatment of urogenital cancer

**Dr. David Deperthes**
**Dr. Laila Barki**

BERTHOLD TECHNOLOGIES

Development of fluorescent / bioluminescent imaging systems for in-vivo application

**Dr. Manfred Hennecke**

FUKUDA DENSHI
FUKUDA DENSHI Switzerland AG

Development of diagnostic ultrasound systems

**Dieter Schweizer**
**Dr. Joël Niederhauser**

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## Evaluate the in vivo detection of minimal residual disease by means of nanoparticles and optoacoustics

2

## Developing an integral in vivo model of minimal residual disease allowing the study of the mechanisms and signatures

**University of Bern**
**Department of Urology**

Prostate cancer progression and bone metastases
Therapy of solid urological tumours and bone metastases

**Prof. Dr. George Thalmann**
**Dr. Marco Cecchini**

**University of Bern**
**Institute of Applied Physics**

Generation and detection of electro-magnetic radiation
Laser systems

**Prof. Dr. Martin Frenz**

The University Of Sheffield.

**University of Sheffield**
**Academic Urology Unit**

Large scale clinical trials in prostate cancer
Mechanisms of progression in prostate cancer

**Prof. Dr. Freddy Hamdy**
**Dr. Colby Eaton**

# promet approach + partners

tp21
technology partner

Project management
Public relations

**Dr. Petra Zalud**
**Dr. Hanno Wittig**

## Developing a therapeutic strategy for the treatment of minimal residual disease in prostate cancer

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## Identifying and validating at least 2 target genes for detection of minimal residual disease in prostate cancer

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**Leiden University Medical Centre**
**Department of Urology**

Long time clinical studies
Treatment of bone metastases from prostate cancer

**Dr. Gabri van der Pluijm**

**Institut National de la Santé et de la Recherche Médicale, U 403**

Pathophysiology of osteoporosis
Diagnosis and care of skeletal metastases

**Prof. Dr. Philippe Clézardin**
**Prof. Dr. Marc Colombel**

**Deutsches Krebsforschungszentrum**
**Biochemical Cell Physiology Group**

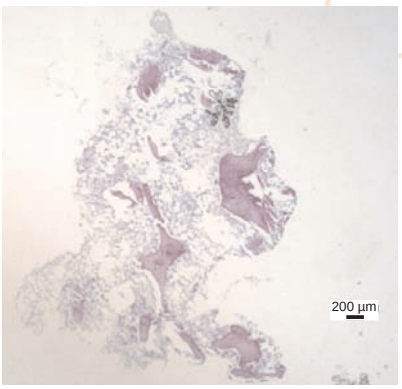
Pathomechanisms of proliferative processes
Tumour-associated bone diseases

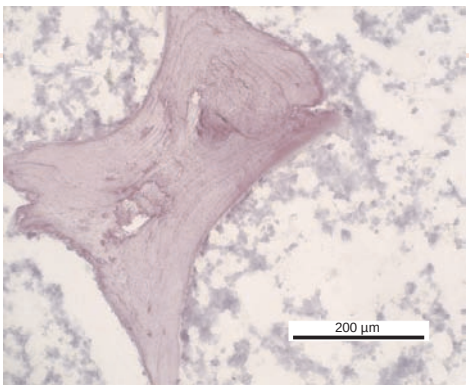
**Prof. Dr. Walter Pyerin**
**Dr. Karin Ackermann**

The overall aim of **promet** is to improve our understanding of the mechanisms and the biology of micrometastases. For this, the micrometastatic cell population needs to be isolated. Until recently, the state-of-the-art of evaluating micrometastatic cells was based on the detection of epithelial cells in circulation. From the development of cancer we have learned that cells may change their phenotype and alter their genetic profile. Thus, cancer cells may not express the same markers when in the tumour and when in circulation, hence during the micrometastatic process. Some of the genes involved in this process are related to stem cells. There is a growing body of evidence that micrometastatic cancer cells have stem cell features called cancer stem cells (CSCs). Stem cells are much rarer than differentiated cells and have self-renewal capacity. This significantly decreases the number of cells responsible for active micrometastatic disease.

The consortium is establishing a method to isolate such cells and has identified several potential markers for the isolation of CSCs that are currently being validated. Once CSCs are isolated they will undergo gene expression analysis with gene arrays and be analysed by proteomics. The consortium intends to identify and validate at least 2 target genes for the detection of minimal residual disease.

Reading the signatures of micrometastasis by genomic and proteomic profiling under stringent conditions with highly selected tissue and processed with pre-established and evaluated techniques is a prerequisite for the understanding of this decisive and often fatal step in the progression of the disease. For genomic profiling, adequate biostatistical analysis is of utmost importance. Proteomic analysis depends on experience and allows the isolation of potential markers and therapeutic targets. By evaluating selected experimental and



# Identifying and validating

clinical samples of blood, bone marrow and tumor tissue, validated and novel markers can be assessed.

Only a close collaboration of large clinical centres, with the necessary turnover and experience, with specialised basic science units will provide the necessary basis for the study of this important clinical process in the biology and natural history of the disease.
In addition, this collaborative network allows for the multi-centre validation of potential markers found by single centre experience in a short period of time. Novel markers can be evaluated in tissues of established tissue repositories and prospectively assessed by the clinicians. Detection of minimal residual disease is critical. Several approaches have been taken in the literature, such as RT-PCR and immuno-histochemistry, which will be validated by the partners of the consortium. New potential markers will be evaluated in samples of primary and secondary prostate cancer, as well as in serum, bone marrow aspirate and urine samples.

**Identifying and validating at least 2 target genes for detection of minimal residual disease in prostate cancer**

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More innovative technologies, such as use of new fluorescent markers or optoacoustical detection of micrometastasis, will be used as well.

We expect to provide one detection assay with the potential for use in clinical practice based on blood, urine or bone marrow aspirates and evidence that optoacoustics can be applied in clinical context.

Adequate experimental models are mandatory for a better understanding of the early steps of the metastatic cascade that lead to colonization and growth of micrometastatic cells, and to analyze the efficacy of novel therapeutic strategies. However, until recently, the majority of the cancer models in vivo were analyzing only late, "macroscopic" stages of the metastatic tumor burden, where most likely the neoplastic growth has become autonomous and interference with the environmental growth support of the tumour is compromised.

Therefore, more sensitive methods to detect and monitor directly metastatic growth in bone marrow/bone of whole animals need to be developed. In vivo expression of reporter genes encoding bioluminescent proteins can be detected externally by sensitive detection systems. Enzyme induced light (bioluminescence) has sufficient intensity to cross animal tissues provided that the endogenous light has a wavelength between 600 and 900 nm. Within this wavelength range, photons are less absorbed by tissue, especially hemoglobin. The consortium is establishing a bio-luminescent system capable of detecting 10 to 20 cells clustered in the bone marrow of animals by enhancing both

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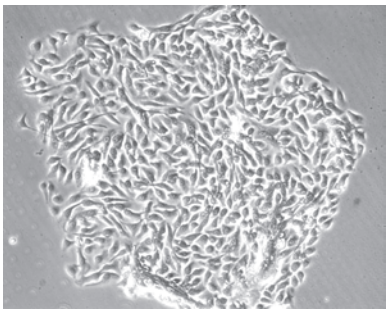
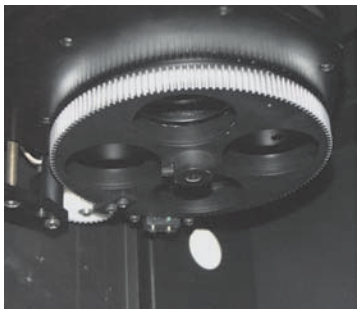
## Developing an integral in vivo model of minimal residual disease allowing the study of the mechanisms and signatures

# Integral in vivo model

the sensitivity of the CCD-camera detectors and the expression of the bioluminescent reporter gene luciferase. It is now possible to follow in real time, non-invasively and repetitively the early settlement of tumor cells in vivo. A major advantage of this system is, that the number of animals used in experiments can be drastically decreased compared e.g. to histology. In addition, detection at two different wavelengths or combining bioluminescence with fluorescence in the same instrument will offer the capability to image two different parameters at the same time, thus offering the possibility to track two different steps of the early bone metastatic process, i.e. tumour growth and tumour cell differentiation.

Fluorescence microscopy offers the potential to follow in real time living cells at high resolution in living animals. Semiconductor based quantum dot (QD) nanoparticles allow simultaneous imaging of different cell populations each tagged with QD nanoparticles emitting different wavelengths. We intend to couple QDs and emission spectrum scanning multiphoton microscopy to develop a means to study adhesion and extravasation in vivo; combined with the whole body bioluminescent reporter imaging this will provide a strong tool to understand the micrometastatic process.





Micrometastases are frequently missed and are elusive to therapy. Detection of minimal residual disease in lymph nodes until now is only possible by microscopy and RT-PCR technology.

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## Evaluate the in vivo detection of minimal residual disease by means of nanoparticles and optoacoustics

Gene amplification techniques, which have a high sensitivity, have been reported to improve diagnostic tests. RT-PCR has been used to detect circulating cells shed by solid tumours; promising results have been reported, but very few studies were able to predict outcome. The consortium will evaluate, in different historical sets of patients who underwent radical prostatectomy with a long follow up, the potential of RT-PCR to predict long term outcome. Immunohistochemical evaluation of factors that contribute to tumour cell survival and progression have been reported to predict outcome and survival. The evaluation of predictive factors in the primary tumour are of interest to clinicians. This simple approach needs to be validated in larger series by the consortium.

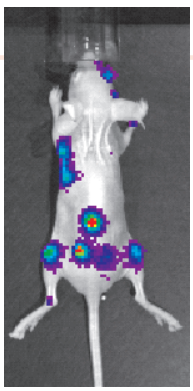
Optoacoustics is a combination of ultrasound with laser technology. Up to now, the main source of contrast in optoacoustic tissue imaging was hemoglobin. Tumour detection, at least in an advanced stage, was possible due to the dense angiogenesis-related network of blood vessels in and around a tumour. Although some of the drawbacks of optical imaging techniques, namely the limited resolution caused by the strong light scattering properties of biological tissue, have been resolved, the imaging of tumours in an early stage is almost impossible due to the lack of selectivity and specificity.

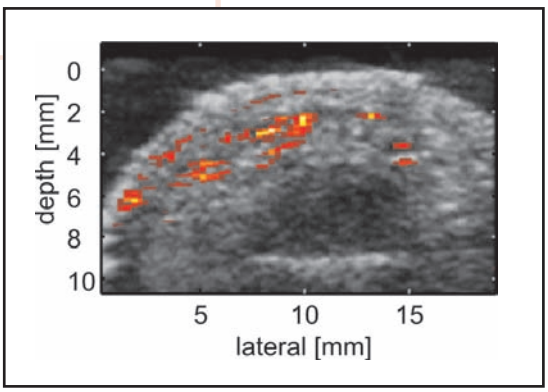
# In vivo detection

The consortium will evaluate antibody coated gold nanoparticles that allow for a more selective and specific detection of tumour cells at an earlier stage.

Development of an optoacoustic biosensor based system to accurately image lymph node metastases represents an important progress in the management of minimal residual disease. The optoacoustic system combines optical excitation of antibodies coupled to gold nanoparticles using an alexandrite laser and detection of the laser generated ultrasonic waves with modified ultrasound detectors. The idea is to merge biological (antibodies) and non-biological (nanoparticles) systems to bioconjugates in order to combine the functionalities of bio-molecules and non-biologically derived molecules for selective imaging tumor tissue structures using optoacoustic techniques. A further objective is to use the strong energy absorption of gold nanoparticles to selectively damage the tumour cells for treatment of minimal residual disease.

A benefit of this approach over photodynamic therapy is that gold nanoparticles are nontoxic and tissue damage only occurs when the temperature is locally raised above approximately 60°C, thus unwanted side effects can be significantly decreased. In close collaboration with Fukuda, a leading producer of medical ultrasound systems who has a long experience in the development and integration of signal processing algorithms into soft - and hardware, and in their combination with an appropriate user interface in commercially sellable ultrasound systems, the irradiation laser and the medical ultrasound system will be combined and optimized for imaging lymph node metastases.



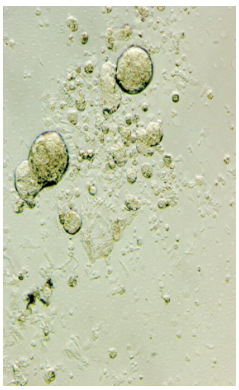


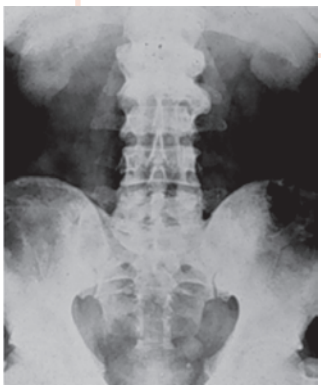
Treatment of minimal residual disease is elusive to therapy as micrometastatic cells may stay dormant (G0) for long periods of time. Therefore current strategies such as chemotherapy have limited effect.

Several innovative preventive and treatment approaches will be established and tested in **promet**. Strategies that impede homing, block invasion, interfere with local growth support, induce cell death or push tumour cells towards differentiation have the potential to efficiently treat minimal residual disease and prevent the development of overt metastases.

Once tumour cells are in circulation, homing to the preferred environment is the first step in the metastatic process. Interference with this process may significantly reduce the number of cells finding a fertile soil. **promet** will evaluate different approaches to block homing of tumour cells to their preferred environment.

For the final development of a metastasis it is essential that the metastatic cells can adapt, and therefore survive and grow, to the specific organ environment that they colonize. Genetic instability within the cancer cell population(s) of the primary tumour is responsible for the acquisition of these new properties. However, acquisition of "all" these properties is a relatively rare event and, consequently, the metastatic process is highly inefficient and only a restricted minority of cancer cells reaching the blood will survive and grow at the distant sites. This suggests that therapeutic interference with the local growth support may be far more efficient than inhibition of earlier steps of the metastatic process, such as cancer cell disaggregation from the primary tumour and intravasation, that seems to be accomplished by a much greater number of cancer cells.





# Therapeutic strategy

Crucial for initiating growth in the bone marrow/bone is the ability by the cancer cells extravasating at this site to establish a cross talk with the stromal compartment of this tissue. Survival and growth promoting molecules locally released during bone resorption/formation are critical for the progression of micrometastatic deposits to overt metastasis. Several putative factors are under evaluation but little is still known on the effector gene pathways that eventually initiate growth in bone micrometastatic cells.

## Developing a therapeutic strategy for the treatment of minimal residual disease in prostate cancer

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Accordingly, we plan to investigate the effect of under- and over-expression of those genes of interest that may encode for growth factor receptor or intracellular signalling molecules previously not investigated in relation to bone micrometastatic growth. Whole body bioluminescent imaging will be essential for tracking and monitoring correctly tumour growth in bone micrometastatic animal models.

Further understanding of these processes and pathways will allow to elucidate this "vicious cycle" of interaction of the tumour cell with its preferred environment and will provide new potential therapeutic targets for this unmet need in prostate cancer therapy.

Different approaches of therapy will be tested, such as potential ligands developed by MedDiscovery.

**promet contact**

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SIXTH FRAMEWORK PROGRAMME

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