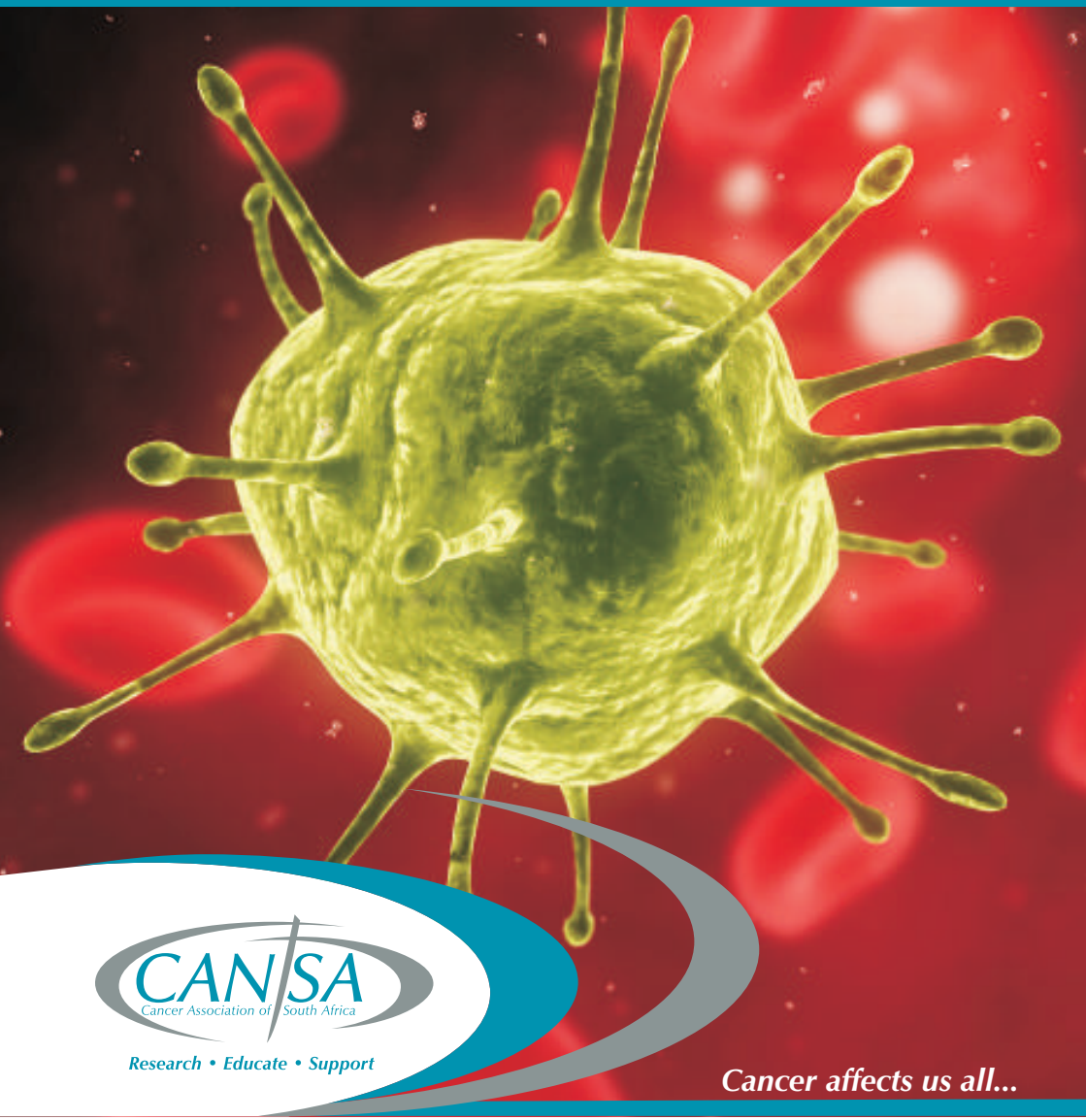
CANSA DETECTIVES

Tracking the Cervical Cancer Virus in South African Men and Women



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CANCER DETECTIVES

Booklet 2

Figure 1: An electron micrograph of human papillomavirus (55mm in diameter) (kindly provided by Dr Linda Stannard)

Cervical cancer is the second most common cancer worldwide in women, after breast cancer. Human papillomaviruses are a major part of a big family of viruses that include many animal viruses, as well as those causing warts and other lesions in humans. HPV ranks as one of the most common sexually transmitted infections worldwide. Specific HPV types cause cancer: there are 15 "high-risk" HPV types causally associated with cervical cancer, as well as with other rarer cancers such as of the penis, of the anus and vulva.

HPV-16 is the dominant type associated with more than half of all cervical cancers worldwide. HPV is also involved in oral tumours and possibly with oesophageal cancer. While HPV-16 is the dominant type detected in oral cancers, unlike cervical tumours, not all of these cancers are associated with HPV infection. It is important to understand that not all people infected with high-risk HPVs will go on to develop cancer: most people will clear their infections without problems.

Prevention of Cervical Cancer

Cervical cancer prevention schemes have traditionally used the Papanicolaou (Pap) smear technique to screen women at regular intervals: cellular changes caused by virus infection on the cervix can be detected by microscopic examination of cells scraped from the cervix. Abnormal Pap smears may be followed up using a more sophisticated test, such as histology on a cervical biopsy (Figure 3).

In South Africa the Pap smear is offered in the public sector clinics 3 times in a woman's lifetime. Women with abnormal smears are referred to tertiary hospitals for further follow-up, including therapy. Cervical cancer takes many years to develop after infection with HPV; this gives an opportunity for cervical screening to detect pre-cancers before the development of invasive cancer, and then the treatment of those women with abnormal Pap smears.

This is a very effective strategy for reducing cervical cancer: however, it has proved a challenge to introduce an effective cervical screening programme into the public health sector in South Africa. As a result, women still do not have access to adequate cervical screening and treatment, and significant numbers are still dying of a preventable disease.

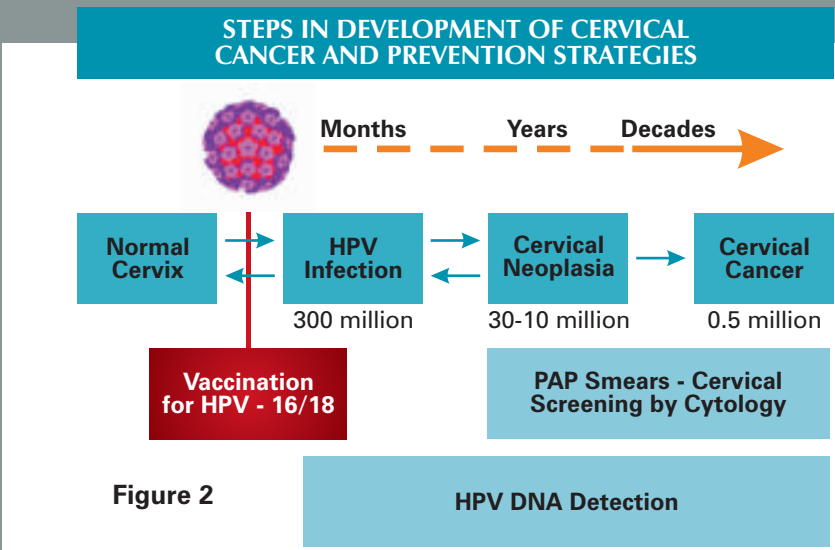

Figure 2

Figure 2: This diagram shows that worldwide most women infected with HPV at the cervix (300 million per annum) will clear their infection and only 10 to 30 million will develop cervical neoplasia or abnormalities, and only 0.5 million will develop cervical cancer per year. Cervical neoplasia can take months to develop after HPV infection, and cervical cancer can take decades. Cervical cell changes are detected by Pap smears, and HPV infections by HPV DNA detection. HPV vaccination against HPV types 16 and 18 is recommended before HPV infection and at the onset of sexual intercourse.

In recent years there has been discussion of screening for HPV DNA to increase the effectiveness of the screening programmes: this would detect the DNA of the HPV types associated with cervical cancer, and identify women at higher risk of cervical disease. This molecular test is easier to implement than cytology, but costs significantly more.

Vaccination is acknowledged as the best strategy to prevent infectious disease. Two prophylactic HPV vaccines - GlaxoSmithKline's Cervarix, and Merck's Gardasil - have recently been introduced into the world market, after extensive clinical trials proved their efficacy and safety. Cervarix vaccinates against HPV-16 and HPV-18 and Gardasil against HPV-16, -18, -6 and -11.

It has been shown that HPV-16/18 cause >70% of cervical cancers worldwide and in more than 60% of South Africans, and HPV-6 and HPV-11 together cause almost 100% of genital warts. Both these vaccines are shown to cross protect to differing degrees from infection to those types which are closely related to the types in the vaccines.

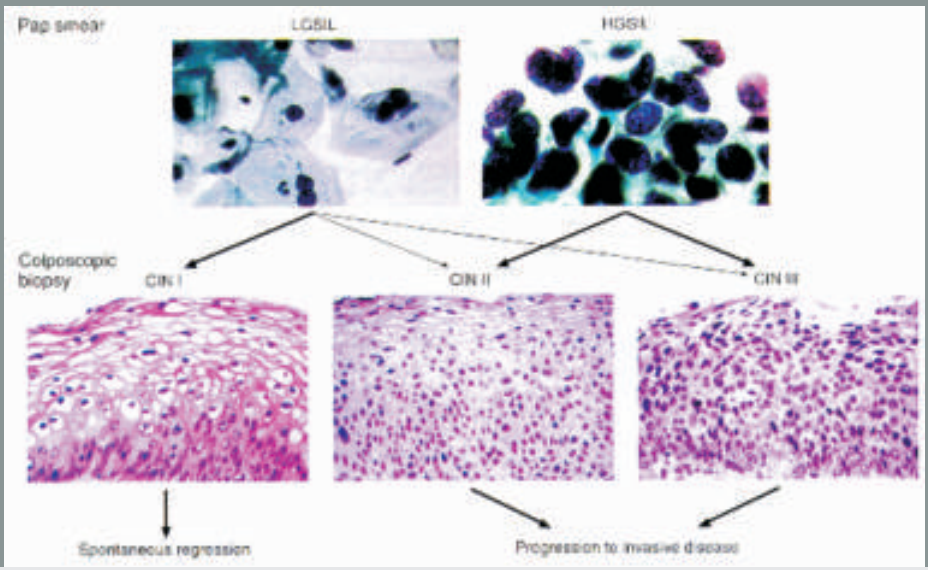

Figure 3: Cellular changes seen at the cervix following Pap smear, LGSIL (Low grade squamous intraepithelial lesions) and HSIL (High grade squamous intraepithelial lesions) or following biopsy (Kindly provided by Professor M. Hagensee, Louisiana State University)

The vaccines protect very efficiently in individuals without prior HPV infection, ideally in those aged 9-14 years, and are less effective in those already exposed to the vaccine-related types. A study into the cost effectiveness of an HPV vaccine offered to South African girls indicated that a current vaccine price reduction of 60% or more would make a vaccine plus screening strategy more cost-effective than a screening only programme.

HPV vaccination will considerably reduce cervical cancer and other HPV-related disease in South African people. The vaccines were licensed for use in South Africa in 2008, but have yet to be introduced into the public sector. In countries such as Australia, where the vaccines have been widely used, there is already a significant impact on the incidence of genital warts in vaccinated women. Although young men were not vaccinated, they also had a significant decrease in genital warts, indicating the value of herd immunity. The present vaccines do not protect against all HPVs that could cause cervical cancer, however, so it is necessary for vaccinated women to continue to engage in cervical screening programmes.

As genital HPV infections are sexually transmitted, those benefiting most from vaccination are girls or boys aged 9-14 years, which is generally before the commencement of sexual activity. This has important implications for vaccination strategies as these will have to be implemented at schools rather than at clinics, and may require parental consent for vaccinating teenagers. Despite general public ignorance about HPV, there has been generally good acceptance of HPV vaccination worldwide.

HIV and HPV

Studies investigating the global distribution of HIV reported that three quarters of the HIV infected people world-wide live in Africa, and most of them are located in sub-Saharan Africa. Infection with both HIV and HPV is therefore highly likely in Africa, and may lead to a serious health crisis – in part, because HPV-associated cancers in AIDS patients occur more frequently than in HIV-negative individuals. In women, HPV-associated cervical, vulva or vaginal cancer and anal cancer and in men, penile and anal cancers are significantly increased in HIV-positive patients compared to HIV-negative patients. HIV-positive women are also four times more susceptible to infection by HPV high risk types. HIV infection and its associated immune suppression enhance progression to high grade cervical disease and decrease the clearance of HPV infection.

HIV-positive women progress to cervical cancer about 10 years earlier than HIV-negative women. HIV-positive men have a higher prevalence of genital HPV DNA and a higher HPV viral load in urine than HIV-negative men. HPV prevalence is greater in HIV infected women with a CD4 cell count $\leq 200/mm^3$ and high plasma HIV RNA levels.

HPV Research at the University of Cape Town

Antibody studies

Present or prior exposure to the virus can be determined by testing for HPV antibodies in serum, cervical secretions or saliva by enzyme-linked immunosorbent assay (ELISA). ELISA testing is used only for research and epidemiological studies as not everyone makes antibodies at detectable levels. UCT were the first group to report using saliva to test for HPV antibodies. In collaboration with the Cancer Epidemiology Research Group (NHLS, Johannesburg) the UCT HPV group showed a statistically significant association with increased anti-HPV-16 IgG antibody (Ab) levels with cancer of the cervix, cancers of other anogenital organs and cancer of the oesophagus.

Vaccine Development Studies

While the commercial vaccines currently available are both blockbusters, with sales of more than US$1 billion a year, there is still room for improvement. Both vaccines are expensive (at least US$240 per 3-dose course in the private sector), and only protect against two of at least 15 high-risk types (16 and 18). Accordingly, with CANSA support in the mid-1990s, our larger group started to investigate the feasibility of both cheaper means of production of conventional vaccines in bacteria and in plants, and the possibility of making "chimaeric" or hybrid vaccines which would be more broadly cross-protective. This work showed sufficient promise that the project attracted two consecutive NRF Innovation Fund investments (1999-2002, 2002-2005, to Anna-Lise Williamson and Ed Rybicki respectively), an investment from DST (SA-Cuba joint projects, PI Ed Rybicki), generated three patents, and subsequent interest from Big Pharma in terms of licensing agreements.

Successes included the demonstration in an animal model system that Bacillus Calmette-Guerin (BCG) could act as a useful vector for a papillomavirus vaccine and that a plant-made papillomavirus antigen was a protective vaccine in rabbits, that HPV virus-like particles (VLPs) could be made in quantity in plant systems and could elicit neutralising antibodies, and that chimaeric HPV VLPs could be made in insect cells. A significant financial return for CANSA was realised by commercial licencing of the patent on the latter vaccine.

Future vaccine research plans include exploring plant production of chimaeric vaccines – funded until 2011 by the DST SA-Cuba project – and attempting to get funding for cGMP manufacture of a plant-produced HPV-16 VLP vaccine for Phase I clinical trial, given considerable international interest in the product.

Cervical Disease and HPV Typing

South African women have a high prevalence of HPV and associated cervical disease including cancer. Cancer of the cervix is the second most common cancer among black South African women, with an age standardized incidence rate (ASIR) of 30 per 100 000 per year. In Finland where the cervical screening programme is well implemented the ASIR is 3.7 per 100 00 per year. It is interesting to note that this is a 70-80% decrease in cervical cancer over 30 years.

Cervical specimens obtained from cervical cancer case-control study (n=1002 control women) performed at UCT were tested to determine the prevalence of HPV infection. In women younger than 30 years, cervical HPV infection was found in 41.8%, this decreased in women aged 30 to 44 years to a low of 18.6% at 45 to 49 years. HPV prevalence then increased slightly in women older than 50 or between 55 and 59 years (23.5% and 21.4%, respectively) (Figure 4). This is very high compared to most countries around the world especially in the older women where HPV prevalence is the range of 5%. HPV-16 and HPV-35 were the most prominent types detected in women with high grade cervical disease.

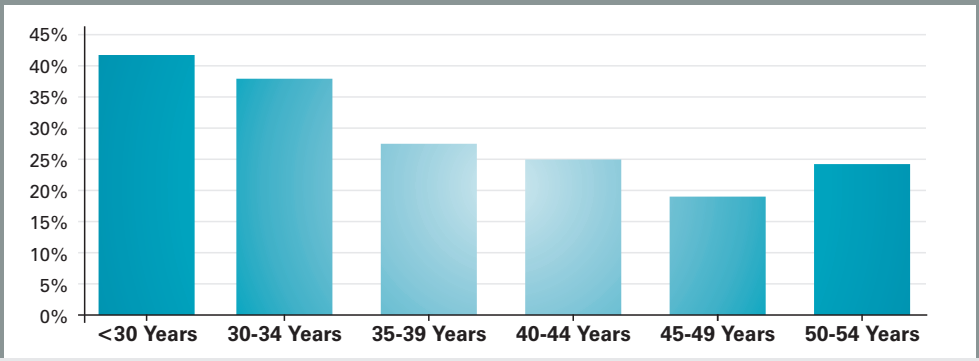

Figure 4: Percentage of Control women (n=1002) HPV positive according to age
Marais et al. (2008) J Clin Microbiol. 46(2):732-9.

The prevalence of HPV in the oral cavity is lower than that observed in the genital tract. In another study done in Cape Town on healthy individuals, oral HPV infection was highest in children (7.9%), followed by adolescents (5.1%), and lowest in adults (3.5%). The predominant HPV type found was HPV-13 followed by HPV-32.

Studies on Couples

Although HPV infection has been studied extensively in women, data on male genital infections and on transmission between couples are limited, particularly in Africa. Anogenital HPV infection in men is largely asymptomatic, but is believed to be responsible for the sustained transmission of HPV to their female partners and thus the perpetuation of HPV in the population. Joel Palefsky (UCSF, USA) stated "Although HPV is transmitted sexually and infects the genitals of both sexes, the cervix remains biologically more vulnerable to malignant transformation than does the penis or anus in men. An understanding of male HPV infection is therefore important in terms of reducing transmission of HPV to women and improving women's health". The incidence of cervical disease is significantly increased in women with partners with penile cancer.

HPV infection has been found in 80% of penile tumours with HPV-16 evident in 69% of these. The prevention of HPV infection in men is considered therefore another way of eliminating cervical cancer in women, as well as penile and anal cancer and genital warts in men. Women with HPV infected male sexual partners are more likely to acquire HPV infection and develop cervical disease. The established risk factors for penile HPV in men are their lifetime number of female sex partners, smoking, and not using condoms. The data on the impact of condoms on HPV transmission in women varies from studies where no association was found to studies where consistent condom use in partners of newly sexually active women reduced the risk of HPV infection and associated disease.

It has also been reported that condom use can protect men from HPV associated penile lesions. High-risk HPV types and their persistence in men are associated with a higher number of sexual partners and abnormal cervical cytology in their partner. A high HPV viral load may enhance viral transmission between partners. Together with HPV-associated lesions, penile diseases may be enhanced by poor penile hygiene.

Recent studies at UCT have investigated the prevalence of genital HPV in sexually active couples and the effect of HIV infection on this and HPV transmission between partners. Couples were recruited from a clinic in Gugulethu, Cape Town, South Africa. Genital samples (a cervical swab in women and penile swab in men) were taken to assess the HPV DNA presence and blood for HIV and HPV antibody testing. Couples were either both HIV-positive, both HIV-negative or HIV-discordant (one partner HIV-positive).

Figure 5: The clinic where study participants were investigated (1) clinic waiting room (2) and clinic reception (3). The clinician in the patient sampling area (4&5) and the research assistant in the laboratory where specimens were processed (6).

HPV genotyping was performed on cervical and penile samples using the Roche Linear Array HPV genotyping test which identifies 37 different HPV genotypes. The prevalence of genital HPV infection of any type was significantly higher in both HIV-positive women (74%) and men (81%); compared to HIV-negative women (37%) and men (49%). HIV-negative men showed a significantly higher prevalence of HPV than HIV-negative women (49% compared to 37%, Figure 6).

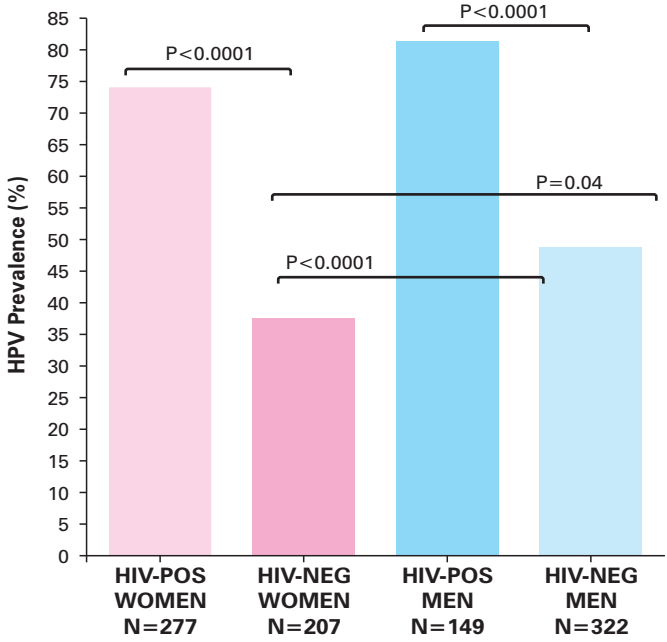

Figure 6: The prevalence of cervical and penile HPV infection according to HIV status (HIV-pos or HIV-neg). The solid red horizontal line in each group indicates the percentage of multiple infections.

HIV-positive women were found to have 3.4 fold higher risk of multiple HPV infection compared to HIV-negative women (50% 139/277 compared to 15% 31/207, P<0.0001). HIV-positive men were found to have 2.2 fold higher risk of multiple HPV infection (66% 99/149 compared to 30% 96/322, P<0.0001; Mbulawa et al., 2010).

HPV transmission between sexually active couples was studied. HPV transmission was defined as the presence of the same HPV type in the partner at next visit initially detected in the index partner. HPV transmission analysis was restricted to couples in which at least one partner was HPV positive at baseline or HPV discordant. Female to male HPV transmission rate per 1000 person-months was more common compared to male to female HPV transmission (28.0 compared to 11.7 rate per 1000 person-months). In male to female transmission events, LR-HPV transmission rate was more common compared HR-HPV transmission rate. In contrast in female to male transmission events, HR-HPV transmission rate were similar (Table 1).

Table 1: Human papillomavirus transmission among heterosexually active couples over 24-month period

	Female to Male		Male to Female	
	TRANSMISSION RATE/1 000 PM	TRANSMISSION EVENTS	TRANSMISSION RATE/1 000 PM	TRANSMISSION EVENTS
Any HPV types	28.0	66	11.7	42
HR-HPV	29.2	38	6.0	9
LR-HPV	26.4	28	15.8	33

HR: high-risk - LR: low-risk

The difference on transmission is at present unclear, however, it could be due to different types could be due to the fact that women were only sampled at the cervix not the vagina while in men samples were from more than one genital site (penile shaft, glans as well as foreskin in uncircumcised men). The risk of HPV transmission to the partner was significantly increased by HIV-positive status and low CD4 counts.

Conclusions from UCT Research

The HPV typing data confirmed that HPV-16 is the dominant type in women with cervical disease indicating that a vaccine that protects from HPV-16 would have a significant impact on cervical cancer in the South African population. HIV co-infection with HPV is a significant problem in South Africa. Women with HIV are more likely to be infected with multiple HPV types, are more likely to share HPV with their partners and more likely to transmit the virus to their partners. As more women receive anti-retroviral therapy the problem of cervical disease in HIV positive women is going to have a significant impact on the health care system. The high prevalence of HPV in men was confirmation of the importance of formulating strategies to deal with HPV in both men and women.

Recommendations

- Expand the present cervical screening programme and improve follow-up and treatment of women with cervical disease.
- Introduce HPV vaccination into schools to at least vaccinate all adolescent girls.
- Manage cervical disease in HIV positive women as part of their overall treatment.

Acknowledgement of major collaborators on HPV projects: David Coetzee, Margaret Hoffman, Jennifer Moodley, Syd Shapiro, Lynette Denny, Jo-Ann Passmore, Leigh Johnson

Key Papers from UCT

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The key scientists that were involved in the human papillomavirus laboratory based research at UCT.

- Professor Anna-Lise Williamson
- Dr Di Marais
- Mr Bruce Allan
- Dr Zizipho Mbulawa
- Ms Anna Salimo
- Dr Vandana Govan
- Dr Inga Hitzeroth
- Professor Ed Rybicki

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Acknowledgements

This booklet was produced and published by the
Cancer Association of South Africa (CANSA)

First published November 2011

Special thanks to Professor Anna-Lise Williamson for telling her story and sharing her records, research and graphics.

CANSA is proud to have been a funder of Professor Williamson's research.

Layout & Design by Limegreen Online Design Print

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