

IMIQUIMOD

Gary A. Richwald

Sexually Transmitted Diseases Program, Los Angeles County Department of Health Services, Los Angeles, California, USA

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Summary

The imidazoquinoline, imiquimod, is a low molecular weight, synthetic immune response modifier that is used for the treatment of external genital and perianal warts. It is formulated in a 5% vanishing cream as Aldara®. This self-applied therapy has shown good efficacy and safety in the treatment of external genital and perianal warts caused by human papillomavirus (HPV) infection. The antiviral mechanism of action of this compound is unlike any other approved antiviral therapy in that it induces the production of antiviral cytokines and cytokines that enhance cellular immunity believed to be necessary for the control or elimination of HPV infection. Imiquimod does not exert its antiviral effects directly on virus-infected cells. Treatment with imiquimod results in resolution of wart tissue and reduction of viral burden. Post-marketing trials using imiquimod demonstrated that patients who experience complete clearance of either new or recalcitrant warts tend to remain clear for longer periods as compared to other treatment modalities. Preclinical data demonstrate *in vitro* and *in vivo* that imiquimod directly induces antiviral and immunomodulating cytokines from monocytes, macrophages and dendritic cells. These immunomodulating cytokines have been shown to potentiate Th1 immunity. Self-application, good tolerability, a unique mechanism of action and a relatively high sustained clearance rate combine to make imiquimod a cost-effective first-line therapy for external genital warts and an appropriate second-line therapy when other treatments are unsuccessful. In small-scale studies requiring replication, imiquimod has also been shown to be effective in the treatment of non-HVP-related skin infections and some dermal neoplasias. © 1999 Prous Science. All rights reserved.

Introduction

There are more than 100 types of human papillomavirus (HPV) already identified in humans (1). Infections with HPV can be asymptomatic, can produce warts or may result in various benign or malignant neoplasias (1). External genital warts (EGWs) are typically associated with HPV types 6 and 11. Studies have shown that these particular HPV types are rarely detected in invasive cervical cancers and are not associated with invasive squamous cell carcinoma of the external genitalia (2-4). Occasionally, EGWs contain HPV types 16, 18, 31 and 45 which have been associated with external genital squamous intraepithelial neoplasia and with intraepithelial dysplasia and squamous cell carcinoma of the vulva, vagina, anus and cervix. There is no evidence to suggest that current treatments for EGWs can fully eradicate HPV, alter the natural history of HPV or affect the development of HPV-related cancer (4).

HPV is the causative agent of genital and perianal warts. By one estimate, at least 10% of sexually active adults aged 15-49 are infected with HPV, yet only 1% display clinical disease in the form of EGWs (5). Among the latter, approximately 20% experience "spontaneous" regression, usually within the first year of having visible warts (6). These data suggest that most people establish appropriate immunological control following infection or disease expression, while others fail to establish or express effective immunity. The development of florid warts in immunosuppressed transplant recipients and persons with

AIDS provides evidence that established immunity can be suppressed or abolished. Because imiquimod induces cytokines that are antiviral such as interferon (IFN)- α and cytokines that augment cellular immunity such as tumor necrosis factor (TNF)- α and interleukin (IL)-12, HPV is an attractive target for immune response modification therapy. EGWs were chosen as the first target condition for the development of imiquimod on the basis of potential for response to immune upregulation, as well as clear clinical end points, dissatisfaction with and limitations of current therapies and recognition of the high incidence of HPV infection in the population (7).

EGWs are one of the most common sexually transmitted diseases in the U.S. (8-10). Comprehensive surveillance data on EGWs are unavailable because HPV infection is not reported to the U.S. Centers for Disease Control and Prevention (CDC). The total annual incidence of new HPV-related EGW cases is estimated to be between 500,000 and 1 million (2).

Epidemiologic studies have estimated that approximately 1-2% of the sexually active population in the U.S. (*i.e.*, 1.4 million) have EGWs (1). New data suggest that 50% or more of the total population are at risk of infection with HPV (11), with a substantially raised estimate of at least 5.5 million new HPV infections in the U.S. each year (7).

Because there is no known cure for HPV, the primary goal of treatment is removal of symptomatic warts (4). A variety of methods have been used for the removal of EGWs, including surgery such as excision, laser vaporization and electrodesiccation or electrocautery, cryotherapy, podophyllin resin, caustic agents such as trichloroacetic acid (TCA), intralesional IFN and patient-applied podofilox (topical solution or gel 0.5%) (12, 13). Although 5-fluorouracil cream has been used to treat EGWs, there are no controlled studies on this agent and it is not approved for this use by the U.S. Food and Drug Administration (FDA); in practice, 5-fluorouracil cream can cause severe local irritation (13, 14).

With the exception of intralesional IFN, all of these treatments are cytotoxic in nature (9, 12, 15). Surgical procedures and cryotherapy are often painful, may result in undertreatment (poor efficacy) or overtreatment (scarring or other complications) if improperly performed and can be expensive, depending on the number of office visits required for the particular procedure and case (2, 13, 14). Podophyllin resin and TCA induce local inflammation, including erythema, erosion and crusting, and require multiple office visits (13). Intralesional IFN requires numerous injections directly into the EGWs, has a high frequency of systemic adverse side effects and is comparatively expensive (13, 14). Podofilox solution or gel produces erosions and burning in most patients, but the recently approved gel form offers increased convenience and ease of application for home use and reduced expense associated with fewer office visits (2, 13, 16).

There is a high frequency of recurrence with most of the current genital wart therapeutic modalities (17). Randomized trials of laser therapy, surgical excision and electrodesiccation demonstrated efficacy rates of 43, 93 and 94%, respectively, and recurrence rates of 95, 29 and 22%, respectively (14). A randomized trial of electrocautery showed 35% efficacy (14). Four randomized trials of podophyllin resin therapy showed 32-79% efficacy with recurrences in 27-65% of the patients. One study of TCA in women showed similar efficacy to podophyllin, and a randomized trial in men demonstrated 81% efficacy with a 36% recurrence rate (14). In 2 randomized trials, intralesional IFN showed 44-61% efficacy, with recurrences in 67% of patients (4). In clinical studies, podofilox (topical solution 0.5%) resulted in 50% efficacy with recurrence in 60% of the patients (18). Podofilox gel had a 39% clearance rate with recurrence in 31% of the patients (19, 20).

The ability of a novel immune response modifier, imiquimod (Fig. 1), to induce significant production of IFN- α by monocytes/macrophages suggests that diseases responsive to recombinant IFN therapy, such as HPV infection, basal cell carcinoma and hepatitis C, may be reasonable clinical targets. The induction of TNF- α , IFN- γ and IL-12 leads to inhibition of IL-5, with animal models demonstrating immune deviation away from Th2 immune responses. This review focuses on the use of imiquimod for the treatment of external genital and perianal warts.

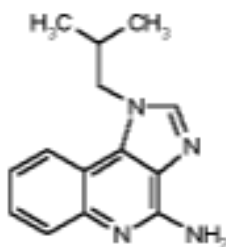


Fig. 1. Chemical structure of imiquimod.

Preclinical Studies

The antiviral effects of imiquimod were first demonstrated in a herpes simplex virus (HSV) guinea pig model (21). Since then, an accumulating mass of data has shown that imiquimod and related imidazoquinolines induce the production of antiviral cytokines, such as IFN- α and TNF- α , as well as cytokines that potentiate Th1 immunity, such as IL-12. Th1 immune responses develop following the production of cytokines such as IL-12 and IFN- α from antigen-presenting cells. In general, the development of antiviral and anticancer immunity requires Th1 responses. These cytokines are produced from monocytes, macrophages and dendritic cells after treatment with imiquimod (22-25). IL-12 and IFN- α bias the development of antigen-specific T cells that predominantly produce IFN- γ . The end result is an immune response that more efficiently eliminates virally infected cells and cancer cells (26, 27). Imiquimod and related imidazoquinolines have been shown to induce long-lasting immunity in numerous virus and cancer animal models.

In vitro studies

In human PBMCs, specifically monocytes, imiquimod induces the production of several cytokines including several subtypes of IFN- α , TNF- α , IL-1, IL-1RA, IL-6, IL-8, IL-10, IL-12p40, granulocyte colony stimulating factor, granulocyte/macrophage colony stimulating factor and macrophage inflammatory protein 1- α (MIP-1 α), MIP-1 β and macrophage chemotactic protein-1 (23, 24). Generally, a low drug concentration (about 0.5 μ g/ml) induces IFN- α and IL-1RA, and the other cytokines are induced with 1-5 mg/ml imiquimod. Most cytokines are detected as early as 1-4 h after stimulation with the drug, and this induction requires both mRNA and protein synthesis (28, 29). Similar findings have been shown *in vitro* in mouse, rat and nonhuman primate systems (30). These cytokines are induced through the activation of transcription factors that bind to the promoter regions of the IFN- α (α 4F1 complexes) and a number of the proinflammatory cytokine nuclear factor κ -B genes (29).

In vivo studies

1) Oral and parenteral

Consistent with *in vitro* findings, when administered orally or parenterally to mice and rats, imiquimod induced increased serum concentrations of IFN- α , TNF- α and IL-6 between 1-4 h after dosing (30). In addition to mice and rats, imiquimod induces IFN in guinea pigs and nonhuman primates. Effective oral doses range from 1-250 mg/kg. Multiple doses of imiquimod on the same day cause augmented IFN- α levels. However, high daily doses can result in a hyporesponsive state characterized by reduced cytokine induction presumably due to downregulation of the imiquimod receptor. Oral imiquimod also causes increased levels of serum 2',5'-oligoadenylate synthetase (2',5'-AS) (31), which is an IFN inducible enzyme shown to be partly responsible for IFN's antiviral properties by inducing apoptosis in virally infected cells (32). A single drug treatment causes elevated 2',5'-AS levels for 3-4 days posttreatment, supporting a 2-3 times per week dosing regimen in efficacy studies (31).

2) Topical

Topical application of the 1% or 5% imiquimod cream to the skin of hairless mice induced increased IFN- α messenger RNA (mRNA) levels and increased protein concentrations of IFN- α and TNF- α in the skin at the treatment site (33, 34). These cytokines were increased between 1-4 h after application. From the same animals, cytokines were not detected from skin on the untreated side of the mice or the site where placebo cream was applied. These data demonstrate the localized effects of imiquimod. Mechanistically, topical treatment of hairless mice with imiquimod caused Langerhans cells in the skin to enlarge, appear activated and migrate from the treatment site to the regional lymph node, implying that imiquimod may enhance antigen presentation to T cells, in addition to inducing local antiviral cytokine production (35).

3) Viral models

As a result of these effects on innate immunity, imiquimod has been shown to be effective in acute viral models where viral clearance is dependent on innate immunity. In herpes simplex virus (HSV)-infected guinea pigs, a single treatment of 2-3 mg/kg of imiquimod given orally, parenterally, intravaginally or topically was protective against primary infection when given 24-72 h before HSV infection (36, 37). In mice, imiquimod caused an increase in survival in Rift Valley Fever virus infection and Banzai virus infection (38). The production of IFN- α is critical for the antiviral effect, at least in the Rift Valley Fever virus model where neutralizing antibody to IFN- α blocked survival induced by imiquimod. Acute antiviral activity was also seen in cytomegalovirus infection models, both in guinea pigs (27) and in mice. The duration of antiviral activity lasted for 3-4 days after oral imiquimod administration and correlated with an elevation in 2',5'-AS activity. Elevated serum 2',5'-AS has been observed 24-72 h post oral treatment with imiquimod in mice, rats, guinea pigs, monkeys and humans

(33). Induction of 2',5'-AS was through the production of IFN- α since IFN- α/β receptor knockout mice did not produce 2',5'-AS following imiquimod treatment (39). Despite imiquimod's ability to induce IFN in virtually every species tested thus far, it did not induce IFN in rabbits. Interestingly, imiquimod was ineffective in eliminating papillomavirus in the rabbit papillomavirus infection model. Overall, these data indicate that IFN is a key antiviral mediator induced by imiquimod.

4) Cancer models

Antitumor activity of imiquimod was also seen in a number of transplantable mouse tumor models (40). When given acutely, the drug was effective at reducing tumor load in mice transplanted with tumors from a number of lines including MC-26 colon carcinoma, B16-F10 melanoma, Lewis lung carcinoma, FCB bladder carcinoma, RIF-1 sarcoma and MBT-2 bladder cell carcinoma. Much of the antitumor effect with imiquimod was again blocked by administration of neutralizing IFN- α antibodies; however, TNF- α also seems to play a role in the antitumor activity in these models. Imiquimod was also effective at inhibiting growth of the human mammary tumor MCF-7 when transplanted into nude mice lacking T cells. These data demonstrate that imiquimod can activate the innate immune system and that T cells are not required for the antitumor effects when imiquimod is administered at the time of tumor transplantation.

5) Acquired immunity

In addition to innate immunity, imiquimod also affects acquired immunity. Although imiquimod does not stimulate T cells directly, it induces T cell cytokine production through the stimulation of antigen-presenting cells. Th1 cytokines, such as IFN- γ , appear to be preferentially induced and Th2 cytokines, such as IL-4 and IL-5, were inhibited in both human and mouse *in vitro* systems. In general, Th2 cytokines are involved in the enhancement of antibody responses and the inhibition of cellular immunity. The production of IFN- γ is due to IL-12 and IFN- α secreted from monocytes and macrophage cytokines (41).

In vivo modulation of acquired immunity with imiquimod has also been shown in animal models. After primary HSV-2 infection in guinea pigs, imiquimod treatment reduced recurrences well after imiquimod treatment had stopped (42). The prolonged effect after treatment was likely due to increased cellular immunity to HSV antigens and HSV infected cells (21, 42-44). In addition, imiquimod demonstrated adjuvant activity in guinea pigs when given along with HSV glycoprotein (45, 46). In mice, imiquimod resulted in rejection of tumors caused by cells which express the HPV type 16 E7 gene. It should be noted that in the E7 immunized mice, imiquimod had no effect on the control tumors. The E7-specific immunity correlated with enhanced delayed-type hypersensitivity that is associated with Th1 immunity. Finally, following elimination of FCB bladder carcinoma cells in mice treated acutely with imiquimod, the mice were totally resistant after 8 months to rechallenge with the same FCB tumor cells but remained sensitive to challenge with a different tumor line (26). The long-lasting immunity observed was likely via cell-mediated immunity. These effects of imiquimod on acquired immunity were further illustrated in immunodeficient mice, where application of imiquimod 5% cream was ineffective in preventing HPV type 11 infection in the human severe combined immunodeficiency mouse model (47).

Safety

Animal studies of acute dermal toxicity, dermal and intravaginal irritation, dermal sensitization and repeat-dose dermal toxicity have not shown imiquimod 5% cream to be a sensitizer and have shown it to be only mildly irritating. Repeat-dose dermal toxicity studies in mice demonstrated that the effects of imiquimod are not systemic, but are local, limited to skin irritation at the dosing site.

Acute systemic toxicity studies with imiquimod were conducted in 3 species (mouse, rat and monkey) using 4 routes of administration (p.o., i.p., s.c. and i.v.). Hypoactivity and lethargy were common reactions. Convulsions frequently occurred at lethal or near-lethal dose levels. The acute oral LD₅₀ for monkeys was > 200 mg/kg, while the acute i.v. LD₅₀ values were in the range of 6-8 mg/kg. Single large dermal doses (2000 and 5000 mg/kg for 24 h) of imiquimod applied to rabbits did not produce any deaths or other signs of toxicity. This indicates a dermal LD₅₀ (median lethal dose) of > 5000 mg/kg.

Repeat-dose oral toxicity studies conducted in rats and monkeys showed that imiquimod produced dose-related changes in lymphoid stimulation, leading to enlargement of the lymphoid organs. The peripheral component of the lymphoid system was also stimulated. At high-dose levels (10-30 mg/kg/day) and after months of daily dosing, overstimulation and downregulation of lymphoid organs occurred in some animals. As imiquimod-induced IFN and other cytokines have a hormone-like control of the lymphoid system, it is postulated that the system would eventually exhibit downregulation and become unresponsive to continued stimulation.

Anemia and thrombocytopenia developed in rats and monkeys given high doses of imiquimod. This was not unexpected, since decreases in erythrocyte and platelet concentrations are known side effects of IFN. Stimulation of the lymphoid system at high doses produced a plasmacytosis in the lymph nodes, spleen and bone marrow. Plasma-cell stimulation correlated with dose-related increases in serum globulin and IgG levels. Decreased serum albumin levels occurred at high doses. Body weight decreased in both rats and monkeys, and death resulted in some rats receiving 10-30 mg/kg/day by the iv., oral or s.c. routes.

Data from recovery animals showed that the adverse effects of long-term oral imiquimod dosing were reversible. In addition, the adverse effects of high imiquimod doses appeared to be pharmacological and not the result of direct imiquimod-induced cytotoxicity leading to cell death.

A general reproduction study in rats showed that imiquimod administration to both males and females before and throughout the mating period, and during gestation and lactation in females, produced clinical signs of toxicity in both parents but did not affect reproductive performance. No adverse effects of imiquimod were observed in regard to mean gestation duration, mean litter size or number of dead pups. Pup survival and growth during lactation were normal. When offspring were mated, no adverse effects were found with respect to their reproductive performance. Imiquimod was not found to be teratogenic in rat or rabbit teratology studies. In rats, at a high maternally toxic dose (28 times the human dose on a mg/m² basis), reduced pup weights and delayed ossification were observed. In developmental studies with offspring of pregnant rats treated with imiquimod (8 times the human dose), no adverse effects were demonstrated. Imiquimod was tested for mutagenicity in eight different *in vitro* and *in vivo* assays. No evidence of mutagenic activity was found in any of the genetic toxicity tests.

Summary

Overall, the results showed that imiquimod's effects on the innate immune response, in particular its ability to induce IFN- α , are largely responsible for its acute antiviral and antitumor effects. However, imiquimod also appears to activate Th1 immunity, probably through the stimulation of monocytes, macrophages and dendritic cells. These results demonstrate the potential of imi-quimod and other imidazoquinolines for treatment of virus infections or tumors in humans.

Human Pharmacokinetics and Metabolism

The immunological activity of imiquimod is thought to be mediated through the induction, both locally and systemically, of cytokines such as IFN and IL-12. Since topical administration of imi-quimod could potentially lead to significant systemic effects if percutaneous absorption were extensive, studies have been conducted to assess the extent and rate of absorption of topical imiquimod.

Tygum *et al.* applied formulated, [¹⁴C]-labeled imiquimod to the forearms of 7 healthy human volunteers for the purpose of assessing the rate and extent of percutaneous absorption (48). After 8 h, strips of skin stratum corneum were collected for analysis. Samples of blood were also collected over a 48-h period and urine and feces were collected throughout the study. The results demonstrated that > 0.2% of the dose was recovered in urine and feces and about 0.5% in the skin stratum corneum. Approximately 97% of the dosed radioactivity was recovered from the dosing site. Consistent with the very low systemic absorption of total radioactivity, measurable serum or urine concentrations of unchanged imiquimod or of the metabolites were not detectable at any of the sampling times. The results indicate that systemic exposure to imiquimod associated with topical dosing was minimal and not likely to result in any systemic safety concerns.

Percutaneous absorption of imiquimod applied daily to patients with anogenital warts was also investigated (49). Imiquimod 5% cream was applied daily to each patient's warts and allowed to remain in place for about 8 h, at which time residual formulation was removed with careful washing. Dosing was continued until complete clearance of the wart had occurred or for a maximum of 16 weeks. Serum and urine samples were analyzed for imiquimod and for two known metabolites. Analyses revealed no measurable (≥ 5 ng/ml) concentrations of imiquimod or of the metabolites in any serum sample. From these data it was concluded that systemic exposure to imiquimod after daily application of the drug to genital/perianal skin was minimal. Although 75% of all patients achieved $\geq 50\%$ reduction in their wart areas, none experienced any serious or clinically significant adverse events, further evidence for the very low but nonetheless therapeutic percutaneous absorption of imiquimod. After single oral doses, < 1% of the dose was recovered in urine as unchanged imiquimod while 10-12 metabolites were detected (mostly as glucuronide conjugates). In heterologous cDNA expression systems and in human liver microsomes, 6 different phase I metabolites, including a primary and a tertiary alcohol, an N-oxide and 3 different phenols were identified (50). The majority of phase I metabolism of imiquimod in these *in vitro* systems was catalyzed by the enzymes CYP1A2 and CYP3A4/5.

Clinical Studies

Current treatment options

Methods for removing EGWs have in the past included surgery, (excision, laser vaporization and electrodesiccation or electrocautery), cryotherapy, podophyllin resin, caustic agents, intralesional IFN and patient-applied podofilox (topical solution 0.5%). The CDC has recently issued revised national guidelines for the treatment of sexually transmitted diseases (4). In the 1998 guidelines, the section on EGWs is now divided into two classes: patient-applied therapies (podofilox and imiquimod) and provider-administered therapies (cryotherapy, podophyllin, TCA, bichloroacetic acid, IFN and surgery). Like the American Medical Association (AMA) consensus statement, the CDC guidelines recommend that physicians have knowledge of and are prepared to supply their patients with at least one patient-applied therapy and one provider-administered therapy (4). This division of treatments reflects the 1997 FDA approval of both podofilox gel (Condylox[®] gel 0.5%) and imiquimod 5% cream.

Efficacy for external and perianal warts

Imiquimod 5% cream (applied topically by the patient 3 times per week or daily) has been shown to have significant efficacy in the induction and maintenance of remission in patients with EGWs and perianal warts. The drug is well-tolerated and has a wide margin of safety. EGWs, the most common viral sexually transmitted disease, were chosen as the first clinical target because injectable IFN- α had demonstrated some benefit and the current therapies did not fully meet patient or physician needs. Patient dissatisfaction with current therapeutic options was significant due to pain, tissue destruction, high recurrence rates, expense and time required for treatment. In addition, current treatments only treat the visible wart symptoms and do not treat the underlying HPV infection. Published results indicate that biopsies of warts from these patients showed little immune recognition, but biopsies from warts undergoing spontaneous regression showed monocytic cellular infiltration and increased Th1 cytokine expression (51, 52). Similar results were seen in patients treated with IFN (52). An immune response modifier that stimulates cell-mediated immunity was considered a good candidate to improve therapy for EGWs.

Two key phase III studies demonstrated the effectiveness of imiquimod 5% cream. The first consisted of a multicenter, randomized, double-blind, placebo-controlled trial comparing both the safety and efficacy of imiquimod 5% and 1% cream with vehicle (13). Patients applied the cream to their warts overnight for 8 h (3 times/ week) until their warts were totally cleared or for a maximum of 16 weeks. The main outcome measurements were the number of patients experiencing the complete elimination of all baseline warts and the recurrence of these warts. In addition, the reduction in baseline wart area, the duration of therapy required to eliminate warts and the frequency and severity of adverse reactions were monitored. Patients who totally cleared their warts were entered into a 12-week follow-up period to monitor recurrence of their warts.

This study (3M Imiquimod Trial 1004) included 180 men and 131 women 18 years or older having 2-50 external anogenital warts. The results are summarized in Tables I and II. Overall, imiquimod 5% cream effectively cleared baseline warts. The 1% imiquimod group was not statistically different from the vehicle group. Interestingly, females had a significantly higher clearance rate and a shorter median time to clearance as compared to males. The better response in females could be due to several factors, including shorter duration of warts prior to treatment in females (3.4 months median) *versus* males (6.7 months median) or better drug absorption in females due to the fact that warts in women are less keratinized than warts in men. Patients who were totally cleared of warts were monitored for recurrences where at least 1 wart qualified as a recurrence. No statistical differences were determined between the groups. It should be noted that the low recurrence rate in the vehicle groups is not surprising since the mechanism of spontaneous clearance has been shown to be due to immune recognition (51, 52). Since the initial clearance rate was highest for the imiquimod 5% group, the sustained wart-free period was also greatest for that group. Treatment was well tolerated. Local erythema was the most common adverse reaction (67, 26 and 24%, respectively) but the majority of patients in each group experienced no or only mild local inflammatory reactions. Approximately 1% of the patients discontinued use due to side effects.

Table I: Percent of patients with clearance of baseline warts following treatment 3 times a week.

Treatment groups	5% Imiquimod (n = 109)	1% Imiquimod (n = 102)	Vehicle (n = 100)
Clearance of baseline warts:			
Intent-to-treat analysis	50*	21	11
Treatment failures analysis	56*	27	14
Recurrences in patients who cleared	13 (6/45)	0 (0/18)	10 (1/10)

* $p < 0.0001$ compared to vehicle-treated group

Table II: Median time to clearance and clearance rate following treatment 3 times a week.

Treatment groups	5% Imiquimod (n = 109)	1% Imiquimod (n = 102)	Vehicle (n = 100)
Median time to clearance	10 weeks	12 weeks	12 weeks
Clearance rate (%) - females	77	46	28
Clearance rate (%) - males	40	10	6

A second phase III trial was conducted in 154 male and 125 female patients with EGWs using daily application of imiquimod 5% or 1% or vehicle with an 8-h application until wart clearance or a maximum of 16 weeks (12, 54). The results are summarized in Table III. Overall, imiquimod 5% cream effectively cleared baseline warts. The daily treatment regimen resulted in an increased rate of total wart clearance when compared to a 3 times per week application regimen. However, daily treatment also resulted in more frequent and more intense local skin reactions.

Table III: Percent of patients with clearance of baseline warts following daily treatment.

Treatment groups	5% Imiquimod (n = 94)	1% Imiquimod (n = 90)	Vehicle (n = 95)
Clearance of baseline warts	71*	16	4
Recurrences in patients who cleared	19 (9/48)	17 (2/12)	0 (0/3)

* $p < 0.0001$ compared to vehicle-treated group

Mechanism of action

The data generated in animal models suggests that imiquimod's antiviral and antitumor effects are largely mediated through the induction of cytokines that drive the innate and cell-mediated immune responses. A clinical study was done to further explore the drug's mechanism of action following topical application of imiquimod 5% cream to EGWs (20). The objective of this study was to determine local and systemic cytokine induction, assess cellular infiltration into the warts and evaluate the effects of imiquimod on HPV DNA and gene expression. This was a phase I double-blind, randomized, parallel-group study using imiquimod 5% cream or placebo applied to wart tissue 3 times a week for up to 16 weeks. Serum and biopsies of warts were taken at predose, after 6 weeks of treatment and at the end of study. As an inclusion criteria, HPV infection was confirmed in the predose biopsy. Biopsies were analyzed by the polymerase chain reaction for HPV DNA (copies/cell) and by reverse transcriptase-polymerase chain reaction for mRNAs to a number of cytokines, cellular markers and viral gene products. Changes from baseline at 6 weeks and at end of treatment were compared between treatments.

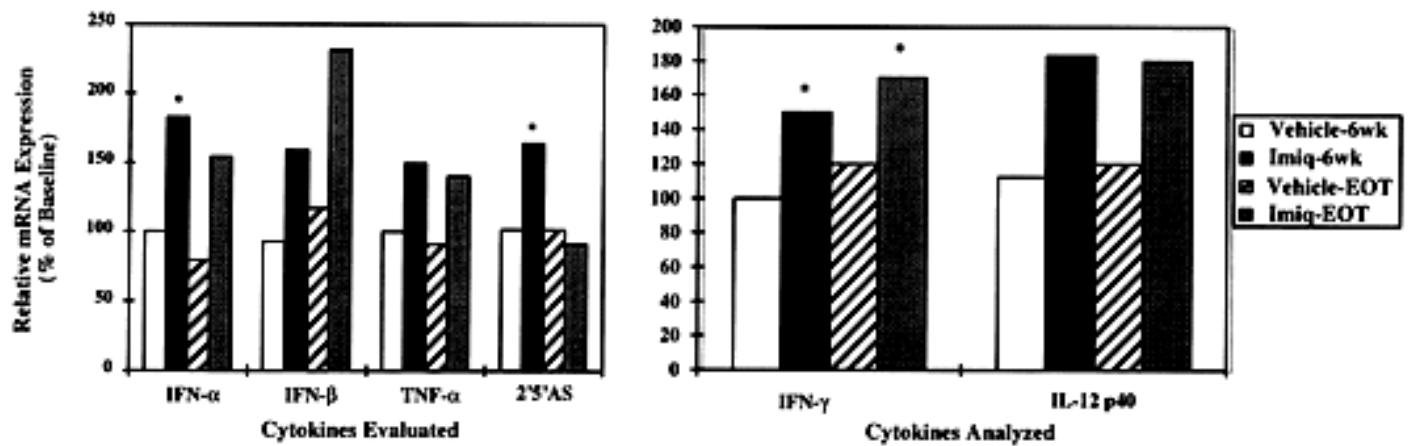


Fig. 2. Induction of various cytokine mRNAs in wart biopsies obtained from patients treated topically with imiquimod 5% cream (trial 1199). Median differences are shown between the imiquimod-treated group at 6 weeks and at the end of treatment (EOT) and vehicle at 6 weeks and at EOT. All measurement criteria for the patients in the vehicle group remained at baseline. Wilcoxon rank sum test comparing the ratio changes from baseline for each cytokine marker between imiquimod-treated patients and vehicle-treated patients at week 6 and at EOT. * $p \leq 0.05$. The p values for IFN- α , 2',5'-AS and IFN- γ at week 6 and IFN- γ at EOT were 0.02, 0.0086, 0.048 and 0.027, respectively.

Results showed that all imiquimod-treated patients had a $\geq 75\%$ reduction in wart area. Cytokine, cellular and HPV mRNAs from wart biopsies were compared at 6 weeks of treatment and at the end of treatment. Imiquimod treatment stimulated significant increases in IFN- α and IFN- α -inducible protein 2',5'-AS (Fig. 2). Both IFN- β and TNF- α were also increased in the imiquimod group but were not statistically different from the vehicle group. Correlating with the increased cytokine mRNAs at the treatment site were increases in mRNAs for CD4 (T helper cells), CD8 (cytotoxic T cells) and CD29 (activated T cells), CD45RO (memory T cells) (Fig. 3). There was a decrease in CD1a mRNA (Langerhans cells). The decrease in CD1a mRNA suggests that Langerhans cells are activated or migrated to the draining lymph node following imiquimod treatment. Again, consistent with the increased CD4 and CD8 mRNAs, there were increases in mRNAs for IL-12p40 and IFN- γ , two cytokines associated with Th1 immunity. Finally, and most importantly, L1, E7 and total HPV mRNAs were decreased following treatment with imiquimod 5% cream (Fig. 4). Coincident with wart regression and diminished virus was a decrease in mRNA expression for markers associated with hyperproliferation (PCNA, *c-myc*) and an increase in markers associated with differentiation (fillagrin, involucrin, p53 and Rb). These changes were likely a result of the reappearance of normal tissue.

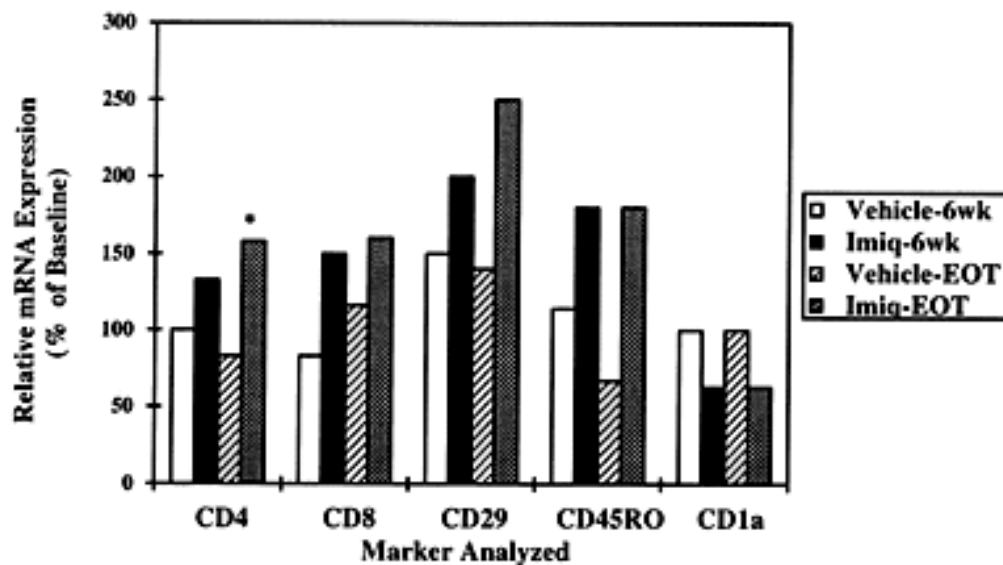


Fig.3. Cellular changes in wart biopsies obtained from patients treated topically with imiquimod 5% cream. Median differences are shown between the imiquimod-treated group at 6 weeks and at the end of treatment (EOT) and vehicle at 6 weeks and at EOT. All measurement criteria for the patients in the vehicle group remained at baseline, except for CD8 which showed some increase over baseline. Wilcoxon rank sum test comparing the ratio changes from baseline for cell surface markers between imiquimod-treated patients and vehicle-treated patients at week 6 and at EOT. * $p \leq 0.05$. The p value for CD4 at week 6 was 0.02.

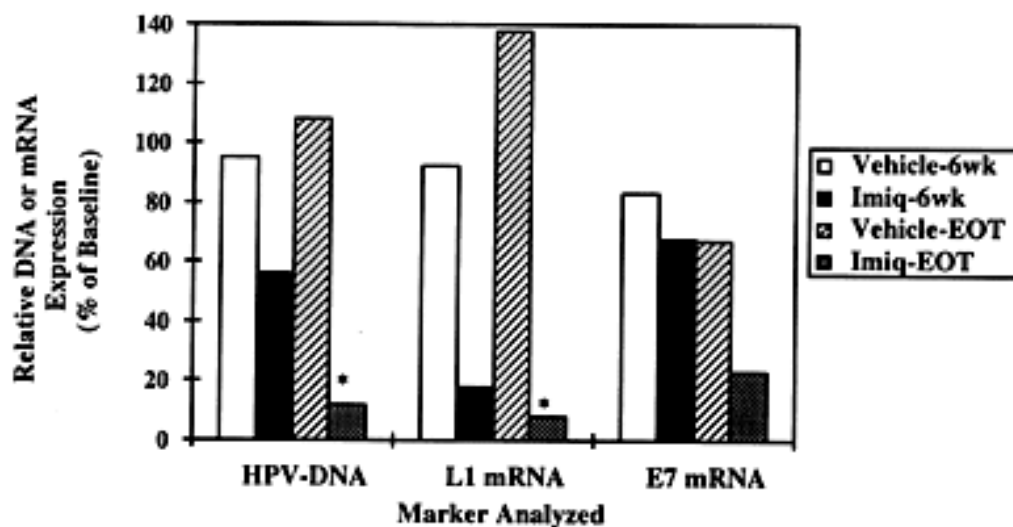


Fig. 4. Effects on HPV DNA and HPV gene expression in wart biopsies obtained from patients treated topically with imiquimod 5% cream. Median differences are shown between the imiquimod-treated group at 6 weeks and at the end of treatment (EOT) and vehicle at 6 weeks and at EOT. All measurement criteria for the patients in the vehicle group remained at baseline or increased. Wilcoxon rank sum test comparing the ratio changes from baseline for HPV markers between imiquimod-treated patients and vehicle-treated patients at week 6 and at EOT. * $p \leq 0.05$. The p values for HPV DNA at week 6 and for L1 mRNA at EOT were 0.0376 and 0.0375, respectively.

These results in humans are consistent with the preclinical results generated with imiquimod in animal models in that imiquimod induces local production of cytokines that are associated with both innate and cellular immunity and which are believed to be necessary for HPV elimination from wart tissue.

The role that innate immunity plays in wart reduction was demonstrated in a vehicle-controlled safety and efficacy trial consisting of HIV-positive genital wart patients (55). Although the primary objective of this multinational, multicenter, double-blind, vehicle-controlled, parallel-group trial was to evaluate the safety of imiquimod 5% cream in HIV-positive patients, a secondary objective was to assess wart clearance and reduction in wart area. A total of 100 patients (97 males and 3 females) were enrolled and treated 3 times per week for up to 16 weeks or until wart clearance. Imiquimod was applied to 65 patients and vehicle to 35 patients. No local skin reactions were seen in a majority of patients and only mild erythema was seen in most of the others. The intent-to-treat analysis of all patients showed that 11% of the imiquimod patients achieved complete wart clearance compared to 6% of the vehicle group, which was not significantly different. However, there was a statistically significant difference between treatment groups for patients who achieved > 50% reduction in wart area: 38% for imiquimod and 14% for vehicle ($p = 0.013$). This was a clinically meaningful reduction in wart area since wart area increases are frequently seen in these patients. These results suggest that even in immunocompromised HIV patients, imiquimod induces an innate response which stops wart growth and causes wart area reduction and may, in part, be mediated by IFN- α . However, the reduced total wart clearance in HIV patients compared to immunocompetent genital wart patients suggests a role for acquired immunity in initial wart clearance as well as in long-term protection from recurrence.

Management of external genital warts and costs

Although there have been a large number of published reports assessing the effectiveness of individual EGW therapies, few are comparative trials required to examine relative effectiveness and recurrence rates after successful treatment (2). Furthermore, when determining overall clinical effectiveness, studies to date have not considered the frequency of recurrences nor the use of a second treatment modality after initial failure to clear warts (56, 57).

The 1997 AMA consensus panel on EGWs defines treatment failure for health care provider-administered therapies as 3 sessions without significant improvement or when complete clearance has not been achieved after 6 provider-administered treatment sessions (2). This guideline was established to prevent extended treatment courses with modalities likely to fail, to reduce unnecessary medical costs and to avoid patient discontinuation. The AMA report also recommends the sequential use of different treatments to debulk and, if possible, clear EGWs initially resistant to therapy.

Despite the challenges of disease presentation, clinician training and awareness and patient preferences, there has been recent progress in the development of comprehensive treatment algorithms for patients with EGWs. A clinical management protocol for patients attending a large public sexually transmitted diseases clinic system followed the CDC and AMA recommendation that patient preference largely determines treatment choice. Imiquimod and cryotherapy are offered as the

patient-applied and provider-administered therapies, respectively. If these modalities are unsuccessful in clearing the warts, a two-stage approach is used and patients are switched to an alternative provider-administered or patient-applied treatment (58).

Pharmacoeconomic models using cost and clinical effectiveness and clinical trial-reported differences in rates of sustained clearance have been proposed (56). In a two-stage therapy model comparing imiquimod and podofilox gel followed by a provider-administered treatment, the lowest cost of care per sustained cleared patient was \$1265, and the highest sustained clearance rate was 61% for patients initially selecting imiquimod (Fig. 5).

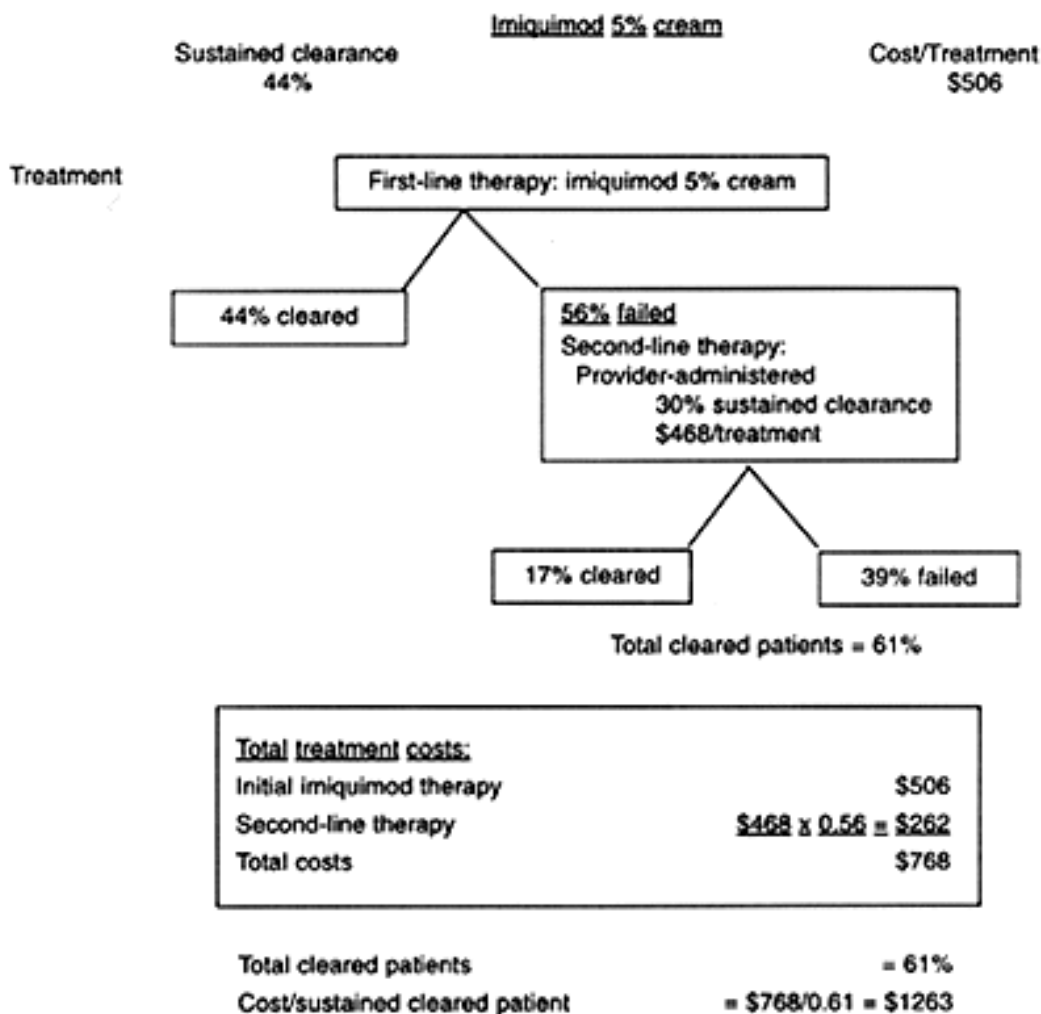


Fig. 5. Flow diagram of the two-stage external genital warts treatment model with imiquimod. Source: Langley, P.C. et al. *The cost effectiveness of patient-applied versus provider-administered intervention strategies for the treatment of external genital warts*. Am J Managed Care 1999, 5: 69-77.

An expanded two-stage model considering both patient-applied and provider-administered treatment options for each stage found that imiquimod followed by TCA for initial treatment failures had the lowest cost per sustained cleared patient and the highest aggregate sustained clearance rate (Paul Langley, personal communication). Although podofilox has a slightly lower price in monthly and treatment period cost times, a two-stage therapy of imiquimod followed by a provider-administered therapy offers better outcomes at a lower unit cost per successful outcome than podofilox. This difference was greatest for females where the sustained clearance rate for imiquimod was 72% compared to 33% for males (59).

Safety

Together with standard assessments of safety and tolerance, specialized human clinical studies were performed in phase I to assess sensitization, cumulative irritation, photoallergenicity, phototoxicity and percutaneous penetration. The results demonstrated that systemic exposure was < 1%, there is no sensitization or photosensitization and imiquimod is less irritating than the commonly used over-the-counter Vaseline Intensive Care Lotion® when applied to nonlesional skin.

However, adverse events were collected in phase II/III EGW trials as both physician observations and patient reported

symptoms. Males and females experienced essentially identical adverse events with the exception that females more often complained of "burning" and males more often showed scabbing. Scarring was not reported, although infrequent (< 1%) instances of hyper- and hypopigmentation were noted. One uncircumcised male who applied the cream daily under the foreskin developed phimosis that responded well to surgical release. Erythema was graded as severe in 4% of patients, with 3% of females showing severe ulceration. Systemic symptoms were minimal to absent, consistent with the pharmacokinetic evidence of minimal systemic exposure, and were probably due to release of locally produced cytokines into the systemic circulation. Only 7 of 421 (1.7%) patients in the imiquimod 5% groups were discontinued from 5 phase II/III safety and efficacy trials due to adverse events, local skin reactions/skin irritation or clinical laboratory abnormalities.

Future uses

Imiquimod 5% cream (Aldara[®]) received approval by the FDA in February 1997 and is currently available in the U.S., Europe, Canada, Mexico, other parts of Latin America and South Africa for the topical treatment of genital and perianal warts. Approvals are expected in additional countries in the near future.

The cytokines induced by topically applied imiquimod include IFN- α , TNF- α and IL-12p40 and indirectly, IFN- β and IFN- γ . Induction of these cytokines stimulates the Th1 cell-mediated immunity. Preclinical data suggests that imiquimod suppresses Th2 immune responses. Therefore, imiquimod should be an effective treatment for chronic virus infections of the skin such as EGWs caused by HPV as well as in common warts, plantar warts, HSV and molluscum contagiosum virus infections (60). Cervical disease caused by HPV might also be treated effectively with imiquimod.

Diseases caused by intracellular bacteria such as leprosy and by intracellular parasites such as leishmaniasis, as well as others caused by intracellular pathogens might also respond to imiquimod treatment. Indeed, *in vitro* studies showed inhibition of *Leishmania donovani* proliferation in imiquimod-treated mouse bone marrow macrophages. Also, topical application of imiquimod cream to mice infected with *L. major* resulted in less severe lesions (61, 62). Finally, more potent analogs of imiquimod were able to inhibit *in vitro* growth of *Mycobacterium avium* in human monocytes (63). Additional studies are needed to confirm these findings.

Potential uses of imiquimod also include ultraviolet-induced skin lesions such as actinic keratosis and skin tumors such as basal cell carcinoma, squamous cell carcinoma and perhaps even melanoma. Clinical results of a small pilot trial of imiquimod 5% cream in patients with the skin cancer, Bowen's disease, showed that 14 of 16 patients cleared their lesions (64). Other skin tumors that might respond include Kaposi's sarcoma and cutaneous T cell lymphoma. A recent pilot study in basal cell carcinoma showed that all patients receiving daily or 3 times per week treatment with imiquimod totally cleared their lesions (65).

Th2 responses were inhibited in preclinical animal models by imiquimod; consequently, atopic-based skin disorders such as atopic dermatitis may also be effectively treated with imiquimod. Other conditions that may respond to topically applied imiquimod include alopecia areata, keloids and cutaneous symptoms of the Th2-mediated autoimmune disease systemic lupus erythematosus. Another possible use for these drugs is as a vaccine adjuvant where Th1 immunity is preferred. Drug application topically or transdermally could be explored with the injectable vaccine. In contrast, skin inflammation due to excessive Th1 responses such as psoriasis and contact dermatitis will probably be worsened by topical treatment with imiquimod. Among drugs, imiquimod is unique in being a topically active cytokine-induced and cell-mediated immunity stimulant. Overall, imiquimod applied topically is an immune response modifier that over time will undoubtedly be proven to be useful in treating a number of acute and chronic conditions of the skin.

Th1 immunity effectively controls virus infections and tumors in most people. Epidemiology studies report that HPV is a frequently occurring infection with 50-75% of sexually active adults demonstrating an antibody response to the virus (1); about 15% of these individuals carry the virus. If the cellular immune response is suppressed due to anti-graft rejection drugs following transplantation, anticancer chemotherapy, acquiring HIV infection or in some cases pregnancy, severe outbreaks of warts can occur.

Some patients develop chronic virus lesions like warts yet most infected people develop an appropriate immune response and have minor or even no symptoms (1). It may be that infected patients with symptoms may have mounted a Th2 response to their infection rather than a Th1 response needed to eliminate the infected cells. A Th2 skewed immune response to the virus may result from a dominant Th2 response to a bacterial infection that was ongoing at the time the virus was first encountered. Consequently, elevated IL-4 levels would suppress the generation of Th1 immunity (66). Conversely, an ongoing Th1 response to a viral infection with IFN- α and IFN- γ induction may prevent the generation of a Th2 response and cause an inappropriate and ineffective immune response to a subsequent bacterial infection. This might explain why patients

with viral pneumonia or influenza sometimes develop bacterial pneumonia which can be severe and even cause death (67).

Interestingly, prolonged Th1 stimulation following treatment with an imidazoquinoline for an acute viral infection may lead to problems with bacterial infections. Furthermore, use of these drugs after serious bacterial infection might downregulate a dominant Th2 response and prevent establishment of chronic virus infections (68). Thus, modifying the immune response through the use of imidazoquinolines could benefit patients with a variety of infections. These drugs offer patients the opportunity for an entirely new approach compared to existing therapeutic options.

Conclusions

Recent advances in the understanding of EGWs and the availability of new patient-applied treatments such as imiquimod have the potential to improve patient care. The introduction of imiquimod cream 5%, a novel topical immune response modifier, is a promising therapeutic development. Although not a cure, imiquimod's efficacy and safety are good compared with that of existing treatments and its recurrence rates are the lowest among current EGW treatments.

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Correspondence: Gary A. Richwald, 3734 Fredonia Drive, Los Angeles, CA 90068, USA.