

Clinical applicability of immunotherapy of cervical intraepithelial neoplasia

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for CIN may currently be imiquimod, because of its established role in other human papillomavirus (HPV)-induced genital conditions, its promising treatment efficacy in high-grade CIN, and its off-label availability. Although imiquimod cannot yet replace the current gold standard treatment for CIN [*i.e.*, large loop excision of the transformation zone (LLETZ)] in all patients, it may be considered in subgroups of patients; for example, young women who may wish to become pregnant in the future, or patients with recurrent CIN lesions in whom a second LLETZ is to be avoided. Immunotherapy of CIN could be extended to post-treatment vaccination, in order to prevent new HPV infections and disease recurrence.

Key words: Cervix; Cervical intraepithelial neoplasia; Large loop excision of the transformation zone; Immunotherapy; Regression; Human papillomavirus

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Core tip: Immunotherapy for cervical intraepithelial neoplasia (CIN) is discussed in light of the natural history of CIN. The pros and cons of the current standard therapy (large loop excision of the transformation zone) and immunotherapy, potential side effects, and available evidence supporting the use of immunotherapy in CIN are addressed.

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Abstract

Immunotherapy for cervical intraepithelial neoplasia (CIN) has not yet reached clinical applicability, but seems sensible and shows promising preliminary results. One of the most promising forms of immunotherapy

IMMUNOTHERAPY OF CERVICAL INTRAEPITHELIAL NEOPLASIA

Cervical intraepithelial neoplasia (CIN) is caused by

infection with human papillomavirus (HPV). Low-grade lesions are primarily caused by low-risk HPV types and are the result of productive infections, in which viral replication takes place^[1,2]. Most of these lesions are effectively cleared by the host immune response^[3]. High-grade lesions are primarily caused by the more oncogenic high-risk HPV types (mainly HPV-16 and -18) and are the result of a transforming infection of cells of the squamocolumnar junction. In these infections, normal viral gene expression is deregulated: Overexpression of early viral genes in the basal cell layers leads to uncontrolled cell proliferation and cell immortalization, and makes the cell susceptible to chromosomal instability. Subsequent viral integration and epigenetic effects further enhance early viral gene expression and genomic instability, resulting in a proliferating cell population with chromosomal aberrations and leading ultimately to cervical carcinogenesis^[1,2]. However, not all HPV infections lead to high-grade CIN, and not all high-grade CIN lesions lead to cervical cancer. Spontaneous regression of high-grade CIN occurs in approximately 20%-40% of high-grade lesions, while approximately 30% of high-grade CIN progresses to cervical cancer^[4-7]. This suggests that the development of HPV-induced cervical pathology not only depends on the cellular changes induced by HPV infection, but is in fact determined by complex interactions among viral factors, functional cellular mechanisms, and the immune system. Clearance of HPV infection and HPV-induced lesions is mediated by the innate and adaptive immune system^[3]. This immune response is a largely local process and depends on the individual characteristics of the host's immune system, but is also influenced by characteristics of the virus and the infectious process. HPV utilizes several effective immune evasion strategies, and infection leads to active downregulation of immune responses. This leads to persistent HPV infections and HPV-induced cervical lesions in a subset of patients. In patients who are unable to clear the infection, HPV resides in the host epithelium for a long time, leading to alteration of cellular processes as previously described, with neoplastic progression as a result.

Ideally, lesions that will regress spontaneously should be differentiated from those that will persist or progress into invasive disease, thus allowing for watchful waiting in the subgroup of patients in which spontaneous regression is expected. Biomarkers could be used to predict the natural history of CIN lesions. Indeed, a recent review identified several promising biomarkers in this regard, but none have yet reached clinical implementation^[8]. As the natural history of high-grade CIN currently remains unpredictable, treatment of high-grade CIN is advised. Currently, the standard therapy for high-grade CIN lesions is surgical excision, which is usually done by large loop excision of the transformation zone (LLETZ). LLETZ is an effective treatment modality, but has two important disadvantages. First, residual and recurrent disease occurs

frequently. Recent studies show residual and recurrent disease rates of 14%-23% after treatment for high-grade CIN or persistent low-grade CIN. Persistent or recurrent high-grade CIN occurred in 3%-10% of these patients^[9-13]. Second, LLETZ is associated with an important long-term complication, namely a two-fold increase in premature birth seen in pregnancies after a LLETZ procedure, most probably as a result of cervical insufficiency^[14,15]. Although some biomarkers may be promising for regression risk prediction, a considerable number of high-grade CIN lesions will not regress spontaneously, making effective treatment modalities necessary. To reduce unnecessary surgical treatment, alternative non-invasive treatment modalities for high-grade CIN are being studied. Since high-grade CIN is the result of an HPV infection that is not adequately cleared by the infected host, immunotherapy of CIN may be an effective alternative to conventional surgical treatment and/or watchful waiting.

Several forms of immunotherapy have been studied for the treatment of high-grade CIN, with varying results. These include both systemic forms of immunotherapy, such as therapeutic vaccines, interferon, and cyclooxygenase-2 inhibitors, and local forms of immunotherapy, such as topical or intralesional interferons and imiquimod^[4,16-21]. Some agents show therapeutic effects, but none have yet reached clinical applicability. This is due to limited evidence, generally modest treatment results, and a high rate of side effects. We currently consider imiquimod (Aldara) to be the most promising form of immunotherapy for high-grade CIN. Imiquimod is a Toll-like receptor agonist with antiviral and anti-tumor properties. It is readily available for off-label use; its efficacy in several HPV-induced genital conditions, such as genital warts and vulvar intraepithelial neoplasia, is already established; and recent studies show promising results in the treatment of high-grade CIN. Lin *et al.*^[19] studied imiquimod treatment of genital HPV infections and both vaginal intraepithelial neoplasia (VAIN) and CIN lesions. They showed significantly more HPV clearance in 26 patients treated with 12 doses of imiquimod cream than in a historic control group (65% vs 30%). A limited number of 6 patients with high-grade VAIN or CIN lesions were treated with imiquimod, of which four (66%) showed disease remission. Treatment efficacy of imiquimod in high-grade CIN was more systematically studied by Grimm *et al.*^[4] in a placebo-controlled randomized controlled trial. They included 59 patients with high-grade CIN, who were treated with one to three applications of imiquimod per week for 16 wk. Significantly more histologic regression and remission was observed in the imiquimod group (73% vs 39%).

Although promising, it is unlikely that imiquimod will completely replace LLETZ as the standard treatment strategy for high-grade CIN. The efficacy of imiquimod and other immunotherapies has not yet reached that of LLETZ. Furthermore, imiquimod treatment is labor-intensive and time-consuming, and

is associated with frequent, albeit generally mild-to-moderate, side effects. Moreover, imiquimod and other forms of immunotherapy may treat current cervical lesions and HPV infections, but do not protect against new infections. The risk of future CIN and cervical carcinoma therefore persists. Recent new insights in the pathophysiology of high-grade CIN suggest that disease recurrence after LLETZ may in fact be the result of incomplete resection of the transformation zone in combination with persistent or new HPV infection. Herfs *et al.*^[22] demonstrated that high-grade CIN may originate exclusively from cells of the squamocolumnar junction. If their observations are correct, disease recurrence could be effectively be prevented by complete resection of the squamocolumnar junction. Alternatively, adjuvant vaccination may provide protection against future infections and disease recurrence. Indeed, the first trial on this topic shows a significant decrease in disease recurrence after LLETZ in combination with quadrivalent HPV vaccination^[13].

In summary, immunotherapy of CIN has not yet reached clinical applicability, but seems sensible and shows promising preliminary results. One of the most promising forms of immunotherapy in CIN may currently be imiquimod, because of its established role in other HPV-induced genital conditions, its promising treatment efficacy in high-grade CIN, and its off-label availability. Although imiquimod cannot yet replace LLETZ as the current gold standard treatment for CIN in all patients, it may be considered in subgroups of patients, such as young women who may wish to become pregnant in the future, or patients with recurrent CIN lesions in whom a second LLETZ is to be avoided. Immunotherapy of CIN could be extended to post-treatment vaccination, in order to promote disease recurrence and prevent new HPV infections.

We advocate further research in the field of immunotherapy in CIN and are currently conducting a trial on the efficacy of 5% imiquimod cream in high-grade CIN [topical treatment with Imiquimod of high-grade CIN (TOPIC) trial; ClinicalTrials.gov identifier: NCT02329171]. This study also includes assessment of side effects, disease recurrence, and quality of life. Further research is also needed on the efficacy of post-treatment HPV vaccination. A combination of immunotherapy for present lesions and vaccination to prevent disease recurrence may provide a good treatment alternative to current surgical treatment for high-grade CIN in selected patients.

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