



Prostate cancer molecular-oriented detection and treatment of minimal residual disease

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In the European Union ~200.000 men are diagnosed with prostate cancer every year and that number is likely to increase due to a growing population at risk due to ageing. Because of 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") at the time of diagnosis/therapy of the primary tumor. Understanding the complex mechanisms of metastasis (circulating tumor cells - micrometastasis - metastasis) 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 metastasis.

The aim of PROMET is to elucidate the mechanisms and the signature of minimal residual disease in prostate cancer and to develop novel therapeutic approaches to prevent the development of minimal residual disease to overt metastasis. In close collaboration of basic scientists with clinical researchers the pathways of minimal residual disease are explored using functional genomics and expression profiling as technology platforms, advanced experimental models of minimal residual disease using bioluminescence, multiphoton microscopy, nanotechnology and optoacoustic technology for detection and treatment. Innovative imaging and therapeutic strategies developed by the industry and selected for their potential to enhance detection and eradicate minimal residual disease are tested in preclinical models for subsequent clinical evaluation.

The goal is to identify at least 2 signal transduction targets and 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 various levels at which we attack the malignant process and validate these at a phenotypic and functional level. We will be developing novel means of detecting and treating minimal residual disease. By integrating a variety of state of the art approaches, we aim at

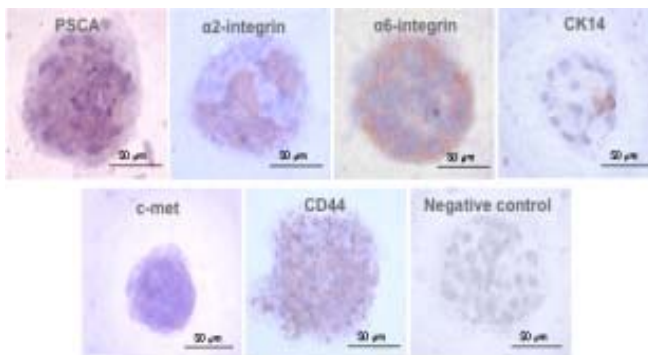
- Identifying and validating at least 2 target genes for detection of minimal residual disease in prostate cancer
- Developing an integral *in vivo* model of minimal residual disease allowing the study of the mechanisms and signatures
- Evaluate the *in vivo* detection of minimal residual disease by means of bioluminescence and nanoparticles with optoacoustics
- Developing a therapeutic strategy for the treatment of minimal residual disease in prostate cancer

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

Why is detection and if possible treatment of minimal residual disease of importance and an unmet need? Treatment of clinically organ confined prostate cancer in curative intent is limited by tumor recurrence in approximately 20 to 30% of patients. At this moment no tools or markers apart from tumor stage and Gleason score system exist to predict tumor behaviour, treatment outcome and prognosis in these patients. This is among other factors due to the incapacity of current staging tools to identify the *metastasis initiating cells* (MICs) or *disseminated tumour cells* (DTCs or occult metastasis).

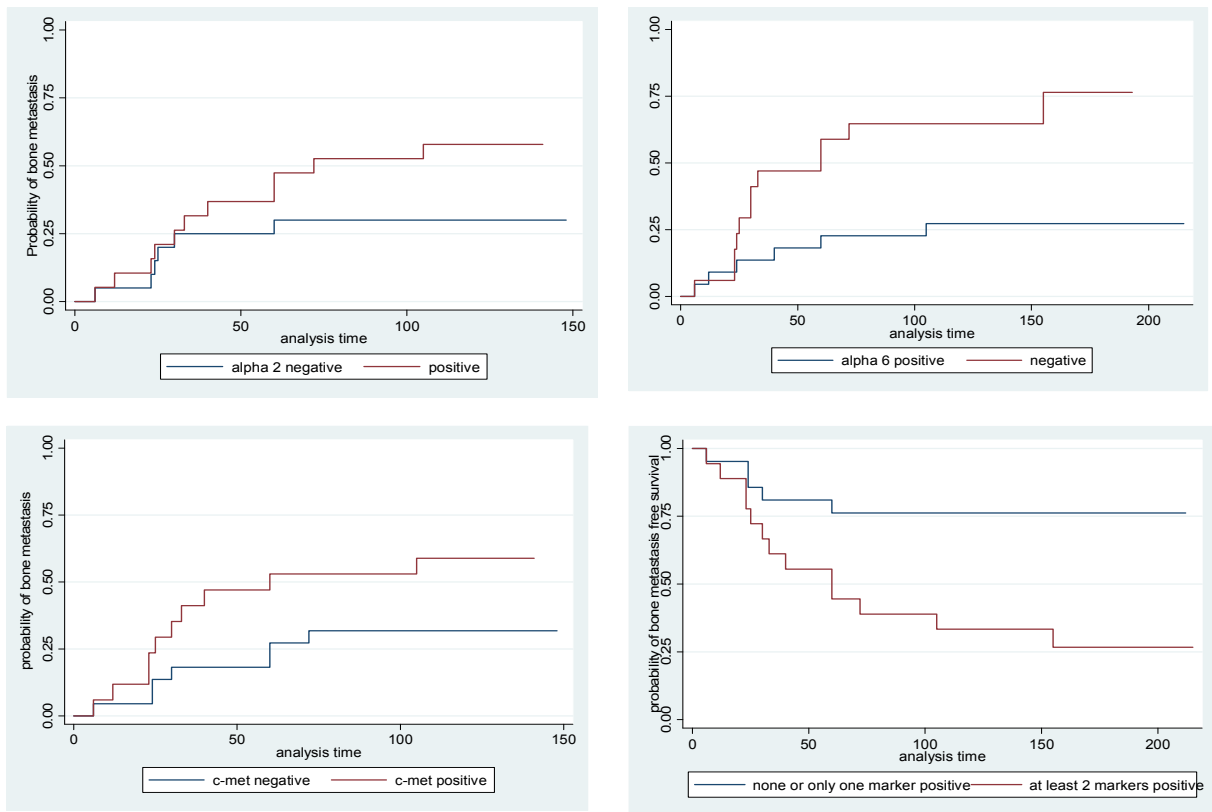
Circulating tumor cells are found early on in disease and represent a heterogeneous population of cells which do not all become MICs as the process of metastases is known to be very inefficient and only very few CTCs develop into overt metastasis. Therefore, one of the main objectives of this proposal became the identification of MICs from the heterogeneous cell population of tumor initiating cells. We hypothesized that in order to survive and initiate a metastasis these cells represent a rare population of *cancer stem/progenitor-like* (CS/PC-like) cells with self-renewal capacity that are exclusively responsible for its growth, local invasiveness and metastatic potential.

Therefore the work of the **PROMET** consortium initially focussed on the determination of potential target genes. By isolating tumor cells from primary tumors of patients operated for prostate cancer able to survive under harsh conditions and to self-renew we were able to evaluate their gene expression with a hypothesis-driven approach. These cells expressed markers consistent with a stem cell/progenitor phenotype. A set of these markers Prostate Stem Cell Antigen (PSCA), $\alpha 2$ integrin, $\alpha 6$ integrin, c-met and CD44 are shown below in an assay of anchorage independent growth which correlates to tumor growth.



These markers were then evaluated in clinical samples of patients with sufficient follow up by immunohistochemistry to assess whether their expression allows predicting outcome. In effect these markers were able to discriminate patients with better outcome and survival. This is currently being verified in a larger independent data set of patients treated at the University of Berne.

Since these identified markers are not suitable for ELISA techniques, we used flow cytometry for quantification of cell phenotype: $\alpha 2^+/\alpha 6^+/c\text{-met}^+$ in the marrow of patients followed for prostatic carcinoma. The test was calibrated to allow detection of 0.001%. In 182 patients there is a significant increase in the percentage of $\alpha 2^+/\alpha 6^+/c\text{-met}^+$ cells in patients having a biological progression (ANOVA: $p < 0,001$). The percentage of cells increases with metastatic risk and is dominant in metastatic patients. Whether this correlates with overall and cancer specific survival requires a longer follow up and confirmation in other patient populations. These studies are ongoing.



Kaplan Meier survival analysis showing bone metastasis free survival and overall survival of patients with high risk primary prostate cancer according to c-met, $\alpha 2$ and $\alpha 6$ integrin expression. Patients were positive if more than 5% of tumor cells stained positively.

On a hypothesis driven basis the Leiden University Medical Center is the first to highlight the functional importance of the ALDH superfamily of enzymes in cancer stem/progenitor cells, tumor growth and the formation of metastases in human prostate cancer. It appears, therefore, that inhibiting ALDH enzyme activity may represent a new therapeutic option for treatment of human prostate cancer but more studies are warranted to address this issue. Furthermore, they demonstrate that the ALDH7A1 isoform is involved both in prostate cancer progression and metastasis.

Another approach taken was to analyze the proteins expressed by prostate cancers into the serum and compare different well defined populations to a control group. By means of iTRAQ proteomics 3 potential proteins of interest as potential markers have been isolated and are currently being evaluated and validated in different clinical sample sets: **Prostate Tumor-Inducing Protein-1 (PTI-1)**, (also known as eukaryotic translation elongation factor 1 alpha 1), which was seen to be over-expressed 1.56 and 1.95 fold in progressing and metastatic samples and **sex (androgen) hormone binding globulin** and **inter-alpha (globulin) inhibitor 3**, which were seen to be over-expressed (1.91 and 1.53 fold respectively), in progressing samples compared to non-progressing samples.

Detection of circulating tumor cells is advocated by different groups and different manufacturers. A frequently used system is the Cell Search System Veridex for analyses of circulation prostate cancer cells from peripheral blood. Leiden University Medical Center tested this system in a small sample set and found that in about 10 % of the prostate cancer patients with organ-confined disease, CTCs can be detected in the blood (< 10 CTCs/7.5 ml of blood). In patients with hormone-refractory prostate cancer >100 CTCs were detected in

7.5 ml of peripheral blood. At present it is unclear whether EpCAM is a suitable marker for tumor-initiating (or rather metastasis-initiating) cells. Furthermore Veridex was not able to meet the promise to generate a bone marrow kit for analyses of the bone marrow aspirates of these patients. As a result this approach was abandoned. LUMC is currently analyzing the collected bone marrow cytopspins by immunohistochemistry instead.

In the same effort to evaluate whether CTCs can be detected UOXF used an automated microscope that has been developed by Ikonosis over the last few years that has the ability to discriminate cells according to immunostaining characteristics. Preliminary work on small numbers of patients showed the system could be used to detect circulating cancer cells in peripheral blood even in early stages of the disease. Results, however, are poorly reproducible at this point and it is likely that many of the targets are actually not CTC. Detection algorithm needs to be improved and molecular detection may also need to use alternative methods than antibodies such as the detection of cytogenetic abnormalities using FISH.

Taken together these data the **PROMET** consortium has been able to demonstrate that **differential gene and protein expression** of minimal residual disease **exists**. There is good preliminary evidence that the presence and risk of minimal residual disease can be recognized and possibly targeted with such marker genes. The work of the consortium thus improved our understanding of the mechanisms of minimal residual disease and has led to the identification of a set of marker genes for the detection of minimal residual disease.

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

Translation of the results from bench to bedside depends on the *in vivo* proof of principal in experimental models. Therefore, one of the main aims of the **PROMET** consortium was to develop tumor models and imaging modalities allowing the monitoring not only of tumor growth and metastasis formation of small prostate cancer cell numbers *in vivo*, but also of synchronous dynamic gene expression in tumors that will lead to a better understanding of processes involved in tumor progression, dissemination and micrometastasis. The knowledge generated by this approach in preclinical models is key to understanding processes involved in minimal residual disease in prostate cancer patients. **PROMET** partners have focused on the development of improving the real-time tracking and detection of human prostate cancer in preclinical models using molecular imaging, in particular imaging of bioluminescence (and fluorescence). Two approaches have been followed; the use state-of-the-art imaging equipment and the development of optimized bioluminescence reporters in prostate cancer cells. Bioluminescent indicators can be used for cell tracking purposes while other bioluminescent (or fluorescent) indicators report a cellular response (e.g. real-time growth factor pathway activity, apoptosis etc.) The simultaneous assessment of two bioluminescent indicators (or combinations of bioluminescence with fluorescence, multimodality imaging) can be used to visualize normal as well as aberrant cellular processes at a molecular-genetic or cellular level of function as well as real-time cell tracking. In prostate cancer metastasis research, whole body bioluminescent and fluorescent imaging techniques have become indispensable tools that allow non-invasive and real-time imaging of gene expression, tumor progression and metastasis, and response to therapeutic intervention.

The development of different animal models addressing different issues of the problem of minimal residual disease using bioluminescence and thus **significantly and drastically decreasing the number of experimental animals** required for experiments has allowed to gain insight into the mechanisms and the gene expression involved. Experimental models

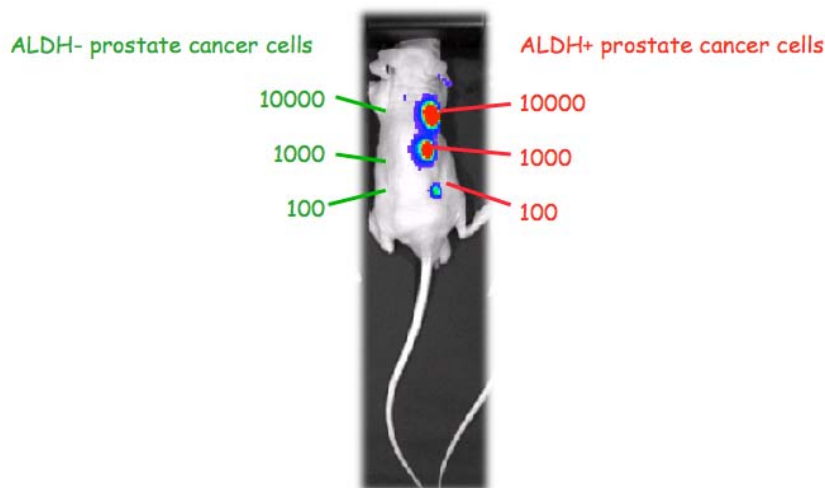


developed and validated **cover hormone-sensitive prostate cancer** and **dormancy**, which did not exist for prostate cancer until now and are important additions to the prostate cancer researchers' armamentarium. In addition we have established a **dorsal chamber metatarsal model for multiphoton fluorescent microscopy** detection and quantification of micrometastases in vivo allowing studying the cellular mechanisms of homing, extravasation and implantation.

The validity of these experimental models could be determined on several occasions. In the **transplantable xenograft model BM18** for instance several genes of interest could be confirmed by UBERN and DKFZ and markers such as *$\alpha 2$ integrin*, *PSCA*, *CD44*, *HEY1*, *SOX2* and *BMI1* were highly enriched under androgen-independent conditions, thus representing S/PC-like cells. Should these findings from gene expression studies be true one would expect such genes to be upregulated in the context of bone. For this we performed induction studies with osteoblast secretomes. Interestingly, with the exception *$\alpha 6$ integrin*, the putative stem cell marker genes *$\alpha 2$ integrin*, *CD44*, *HEY1*, *SOX2* and *PSCA*, shown above to be upregulated in androgen-independent cells (likely CSCs), become upregulated also in the various CaP cell types tested in these induction experiments with the osteoblast secretomes.

Finally, we could confirm by overlapping results from *in-vivo* and *in-vitro* data that the comparative evaluation of expression profiles of C4-2B4 and BM18 models appears to promise a number of highly interesting insights into the prostate cancer process and the genes related to it and thus of importance for the evaluation of both marker genes and potential therapeutic/preventive measures. The evaluation process is still in progress and should provide additional material for publication and/or novel experimental set-ups and grant applications.

Selection of tumor cells on the basis of ALDH+ vs ALDH- selects for cells with self renewal capacity and increases their tumorigenic and metastatic potential. ALDH+ cells may grow tumors at a dilution of approximately 100 cells.



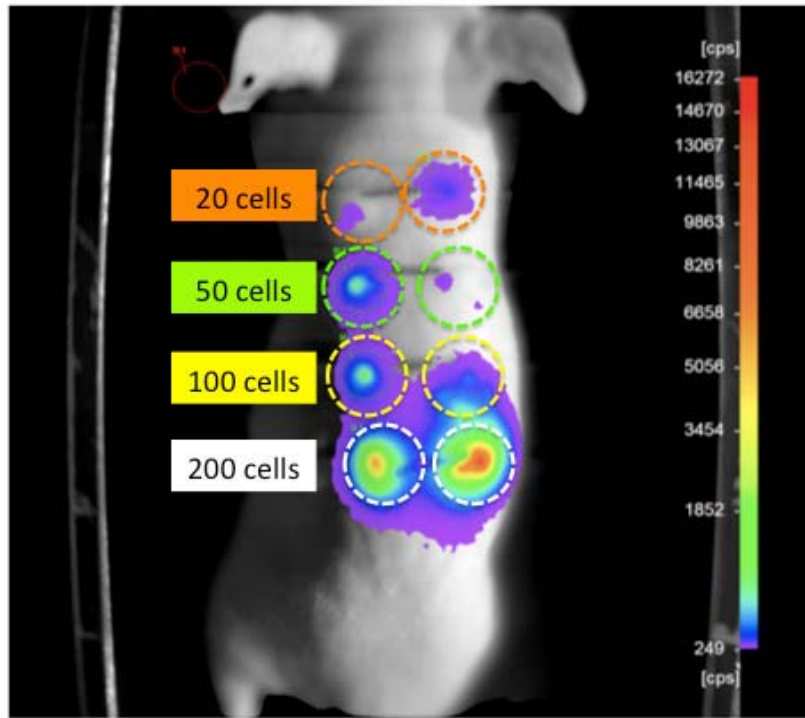
One of the characteristics of mesenchymal stem cells derived from the bone marrow is the capacity to fuse with other cell populations and initiate transdifferentiation. This has been shown to be an integral part of the process of tissue regeneration. In studies to identify cells with stem cell like phenotypes we tested the hypothesis that subpopulations with fusogenic capabilities are present within the PC3 human prostate cancer cell line. This was done by stably transfecting PC3 populations with constructs allowing either the expression of red fluorescent protein (RFP) and hygromycin resistance genes or (GFP) with neomycin resistance genes. These populations were mixed together and allowed to grow in flasks for

48h. Cultures were then treated with lethal doses of both antibiotics. Resistant clones containing cells expressing both RFP and GFP were observed in cultures after 3 weeks. Estimates of the rate of productive fusions were that one proliferating clone of fused cells was generated per 100,000 interacting cells. The fused clones were isolated and characterized. Fused cells were synkaryons of approximately twice the cellular volume of either parent strain with karyotypes of 140-150 chromosomes (cf parent PC3 ~70-80 chromosomes). This increase to near double chromosome numbers further supports the concept that fusion has occurred in these cells. Fused populations proliferated at the same rate as the fastest growing parental population both in terms of log phase growth in large cultures and in monolayer cloning assays although colony sizes of fused populations were significantly larger. The most striking feature of fused populations was the behaviour of cells when the selection pressures of growth in semi-solid agar or growth in immunodeficient animals. Fused cells cloned in agar at 5x the frequency of either parent population and formed tumours in mice more quickly and with higher frequency than either parent cell strain. These results suggest that fusion between a specific rare population of prostate cancer cells results in genetic variation that could be a key functional part of the metastasis initiating cell phenotype.

Evaluate the *in vivo* detection of minimal residual disease by means of bioluminescence and nanoparticles with optoacoustics

Leiden University Medical Center has generated a number of prostate cancer cell lines (PC-3M-Pro4, C4-2B4 and VCaP) combining different **bioluminescent indicators** that allow – thanks to the different wavelengths emitted by these indicators - simultaneous assessment of different types of luciferases, e.g. *Photinus pyralis* (firefly) luciferase and its codon-optimized form luciferase2 (Fluc and Fluc2 resp), a transmembrane form of *Gaussia* luciferase (TM-Gluc) and *Renilla reniformis* luciferase (Rluc) *in vitro* and *in vivo*. While firefly luciferase uses luciferin as a substrate, coelenterazine is the luciferin substrate used in *Gaussia* luciferase and *Renilla reniformis* luciferases. This offers the possibility to administer two different substrates to mice containing human prostate cancer cell expressing Fluc and either Rluc or Gluc. This offers interesting possibilities to measure the activities of both luciferases, one for cell tracking, the other for measurement of pathway activity.

One aim of the consortium was to **detect tumor cells at the level of 1-20 cells**. BERTHOLD has further improved conventional CCD cameras and developed emCCD cameras, where the signal can be amplified up to 1000 times. The combined use of new equipment and codon-optimized luciferases (luciferase2) has led to strongly enhanced detection of small cell numbers *in vivo*. In a cell titration experiment *in vivo*, UBERN could detect by these improvements as few as 20 PC-3M-Pro4 Fluc2 cancer cells injected subcutaneously (see below) with a correlation coefficient of 0.99 between 20 and 200 cells and with good reproducibility. Most likely, this detection limit could be even improved in a real micrometastatic deposit, where cells are in mutual contact and, thus, represent a more concentrated source of photon emission. Furthermore, the cells are more closely in connection with the microvasculature from where the substrate D-luciferin diffuses.



Photon emission by PC3Mpro4 Fluc2 cells *in vivo*. Ten microliters of cells suspended in Matrigel® were injected subcutaneously at the cell density indicated, each density at duplicate sites of the dorsal skin of a Balb/c *nu/nu* mouse.

The collective effort of **PROMET** partners has resulted in strong and significant improvements (>650 x) in the real-time tracking of human prostate cancer cells in preclinical models. The implementation of more sensitive, *in vivo* molecular imaging has paved the way to a better understanding of organ colonization, minimal-residual disease, the role of cancer stem/progenitor-like cells and drug response.

The optoacoustic approach proposed for the detection and treatment of micrometastases has proven more complex than anticipated by the consortium and other groups using nanoparticles, which is reflected in the literature with few really reproducible results. Robust and consistent production of functionalized antibody coated gold nanoparticles is a challenge not yet resolved. Furthermore, current imaging systems and algorithms are insufficient and need to be improved and adequate experimental models need to be established to set up this technique at a level allowing clinical testing and application. As imaging quality depends on the sample orientation, strong background in the depth of the tissue is deleterious for the detection of minimal residual disease. Based on these unmet issues we decided to develop in close collaboration with the industry (Fukuda Denshi) a **novel optoacoustic instrument** that allows to alternately record optoacoustic and ultrasound imaging and improved the hardware by decreasing reconstruction artifacts by different algorithms and displacement compensated averaging (DCA). These proposed methods are computationally inexpensive and make real-time imaging possible. Furthermore, we have been able to demonstrate that functionalized gold nanoparticles work as contrast agent for optoacoustics. However, these

results are difficult to reproduce, due to **variability of the functionalized nanoparticles**. As many companies are working on the production of nanoparticles for *in vivo* use, also with antibody coating, we decided not to put any effort in their production and improvement of coating methods, still mostly inconsistent.

In order to decrease background and develop suitable scanning procedures to improve our image quality we developed two **highly sophisticated tissue phantoms** which are elastic and provide both, optical and acoustic contrast. Working with these tissue phantoms we were able to reduce the systematic background, which decreases the background level by about 60%, leading to strongly improved contrast of embedded absorbing structures. In addition we could enhance 3D imaging and tissue localisation.

Nanoparticle behavior in biological systems is as well as the optoacoustic effect on gold nanoparticles on the cellular level remain unclear. It is not clear how cells that take up nanoparticles are detected. Is this dependent on the nanoparticle itself or are reactions of the cell microenvironment responsible for detectability? This is of major importance for modeling of algorithms and improvement of *in vivo* detection.

Using electron and confocal microscopy we asked the question what happens when a single nanoparticle is hit by a short laser pulse and does the irradiation threshold for cell death depend on the number of particle bound to or taken up by a single cell? For this purpose, we utilized macrophages in a dedicated microscopic setup for controlled irradiation of single particles and cells. Spherical gold particles of 90 nm diameter in suspensions down to a concentration of 10^8 particles/ml were used. The results obtained from the single particle experiments provide a coherent picture of bubble formation. The experiments revealed that **cell damage** is obtained if the delay between the two distinct pressure transients is between 0.6 to 0.8 μs , which correspond to a bubble diameter of about half the size of the cell. The lowest threshold for cell damage of about 40 mJ/cm^2 was found for the highest nanoparticle concentrations, which however is still above the ANSI limit for skin exposure, 20 mJ/cm^2 for these laser parameters. That means that a non-invasive application using aggregated particles within cells also requires more efficient absorption or methods to enhance vapor bubble formation.

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

Micrometastasis is an impediment to the development of effective cancer therapies. The Universities of Sheffield, Leiden and Berne have implemented and optimized a number of preclinical models of prostate cancer growth, progression and (micro)metastasis that can be used to for real time cell tracking, tumor growth and drug response. Preclinical models encompass a **dorsal-skin fold chamber model** (DSC) as a new model for real-time tracking of prostate cancer colonization of bone *in vivo* using fluorescence microscopy, optimized preclinical xenograft models of intra-prostatic growth and tumor progression and two models of (bone) metastasis, (intra-bone growth, intra-cardiac inoculation) using sensitive non-invasive real-time bioluminescence and fluorescence imaging.

A number of **experimental therapeutic strategies** have been evaluated in these preclinical models. The anti-resorptive bisphosphonate zoledronic acid (Zometa™) was administered to mice, thus lowering bone-turnover prior to inoculation of human prostate cancer cells (PC3, PC3MPro4) as compared to treatment of established bone metastases. Lowering osteoclastic bone resorption by bisphosphonate treatment significantly inhibited the homing and subsequent growth of human prostate cancer cells in all tested preclinical models (preventive setting). These data provide the rationale for **targeting of the supportive bone microenvironment**. In addition early treatment with bisphosphonates allows decreasing the



number of metastases and the future tumor load.

In addition a non-peptide antagonist of αv integrins was evaluated in a preventive and curative protocol. Our data indicate for the first time that **αv -expression mediates invasiveness** (via an epithelial-to-mesenchyme transition) and contributes to the acquisition of a cancer stem/progenitor phenotype in human prostate cancer cells with metastasis-initiating features. Our studies provide the rationale for the use of αv -integrin antagonists in the treatment of advanced human prostate cancer. It was found that inhibition of αv integrins affects both cancer cell and the supportive stroma. Integrin-mediated adhesion and migration of human prostate cancer was strongly inhibited *in vitro*. Furthermore, targeting of αv integrins leads to **inhibition of osteoclastic bone resorption** (osteoclasts) and angiogenesis. The inhibitory effects of the αv integrin antagonist was most profound in a preventive setting (continuous administration) followed by the curative protocol (inhibition of established bone metastases).

Evidence is mounting that the entire population of tumor cells in human prostate cancer arises from a small number of cells, the **cancer stem/progenitor cells (CSCs) or tumor-initiating cells (TICs)**. Whether this subpopulation of cancer cells also contributes to metastatic behaviour in human prostate cancer remains poorly understood. A putative TIC phenotype, CD44⁺/ $\alpha 2\beta 1^{\text{high}}$ /CD133⁺, has been identified in prostate cancer (Collins et al 2005). However, because of the heterogeneous nature of solid cancers, the reliability of using cell surface markers as the sole way to isolate TICs remains controversial. A complementary strategy for identifying TICs involves measurement of **aldehyde dehydrogenase (ALDH) activity**. The data from Leiden University Medical Center is the first to highlight the functional importance of ALDH enzymes in cancer stem/progenitor cells, tumor growth and the formation of metastases in human prostate cancer. ALDH is a detoxifying enzyme which has important functions in the development of epithelial homeostasis, and as a result, deregulation of this class of enzymes has been implicated in multiple cancers. ALDH activity is important for drug resistance, cell proliferation, differentiation, and response to oxidative stress. It is becoming increasingly clear that ALDH activity can be used, either alone or in combination with cell surface markers, to identify TICs. It appears, therefore, that inhibiting ALDH enzyme activity may represent a new therapeutic option for treatment of human prostate cancer but more studies are warranted to address this issue. Furthermore, they demonstrate that the ALDH7A1 isoform is involved both in prostate cancer progression and metastasis.

Med Discovery has advanced the development of its **recombinant KLK2 inhibitor** as potential prostate cancer therapy. In order to prepare a regulatory package and progress the molecule into the clinic, Med Discovery has completed earlier non-clinical studies with GLP pre-clinical toxicology testing (dose range finding, toxicokinetics and 28 day repeat toxicology and safety, immunogenicity) and prepared a clinical plan to support the early clinical trials. The preclinical results obtained should allow Med Discovery starting the early clinical development of the drug in 2011.

After a mixed Single Ascending Dose/Multiple Ascending Dose (SAD/MAD) phase I study in healthy volunteers to assess the safety of MDPK67b and to generate pharmacokinetic data in humans a **phase II study in asymptomatic prostate cancer patients** will be launched.

Functional significance of candidate marker genes emerging out of WP 1

Genes identified within the context of the research of the **PROMET** consortium need not only be assessed for their potential as markers but also for their functional impact. The functional involvement of a number of these cell-surface molecules previously identified a gene



signature representative of prostate cancer ($\alpha 2$, $\alpha 6$ and αv integrins) and intracellular enzymes (aldehyde dehydrogenases, ALDH) along the metastatic cascade of prostate cancer is unclear and have been subject to study during the last **PROMET** period using lentiviral knockdown of selected target genes and evaluation in the established preclinical models of prostate cancer by molecular imaging and histochemical analysis, as well as in the dorsal Boyden chamber model established in Sheffield.

Leiden University Medical Center has performed **lentiviral knockdown studies** of a number of target genes that have been identified in WP1&2. The following knock-down studies have been performed in PC-3M-Pro4 Fluc cells:

-the aldehyde dehydrogenases (ALDH) ALDH4A1, ALDH7A1, ALDH9A1.

-the integrin adhesion receptors $\alpha 2$, $\alpha 6$ and αv .

Leiden University Medical Center has been able to generate PC-3M-Pro4 Fluc clones with stable knockdown of integrin αv and ALDH4A1, ALDH7A1 ALDH9A1. Despite several attempts knockdown of $\alpha 2$ and $\alpha 6$ integrins could not be accomplished. We believe this could be due, at least in part, either to off-target effects or for the critical role of these proteins for prostate cancer cell survival. This implies a critical role of these integrins in cell growth *in vitro* and may be indicative of stem/progenitor properties that are required for survival.

Leiden University Medical Center demonstrated, in close collaboration with the University of Bern and Sheffield that a **subpopulation of ALDH^{hi}/CD44⁺/ $\alpha 2$ ⁺/ $\alpha 6$ ⁺/ αv ⁺ human prostate cancer cells** exist that display stem/progenitor properties. Furthermore, targeting of αv -integrins by a non-peptide αv -integrin antagonist resulted in strong inhibition of bone metastasis in preclinical models. Upon αv -integrin expression knockdown, a concomitant decrease of prostate cancer stem/progenitor markers (integrin $\alpha 2$, CD44v6, and ALDH) and EMT-associated genes (Snail, Snail2, and Twist) was observed. In addition, knockdown of αv -integrin in human prostate cancer cells diminished their clonogenic and migratory properties *in vitro*

ALDH has 18 isoforms. Several candidate isoforms were selected. ALDH7A1 is highly expressed in clinical samples of primary prostate cancer and matching bone metastases. PC-3M-Pro4 Fluc clones with stable ALDH7A1 knockdown by shRNA technologies using a MISSION library resulted in diminished clonogenicity, migration and the formation of distant metastases. Our data are the first to highlight the functional importance of ALDH enzymes in tumor growth and the formation of metastases in human prostate cancer. It appears, therefore, that inhibiting ALDH enzyme activity may represent a new therapeutic option for treatment of human prostate cancer but more studies are warranted to address this issue.

Major achievements of the project to the state of- the-art

The major achievements of the promet project are

- Identifying the stem cell/progenitor like cell population as the metastasis-initiating cell of minimal residual disease in prostate cancer
- Developing experimental models of androgen-dependence and dormancy
- Significant improvement of *in vivo* imaging using high-end imaging material and varying flourochromes for minimal residual disease
- Determination of potential therapeutic approaches for minimal residual disease in prostate cancer leading to a phase I/II clinical trial