Publishable Summary I: Main findings

Separately uploaded into Research Participant Portal

A summary description of project context and objectives

Scientific background of METOXIA

The METOXIA project has an over-all focus on the increased understanding of the regulatory mechanisms which help cancer cells survive under unfavourable micro-environmental conditions characterized by low oxygenation (denoted hypoxic areas). Solid cancers generally contain areas with lower levels of oxygen than what is usual in normal tissues and thus, the cancer cells learn how to adapt to such conditions. The METOXIA project furthermore aims to use the increased knowledge of the protective regulatory mechanisms to develop new principles for cancer detection and treatment: Since only cancer cells and not normal cells experience hypoxia one can develop cancer-specific detection and treatment by attacking molecules specific to the protective regulatory mechanisms activated by the cells under hypoxic conditions.

It has long been known that the problems created by hypoxic areas in solid cancers are serious for the patient: In short, cancer cells in such hypoxic micro-environments represent a reduced chance for successful treatment. With respect to radiotherapy the problem relates to increased dose tolerance for individual cells under hypoxic as compared to well-oxygenated conditions while for chemotherapy there is in some cases both increased dose tolerance and reduced drug access to the hypoxic as compared to well-oxygenated areas. Even if the patient is treated with surgery alone there are indications that the amount of tumour tissue which is hypoxic correlates negatively with the outcome for the patient. Recent research has furthermore shown that variable hypoxia in tumours is one of the major drivers of metastatic spread of cancer, the major cause of death by the disease. Thus, **hypoxia is responsible for a double effect of reducing the potential of a successful treatment of the cancer patient: Resistance to treatment and ability to spread to distant parts of the body.** The positive side of this problem is that the very low level of oxygen found in solid tumours is specific to cancer. Therefore, **if one could develop new methods to specifically detect and inactivate cells in hypoxic areas one might obtain a cancer-specific effect, selective for the most harmful of the cancer cells.** In the original call which METOXIA answered it was made clear that the research should not be limited to pre-clinical biological models, but should include proof-of-principle clinical research. As part of the consortium some of the leading cancer clinics in Europe are partners.

Main Objectives of METOXIA

On basis of the clinical problems raised by hypoxic tumour micro-environments the work within METOXIA encompasses increasing the knowledge concerning the metastatic process in the hypoxic micro-environment at the molecular level in order to develop novel strategies for modification of this micro-environment and thus improve the efficacy of chemotherapy and radiotherapy. Major objectives are:

- Gain new knowledge about molecular mechanisms behind hypoxia-driven metastasis in order to reduce metastatic spread and increase patient cure rate.
- Develop improved methods to detect the propensity of a cancer to metastasise before the metastatic spread has become manifest clinically.
- Develop new treatment management of metastatic disease.
- Develop new methods to increase the effectiveness of treatment in hypoxic areas of the primary tumour.
- Development of new methods for detection/imaging of hypoxic areas.
- Generate pre-clinical models for the study of the role of hypoxia in metastases.
- Development of new tools for monitoring and control of peri-cellular micro-environment *in vitro*.

The partner organisation

There are at present 21 partners in the consortium. The work was partitioned in 8 Work Packages (WP) of which one (WP9) relates to management and one (WP6) is empty (the activity originally included in WP6 was during the 2nd period amendment moved into WP5, WP7 and WP8 after a decision made at the 2nd General Assembly in June

2010). This reorganization turned out to be a success and no further reorganization of the consortium has been done during the 3rd, 4th and 5th periods.

Largely the non-management WPs can be divided into 2 main groups (WP6 is now empty):

- Work involving cancer patients (WP1, WP5 and WP8).
- Work involving primarily fundamental research, divided into two sub-areas:
    1. Studies of primarily biological nature (WP2, WP3, WP4)
    2. Development of more technical nature (development of equipment for monitoring and control of micro-environmental parameters related to hypoxia) (WP7).

The WEB-site of METOXIA is on the following address: http://www.metoxia.uio.no/ In line with the consortium agreement some of the reported material is confidential. The WEB-site thus is divided into an open area accessible for all and a restricted area accessible for partners only.

The work performed since the beginning of the project and the main results achieved so far

Over the 5.5 year total duration of the METOXIA project 105 scientific deliverable-reports have been completed and, in addition also 18 management deliverables. Thus, considering the amount of work here reported only some major findings can be dealt with in a summary.

The line of research which has led to the most optimistic results concerning the possibility to develop a new drug for cancer therapy has the following background: The hypoxic micro-environment in solid cancers influences several regulatory cascades in cells. Since cancer cells in solid tumours experience such conditions over a long time these regulatory mechanisms represent a selective pressure of cancer cells into more hypoxia-tolerant phenotypes. This development correlates with a higher degree of malignancy and furthermore, with higher metastatic potential. For development of new diagnostic and treatment concepts it is important that these findings are connected to knowledge of hypoxia-regulated molecular pathways with which we can interfere. Even a small reduction in oxygenation results in regulatory consequences for a large part of the genome of our cells. There are several key regulators of these cascades, but the one responding to the smallest reduction in oxygenation is the protein HIF (Hypoxia Inducing Factor). This protein is what we denote a transcription factor; meaning that it can regulate activation of certain genes on DNA. It turns out that HIF-regulation involves a large number of genes and several processes which are vital for the cells and tissues to survive hypoxic conditions: Cell metabolism, energy production, angiogenesis (*i.e.* formation of blood vessels), extracellular matrix properties (EMT-epithelial mesenchymal transition) and pH-control. All these have been thoroughly studied within the METOXIA programme and valuable new knowledge has been accumulated. Notice however, that since HIF itself regulates so many processes it can hardly be considered as a target for treatment. Attacking HIF might affect too many normal processes and might not be cancer-specific. The focus for development of new cancer therapy therefore is not primarily on this central regulator, but rather on several of the cascades it regulates. One such mechanism has been developed into METOXIA-patented products of potential value as new anti-cancer drugs. This relates to certain small-molecular inhibitors of the HIF-regulated protein Carbonic Anhydrase IX (CAIX) involved in cellular uptake of CO_2 for bio-mass synthesis and pH-regulation. These CAIX-inhibitors have in pre-clinical experiments shown a great potential for specific treatment effects on reduction of the metastatic potential of the primary tumour and also to some extent seem to increase the effect of traditional radiotherapy and some chemotherapy.

Two arms of development have been worked out

a) Several METOXIA-partners collaborate on the development of small-molecular sulfamate compounds (patented). These include chemicals with high specificity to the active site of the CAIX-protein. By specifically inhibiting CAIX our findings indicate that we have not only new possibilities for cancer treatment, but also new diagnostic principles. Thus, the METOXIA results point to new possibilities for drug development. Still, we need to add that we have also experienced the threshold for such development through our contact with large pharma: The practical problems related to development of hypoxia-specific treatment seem to exceed those of more traditional drug development. The reason for this is that the killing

of hypoxic cells alone by no means can be expected to have a major influence on the primary tumour. Hypoxic cells proliferate less than well-oxygenated cells (i.e. they do not give rise to more cells as long as they are hypoxic). The problem they create is resistance against traditional treatment and increased ability to form distant metastasis. Thus, they are expected to be responsible for relapses after completed treatment. Development of a new hypoxia-specific agent therefore will have to be done in combination with traditional treatment and in the short run it may become difficult to observe an extra effect on the primary tumour compared to the effect of the traditional treatment alone.

b) As a possible improvement of the combination treatment a sub-group of METOXIA partners have synthesized a group of chemicals (patented) having a dual effect: These chemicals both have the ability to inhibit CAIX and to increase radiosensitivity of hypoxic cells. To follow up the development of these chemicals a new company (an SME) was founded shortly before the end of METOXIA and the aim is to perform preclinical and clinical studies of the effect of these compounds given simultaneously with radiation therapy.

Targets other than the HIF-regulated pathways have also been investigated and some found to be of great potential interest as targets for hypoxia-specific markers and treatment. Two targets of interest related to pH-regulation and metabolism are the lactate transporter MCT4 and its subunit CD147/Basigin. Our partner 13/CNRS has tested an MCT4-inhibitor produced by AstraZeneca and confirmed its specificity for MCT4 and reports this compound to be ready for clinical trials in the near future. Also the two stress-related processes initiated under hypoxic conditions denoted unfolded protein response (UPR) and the mammalian Target of Rapamycin (mTOR) have been investigated by several partners with 20/GROW-UM as most central. Both processes act as hypoxia sensors in the cells. UPR is a process which leads to an accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum while mTOR is a protein kinase which regulates cell growth. In a collaboration between 20/GROW-UM, 2/MAASDTRO and 5/UOXF.BP it was shown that hypoxia activation of UPR induces expression of the metastasis-associated gene LAMP3, thus identifying LAMP3 as a new candidate biomarker of UPR activation by hypoxia in tumours and a potential mediator of hypoxia-induced metastasis. Regarding mTOR-inhibitors, these have been introduced as anti-cancer drugs for renal cell carcinoma (tensirolimus and everolimus).

Another field which has led to filed patent applications is the induction (and also counteraction) of radiation-induced resistance to chemotherapeutic compounds by low dose-rate irradiation. During the 3rd and 4th periods this research at 1/UIO indicated that radiosensitivity of cells can be modified even several hours after the cell was irradiated and that the effect is easily induced also in animals. The relevance to METOXIA stems from our finding that the mechanism activated by low dose-rate irradiation is also activated by variable hypoxia. Thus, we have the possibility to attack mechanisms responsible for adaptation to chemotherapy resistance during hypoxic conditions. So far we do not have a patented compound for this action, but in the 5th period we have shown in animal experiments that the protein to inhibit is transforming growth factor β3 (TGF-β3), and we have shown that at least one commercial chemical, not developed into a drug can inhibit TGF-β3 and counteract radio- and chemo-resistance.

Various new biological test models as well as technical measurement devices have been developed. **New *in vitro* 3D-models as based on purified alginates** for pre-clinical studies of efficiency of new modalities have been taken into use towards proof-of-principle testing of treatment benefit. Also **new animal models for pre-clinical studies** of efficiency of new modalities have been developed towards proof-of-principle testing of treatment benefit. Special emphasis has been placed on development of CAIX knock-down models so that the effect of CAIX-inhibitors can be assured to result from a specific effect on the CAIX-protein and not result from some unspecific toxicity. For the testing of effects on metastasis orthotopically implanted tumours have been used. **Development of new sensor- and micro-fluidic technology and instrumentation for detection and control of oxygen** and other substances in the cell and tissue micro-environments are being developed. A disposable cell culture flask with a sensors molded into the flask bottom has been developed.

A central clinical investigation is the study of micro-metastases (i.e. circulating cancer cells) as a means for individual evaluation of the metastatic potential of cancers in patients. Also our effort to develop a comprehensive classification of tumour hypoxia, anoxia and reoxygenation has been highly prioritized as it aims to allow clinicians to predict response to targeted agents which include those activated by hypoxia. In this connection **detection and visualization of hypoxia in patient tumours are highly prioritized** and a promising new method passed phase I study during the 3rd period and protocols have been completed during the 4th period for this to continue with 3 phase II studies which has been ongoing during the 5th period of METOXIA. This method involves non-invasive PET imaging of tumour hypoxia by use of a radioactive tracer denoted [^{18}F]HX4, a member of the 2-nitroimidazole family of chemicals. The special quality of these chemicals, denoted bioreductive compounds, is that they are chemically modified under hypoxic conditions and thereby can represent both chemotherapeutic drugs specific to hypoxic cells and also offer PET-imaging of reactive activity. This particular study was not included in the plans of METOXIA from the start, but is an example of the importance of amendments of new possibilities coming up during the development of the project.

Although the idea of targeting a sub-set of hypoxic tumour cells to improve treatment outcome is intriguing, clinical studies proving that not only do hypoxic intervention work but it does so only in patients with hypoxic tumors has not been conducted until now. In this context, the development of a hypoxia gene signature at 6/AUH,AS which, when tested in material from a large randomized phase III study, was able to identify those HNSCC patients which benefit from hypoxic intervention with Nimorazole (radiation sensitizer) was an important step forward. As a direct spin-off, several multi-center clinical projects that will further clarify the full potential of this gene signature to identify patients suitable for hypoxic intervention has been launched.

Also development of bone-seeking α-particle-emitting radio-nuclides (Alpharadin®) for localized treatment of bone metastases has been involved in METOXIA. As α-particles deliver their energy concentrated (i.e. with high LET) this type of radiation is equally effective under hypoxic as under aerobic condition and is thus expected to abolish the radioprotective effect of hypoxia. This project was started before METOXIA by the Norwegian company Algeta. A global phase III clinical trial (ALSYMPCA) with Alpharadin® in patients with castration-resistant prostate cancer (CRPC) and bone metastases was successfully completed in June 2011. Algeta was recently bought by Bayer Pharma AG for more than 2 billion euros and Alpharadin is presently manufactured under the trade name Xofigo.

METOXIA furthermore takes part in the large-scale randomized trial started several years ago in the Netherlands comparing Accelerated Radiotherapy (AR) with Accelerated Radiotherapy plus Carbogen and Nicotinamide (ARCON) in laryngeal cancer. In this large multi-centric trial METOXIA has taken part in the evaluation of consequences of Carbogen and Nicotinamide in relation to degree of tumour hypoxia. Despite lack of benefit in local tumour control for advanced laryngeal cancers the results are promising, indicating a significant gain in regional control rate, with equal levels of toxicity, and even indicating increased disease-free survival for the patients having the most hypoxic tumours.

The expected final results and their potential impacts and use (including socio-economic impact and the wider societal implications of the project so far)

The METOXIA consortium answered a call which was ambitious, aiming to translate knowledge on the molecular machinery responsible for survival and spread of metastatic tumour cells under hypoxic conditions into innovative and validated molecular targets with therapeutic applicability that target the cancer cell or tumour stroma. The wording here can hardly be understood in any other way than to accept that the consortium also would have to protect its IP with the aim to try and secure a potential drug development. The level of ambition was furthermore strengthened by the statement that the consortium should include participants with ample clinical expertise to guarantee a clinical proof-of-principle. The METOXIA-consortium met all these premises. It was a large consortium, involving expertise over such a broad range as clinical and experimental medicine, molecular biology, synthetic chemistry, biophysics and electronics. As indicated above several product developments are successful as planned. The greatest challenge is however the aim for the research to result in a new drug introducing a new principle into cancer therapy. As the project stands by the end of METOXIA there are patents in international phase which can be further developed. We now see the possibility that inhibition with small molecules of a mechanism in the molecular machinery causing hypoxic cancer cells to survive and spread may come as far as to clinical tests in the

relatively near future. There are several challenges to face before one reaches this goal, but we hope we have laid an important basis for the development. During the 3rd and 4th periods we sub-contracted the company Cyprotex Discovery Ltd, to perform professional preclinical testing on 8 selected compounds from our patented sulfamates and dual-activity compounds. We have used these data together with our various partner's effect testing in animal- and *in vitro*-models to complete a report which the patent owners can use for further development of the compounds in collaboration with large pharma industry. Our view is that the potential social-economic impact of a new low-toxicity cancer treatment, halting or even stopping metastasis and improving the effect of conventional therapy can hardly be over-estimated. In our clinical programme we have cancers of the lung, breast, prostate, colon-rectum and head-neck; altogether some of the dominating cancer types in the world. So far we have reason to expect, although this has not been proven, that at least some patients within all these groups may benefit from a hypoxia-specific drug. We expect that the new knowledge brought up by the METOXIA-consortium will be of utmost value for future pharmaceutical development within this field.

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