**Summary of participants**

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Management of the project

Background

With the advent of Omics and other High Throughput Screening (HTS) technologies our insight into the pathophysiology of disease has significantly increased over the last decade. Integrative research concepts and methodologies such as Systems Biology geared towards deriving molecular models of disease, and Systems Medicine aimed at linking such models with clinical and other descriptors of disease phenotypes, opened the avenue towards truly grasping the complexity of chronic diseases. These developments in turn revealed major shortcomings in predicting clinically relevant outcomes such as diagnosis and prognosis and rarely influence treatment decisions. Thus, currently, complex molecular information at the individual patient level still has limited clinical utility.
The major element missing for truly ‘personalized medicine’ is integration of a conceptual as well as technological framework. DIPROMON aims to establish such a prototype framework.

We have selected bladder cancer as our ‘proof of concept’ scenario. Bladder cancer (or more precisely urothelial carcinoma of the urinary bladder (UCB)) has an estimated global prevalence of 2.7 million. UCB results in significant morbidity and mortality, with overall 5-year survival rates of only 57% and 47% for men and women, respectively, when presenting with muscle-invasive disease. In addition to its impact on patients, the disease presents a significant economic burden on health systems with a mean estimated treatment and surveillance cost of about USD 200,000 per patient from the time of diagnosis, making it the most expensive of all human cancers to treat from diagnosis to death.

Biomarkers of UCB with predictive and prognostic utility and which can guide treatment decisions are urgently needed for a number of reasons:
- Firstly the disease has two distinct identities. Most commonly it presents with superficial disease (stages Tis, Ta, T1) which may be relatively non-aggressive (papillary) and unlikely to cause morbidity. In contrast a proportion of patients present with high grade (non-papillary) disease characterized by a propensity to recur, invade and metastasize.
- Secondly a proportion of patients will progress locally and/or systemically (i.e. develop T2-T4 disease) requiring bladder removal (cystectomy), radiotherapy or chemotherapy. No treatment in the last decade has made significant improvements in patient survival; furthermore no predictive biomarkers can guide the physician regarding which patients may have any benefit from systemic chemotherapy (in the neoadjuvant, adjuvant or palliative setting). Superficial bladder cancer is treated with intravesical bacilli calmette Guerin (BCG). BCG may control disease temporarily (reduce relapse rate) but is associated with significant toxicity and patient morbidity. No biomarkers predict relapse post BCG, so patients undergo repeated cystoscopic examinations. The proportion of patients who go on to develop invasive and metastatic disease has not changed over the last decade and comprises around 15-20% of patients presenting with superficial disease. Once bladder cancer reaches...
this stage the options are cystectomy, radiotherapy or chemotherapy. Although the stage and grade of the cancer on immunohistochemistry correlates with its aggressiveness, there are no useful molecular biomarkers or other measures in current practice which can guide the clinician in terms of likelihood of response to chemotherapy, duration of response and likelihood of reduced survival.

Currently, most patients experience a similar pathway following the occurrence of haematuria (either macro or microscopic) involving: urine cytology, upper tract imaging, and cystoscopy, followed by the resection of any detected lesions for pathological diagnosis, staging and initial treatment. Urine cytology has a high specificity (78-100%), although it lacks robust sensitivity (12.2-84.6%), especially for low and intermediate grade tumours, and is operator dependant. A number of urinary biomarkers have also been utilized for diagnosis and surveillance (including FISH, ImmunoCyt and NMP22), but no marker has yet been shown to decrease the need for cystoscopy. Although some of these markers have a higher sensitivity compared with cytology, neither urinary cytology nor urinary biomarkers can safely replace cystoscopy at the current time.

The DIPROMON Concept

DIPROMON holds the potential for characterising patients beyond the current predictors which are based on histological appearance and traditional staging. Providing individuals with personalised risk profiles will have enormous benefits not only to delinate patients at highest risk of disease dissemination and death, but also aid in the prediction of relapse and thereby potentially reduce the need for cystoscopic surveillance.

Our platform integrates large scale Omics profiles leading to delineation of a molecular disease map, from there selecting a biomarker set. A flexible, multimarker technology platform will be further developed and integrated for allowing measuring such marker profiles in a clinical setting. Statistical modelling will integrate the multimarker profile with the ‘gold standard’ clinical descriptors (such as tumour stage and grade) for coming up with a rulebased decision set for predicting outcome with a
given therapy. Although the current patients evaluated in DIPROMON are receiving standard of care, this rule set may be objectively validated in a future prospective clinical study.

The main objectives of DIPROMON are therefore as follows:

- Design of a novel patient stratification concept
- Building the tools, technologies and procedures to apply this concept to the clinical context
- Validating the concept in patients with bladder cancer
DIPROMON partners

1) University of Surrey, Financial Co-ordinator
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The University of Surrey is a thriving, campus-based academic community where inspiring intellects gather to create new understanding, share advanced knowledge and use award-winning expertise to address the world’s most urgent problems.

It has some of the best new academic, library, sports, arts and accommodation facilities of any university in the UK, as well as one of the most envied Professional Training placement programmes in the world. This combination of prestige, expertise, resources and lifestyle is unique, and it’s all to be found here in the beautiful countryside of the Surrey Hills just half an hour from central London.
The Oncology Group has been established since 2006 and specialises in translational research in urological cancers. This includes novel biomarker evaluation, tumour targeting through transcription (homeobox gene targeting), immunotherapy and oncolytic virotherapy, the conduct of novel cancer therapy studies with the emphasis on biomarkers and the development of orthotopic models of bladder cancer. The group is 24 strong and includes two specialist uro-oncologists, 3 molecular oncologists, research assistants, postgraduate students, research nurses and individuals dedicated to sample procurement and biobanking. The University laboratories provide state of the art facilities for biobanking and molecular analysis. The University of Surrey will be responsible for the procurement and storage of all samples prior to shipping to partners. The group have extensive experience in translational oncology, phase I, II and III clinical trials and biobanking human samples.

Co-ordinators

Prof Hardev Pandha, MD PhD (M) is the head of the group and a clinician scientist, combining his role as laboratory head with that of a specialist medical oncologist. He has conducted over 50 clinical trials at the phase I, II and III stages. His research funding has included an FP6 award (SAGE) generating anti-cancer antibodies in plants. Prof Pandha has supervised 12 postgraduate degrees (MD and PhD) and is an examiner for MSc and PhD. He will supervise all the work undertaken at the P1-SURREY site.

Dr. Richard Morgan, PhD (M) is a senior lecturer in molecular oncology and has
extensive experience in molecular biology. He will undertake experimental work with collected tissue in conjunction with partners. He has extensive experience in cell culture, gene cloning, microarray, quantitative PCR, siRNA knockdown, ELISA as well as protein based assays.

Mr Simon Bott, MD (M) is a consultant Urologist at Frimley Park Hospital in Surrey, with extensive clinical experience in bladder cancer and previous bladder biomarker studies. He will provide access to patients and also expert analysis on the relevance of DIPROMON to bladder cancer patient scenarios.

2: Emergentec biodevelopment GmbH (EMTEC)
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Emergentec, established in 2002, develops and offers best-of-breed software, know-how and workflows for data driven biotech and pharma R&D. Our solutions are geared towards next level biomarkers and targets, patient stratification, and drug repositioning – key elements for improving diagnosis and therapy of human diseases.

Our products and business solutions address three major aspects in data-driven Life Sciences: management, integration, and analysis of data and information – with clear focus on candidate finding and lead development in the realm of diagnosis, prognosis and therapy of human disease. We offer software, know-how driven services, and analysis contract research on the basis of our lead platforms BASE, BIO and UNITS, see www.emergentec.com for further details.
Within DIPROMON we further develop and apply our key platforms for organizing large scale bladder cancer multi-Omics profiling data (via BASE), from there utilizing our UNITS concept for biomarker candidate finding and evaluation regarding i) diagnosis of recurrence, ii) diagnosis at initial presentation, and iii) prognosis of disease progression and optimized therapy regimes, complemented by utilizing our BIO annotation platform for optimal biomarker candidate selection.

3) **AXO Science SAS**

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The core expertise of AXO Science is to develop and perform cost efficient, high-throughput and multiparametric analysis. More precisely, the proposed technology uses an innovative microarray format based on a patented technology which enables the immobilization of up to 200 probes at the bottom of a 96-well plate (100 spots for a 384-well plate). This density of probes can only be achieved thanks to the specially developed adhesive surface on which probes are spotted and which is subsequently assembled with bottomless microtiter plates.

For quantitating multimarker profiles, AXO Science will establish a modular assay platform allowing highly standardized measurement of sera and urine. The developed platform, unique thanks to its flexibility and automation in the field of
diagnostic microarrays, will have to generate a simple multiplex identification in a high-throughput format.

The added value of the AXO Science technology put in place in DIPROMON in comparison to existing technologies includes:

- A high density of probes
- The use of a classical microtiter plate format enabling the easy automation of the developed multiplexed high-throughput assays
- A quantitative detection of the level of interactions on each spot using a cost efficient optical imaging
- The possibility to use blood or sera samples without any pre-treatment and to integrate in the robotic platform any sample pre-treatment step (filtering, DNA extraction, PCR…)

**AXO Science HIFI Technology**

By combining latest spotting techniques, fully robotized platform and new optical reading systems, AXO Science *HIFI Technology* opened a new era in the large scale screening of several parameters for numerous patients.

*HIFI Technology* shows several assets:
fully robotized platform for multiplexed high-throughput assays can be adapted to any DNA, protein and peptide based assays

- up to 100 molecular probes can be immobilized in each well
- classical 96 or 384 wells format, processable in any automated laboratory system
- cost-efficient optical detection of the positive results
- quantitative detection of the level of interactions on each spot
- protocol increasing the interactions between targets and immobilized probes and lowering the assay background
- overall process is fast and cost efficient

AXO Science HIFI Technology can be adapted to many molecular based diagnostic applications (blood genotyping, cancer diagnostic, pathogen detection etc.)

Multiplexed assays bring the ability to profile multiple molecules from a single sample, in a single assay and are the next generation of bioanalytical systems. They offer many advantages:

- Generate more information on interrelationships between related analytes within a sample with better correlation
- Decrease precious sample volume requirement
- Reduce assay reagent, expense and labor
LIONEX is growing biotech company with exceptional commitment to research and product development to combat tropical diseases such as TB, HIV and malaria. Immunotherapy of Urinary Bladder Cancer is an additional area of research where significant progress has been made. One product of LIONEX is ready for clinical trials. Based on decades of experience and expertise, LIONEX has developed and produced a large number of recombinant antigens suitable for developing diagnostic tests, vaccines and adjuvants. LIONEX has excellent facilities and expertise including P2 and access to P3 facilities using expression systems such as *E.coli*, Baculoviruses, mycobacteria and Eucaryotic systems. More than 100 recombinant proteins of *M. tuberculosis* have already been produced by LIONEX and are available in significant quantities. This fact shall be of considerable advantage for developing diagnostic products. A series of rapid tests and ELISA kits are available for diagnosis and for measuring immune response in vaccine development studies. Several of the LIONEX products are currently in development as therapeutics for Tuberculosis and Cancer. Intensive research during the last four years has resulted in highly promising and safe drug candidates with strong bactericidal activity against drug resistant *M. tuberculosis*. 
The AIT Austrian Institute of Technology is an Austrian research company with an European structure and Austria’s largest non-university research institute for applied research. AIT provides research and technological development to realize basic innovations for the next generation of infrastructure related technologies in the fields of health & environment, energy, mobility and safety & security. These technological research areas are supplemented by the competence in foresight & policy development.

The Health & Environment Department uses its expertise in three sectors, namely environment, life style & nutrition and health & predisposition to develop technologies and solutions that are designed to improve health and quality of life for everyone. We combine modelling and simulation, sensor and ‘omics’ technologies as well as system integration with longstanding regulatory know-how to create innovative solutions for our customers and partners in specific research fields.

The main role of AIT in DIPROMON is assay development and validation for rapid multiplex detection of protein biomarkers in urine to support treatment decisions in bladder cancer. AIT has extensive experience in the development of protein biomarker arrays for medical diagnostics, and is currently running a multi-national project on the development and validation of a point-of-care device for sepsis (www.valipro.eu). The on-chip assays will be established on proprietary ARChips Epoxy using standard microarray technology and transferred to the AxoScience platform to run the clinical trials.
OnkoTec develops systems for fast, inexpensive and reliable diagnosis of different diseases, based on automatic analysis of released cells and protein biomarkers from body fluids.

We develop the **Onkovidon family of markers**, e.g. **Onkovidon**\textsuperscript{green}, a long term life cell lipid stain, the novel near-infrared dyes, **Onkovidon**\textsuperscript{NIR}, for specific staining of tumor tissue, and a novel staining kit, **Onkovidon**\textsuperscript{plus}, for fluorescence cytodiagnosis. The **OnkoCell-C instrumentation family** is a diagnostic device for use in automated and reliable fluorescence cytodiagnosis or in other applications that require scanning larger areas with high microscopic resolution. Within DIPROMON we will combine fluorescence cytology with specific biomarker detection for reliable recurrence tests of urothelial bladder carcinoma.

The imaging unit **OnkoCell-C** (left) and urothelium cells (right) stained with **Onkovidon**\textsuperscript{dual}