Cervical dysplasia: encapsulated DNA treatment shows promise

Martina Habeck, freelance writer

In a Phase 2B clinical study, a DNA therapy that kills cells infected with human papilloma virus (HPV) has been shown to resolve cervical lesions in 70% of women under 25 years of age.

Infection with HPV accounts for a third of all sexually transmitted disease (STD) cases – some 5.5 million new genital HPV cases occur each year in the USA alone [1]. There are many forms of HPV and it can cause various diseases of the genital tract. The HPV 6 and HPV 11 subtypes are primarily associated with more benign lesions, such as genital warts, whereas the high-risk subtypes, such as HPV 16 and 18, can cause cervical and anal dysplasia, which can progress to cancer.

The idea of a therapeutic vaccine to treat these conditions has been explored for some time. 'The vast majority of people who get infected with oncogenic HPV do clear the virus', says Stephen Tyring, professor of microbiology and immunology at the University of Texas Medical Branch, TX, USA (http://www.utmb.edu). 'To make the body recognize the infectious virus and clear it, to use the body's own immune system, would be an ideal type of therapy.'

Four companies have active drug development programmes in this field, with therapeutic vaccines in the clinic (most have completed Phase 2). The four firms tend to concentrate on different indications, but their test compounds all target the activity of the viral proteins E6 and E7, which are synthesized early during infection and play a key role in cell transformation.

Whereas Stressgen (http://www.stressgen.com), Medigene (http://www.medigene.com) and Xenova (http://www.xenova.co.uk) use protein-based approaches, Zycos (http://www.zycos.com) works with DNA-encoding regions that include immunogenic peptides that are derived from E6 and E7. This approach has the advantage that DNA therapies are much easier to manufacture under GMP conditions than are protein-based treatments, says Lutz Gissmann of the German Cancer Research Center (http://www.dkfz-heidelberg.de/).

Development of ZYC101a

ZYC101a, the lead candidate from Zycos in the HPV therapeutic vaccine programme, contains plasmid DNA encoding immunogenic regions from the E6 and E7 proteins. The plasmid is encapsulated within polymeric microparticles of 1–2 μm.

To identify the immunogenic sequences of E6 and E7, the investigators stripped all of the antigens from cells synthesizing HPV-16 or HPV-18 proteins. The peptides were then purified and sequenced using HPLC and mass spectral analysis. Regions of viral DNA encoding the sequences of those epitopes that were derived from the E6 and E7 proteins were then incorporated into a plasmid with a cytomegalovirus promoter.

To activate the immune system more effectively, the investigators formulated the plasmid DNA within microparticles made of PLG (poly[D,L-lactide-co-glycolide]). 'We wanted the DNA to go to professional antigen-presenting cells [APCs]', says Mary Lynne Hedley, Vice President of Research at Zycos. 'We felt that if we can make our DNA look like a little particle, then we could harness the natural activity of the APC, that is phagocytosis by monocytes that would subsequently differentiate into professional APCs.' The biocompatible and biodegradable polymer PLG was chosen because it is already approved by the FDA for the delivery of sustained-release formulations such as Lupron Depot® (Genentech; http://www.gene.com) and Nutropin Depot® (TAP; http://www.tap.com).

Proof of concept

According to Hedley, preclinical experiments indicated that the approach might indeed be successful. Using an *in vitro* assay for interferon γ secretion, the scientists verified that human T cells recognize the peptides encoded by the drug. Experiments in animal models suggested that the agent might also elicit an immune response *in vivo*: When the investigators repeated the *in vitro* test with lymphocytes isolated from ZYC101a-treated mice, they found the same stimulation of T cells. Finally, studies using a mouse tumour model that synthesizes the E7 protein of HPV and eventually kills the animals demonstrated that the drug could protect against challenge of animals with the tumour. This result suggested that the agent might be effective in treating cervical dysplasia.

Clinical evidence

Following a promising dose-escalation study [2] with a formulation similar to ZYC101a, the company conducted a Phase 2B study that included 161 women with high-grade dysplasia. The study participants were randomized

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to placebo, 100 μg ZYC101a or 200 μg ZYC101a. Treatment was administered three times at three-week intervals as intramuscular injections. After six months, the patients underwent Loop electrosurgical excision procedure (LEEP) surgery and the cervical tissue was evaluated.

At the DNA Vaccines 2002 conference in Edinburgh, UK (23–25 October 2002), Hedley presented preliminary results that showed that, in the study population as a whole, lesions were resolved in 43% versus 27% of women (ZYC101a versus placebo; $p = 0.12$), whereas in women <25 years of age, the lesions resolved in 70% versus 23% of women (ZYC101a versus placebo, $p < 0.01$). The results are encouraging, says Tyring. He believes that 'the success of a therapeutic vaccine will stimulate other people to work on this area with more intensity'.

There is a great need for a medical alternative to currently available treatment options. At present, women with high-grade cervical dysplasia undergo surgery to remove the diseased tissue. However, surgical methods, such as LEEP, do not always clear the disease, and they can lead to complications during pregnancy. This is a crucial problem because more and more women between 12 and 25 years of age present with the disease, relates Hedley.

Future work

Zycos now wants to confirm these data in younger women and find out why the regression of lesions was not significant in women over 25 years of age. 'There should not be a magic cut-off at 25', says Hedley. 'Perhaps we can see some efficacy in those older women as well, potentially by changing the route of injection.'

Hedley and colleagues also want to explore the efficacy of the drug in other indications. Although cervical dysplasia is mainly associated with HPV-16 and HPV-18, 20 different HPV subtypes were present at baseline in the study population. ZYC101a cross-reacted with most of these subtypes.

This result suggests that the agent, or a similar formulation, could be beneficial in treating genital warts, anal dysplasia and even cervical cancer. 'I think what we are seeing is the tip of the iceberg', says Hedley. 'HPV causes so many [conditions] that once you have this initial efficacy, this really opens up a large possibility for you.'

References

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2 Sheets, E.E. *et al.* Immunotherapy of human cervical high-grade cervical intraepithelial neoplasia with microparticle delivered HPV 16 E7 plasmid DNA. *Am. J. Obstet. Gynecol.* (in press)

New immunotherapy triple strategy for AML

Kathryn Senior, freelance writer

A patient's own blood cells could be manipulated to 'mop up' residual malignant cells that might remain after standard treatments for acute myeloid leukaemia (AML), according to a study published in late October 2002 [1]. Edward Ball's group at the UCSD Cancer Center (University of California at San Diego, CA, USA; http://cancer.ucsd.edu/) treated the AML cells of patients to turn them into antigen-presenting cells and expanded T cells with anti-AML specificity during *in vitro* culture. 'The basic idea was to make the AML cells more visible to the patient's immune system, and to boost specific immunity to AML cells at the same time', explains Ball.

Immune response important in AML recovery

AML is a difficult disease to treat. Standard therapy is usually high-dose chemotherapy, with haematopoietic stem cell transplantation (HSCT). It has been known for some time that the immune system is important in the eradication of residual leukaemia after HSCT. Ironically, the removal of T cells from the allogeneic graft, done to reduce graft-versus-host-disease (GVHD), is associated with an increased incidence of leukaemia recurrence, although patients who experience GVHD tend to have a reduced risk of relapse. 'Attempts have been made to enhance

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