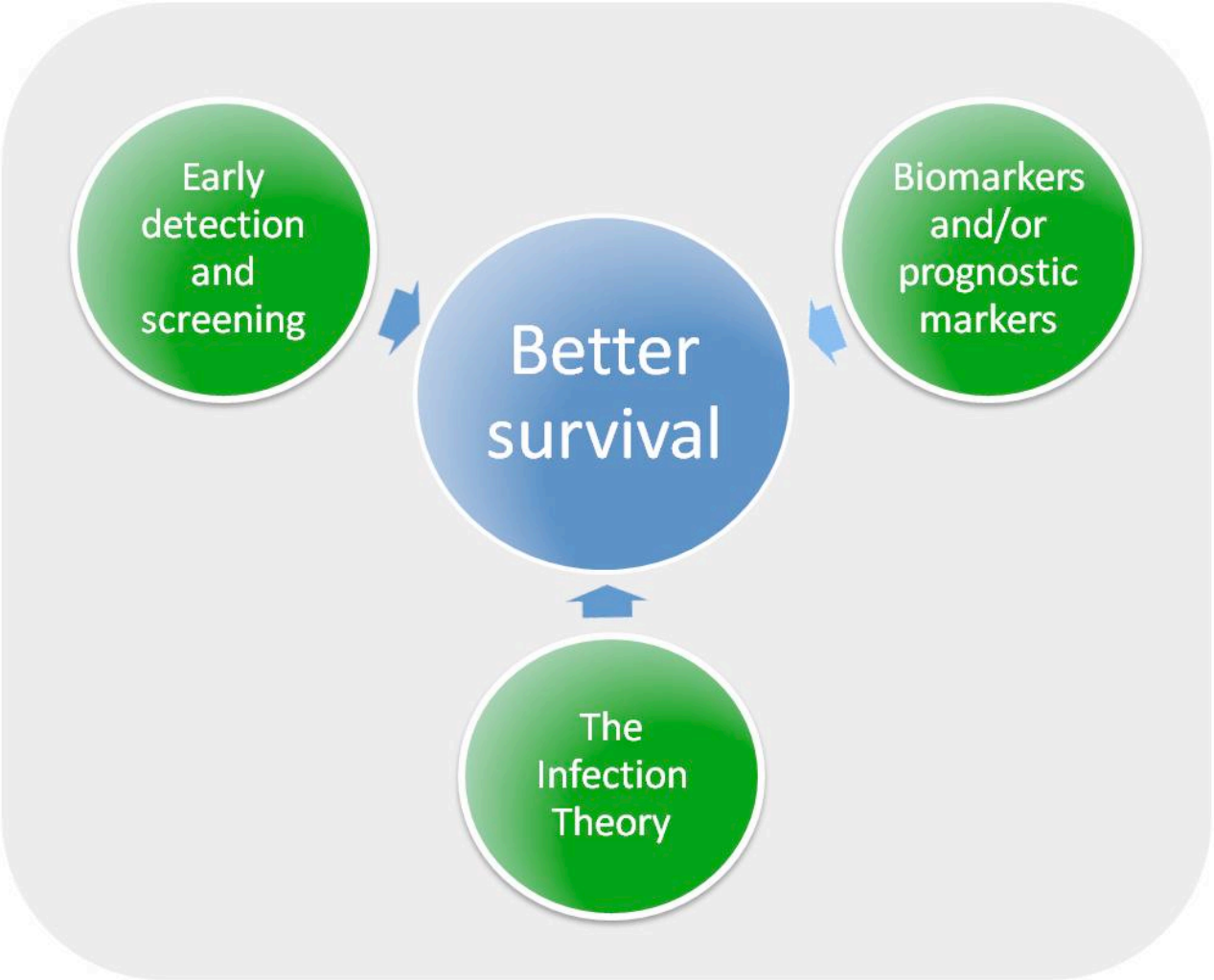
Mermaid III

The challenge of ovarian cancer:

Screening, early diagnosis and identification

of high-risk women



Mermaid

The idea for Mermaid arose in the year 2000. The vision was to secure funding for front-line research for abdominal cancer in women. We can now report that the initiative has been a great success. Research on cervical and ovarian cancer has been supported with over DKK 45 million. More than 60 scientific articles in international journals have reported the important new findings that these efforts have produced.

MERMAID I focused on ovarian cancer. The identification of new genetic and molecular factors has contributed to a better understanding of the development of the disease and its course. At the same time, the initiative has been taken to establish an international consortium, The Ovarian Cancer Association Consortium, covering more than 30 different, highly esteemed research groups from around the world. Mermaid I has thus ensured a contribution to better survival for this group of women.

The point of departure of MERMAID II was research on cervical cancer. Notable results have been achieved in just a few years: identification of virus types that pose an especially high risk of the development of cancer, which makes it possible to identify – and thus help – women at particularly high risk.

Further information about MERMAID I and MERMAID II on the webside: www.mermaidprojektet.dk).

MERMAID III

MERMAID III is the latest project and is outlined below. The focus is again on ovarian cancer, as the form of cancer in women with the highest mortality rate.

The first breakthrough

Ovarian cancer is the form of abdominal cancer that represents the commonest cause of death among women in Europe and the USA. In 2015, for example, 140 000 women died of this serious disease. 10 years ago, Danish women had the highest risk of contracting the disease and at the same time the lowest chances of survival. These facts prompted a number of research and development initiatives – including the Mermaid I project – aimed at improving all aspects of the course of the disease in women: from the time that they observe the first symptoms at home through to the diagnostic phase, therapy and ultimately the rehabilitation phase (Figure 1). We can now see the results of the multifaceted initiatives. The latest reports show that survival in these women has improved by 8-10%. Denmark and Finland are now the two Nordic countries with the best survival, and in the coming years survival will probably improve a further 10% so that we will reach a level on a par with the best centers in the world.

Even with these improvements, however, it will still be only one in every two women who will survive the disease.

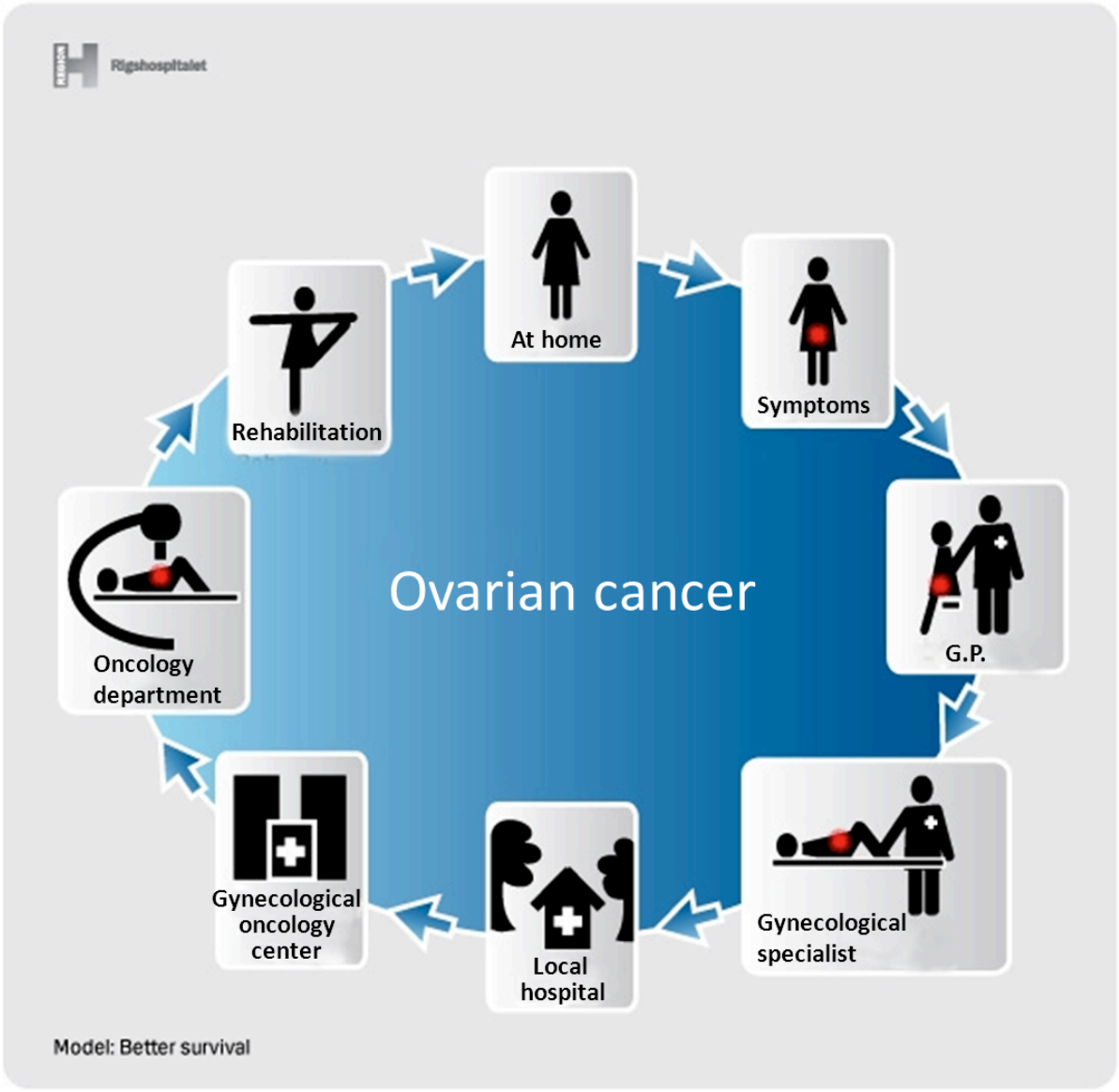


Figure 1: Over the past 10 years, research initiatives have focused on better therapy in the form of optimal surgery via centralization, better diagnosis, etc.

The decisive breakthrough for survival

We do not know what causes ovarian cancer. The disease does not usually produce symptoms until it is at an advanced stage, which explains the high mortality rate. A decisive breakthrough to improve survival will be early detection and diagnosis. If we can identify all women by the earliest

stage, survival will reach as high as 90%. In other words, with a completely optimal situation, we will, purely in Denmark, be able to save the lives of more than 200 women each year.

Technological development within, among other things, the field of molecular biology provides real hope that we can achieve our aim of better survival for these women. The present project proposal is based precisely on these unique opportunities and addresses the problem from a number of angles, see Figure 2.

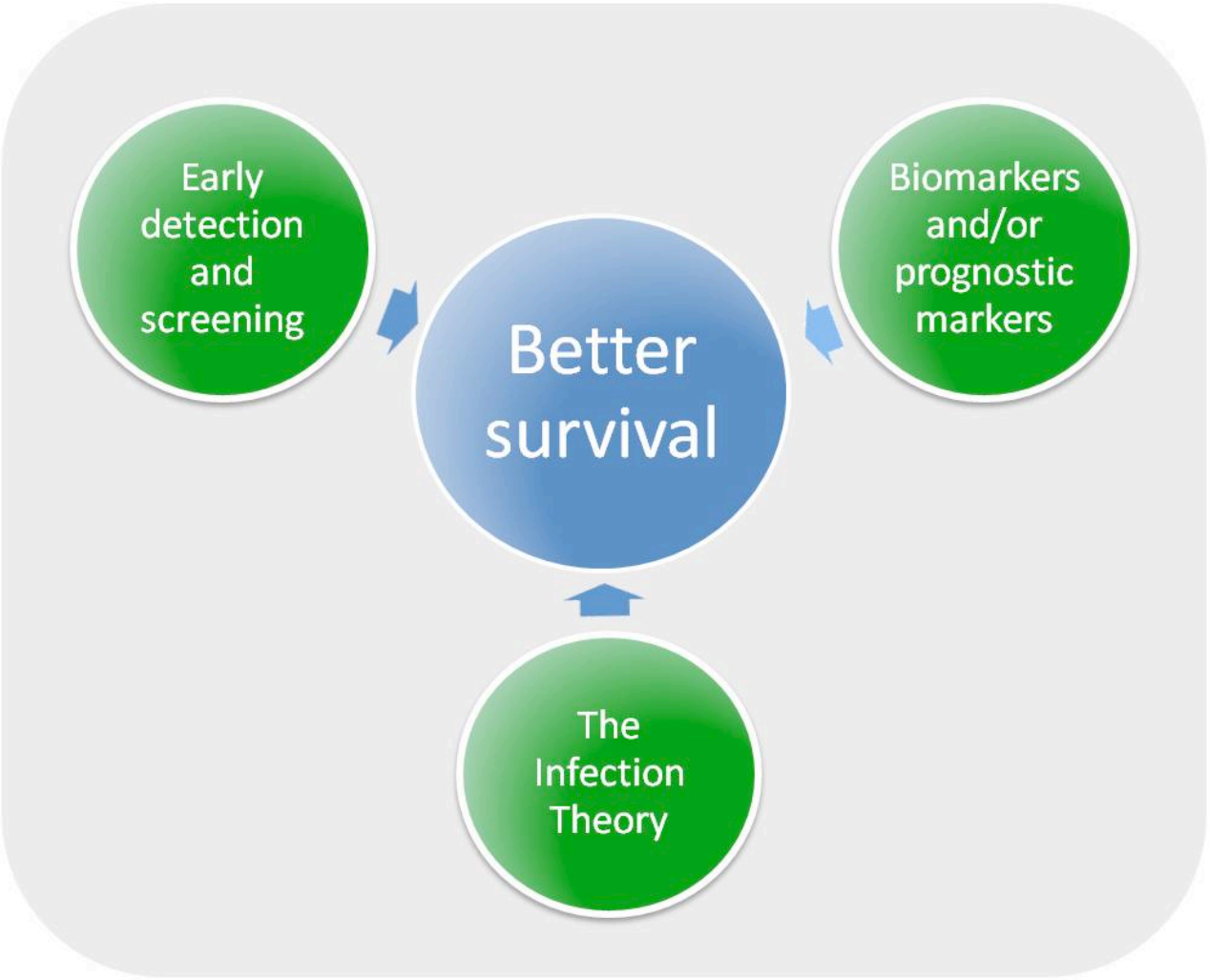


Figure 2: Achieving a marked improvement in survival for women with ovarian cancer necessitates addressing the problem from a number of angles.

Mermaid III for better survival.

The above is the point of departure for Mermaid III. The combination of a team of researchers who have detailed experience and knowledge within the field, existing biobanks and databases

and new technological advances makes the present project blueprint realistic and provides a much more advanced starting point than when Mermaid I started. The project will focus on finding the cause of the disease, early diagnosis, screening and the identification of high-risk women. All efforts are aimed, at best, at preventing the development of ovarian cancer or making the diagnosis early enough for the woman to survive.

The three action areas are briefly outlined below.

Early detection and screening

DNA from routine cervical cytology samples - the value in early detection or screening for ovarian cancer

Identification of gene changes and other biomarkers for use in screening for ovarian cancer or for identifying women at high risk of developing ovarian cancer is the first approach to meeting the challenge. From biological material, such as cell samples from the cervix, it is possible to extract DNA (hereditary material), and this forms the basis for being able to identify potential changes in various genes and also various biomarkers. A provisional investigation has indicated that, for example, mutations in the p53 gene can be detected in cell samples from the cervix taken during the routine screening for cervical cancer in which a very large proportion of Danish women take part. The p53 gene is a tumor suppressor gene, i.e. if a cell has sustained DNA damage, p53 ensures that the cell cannot divide and form new cells until it has been attempted to rectify the defect. We know that mutations in the p53 gene have taken place in a very large proportion of patients with ovarian cancer. This marker has therefore been chosen as the first for testing whether we can detect ovarian cancer early enough for virtually all women to survive, or whether we can identify women who are at particularly high risk of developing ovarian cancer, thus enabling them to be monitored closely and, where appropriate, undergo risk-lowering surgical removal of their ovaries.

In the proposed MERMAID III project, the collection of samples will include pathology departments in Denmark, where cell samples from the cervix are taken using a liquid-based method. We propose collecting samples from more than 100 000 Danish women to obtain substantial biological material from which DNA/RNA can be extracted.

Data from this large group of women will be regularly linked to the Pathology Database (a register containing the results of all histological investigations conducted in Denmark) to identify women who have developed ovarian cancer. The cell samples from these women with ovarian cancer and also samples from a select group of women who have not developed ovarian cancer will be tested for changes in various genes such as p53, but also other potential markers such as KRAS, changes in methylation patterns and various micro-RNA will be investigated and validated. The potential markers included in the project will be selected on the basis of results from the most renowned medical centers across the world. It is also a strength of the project that findings from the “Pelvic Mass” study can be evaluated in this unique biological material from women before they develop cancer.

This nationwide collection of DNA material from healthy women will become internationally unique biological material for testing biomarkers for ovarian cancer and also offers substantial research potential in relation to other forms of cancer in women.

Biomarkers for ovarian cancer and/or prognostic markers
Beyond present knowledge of the ovarian cancer disease

Markers are specific signal substances that are released from the cancer tumor and which can be detected in the blood using sensitive measurement methods. This will therefore be crucial if we are to be able to demonstrate a marker that is exclusively produced in ovarian cancer. This will allow early detection during, for example, screening and better diagnosis. Detailed knowledge of the human genome and the genetic processes has yielded a range of new marker candidates. The new markers are involved in the biological processes associated with cancer development (MicroRNA), may directly inhibit cancer (P53) or directly serve as an on/off button for cancer development (KRAS). The investigations will be conducted on existing material from our "Pelvic Mass" ovarian cancer biobank.

Epigenetics is the study of hereditary aspects in the cells other than those brought about by DNA. This research discipline started in 2011 and is thus one of the newest within cancer research with particularly high expectations for scope for screening. These analyses are performed by investigating 'DNA methylation changes' and are implemented at the Molecular Unit, the Pathology Department, Herlev Hospital.

One of the fundamental prerequisites for understanding the cause and development of cancer is elucidation of the underlying changes in the genome (DNA). Currently only around 5% of cases of ovarian cancer are explained by known changes in DNA (Breast Ovarian Cancer genes - BRCA). Based on genealogical trees in families with ovarian cancer, it can be calculated that a further 5-10% must be due to other more commonly occurring unknown genetic DNA changes. The other approx. 85% of ovarian cancer cases arise sporadically, probably as a result of rarer unknown DNA changes.

Our lack of knowledge of further mutations is due to the fact that we previously did not have technology for investigating the genes within a given time and within financially reasonable constraints. The great wish for the future is that we can clarify all the genes that are important for the development of ovarian cancer. This calls for each patient's total, million-strong DNA structure (genome) to be described down to the individual DNA building blocks. In this way, the underlying DNA changes can be described and form the basis for all the research disciplines of diagnosis, screening, prognosis and new biologically based therapies.

The prospects of this research being capable of being implemented are great as regards requirements for a large number of patients, a high-quality biobank, the necessary data, academic personnel engaged in molecular biology and the latest technology within this area. Technologically, a platform has just been developed that makes it possible to elucidate the entire genetic "map" for each patient. Because the platform heralds a new genetic era, this technology has been dubbed *Next-generation sequencing.*

The infection theory

The infection theory is based on the hypothesis that ovarian cancer is due to an infection, whether viral or bacterial, that is transmitted from the vagina to the abdominal cavity via the uterus and uterine tubes.

The cause of ovarian cancer has never been demonstrated, but epidemiological studies have uncovered possible factors (removal of the uterus, sterilization and many pregnancies) that may cut the risk of the potential development of ovarian cancer. A feature common to the risk-reducing factors is that they break the link between the woman's vagina and abdominal cavity, and thus eliminate the possibility of an infectious agent reaching the mucosal membrane in the abdominal cavity where ovarian cancer arises. An interesting observation in this context is that primary peritoneal cancer (cancer of the mucosal membrane in the abdominal cavity), which is analogous to ovarian cancer, has never been detected in a man, where the anatomical difference means that it is not possible for a bacterium or virus to reach the abdominal cavity from outside. In more and more cancer diseases, a link is being detected with an earlier infection.

We will first of all study 200 women with ovarian cancer from the "Pelvic Mass" project at the Rigshospitalet and Aarhus University Hospital, Skejby. Tissue samples will be investigated for possible viral and bacterial infection. Newly developed methods in the field of molecular biology mean that we are nowadays much better able to detect even very small quantities of viruses and bacteria. Viruses can be detected using micro array methods, which will be employed in collaboration with colleagues in the USA. The bacterial studies, which can detect even small quantities of bacterial DNA, will be conducted in Denmark.

If in this way we obtain knowledge of the natural history of the disease, this will open up completely new scope for diagnosis and therapy. The whole of research into cervical cancer serves as a comparison, in which we now know that a virus is the cause and we can vaccinate against the disease.

Mermaid III for better survival.

The research plan described opens up the possibility that purely in Denmark we can save 200 women's lives by detecting ovarian cancer at a stage when the disease is curable, or – even better – completely prevent the disease. The teams of researchers involved all have extensive experience of research at the highest international level within their respective areas of interest. With its databases, nationwide screening and personal identification number system, Denmark provides a unique point of departure for these studies.

The following are responsible for the three projects: Professor Susanne Krüger Kjær, University of Copenhagen, Rigshospitalet, Professor Claus Høgdall, University of Copenhagen, Rigshospitalet and Professor Jan Blaakær, Århus University Hospital.

The project will therefore be able to make a solid contribution in the fight against cancer.