Topical Imiquimod: Mechanism of Action and Clinical Applications

David Vidal*

Department of Dermatology, Hospital Dos de Maig, Barcelona, Spain

Abstract: Imiquimod is a synthetic imidazoquinoline heterocyclic amine of 240.3 Da (C14H16N4). Imiquimod is a cytokine inducer and a modifier of the innate immune response, as well as acquired antiviral and antitumor immune responses. Imiquimod 5% cream has proven to be an effective treatment for external genital warts, superficial basal cell carcinoma, and actinic keratosis.

Keywords: Imiquimod, imidazoquinolone, immune response modifier, toll-like receptor, skin infection, skin cancer.

1. IMIQUIMOD

Imiquimod (IM) is a synthetic compound belonging to the imidazoquinolone family of drugs. The chemical formulation of IM is 1-(2-methylpropyl)-1*H*-imidazo[4, 5-*c*]quinolin-4-amine (C14H16N4), and has a molecular weight of 240.3 Daltons (Figure 1). IM is a crystalline, odorless solid that varies from white to off-white in color. It is a stable compound, hardly soluble in aqueous systems and most organic solvents. IM sublimes at a melting point of 297°C to 299°C. The constant ionization for IM is about 7.5.

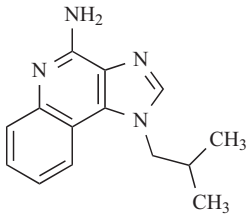

Fig. (1). Imiquimod 1-(2-methylpropyl)-1*H*-imidazo[4,5-*c*]quinolin-4-amine, C14H16N4, 240.3 Da.

IM 5% cream is packaged in single-use sachets containing 250 mg of cream and 12.5 mg of IM. IM 5% cream is marketed in 12 sachet boxes (Aldara™, 3M Pharmaceuticals, St. Paul, MN, USA). Each gram of the 5% cream contains 50 mg of imiquimod in an off-white oil-in-water vanishing cream base consisting of isotearic acid, cetyl alcohol, stearyl alcohol, white petrolatum, polysorbate 60, sorbitan monostearate, glycerin, xanthan gum, purified water, benzyl alcohol, methylparaben, and propylparaben [1]. One sachet of cream may be applied to a skin area of up to 386 square cm [2].

Studies on acute dermal toxicity, dermal irritation, dermal sensitization, and repeat-dose dermal toxicity have demonstrated that IM 5% cream is slightly irritating. IM has no detectable potential for inducing either photocontact allergy or phototoxicity in humans. However, IM reduced the time to skin tumor formation in an animal photocarcinogenicity study, but did not enhance ultraviolet radiation induced damage to human epidermal cells or DNA [3]. According to the results of five *in vitro* and three *in vivo* genotoxicity tests, IM showed no mutagenic or clastogenic potential. No appropriate or well-controlled studies have been conducted in pregnant women; hence, IM should only be used in pregnancy if potential benefits surpass potential risks to the fetus (pregnancy category C). Furthermore, it is not known whether topical IM is excreted in breast milk [1].

IM 5% cream is a patient-applied therapy for external use only. Before applying the cream, the patient should clean the treatment area and dry it thoroughly. Enough cream should be applied to cover the treatment area, including one centimeter of surrounding skin. The cream should be rubbed into the treatment area until absorbed (i.e., the cream is no longer visible), and the treatment area should not be bandaged or otherwise covered or wrapped so as to be occlusive. IM must be applied before normal sleeping hours and left on the skin for 8 hours. After the treatment period, the cream should be removed by cleaning the treated area with mild soap and water. Minimal systemic absorption of IM through intact skin occurs during treatment with topical IM.

The most frequently reported adverse reactions are skin and application site reactions, although some patients develop systemic reactions. Typically, these reactions are more frequent and intense as dosing frequency increases, and decrease in intensity or resolve after cessation of IM therapy. Local skin reactions are frequent, generally mild and well tolerated, and may extend beyond the application site onto the surrounding skin. However, treatment should be discontinued for some time in some patients due to the intensity of local reactions. These skin reactions include erythema, edema, induration, vesicles, pustules, erosion, excoriation, ulceration, weeping, exudate, flaking, scaling, dryness, scabbing, crusting, itching, soreness, and burning. Localized hyperpigmentation, hypopigmentation and vitiligo may follow IM therapy, and these changes may be permanent. Rarely, IM may trigger or exacerbate skin inflammatory conditions such as psoriasis, pemphigus, aphthous ulcers, and angioedema. Systemic reactions that may be related to IM 5% cream are fatigue, fever, malaise, pain, myalgia, arthralgia, headache, nausea, diarrhea, and influenza-like symptoms.

*Address correspondence to this author at the Department of Dermatology, Hospital Dos de Maig 301, Barcelona 08025, Spain;
E-mail: 33053dvs@comb.es

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2. MECHANISM OF ACTION

IM was originally developed for use as a nucleoside analog, but IM does not have nucleoside-like activity. Currently, IM is the first marketed member of a new class of drugs called immune response modifiers. *In vitro* IM has no direct antiviral activity in cell culture and does not induce nonspecific cytolytic destruction. Nevertheless, recent studies have shown that IM has relevant inherent proapoptotic activity against tumor cells.

In vitro and *in vivo* studies have shown that topical IM primarily affects the skin and its immune system. IM is a toll-like receptor (TLR) agonist, mainly TLR-7 but also TLR-8, that enhances both the innate and acquired immune responses, leading to a strong antiviral and antitumoral activity [4]. TLR are a family of pattern recognition receptors that may trigger innate immune responses and affect subsequent adaptative immune responses. TLR are transmembrane proteins expressed on immune cells, and 10 TLR are currently known to be expressed in humans. IM binds to the TLR7 on the cell surface of professional antigen-presenting cells such as monocytes, macrophages, and plasmacytoid dendritic cells [5]. When TLR7 is activated by IM, the intercellular domain of the TLR7 triggers a MyD88-dependent signaling pathway that activates the signal transducer and the activator of transcription 1 (STAT-1) and, ultimately, leads to the nuclear factor-kappa B (NF-κB) and the interferon transcription factor. These factors modulate the mRNA expression of many immunomodulatory genes. Interferon-alpha (INF-α) is the main cytokine induced by IM, it also induces tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 alpha, IL-1 beta, IL-1 receptor antagonist, IL-6, IL-8, IL-10, IL-12, granulocyte-macrophage colony stimulating factor (CSF), granulocyte CSF, and macrophage inflammatory protein-1 alpha [6, 7]. Most of these cytokines display proinflammatory activity and promote a T helper cell type 1 (Th1) adaptative immune response. Moreover, IM also inhibits the production of IL-4 and IL-5, thus suppressing the development of a T helper cell type 2 (Th2) adaptative immune response [8]. *In vitro* studies have found induction of cytokines by IM as early as 1 to 4 h after stimulation with IM in human peripheral blood mononuclear cells [9]. Cytokine synthesis is induced dose-dependently by IM and the maximum peak production occurs 8 hours after topical application.

IM enhances the activation of Langerhans cells (LC), i.e. bone marrow-derived epidermal dendritic cells that represent the major antigen-presenting cells in the skin. Activated LCs take up and process local viral and tumoral antigens within the epidermis and migrate to the draining regional lymph nodes [10]. Within the nodes, LCs present processed antigens to naive CD4 T lymphocytes using MHC class II molecules. These T lymphocytes develop clonal expansion and differentiate to memory and activated T cells that return to the dermis, where they express Th1 cytokines (INF-α, INF-γ and TNF-α). IM is also a potent activator of cytotoxic T lymphocytes (CD8+, perforin +, granzyme B +) [11], macrophages and B lymphocytes [12] leading to infiltration of the treated tissue by lymphocytes and macrophages.

Reviewing the specific mechanism of action of IM for genital [13, 14] and common warts [15], IM decreases HPV DNA and mRNA expression for both early and late viral proteins and enhances the expression of 2', 5' oligoadenylate synthetase. The analysis of IM-treated warts demonstrated that patients achieving complete clearance with IM presented high mRNA levels of STAT1 and interferon response factor 1 prior to IM treatment [16]. Anogenital warts unresponsive to IM showed low densities of dermal dendritic cells and an apparent lack of LC activation, thus suggesting that both intraepidermal and intradermal compartments of antigen-presenting cells might be affected in IM-resistant lesions [17].

The specific mechanism of action of IM for skin cancer has been studied in basal cell carcinoma (BCC) [18], actinic keratosis (AK) [19] and melanoma [20, 21]. Several immune-mediated tumor destruction mechanisms are likely to be involved in this mechanism and may be responsible for the clearance of the tumors. In cutaneous malignancies, IM rapidly increases the peritumoral inflammatory infiltrate, with a significant number of CD4+ T-helper mixed with dendritic cells, CD8+ cytotoxic cells, CD68+ macrophages and CD20+ B lymphocytes [22]. Most CD8+ T cells express cytotoxic granules, T cell-restricted intracellular antigen and granzyme B, thus suggesting that the cytotoxic T-cell-mediated immune response is likely to be mediated by CD8+ T lymphocytes [23]. The analysis of IM-treated BCC demonstrated that tumors that cleared with IM were surrounded by many dermal dendritic cells prior to treatment, whereas imiquimod-resistant BCC had fewer dermal dendrocytes before IM treatment [24]. *In vitro*, IM induces apoptosis in melanoma [25], squamous cell carcinoma, human epithelial (HeLa S3), and human keratinocyte cell lines (HaCaT) [26, 27]. *In vivo*, IM increases apoptosis of basal cell carcinomas and melanomas. Apoptosis, which is programmed cell death or cellular suicide, is a physiological process by which nonviable cells are eliminated. Apoptosis acts as a basic cellular process in the regulation of cell population, both in the morphogenesis and homeostasis of tissues. Moreover, apoptosis is a strictly regulated process, and many genes and proteins participate in its control. At the same time, most genes involved in the control of apoptosis also participate in the mechanisms regulating the cellular cycle. The alteration of these genes may lead to carcinogenesis. The main pro-apoptotic pathways are the death receptors, P53, Bax and the activated cytotoxic cells, through the release of granules of granzyme B. The main anti-apoptotic protein is the Bcl-2, which interferes with Bax in the mitochondria. *In vivo*, IM downregulates Bcl-2 expression and increases apoptosis of BCC cells. Furthermore, imiquimod does not seem to significantly modify p53 expression, or cell proliferation in BCC cells. Therefore, BCC cells become more susceptible to apoptosis through a decreased Bcl-2 expression following treatment with imiquimod [28]. There is controversy on whether IM induces or not the expression of Fas on BCC cells thus making BCC cells susceptible to Fas-FasL-mediated apoptosis [29, 30]. *In vitro* studies with melanoma and SCC cell lines suggest that IM activates several caspases and the Bcl-2-dependent cytosolic translocation of cytochrome c. This activation is independent of the membrane-bound death receptors Fas, TRAIL and TNF receptors [31].

High-density oligonucleotide arrays have been used to determine gene expression profiles of BCC treated with IM. It induces genes involved in different aspects of immune response, modulates the expression of genes involved in the control of apoptosis and oncogenesis, and induces the expression of opioid growth factor receptor (i.e., a molecule with immunomodulatory and antiproliferative activity) [32].

The inhibition of angiogenesis is also involved in the antitumor activity of IM. This drug is a strong inhibitor of tumor cell-induced angiogenesis. The antiangiogenic effect of IM is mediated by IL-18, probably through promoting the production of INF-γ, the most important inhibitor of angiogenesis [33, 34].

3. CLINICAL APPLICATIONS

IM 5% cream has proven to be a safe and effective treatment for external genital and perianal warts, superficial basal cell carcinoma (BCC) and actinic keratosis (AK) in randomized, double-blind, vehicle-controlled trials. IM is currently approved for the treatment of these three conditions, but off-label applications of IM have demonstrated its efficacy for many other skin conditions (Table 1).

Table 1. Skin Disorders Treated with Topical Imiquimod

Official licensed indications
External genital and perianal warts (RCT)
Superficial basal cell carcinoma (RCT)
Actinic keratosis (RCT)
Unlicensed indications
Common, plantar and flat warts
Molluscum contagiosum (RCT)
Cutaneous leishmaniasis (RCT)
Bowen´s disease
Actinic cheilitis
Vulvar intraepithelial neoplasia
Nodular basal cell carcinoma (RCT)
Infiltrative basal cell carcinoma
Mycosis fungoides
Lentigo maligna
Melanoma metastasis
Extramammary Paget's disease
Hemangioma
Prevention of keloids after surgery

RCT: effectiveness based on randomized controlled trials.

IM is indicated for the treatment of external genital and perianal warts (condyloma acuminata) in individuals aged 12 years and over. IM must be applied at night, 3 times per week, for a maximum of 16 weeks, or until the warts disappear [35, 36]. The clearance rates range from 50 to 80%, and IM is associated with lower recurrence rates when compared to other current therapies.

Although the use of IM for managing other skin infections has not yet been approved, there is a growing body of evidence supporting the efficacy of IM in the treatment of other HPV-related diseases such as common warts, subungual and periungual warts, plantar warts, flat warts, lip papillomatosis, epidermodysplasia verruciformis, and genital and anal intraepithelial neoplasia.

A randomized controlled trial has assessed the efficacy of IM in molluscum contagiosum (MC) [37]. MC is an infection caused by a poxvirus that causes small, umbilicated, raised papules in the epidermis. IM is safe, well-tolerated and with high clearance rates in children with MC. There are also case reports of herpes simplex successfully treated with IM. However, a randomized controlled trial failed to show any benefit of IM for the treatment of recurrent genital herpes [38].

IM has been used in cutaneous leishmaniasis with some success. Current treatments for cutaneous leishmaniasis are limited by their toxicity, high cost, discomfort and drug resistance. A combination therapy with topical IM and parenteral meglumine antimoniate has demonstrated high efficacy in a randomized clinical trial [39].

IM is indicated for the topical treatment of biopsy-confirmed, primary superficial BCC in immunocompetent adults, when surgical methods are less appropriate. BCC is the most common human cancer, and its incidence is increasing worldwide. BCC is a slow-growing, locally invasive malignant epidermal skin tumor. BCC may be divided into three common growth patterns: superficial (15%), nodular (75%) and infiltrative (5%). While excisional surgery is the standard management for BCC, topical IM has the advantage of leaving no cosmetic trace after eradication of the lesion. IM has shown efficacy against BCC when applied 5 times per week for 6 weeks. Most BCC significantly decrease in size or achieve complete remission with IM. The clearance rates range from 70 to 100% depending on both the size and the histological pattern of the tumor [40, 41]. Higher erosion and crusting severity is generally associated with higher clearance rates. In addition, the efficacy of IM in BCC has also been demonstrated in patients with basal cell nevus syndrome [42] and xeroderma pigmentosum [43]. Few recurrences have been reported during the 2-year follow-up studies, although the results of the 5 year follow-up studies have not yet been published.

IM is indicated for the topical treatment of clinically typical, nonhyperkeratotic, nonhypertrophic actinic keratoses (AK) on the face or scalp in immunocompetent adults. AK are the most common form of in situ squamous cell carcinoma and are caused by chronic sun exposure. AK are premalignant lesions that may progress to squamous cell carcinoma. IM administered twice per week for 16 weeks is an effective and well-tolerated treatment for AK [44, 45]. Subclinical AK lesions may become apparent in the treatment area during treatment with IM. Most patients achieve complete clinico-pathological tumor clearance (from 60 to 100%) and an excellent cosmetic result. Long-term follow-up data showed a low incidence of new AK lesions following treatment with IM. Although it has not been approved, a cycle dosing regimen may be also effective. Cycle therapy consists of once-daily application of IM, 3 times a week for 4 weeks, followed by a rest period of 4

weeks. The cycle may be repeated if any AKs remain after completing an 8-week cycle [46].

Among other in situ squamous cell carcinoma successfully treated with IM we include: Bowen´s disease [47], actinic cheilitis [48], and genital and anal intraepithelial neoplasia [49].

The immune system plays a crucial role in the development and pathogenesis of skin cancer, and IM increases the host's immune response against different types of cancer cells. Open controlled trials and case series support the use of IM in selected cutaneous malignancies, such as lentigo maligna, melanoma metastasis, T cell cutaneous lymphoma and extramammary Paget's disease.

Several case series of complete regression of lentigo maligna (LM) after IM treatment have been reported. LM is an *in situ* melanoma that most commonly appears on the sun-exposed skin of the head of elderly patients. Complete removal of LM may be difficult due to its occasional extensive subclinical extension. IM may be used for LM in elderly patients who are not good candidates for surgery and/or require less aggressive interventions. However, a rigorous post-treatment follow-up is mandatory, because long-term recurrence rates after treatment with IM are yet unknown [50].

IM may clear clinically and histologically malignant melanoma skin metastasis [51] but fails to prevent lymphogenous metastatic spread [52].

Mycosis fungoides (MF) is the most common form of cutaneous T cell lymphoma. Treatment of MF with IM is well tolerated and associated with a histological and clinical response rate of 50% in open label studies [53, 54]. Nonregressing primary cutaneous CD30+ T-cell lymphoma has also been successfully treated with IM [55].

IM offers a minimally invasive option for the treatment of cutaneous extramammary Paget's disease, an uncommon neoplasm found in the genital, anorectal, or axillary area. The treatment-associated morbidity is minimal compared with more invasive therapies, and self-application by the patient improves convenience and appeal [56].

The effect of IM on innate and cell-mediated immunity highlighted the possibility of using topical IM in immunosuppressed patients. Solid organ transplant recipients and HIV+ patients are a growing population at increased risk for developing cutaneous infections and skin cancer, thus resulting in significant morbidity and mortality. In immunosuppressed patients, IM has proven to be effective for external anogenital warts, common warts, MC, herpes simplex, BCC [57], AK, Bowen's disease and anal intraepithelial neoplasia.

IM may be the treatment option for dermatological conditions in which the immune system is thought to play a role in the regression of the disease, such as keratoacanthoma, hemangioma and Kaposi´s sarcoma. IM may also be a successful treatment option in conditions in which the exogenous IFN-α has shown utility. For example, IFN-α injections decrease the recurrence rate of excised keloids, an observation that has also been reported with IM therapy [58].

Finally, IM has already been used as a successful treatment for invasive cutaneous squamous cell carcinoma, trichoepitheliomas, porokeratosis of Mibelli, angiolymphoid hyperplasia with eosinophilia, vulvitis circumscripta plasmacellularis, chronic discoid lupus erythematosus, localized morphea, granuloma annulare, granuloma faciale, silicone granuloma, and chronic interdigital tinea pedis. Future IM indications may be cutaneous fibromatoses, mastocytosis and tattoo removal.

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ABBREVIATIONS

AK	=	Actinic keratosis
BCC	=	Basal cell carcinoma
CSF	=	Colony stimulating factor
HIV	=	Human immunodeficiency virus
HPV	=	Human papilloma virus
IFN	=	Interferon
IL	=	Interleukin
IM	=	Imiquimod
LC	=	Langerhans cell
LM	=	Lentigo maligna
MC	=	Molluscum contagiosum
MF	=	Mycosis fungoides
MHC	=	Major histocompatibility complex
NF-κB	=	Nuclear factor-kappa B
STAT-1	=	Signal transducer and activator of transcription 1
TLR	=	Toll-like receptor
TNF	=	Tumor necrosis factor
TH1	=	T helper cell type 1
TH2	=	T helper cell type 2

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