Nonsurgical Innovations in the Treatment of Nonmelanoma Skin Cancer

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ABSTRACT

Basal cell carcinoma and squamous cell carcinoma are the most frequent types of cancer in the United States and represent 75 percent and 20 percent, respectively, of all nonmelanoma skin cancers. Since ultraviolet radiation is implicated in their development, photoprotection is fundamental in their prevention. Additional preventive measures include identifying high-risk individuals for early detection along with using agents, such as retinoids, that are effective in decreasing the risk of premalignant cells further developing into carcinomas. Newer agents achieving this goal include perillyl alcohol, T4 endonuclease 5, DL-α-tocopherol, and α-difluoromethylornithine. Procedural modalities are currently the standard of treatment, but recent evidence has consistently shown that newer (nonsurgical) therapies, such as interferon, imiquimod, retinoids, and 5-fluorouracil, can be used effectively either as monotherapies or as adjuvants to those surgical modalities for the treatment of superficial nonmelanoma skin cancers and premalignant lesions. These newer therapies have achieved significant reductions in morbidity and mortality. Procedural modalities that have been evolving into important tools for the treatment of actinic keratosis and nonmelanoma skin cancers include photodynamic therapy and lasers. Nonsurgical therapies currently proving to be effective in clinical trials include ingenol mebutate and cyclooxygenase-2 inhibitors. Agents that are showing promising results in early phases of clinical trials include betulinic acid; hedgehog signaling pathway inhibitors, such as cyclopamine and GDC-0449; α-melanocyte–stimulating hormone analogs, such as afamelanotide; epidermal growth factor receptor inhibitors, such as gefitinib and erlotinib; anti-epidermal growth factor receptor monoclonal antibodies, such as cetuximab and panitumumab; and the 5-fluorouracil prodrug capecitabine. (*J Clin Aesthetic Dermatol.* 2010;3(6):20–34.)

Nonmelanoma skin cancer (NMSC) represents the most common form of cancer in humans, with an estimate of more than 1,000,000 new cases and 1,000 deaths in the United States in 2009.[1–3] The two subtypes associated with ultraviolet radiation (UVR) as a major contributory factor, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), account for 75 percent and 20 percent of the cases, respectively.[2,4,5] Although the relative mortality is low (0.1%), NMSCs may cause considerable morbidity, particularly in visible areas, such as the head and neck, with consequent unacceptable cosmetic outcomes and/or functional impairments, causing direct and indirect costs of management in the order of billions of dollars annually.[2–6] Most cases can be diagnosed clinically. Newer, noninvasive diagnostic tools, including dermoscopy, high frequency ultrasound, and confocal microscopy, may help in the diagnosis; however, the histopathological evaluation remains the gold standard for diagnosis.[7,8] Current procedural modalities, such as Mohs micrographic surgery, regular excision, cryosurgery, curettage and electrodessication, and radiation therapy, as well as nonsurgical modalities (indicated as monotherapy or as adjuvants), including interferon (IFN), imiquimod, retinoids, and 5-fluorouracil (5-FU), have demonstrated to be effective for the treatment and prevention of NMSC.[5,6,9,10] Our focus is to describe new developments in the prevention and treatment of NMSC. Some considerations are taken in regard to actinic keratoses (AKs), which represent the initial intraepidermal manifestation of keratinocyte abnormal transformation that may potentially progress to SCC.[11]

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PREVENTION

The approach to NMSC prevention begins with the identification of high-risk individuals. Individuals with UVR-related skin cancers (i.e., BCC and SCC) usually have the following qualities: Fitzpatrick I–II skin phototype; male gender; older age (40–79 years old); history of chronic UVR exposure; living in lower latitudes (closer to the equator); predisposal to genetic disorders, such as xeroderma pigmentosum (XP), basal cell nevus syndrome (BCNS), epidermodysplasia verruciformis, and albinism; immuno-suppression; status post-organ transplantation; exposure to ionizing radiation, coal tars, soot, petroleum oils, polycyclic aromatic hydrocarbons, and arsenic; burn scars; and infection with human papillomavirus types 16, 18, 30, and 33 (SCC).[2,11,12]

Primary prevention includes sun-protective behavioral measures, such as avoidance of excessive sun exposure, particularly between 11 a.m. and 2 p.m.; avoidance of artificial UV sources, such as tanning beds and prolonged UV treatments; application every 3 to 4 hours of a broad-spectrum sunscreen with UVB protection of at least 30 sun protection factor (SPF) and high and extended UVA protection; reapplication of sunscreen in cases of excessive sweating or swimming; and the use of protective clothing.[4,6,11–15]

Secondary prevention includes a full body examination for early detection and several treatment modalities that may prevent further development and recurrence. Among these treatments, topical and systemic retinoids have demonstrated their efficacy in decreasing the risk of developing BCC and SCC.[5,16–18] Retinoids induce apoptosis, arrest growth, stimulate differentiation of tumor cells during carcinogenesis,[19–21] and downregulate the overexpression of cyclooxygenase-2 (COX-2) induced by UVR, causing a decrease in prostaglandins, which are increased in NMSC.[22–25] Isotretinoin and acitretin are the most common systemic retinoids used for NMSC chemoprevention.[26,27] They may decrease the morbidity and mortality seen in patients with single, high-risk, and multiple primary cancers, particularly in those with organ transplants, immunosuppression, xeroderma pigmentosum, and BCNS.[5,26,28,29] Several studies have demonstrated the efficacy of topical all-trans-retinoic acid (tretinoin) for the treatment of AKs, thus preventing their progression to SCC.[9,29–36]

The consumption of a low-fat diet has also been associated with a reduction in the number of AKs in individuals with a history of NMSC[37,38] and in animal models.[39] Current evidence does not support the association of fat intake with the development of BCC.[39]

Newer agents currently in development or being studied for the prevention of NMSC include the following:

Perillyl alcohol (POH)—a hydroxylated monoterpene found in essential oils of plants, including citrus peels, mints, and celery seeds[40] with antitumor activity in UV-induced skin carcinogenesis[41] inducing apoptosis, and suppression of inflammation, oxidative stress, the activity of ornithine decarboxylase, thymidine incorporation into deoxyribonucleic acid (DNA), the Ras pathway, and alteration of the Bax:Bcl-2 ratio in mice skin.[42–45]

T4 endonuclease 5 (T4N5)—an enzyme involved in the reparation of cyclobutane pyrimidine dimers generated in the DNA molecule after exposure to UVR, which have been associated with the generation of NMSC.[46–48] A topical formulation has been developed and T4N5 has been found within the cytoplasm and nucleus of epidermal Langerhans cells and keratinocytes in preliminary studies. Early clinical studies have demonstrated its efficacy (i.e., reduction of 68% of AKs in 30 patients with XP compared with placebo) with good tolerability in the prevention of NMSC.[46,47,49] Two well-controlled studies, a Phase 3 and a Phase 2b, are currently being conducted in XP and in renal transplant recipients, respectively, to further evaluate T4N5's safety and efficacy.[50,51]

DL-α-tocopherol—the active component in vitamin E and a well-known antioxidant that, when topically applied in mice, has been found to prevent cancer and decrease UVB-induced cytokine stimulation and inflammation.[52–55] However, DL-α-tocopherol has failed to demonstrate any significant protective effect in well-controlled clinical trials.[55]

α-difluoromethylornithine (DFMO)—an irreversible inhibitor of ornithine decarboxylase, the rate-limiting enzyme in polyamine synthesis.[56,57] Polyamines (e.g., putrescine, spermidine, spermine) bind to DNA, ribonucleic acid (RNA), and phospholipids and control DNA replication, transcription, and translation. They are imperative for normal cellular proliferation and differentiation[58,59] and are upregulated during the promotion phase of chemically and UVB-induced skin carcinogenesis models.[29] DFMO 10% ointment was applied to one forearm each of 48 patients with moderate-to-severe AKs for six months in a randomized, controlled trial (RCT).[60] A significant reduction in the number of AKs was found in comparison to placebo, with a small percentage of patients reporting moderate-to-severe local adverse events (AEs). In a follow-up study,[61] a significant reduction in the levels of p53 was found, while levels of proliferating cell nuclear antigen (PCNA) and the apoptotic rate were not significantly altered in the DFMO-treated skin. Another RCT[62] reported significantly less new NMSCs in 291 patients with a history of previous NMSC who received oral DFMO for 4 to 5 years compared with placebo. Current RCTs are comparing the efficacy of DFMO with or without triamcinalone in the prevention of NMSC in patients with AKs[63] and the effectiveness of DFMO in preventing skin cancer in patients who have previously received treatment for early-stage skin cancer.[64]

INTERFERON

Interferon (IFN) works by binding to receptors located on target cells. The exact mechanism of action of these cytokines remains unclear, but interferons are known to have many important effects for the treatment of skin cancer, including antiproliferative effects (i.e., inhibition of mitosis and growth factors, activation of pro-apoptotic genes, and promotion of antiangiogenic activity) and upregulation of the immune system in the skin.

Intralesional IFN-α2b injections three times per week (3x/wk) for 2 to 3 weeks have shown to be effective for the

treatment of AKs compared with placebo.[65] Limitations of intralesional IFN's clinical use in AKs include multiple injections and many office visits. The topical application of IFN-α2b gel 30 million IU/g four times daily for four weeks obtained clinical, although nonsignificant, improvement of AKs compared to placebo.[66]

Intra- and perilesional IFN represent effective nonsurgical alternatives to treat BBCs, obtaining clearance rates between 70 to 100 percent.[67,68] Its use is limited by its cost, safety profile, and the inconvenience of returning to a physician's office for multiple injections.[69,70] A multicenter RCT where IFN-α2b was used to treat 172 patients with biopsy-proven BCC found the optimal dose to be 1.5 million IU intralesionally administered 3x/wk for three weeks. Significant clinical and histological clearance was obtained when compared with placebo.[71] Similar doses used for recurrent or morpheaform BCCs failed to demonstrate clinical efficacy.[72] Twenty BCCs received treatment with intralesional IFN-α2b 3x/wk for three weeks at a dose of 1.5 million IU for lesions less than 2cm in diameter and three million IU for lesions 2cm or greater in diameter. More than half of lesions completely responded clinically and histologically at eight weeks of follow up. In those lesions that completely responded, only one recurrence was reported at five years of follow up.[73]

Intralesional IFN has also shown efficacy for the treatment of SCCs. A total of 33/34 SCCs localized to sun-exposed areas showed complete histological response after treatment with intralesional IFN-α2b at a dose of 1.5 million IU, 3x/wk for 3 weeks.[74] The same regimen was used in 27 invasive and seven *in-situ* SCCs, ranging in size from 0.5 to 2.0cm, obtaining clinical and histological clearance rates (CRs) in 97 percent at 18 weeks, with a 96.2 percent CR in the invasive lesions. Almost 94 percent of patients and investigators rated the cosmetic results as "very good" or "excellent."[75] In a study evaluating transplant-associated metastatic SCC, a combination therapy with retinoids and IFN-α led to a low number of complete responders.[76] A combination of IFN-α and IFN-γ applied peri- and intralesionally 3x/wk for three weeks was used for the treatment of 12 BCCs and four SCCs in 16 elderly patients with extensive and recurrent BCCs or SCCs who had previously failed other treatments. Almost half of the patients had complete response to the treatment.[77]

AEs from IFNs are usually dose-dependent and will remit after dose-reduction or cessation of therapy. They include anorexia, influenza-like symptoms, skin necrosis at injection site, rhabdomyolysis, hypotension, arrythmias or tachycardia, neurological effects, gastrointestinal disturbances, erectile dysfunction, fatigue, hepatotoxicity, hematological toxicity (leukopenia, thrombocytopenia, anemia), hypothyroidism, and ocular toxicity. Cases of exacerbation of psoriasis and induction of *de novo* psoriasis and psoriatic arthritis have been reported.[78] Interferon use during pregnancy has not formally been studied; however, it has been administered to rhesus monkeys at doses equivalent to those administered in humans and has abortifacient effects.[79]

5-FLUOROURACIL

Topical 5-FU decreases cell proliferation and induces cellular death, particularly in cells with high mitotic rates, through inhibition of thymidylate synthetase, which interferes with DNA synthesis.[80,81] Evidence suggests that 5-FU used as a topical chemotherapeutic agent in NMSC has been effective for the treatment of superficial BCC (sBCC), *in-situ* SCC, and AKs. Due to lack of penetration through the dermis, 5-FU is generally not recommended for invasive BCCs and SCCs.[5,81] Several studies have reported the efficacy of topical 5-FU 5% ointment for the treatment of NMSC, with most patients rating their cosmetic results as "good" to "excellent." However, high recurrence rates have been frequently reported.[82,83,85]

A total of 103 patients with invasive BCCs were treated with 5-FU in concentrations from 1 to 5% twice daily for a range of 1 to 5 weeks. Although in 25 percent of the cases the treated area had normal appearing skin, and the remaining ulcerated cases had minimal neoplastic tissue, all cases presented histological evidence of invasion after microscopically controlled excision. 5-FU controlled the superficial part of the BCCs, leading to a relatively benign clinical appearance of the tumors, causing delays in definitive treatment for up to 18 months.[84] Several studies have compared the efficacy, safety, and cosmetic outcomes of 5-FU and photodynamic therapy (PDT) for the treatment of Bowen's disease (BD) in both immunocompetent[86] and organ transplant patients.[86–88] In both groups of patients, PDT demonstrated superior efficacy, less recurrences, and less AEs during treatment and after 12 months of follow up.[87,88] In addition, PDT obtained better cosmetic outcomes than 5-FU. Hamouda et al[88] treated 10 patients with multiple unresectable SCCs in XP patients with 5-FU twice daily for an average of six months. Although clinical superficial regression was reported in most of the patients, post-treatment biopsies in five patients showed clearance of the epidermal ulceration in one patient and the persistence of deep dermal components in the remaining four samples.

A total of 14 elderly patients with biopsy-proven aggressive, multiple or recurrent SCCs that had failed prior standard treatments (including topical 5-FU) were treated with oral 5-FU 175mg/m^2 daily for three weeks every five weeks.[89] Oral 5-FU was effective and well tolerated, although larger studies are needed to evaluate its real clinical value.

In regard to the topical treatment of AKs, 5-FU has demonstrated high efficacy rates[90–94]; however, recurrences are frequently reported,[95] and low adherence due to AEs has been related to 60 percent of treatment failures.[96] Topical 5-FU has the advantage of also treating clinically undetectable (subclinical) AKs.[93] The combination of 5-FU and imiquimod has resulted in faster responses and fewer AEs.[97] Current studies are evaluating the role of 5-FU in the prevention of new NMSC on the face and ears.[98]

IMIQUIMOD

Imiquimod stimulates the innate- and cell-mediated immune responses, potentiating their antiviral, antitumoral, and immunoregulatory properties. Imiquimod 5% cream is

approved by the United States Food and Drug Administration (FDA) for the treatment of external genital and perianal warts; nonhyperkeratotic, nonhypertrophic AKs; and sBCC mostly in patients in whom surgery is not an option.[99] A new concentration of imiquimod (i.e., 3.75%) has been approved by the FDA for the treatment of AKs on the face or balding scalp and will be available soon in US and Canadian markets. In addition, generic imiquimod 5% cream is currently available.

The antitumoral mechanisms of action of imiquimod include the following:

1. Induction of the production of cytokines, including IFN-α, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-2, IL-6, IL-8, and IL-12, by human peripheral blood mononuclear cells
2. Stimulation of monocytes, macrophages, and toll-like receptor (TLR) 7 and 8-bearing plasmacytoid dendritic cells (DCs) and epidermal Langerhans cells. The stimulation of TLRs induces the production of pro-inflammatory cytokines involved in the modulation of the innate immune system[100]
3. Stimulation of the production of IL-6, IL-8, and IFN-α, by keratinocytes resulting in a Th1-dominant response[100–106]
4. Increase in the levels of type I IFN, which improves the response to endogenous IFN-α, usually low in AKs. Suppression of type I IFN signaling proteins is an early event leading to SCC[107]
5. Induction of FasR (CD95), a member of the tumor necrosis receptor family, involved in apoptotic pathways
6. Induction of pro-apoptotic pathways associated with the B cell lymphoma/leukemia-2 (Bcl-2)-associated X (Bax) protein[108,109]
7. Induction of caspases 9 and 3, which have been linked with stress signaling, mitochondrial death pathways, and apoptosis[110]
8. Induction of E-selectin on blood vessels of invasive SCCs, which is a ligand for lymphocyte antigen expressed by skin resident T-cells that are in charge of immunosurveillance, and is usually absent in SCCs[9]
9. Reduction of T-regulatory cells (expressing the transcription factor FOXP3) infiltrating SCCs. These FOXP3+ T-regulatory cells cause suppression and impairment of effector T-cells (responsible for immunosurveillance), surround the tumor, and prevent T-effector cells from reaching the tumor[9]

Imiquimod, as topical therapy for AKs, has the advantage of treating multiple lesions including subclinical AKs. CRs of AKs treated with imiquimod range from 45 to 85 percent,[108,111–116] with recurrence rates up to 16 percent after 18 months of treatment and approximately 20 percent at 24 months follow up.[111,117]

Actinic keratosis. A total of 829 patients with a total of 7,427 AKs were treated with imiquimod 5% cream 3x/wk for four weeks followed by a four-week rest. A second four-week treatment period was applied in cases of remaining lesions. Imiquimod obtained high clearance rates and reduced 85 percent of the total number of lesions.[118]

In several multicenter, prospective, double-blind RCTs, imiquimod 5% cream was evaluated in a total of 1,493 patients with multiple AKs on the face or balding scalp. Imiquimod was applied daily, 2 to 3x/wk for a maximum of 24 weeks.[115,116,119–121] Overall, 3x/wk regimens result in a higher clinical response than 2x/wk. The median percentage reduction in the number of AKs from baseline was greater than 86 percent with 3x/wk regimens and 83.3 percent with 2x/wk applications.[120] Most common AEs included local skin reactions, such as erythema, scabbing/crusting, flaking/scaling/dryness.

A RCT was conducted applying imiquimod or vehicle twice weekly for eight weeks following 3- to 5-second cryotherapy of target AKs within a 50cm^2 field on the face or scalp. At 12 weeks, the CR was similar for imiquimod and vehicle, but fewer total AKs were noted with imiquimod. A progressive reduction in subclinical AKs with imiquimod compared with a progressive increase with vehicle was noted. In addition, more subjects treated with imiquimod achieved clearance of subclinical AKs than with the vehicle. Imiquimod post-cryotherapy may increase clearance of subclinical and total AKs.[122]

In a multicenter RCT,[123] 43 post-transplant patients receiving immunosuppressive therapy within the prior six months concomitantly presenting with AKs were treated with imiquimod or vehicle 3x/wk for 16 weeks. Imiquimod proved to be effective and well-tolerated for the treatment of AKs in immunocompromised patients.

Two concentrations (3.75% and 2.5%) of imiquimod cream have been studied in order to optimize its topical use. Imiquimod 3.75% applied daily for two-week treatment cycles with two weeks without medication in between cycles, for four months, maintained the efficacy achieved by 5% imiquimod and was well-tolerated, when measured by median percent reductions in the number of AKs.[124] Currently, clinical studies are assessing AK treatments using imiquimod 3.75% cycle dosing as field- and lesion-targeted treatment.[124] Furthermore, in four Phase 3 studies, treatment of 5 to 20 AKs on the entire face or balding scalp was evaluated using imiquimod 2.5% and 3.75% creams applied daily in two cycles of either 2 or 3 weeks, separated by a matching no-treatment interval (2/2/2 or 3/3/3). More than 40 percent of the patients who achieved a complete response at eight weeks post-treatment with the 3.75% cream maintained a recurrence-free status after 12 months of follow up. Complete responders who had recurrences at the follow-up visit had less lesions compared with patients treated with placebo.[125]

Superficial basal cell carcinoma (sBCC). As a non-invasive treatment modality for sBCC, imiquimod may have some advantages over surgical procedures, especially in regard to cosmetic outcomes.[147] RCTs have been conducted using imiquimod 5% cream for histologically confirmed nodular BCC (nBCC) and sBCC. Various treatment regimens were applied in these studies (for example: twice-daily, once-daily, 5x/wk, 3x/wk), achieving respons-

es up to 100 percent. Regimens with more frequent imiquimod applications (once daily and twice daily) were associated with the best responses. Once-daily or 5x/wk application groups had the highest efficacy rates with acceptable safety profiles.[148,149] Most common AEs included local skin reactions.

In RCTs evaluating a total of 890 patients with histologically proven sBCC who received treatment with imiquimod 5% cream 5x/wk or 7x/wk for six weeks or placebo,[145,150] it was shown that imiquimod-treated groups obtained significantly better clinical and histological responses. Local skin reactions were reported more often in the 7x/wk than in the 5x/wk imiquimod-treated group.

Nodular basal cell carcinoma (nBCC). In two Phase 2 studies, the highest response rate achieved was 76 percent using imiquimod 5% cream twice daily for 12 weeks.[151] The responses in these studies were not as high as those seen in studies where sBCC was treated with imiquimod 5% cream. This type of treatment modality applies better for those patients in which surgery, radiotherapy, or cryotherapy is not an option. In a RCT,[152] 93 patients with biopsy-proven nBCC applied imiquimod 5% cream in several different treatment regimens for six weeks. At the end of treatment, more frequent applications (i.e., 3x/wk) under occlusion achieved better results than less frequent applications (i.e., 2x/wk); however, no significant differences were found between the groups. An evidence-based systematic review supports the use of topical imiquimod as monotherapy for sBCC and SCC *in situ*. The use of this modality may be limited to patients with small tumors in low-risk locations who cannot undergo surgery or other treatment regimens for which long-term clearance rates have been determined.[153]

RESIQUIMOD

Resiquimod is a TLR 7/8 agonist that has similar effects as imiquimod on monocytic cells, although it is 10 to 100 times more potent than imiquimod.[154–156] In addition, it induces IL-1 receptor antagonist (IL-1ra), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemotactic protein (MCP-1).[156] A total of 132 patients with 4 to 8 AKs on the face or the balding scalp applied resiquimod at different concentrations once daily, 3x/wk for four weeks obtaining complete responses ranging from 40.0 to 74.2 percent. No statistical difference was found among the various concentrations of resiquimod, but better tolerance was seen with lower concentrations.[157]

INGENOL MEBUTATE

Ingenol mebutate (IM) is an extract from the plant *Euphorbia peplus*, also known as milkweed, which has been used for several years as a home remedy for skin conditions, such as AKs and skin cancers.[158–161] It has a dual mechanism of action first causing chemoablation and disruption of the plasma membrane with loss of the mitochondrial membrane potential and mitochondrial swelling, specifically targeting dysplastic keratinocytes, leading to fast cell death by primary necrosis. It also induces rapid healing and restoration of the normal clinical and histological morphology.[158] This necrosis allows IM to remain active in the presence of apoptosis-resistant tumor cells.[162,163] Second, IM induces neutrophil infiltration and the production of tumor-specific antibodies and pro-inflammatory cytokines, leading to an antibody-dependent cellular cytotoxicity that eliminates residual cells.[164] IM has been evaluated in several controlled studies for the treatment of AKs. A total of 280 patients with AKs have been treated with topical IM gel at concentrations of 0.025% and 0.05% achieving significantly better clinical results than the vehicle.[159,165] Common AEs included erythema, flaking/scaling, and crusting. There were no reports of scarring. Current Phase 3 studies are evaluating IM topical gel at 0.015% for the treatment of AKs located on the face and scalp[166,167] and 0.05% for non-head locations.[168,169]

CYCLOOXYGENASE-2 INHIBITORS

Cyclooxygenase (COX) is the rate-limiting enzyme in the pathway of synthesis of prostaglandins from arachidonic acid. Increased production of prostaglandins may be associated with the development of UV-induced NMSC.[170] Prostaglandin E2 (PGE2) has also been implicated in the induction of bcl-2 gene expression, IL-6, and the inhibition of apoptotic pathways. It has been determined that of the two COX isoforms, COX-2 is overexpressed in several human neoplasias, including colon carcinoma, SCC of the esophagus, and skin cancers (AKs, melanoma, and NMSC), and in metastasic murine mammary tumor cells.[171,172] UVB is a well-known factor capable of inducing COX-2 in humans and in animal models. In the latter, a decrease in the number of skin tumors has been obtained after the administration of a selective COX-2 inhibitor.[22,23,173] Several clinical trials have evaluated the efficacy of selective COX-2 inhibitors for the chemoprevention of AKs and NMSC.[174–179] Topical diclofenac 3.0% gel in hyaluronan 2.5% gel, which reduces diclofenac's epidermal diffusion, has shown to be safe and effective in the treatment of AKs. A combination of diclofenac gel and cryosurgery demonstrated to be superior to cryosurgery alone in a multicenter RCT.[180] As monotherapy, diclofenac/hyaluronan topical gel was applied twice daily to 195 patients with AKs in a RCT, achieving significantly better clearance scores than placebo after 60 days of treatment.[181] A recent study showed that short-term nonsteroidal anti-inflammatory drug (NSAID) use (<5 years), but not long-term use, had significant protective effect for SCC and BCC compared with non-NSAID users. Non-aspirin NSAID use showed significant protective effects against BCC. This effect was not seen with aspirin.[182] In a In a small series, diclofenac has been shown to be clinically and histologically effective and well-tolerated for the treatment of Bowen's disease.[183–185] Animal studies have shown that other selective COX-2 inhibitors, such as celecoxib, and nonselective COX inhibitors, such as indomethacin, are also effective for the chemoprevention

of skin cancers.[22,25,186] Currently, clinical trials are evaluating the use of celecoxib for the prevention of AKs[187] and for the prevention of BCCs in patients with BCNS.[188]

BETULINIC ACID

Betulin, betulinic acid, oleanolic acid, lupeol, and erythrodiol are pentacyclic triterpenes that are contained in the outer bark of birch (Betula alba cortex) and are found to have antiviral, antimicrobial, hepatoprotective, and antitumoral effects.[189,190] Importantly, they promote keratinocyte differentiation and induction of cytotoxic, antiproliferative, and apoptotic effects on tumor cells.[191] In a randomized trial, Huyke et al[191] treated 45 patients (with <10 AKs each) with either a topical betulin-based oleogel twice daily, croyotherapy, or a combination of the two. Treatment with betulin-based oleogel was well-tolerated and showed efficacy in treating AKs. Further controlled studies on larger sample sizes investigating the use of betulinic acid for the treatment of NMSC are warranted.

CYCLOPAMINE

Cyclopamine is a steroidal alkaloid extracted from the corn lily *Veratrum californiucum*, which is very common in subalpine meadows.[192] It has shown inhibitory activity against the hedgehog family of intercellular signaling proteins. This membrane complex, particularly the Sonic (hedgehog) (SHH) subtype, present in mammals, has recently been implicated in the development of BCC in the white population.[193–195] The hedgehog signaling pathway is modulated by two transmembrane proteins, the tumor suppressor patched (PTCH), and the proto-oncogene smoothened (SMO). The signaling is activated when the SHH protein binds directly to PTCH receptor (encoded from the PTCH-1 gene), causing its inhibition and further repression of the expression of the target genes.[193,195] When the SHH protein is not present, PTCH-1 inhibits the activity of smoothened (SMO), which is a key activator of the pathway, thus preventing the expression of target genes, such as glioma transcription factor-1 (GLI-1).[195,196] Binding of SHH to the PTCH receptor eliminates that inhibition of PTCH on SMO, resulting in a dysregulated hyperactivity of the hedgehog pathway.[193] Good examples of the activation and dysregulation of the hedgehog pathway represent human sporadic development of BCCs, and BCNS, where germ-line mutations in the PTCH-1 gene or in any of the components of the hedgehog pathway contribute with their development.[196–198] Other neoplasias that have been related to mutations in the hedgehog signaling pathway include breast carcinomas, odontogenic keratocysts, medulloblastomas, sebaceous nevus, trichoepitheliomas, fibrosarcomas, and dermatofibromas.[193,195] Clinical studies are needed to evaluate the efficacy and safety of topical cyclopamine for the treatment of NMSC.

GDC-0449

GDC-0449 is another hedgehog pathway inhibitor specifically targeting the SMO membrane protein. It is more potent and has more favorable properties than cyclopamine.[196,199] Studies in animal models of medulloblastoma and xenograft models of human colorectal cancer and pancreatic carcinoma have demonstrated GDC-0449's antitumoral activity.[199,200] A case of a 26-year-old patient with the diagnosis of cerebellar medulloblastoma resistant to multiple therapies with further systemic metastatic disease received treatment with a daily oral dose of 540mg of GDC-0449 for several months.[201] Although the response (tumor regression) was impressive and fast, it was transient and incomplete, and the patient died afterwards. A Phase 1, multicenter, open-label study was conducted to evaluate safety and tolerability (Stage 1) and pharmacokinetics (Stage 2) of GDC-0449 in patients with metastatic or locally advanced BCC who have failed standard therapy.[199] A total of 33 patients were enrolled: 18 with metastatic tumors and 15 with locally advanced BCC. GDC-0449 had antitumor activity in those patients with advanced BCC. The most frequent AEs included fatigue, muscle spasm, weight loss, and hyponatremia. Current clinical Phase 2 studies are being conducted to evaluate safety and efficacy of GDC-0449 for the treatment of BCNS and sporadic BCCs.[202,203]

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) represents a noninvasive option for the treatment of numerous, thin, nonhyperkeratotic AKs.[204,205] Aminolevulinic acid (ALA) or methylaminolevulinate (MAL) is first applied to the skin at the target area, where it is absorbed and accumulates inside dysplastic and neoplastic cells up to 10 times faster than in normal cells.[206–209] It is converted to a potent photosensitizer, protoporphyrin IX, which, when exposed to a light source, activates and leads to the formation of reactive oxygen species and destruction of dysplastic cells.[11] When undergoing PDT, patients often complain of stinging, burning, and itching at the site of treatment. Erythema, scaling, and crusting may be evident after treatment, but usually the area heals with no evidence of scarring.[5]

Field-directed therapy for the prevention of new cancer formation may be beneficial to individuals with multiple precancerous lesions. In a study by Apalla et al,[210] 45 patients previously diagnosed with NMSC, including BCCs and SCCs, on the face or scalp, with AKs located symmetrically in the same region, were treated with PDT in order to evaluate its effect on the formation of new NMSCs. Two treatment cycles of ALA-PDT, one week apart, were used on one side of the face, while placebo-PDT was applied similarly to the other side of the face. It was found that the ALA-PDT-treated areas were significantly delayed in the mean time until appearance of new lesions and had a decreased number of new lesions at three months and at 12 months of follow up compared to placebo, demonstrating the potential use of PDT for NMSC prophylaxis.

In an investigator-blind RCT,[209] 243 patients with a total of 1,403 AKs on the face and scalp received either ALA or vehicle followed by 14 to 18 hours of incubation and exposure to blue light. A significant difference in clearance rate was seen when compared to placebo at 12 weeks of follow up. A double-blind, multicenter RCT211 evaluated the use of two treatments of MAL, red light PDT, one week apart,

in 80 patients with AKs on the face or scalp. Three months post-treatment, this regimen was significantly better than placebo in clearing lesions. In a multicenter, randomized, open study, 211 patients with 413 total AKs were treated with different MAL-PDT regimens, and a single treatment of MAL-PDT was found to be as effective as two treatment sessions, one week apart for thin AKs; two treatment sessions are recommended for thicker or nonresponsive lesions.[212]

When comparing PDT to cryotherapy, results have varied, but cosmesis is found to be superior with PDT.[206,213] When compared to 5-FU for the treatment of AKs on the face or scalp of 36 patients,[214] equivalent efficacy was found; however, ALA-PDT was better tolerated than 5-FU. In 30 patients with a total of 256 AKs on the dorsal hands or forearms, two sessions of ALA-PDT, 15 days apart, were compared to imiquimod 5% cream daily, 3x/wk for four weeks, with a four-week break and another cycle if CR was not achieved. The CR rates were significantly higher for the imiquimod-treated group at one month. At six months, the CR rates were also higher, but did not reach significance. A larger number of patients preferred PDT in regard to procedure and in terms of efficacy.[215] The efficacy of two sessions of ALA-PDT one month apart followed by either imiquimod 5% cream or vehicle applied daily, 2x/wk for 16 weeks, starting at Month 2 were evaluated in a double-blind, split-face RCT.[216] Median lesion reduction at one year was significantly greater on the imiquimod-treated side of the face than on the vehicle-treated side.

PDT also represents a treatment option for superficial NMSCs. The depth of penetration makes it less successful for lesions such as nBCCs, although it still remains an option. Past studies have shown that fractionated PDT leads to higher responses than continuous illumination PDT.[8,9] When ALA-PDT with a fractionated illumination scheme was used to treat 552 lesions (430 sBCCs, 20 nBCCs, 32 BDs, 70 AKs), a CR was seen in 95 percent of lesions at an average follow up of two years with a "good" to "excellent" cosmetic outcome in 95 percent of lesions. For lesions larger than 2cm, the CR rate at two years was 89 percent (n=57).[217]

One disadvantage of PDT is the need for multiple office visits to receive the treatment. In an open pilot study, 12 patients with BD and sBCC <2cm in diameter were given two PDT treatments, one month apart, using a lightweight, organic, light-emitting diode (OLED) as a light source suitable for ambulatory PDT. At one year follow up, seven patients remained clear of lesions and all subjects scored the pain level as less than 2, using a numerical rating score of 1 to 10. Routine PDT light sources scored a 6 on the numerical rating scale. Ambulatory PDT may represent a less painful and more convenient method of treatment for NMSC patients.[218]

Currently, there is a Phase 2 trial underway to determine the safety and efficacy of ALA-PDT versus vehicle-PDT for the treatment of AKs and reduction of new NMSC of the scalp or both forearms in solid organ transplant recepient subjects on chronic immunosuppressive therapy.[219]

LASERS

Lasers induce coagulative necrosis, ablation, and hyperthermia, leading to tumor destruction. Many studies have provided evidence that lasers represent a new, effective treatment option for the management of NMSCs.[220–224] A single pass with a CO_2 laser may be used to destroy lesions in the superficial epidermis that are at risk of becoming AKs or SCCs and possibly BCCs.[225]

CO_2 laser wavelengths are absorbed by water in the epidermis, resulting in nonspecific tissue destruction. CO_2 lasers have been used to treat sBCCs and Bowen's disease. The use of superpulsed CO_2 lasers was studied in 44 patients with Bowen's disease. The total response rate was 97.7 percent, and clearance after one treatment was achieved in 86.3 percent of patients. A total of five patients achieved clearance after more than one treatment and one patient did not respond. Three patients showed evidence of recurrence at six months, four months, and at one year and five months, with an unknown evolution time. One patient had a serious AE, the formation of a keloid, after treatment. Thirteen other patients experienced minimal local AEs, such as erythema and hypo/hyperpigmentation.[226]

In a study by Moskalik et al,[227] the use of neodymium (Nd) lasers for the treatment of facial skin cancers was assessed. In 3,461 patients with 3,534 BCCs and 90 SCCs, pulsed Nd and neodymium-doped yttrium aluminium garnet (Nd:YAG) lasers were used, and patients were followed for three months to five years. Recurrences were seen in 1.8 percent of patients with BCCs treated with pulsed Nd laser, in 2.5 percent of patients with BCCs treated with Nd:YAG laser, and in 4.4 percent of patients with SCCs treated with pulsed Nd laser. Irradiation using Nd lasers represents a useful way to treat facial skin cancers.[227]

AFAMELANOTIDE (CUV1647)

UVB induces the synthesis of melanin in melanocytes through the release of a-melanocyte stimulating hormone (a-MSH) from cutaneous melanocytes and keratinocytes. This effect is produced after UVB has already caused substantial damage to keratinocytes.[228] The increase in melanin may have protective effects against UVB. Afamelanotide (CUV1647) is an analogue of a-MSH with higher potency and longer action than the naturally occurring hormone.[229] It is currently being studied to evaluate its efficacy in reducing the number of AKs and SCCs in organ transplant recipients on chronic immunosuppressive therapy.[230]

EPIDERMAL GROWTH FACTOR RECEPTOR INHIBITORS

The epidermal growth factor receptor (EGFR) gene codes for a transmembrane tyrosine kinase receptor related to cellular proliferation and epithelial development.[231] Its overexpression has been associated with non-small-cell-lung cancer, colorectal cancer, SCC of the head and neck, and SCC.[231–233] Several antitumoral agents that block this receptor that have been developed for the treatment

of those cancers and are currently in different phases of clinical evaluation for the treatment of NMSC, include gefitinib[234] and erlotinib.[235–238] In addition, anti-EGFR monoclonal antibodies have been developed and are currently in clinical trials, including cetuximab[239] and the fully human panitumumab.[240–244]

CAPECITABINE

Capecitabine is an oral prodrug of 5-FU that has demonstrated efficacy for the treatment of SCC, particularly in combination with IFN. As a monotherapy, it was administered orally at a dose of 1g/m^2 divided into two daily doses on Days 1 through 14 of a 21-day treatment cycle in 14 organ transplant recipients with recurrent SCCs and BCCs. Capecitabine significantly decreased the SCC recurrence rates in these patients.[245] Further studies are needed to determine its role in the treatment of NMSC.

CONCLUSION

The incidence and prevalence of NMSC will continue to rise due to several factors including, but not limited to, the increase in human lifespan, early detection, and awareness campaigns. Preventive measures include sun protection and consumption of low fat diets, along with retinoids and newer agents, such as perillyl alcohol, T4N5, DFMO, and DL-a tocopherol. Although surgical modalities are considered the standard of care for the treatment of NMSC, newer nonsurgical agents targeting key cellular receptors or immunological responses have considerably reduced morbidity and mortality and increased the quality of life of patients. Some of these agents, including imiquimod, IFN, and 5-FU, have consistently demonstrated substantial efficacy when used as monotherapies and in combination with surgical modalities. PDT and lasers have also shown to be effective alone or in combination with topical immunomodulators for the treatment of NMSC and premalignant lesions. Clinical trials evaluating newer treatment options, such as IM, COX-2 inhibitors, betulinic acid, cyclopamine, GDC-0449, resiquimod, afamelanotide, EGFR inhibitors, and capecitabine are obtaining promising results.

REFERENCES

1. Skin cancer. National Cancer Institute. http://www.cancer.gov/cancertopics/types/skin. Accessed on: March 4, 2010.
2. McGuire JF, Ge NN, Dyson S. Nonmelanoma skin cancer of the head and neck I: histopathology and clinical behavior. *Am J Otolaryngol*. 2009;30(2):121–133.
3. Seidler AM, Bramlette TB, Washington CV, et al. Mohs versus traditional surgical excision for facial and auricular nonmelanoma skin cancer: an analysis of cost-effectiveness. *Dermatol Surg*. 2009;35(11):1776–1187.
4. King SC, Chen S. Analyzing the cost of preventing nonmelanoma skin cancer. *J Invest Dermatol*. 2009;129(12): 2745–2746.
5. Neville JA, Welch E, Leffell DJ. Management of nonmelanoma skin cancer in 2007. *Nat Clin Pract Oncol*. 2007;4(8):462–469.
6. Ho T, Byrne PJ. Evaluation and initial management of the patient with facial skin cancer. *Facial Plast Surg Clin North Am*. 2009;17(3):301–307.
7. Ulrich M, Stockfleth E, Roewert-Huber J, et al. Noninvasive diagnostic tools for nonmelanoma skin cancer. *Br J Dermatol*. 2007;157 Suppl 2:56–58.
8. Ahlgrimm-Siess V, Hofmann-Wellenhof R, Cao T, et al. Reflectance confocal microscopy in the daily practice. *Semin Cutan Med Surg*. 2009;28(3):180–189.
9. Berman B, Viera M, Amini S, Valins W. Immune response modulators in the treatment of skin cancer. In: Rigel et al, ed. *Cancer of the Skin*. 2nd Edition. Chapter 44. In press.
10. Ge NN, McGuire JF, Dyson S, Chark D. Nonmelanoma skin cancer of the head and neck II: surgical treatment and reconstruction. *Am J Otolaryngol*. 2009;30(3):181–192.
11. Berman B, Amini S, Valins W, Block S. Pharmacotherapy of actinic keratosis. *Expert Opin Pharmacother*. 2009;10(18):3015–3031.
12. Lee DA, Miller SJ. Nonmelanoma skin cancer. *Facial Plast Surg Clin North Am*. 2009;17(3):309–324.
13. Nash JF, Tanner PR, Matts PJ. Ultraviolet A radiation: testing and labeling for sunscreen products. *Dermatol Clin*. 2006;24(1):63–74.
14. Moyal DD, Fourtanier AM. Broad-spectrum sunscreens provide better protection from solar ultraviolet-simulated radiation and natural sunlight-induced immunosuppression in human beings. *J Am Acad Dermatol*. 2008;58(5 Suppl 2):S149–S154.
15. Tuchinda C, Lim HW, Osterwalder U, Rougier A. Novel emerging sunscreen technologies. *Dermatol Clin*. 2006;24(1):105–117.
16. Moon TE, Levine N, Cartmel B, et al. Effect of retinol in preventing squamous cell skin cancer in moderate-risk subjects: a randomized, double-blind, controlled trial. Southwest Skin Cancer Prevention Study Group. *Cancer Epidemiol Biomarkers Prev*. 1997;6(11): 949–956.
17. Evans TR, Kaye SB. Retinoids: present role and future potential. *Br J Cancer*. 1999;80(1-2):1–8.
18. Lippman SM, Lotan R. Advances in the development of retinoids as chemopreventive agents. *J Nutr*. 2000;130(2S Suppl):479S–482S.
19. Niles RM. Recent advances in the use of vitamin A (retinoids) in the prevention and treatment of cancer. *Nutrition*. 2000;16(11–12): 1084–1089.
20. Nicholson RC, Mader S, Nagpal S, et al. Negative regulation of the rat stromelysin gene promoter by retinoic acid is mediated by an AP1 binding site. *EMBO J*. 1990;9(13):4443–4454.
21. Fanjul A, Dawson MI, Hobbs PD, et al. A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. *Nature*. 1994;372(6501):107–111.
22. Pentland AP, Schoggins JW, Scott GA, et al. Reduction of UV-induced skin tumors in hairless mice by selective COX-2 inhibition. *Carcinogenesis*. 1999;20(10):1939–1944.
23. Buckman SY, Gresham A, Hale P, et al. COX-2 expression is induced by UVB exposure in human skin: implications for the development of skin cancer. *Carcinogenesis*. 1998;19(5):723–729.
24. Müller-Decker K, Reinerth G, Krieg P, et al. Prostaglandin-H-synthase isozyme expression in normal and neoplastic human skin. *Int J Cancer*. 1999;82(5):648–656.
25. Fischer SM, Lo HH, Gordon GB, et al. Chemopreventive activity of celecoxib, a specific cyclooxygenase-2 inhibitor, and indomethacin against ultraviolet light-induced skin carcino-

genesis. *Mol Carcinog*. 1999;25(4):231–240.
26. Campbell RM, DiGiovanna JJ. Skin cancer chemoprevention with systemic retinoids: an adjunct in the management of selected high-risk patients. *Dermatol Ther*. 2006;19(5):306–314.
27. Otley CC, Stasko T, Tope WD, Lebwohl M. Chemoprevention of nonmelanoma skin cancer with systemic retinoids: practical dosing and management of adverse effects. *Dermatol Surg*. 2006;32(4):562–568.
28. Stratton SP, Dorr RT, Alberts DS. The state-of-the-art in chemoprevention of skin cancer. *Eur J Cancer*. 2000;36(10): 1292–1297.
29. Einspahr JG, Stratton SP, Bowden GT, Alberts DS. Chemoprevention of human skin cancer. *Crit Rev Oncol Hematol*. 2002;41(3):269–285.
30. Bollag W, Ott F. Retinoic acid: topical treatment of senile or actinic keratoses and basal cell carcinomas. *Agents Actions*. 1970;1(4):172–175.
31. Barranco VP, Olson RL, Everett MA. Response of actinic keratoses to topical vitamin A acid. *Cutis*. 1970;6:681–685.
32. Bollag W, Ott F. Vitamin A acid in benign and malignant epithelial tumours of the skin. *Acta Derm Venereol Suppl (Stockh)*. 1975;74:163–166.
33. Kurka M, Orfanos CE, Pullmann H. Vitamin A acid for the topical management of epithelial neoplasms. Combination with 5-fluorouracil. *Hautarzt*. 1978;29(6):313–318.
34. Kleinsmith DA, Thomas L. Retinoic acid in the treatment of actinic keratoses. *J Dermatol Surg Oncol*. 1988;14:103.
35. Misiewicz J, Sendagorta E, Golebiowska A, et al. Topical treatment of multiple actinic keratoses of the face with arotinoid methyl sulfone (Ro 14-9706) cream versus tretinoin cream: a double-blind, comparative study. *J Am Acad Dermatol*. 1991;24(3):448–451.
36. Alirezai M, Dupuy P, Amblard P, et al. Clinical evaluation of topical isotretinoin in the treatment of actinic keratoses. *J Am Acad Dermatol*. 1994;30(3):447–451.
37. Black HS, Herd JA, Goldberg LH, et al. Effect of a low-fat diet on the incidence of actinic keratosis. *N Engl J Med*. 1994;330(18): 1272–1275.
38. Ibiebele TI, van der Pols JC, Hughes MC, et al. Dietary fat intake and risk of skin cancer: a prospective study in Australian adults. *Int J Cancer*. 2009;125(7):1678–1684.
39. Black HS, Lenger WA, Gerguis J, Thornby JI. Relation of antioxidants and level of dietary lipid to epidermal lipid peroxidation and ultraviolet carcinogenesis. *Cancer Res*. 1985;45(12 Pt 1):6254–6259.
40. Belanger JT. Perillyl alcohol: applications in oncology. *Altern Med Rev*. 1998;3(6):448–457.
41. Crowell PL. Prevention and therapy of cancer by dietary monoterpenes. *J Nutr*. 1999;129(3):775S–778S.
42. Barthelman M, Chen W, Gensler HL, et al. Inhibitory effects of perillyl alcohol on UVB-induced murine skin cancer and AP-1 transactivation. *Cancer Res*. 1998;58(4):711–716.
43. Chaudhary SC, Alam MS, Siddiqui MS, Athar M. Perillyl alcohol attenuates Ras-ERK signaling to inhibit murine skin inflammation and tumorigenesis. *Chem Biol Interact*. 2009;179(2-3):145–153.
44. Stratton SP, Saboda KL, Myrdal PB, et al. Phase 1 study of topical perillyl alcohol cream for chemoprevention of skin cancer. *Nutr Cancer*. 2008;60(3):325–330.
45. Stratton S. Phase 2a randomized, placebo-controlled, double-blind trial of topical perillyl alcohol in sun damaged skin. ClinicalTrials.gov Identifier: NCT00608634. http://clinicaltrials.gov/ct2/show/NCT00608634. Accessed on: March 4, 2010
46. Cafardi JA, Elmets CA. T4 endonuclease V: review and application to dermatology. *Expert Opin Biol Ther*. 2008;8(6):829–838.
47. Yarosh D, Klein J, O'Connor A, et al. Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study. Xeroderma Pigmentosum Study Group. *Lancet.* 2001;357(9260):926–929.
48. Yarosh DB. Enhanced DNA repair of cyclobutane pyrimidine dimers changes thebiological response to UV-B radiation. *Mutat Res*. 2002;509(1-2):221–226.
49. Zahid S, Brownell I. Repairing DNA damage in xeroderma pigmentosum: T4N5 lotion and gene therapy. *J Drugs Dermatol*. 2008;7(4):405–408.
50. Yarosh D et al. A randomized, double-blind, multi-center clinical study to test the safety and efficacy of T4N5 liposome lotion on patients with xeroderma pigmentosum in the protection against actinic keratosis. ClinicalTrials.gov Identifier: NCT00002811. Available at: http://clinicaltrials.gov/ct2/show/NCT00002811. Accessed on: March 4, 2010
51. Elmets CA et al. A phase IIb randomized, double-blind, placebo-controlled clinical trial of topical bacteriophage T4 endonuclease V in renal allograft recipients with a history of non-melanoma skin cancer. ClinicalTrials.gov Identifier: NCT00089180. Available at: http://clinicaltrials.gov/ct2/show/NCT00089180. Accessed on: March 4, 2010
52. Fryer MJ. Evidence for the photoprotective effects of vitamin E. *Photochem Photobiol.* 1993;58(2):304–12.
53. Rhie G, Shin MH, Seo JY, et al. Aging– and photoaging-dependent changes of enzymic and nonenzymic antioxidants in the epidermis and dermis of human skin *in vivo*. *J Invest Dermatol*. 2001;117(5):1212–1217.
54. Gensler HL, Magdaleno M. Topical vitamin E inhibition of immunosuppression and tumorigenesis induced by ultraviolet irradiation. *Nutr Cancer*. 1991;15(2):97–106.
55. Foote JA Ranger-Moore JR, Einspahr JG, et al. Chemoprevention of human actinic keratoses by topical DL-α-tocopherol. *Cancer Prev Res* (Phila, Pa). 2009;2(4): 394–400.
56. McCann PP, Bitonti AJ, Pegg AE. Inhibition of polyamine metabolism and the consequent effects on cell proliferation. In: L Wattenberg, ed. *Cancer Chemoprevention*. Boca Raton, FL: CRC Press;1992:531–539.
57. Pegg AE. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res*. 1988;48(4):759–774.
58. Pegg AE, Madhubala R, Karneji T, et al. Control of ornithine decarboxylase activity in difluoromethylornithine-resistant L1210 cells by polyamines and synthetic analogues. *J Biol Chem.* 1988;263(22):11008–11014.
59. Elmets CA, Athar M. Targeting ornithine decarboxylase for the prevention of nonmelanoma skin cancer in humans. *Cancer Prev Res* (Phila, Pa). 2010;3(1):8–11.
60. Alberts DS, Dorr RT, Einspahr JG, et al. Chemoprevention of human actinic keratoses by topical 2-(Difluoromethyl)-dl-ornithine. *Cancer Epidemiol Biomarkers Prev*. 2000;9(12):1281–1286.
61. Einspahr JG, Nelson MA, Saboda K, et al. Modulation of biologic endpoints by topical difluoromethylornithine (DFMO) in subjects at high-risk for nonmelanoma skin cancer. *Clin Cancer Res*. 2002;8(1):149–155.
62. Bailey HH, Kim K, Verma AK, et al. A randomized, double-blind, placebo-controlled phase 3 skin cancer prevention study of {alpha}-difluoromethylornithine in subjects with pre-

vious history of skin cancer. *Cancer Prev Res* (Phila Pa). 2010;3(1):35–47.
63. Alberts DS. Phase IIB randomized, double-blinded, placebo controlled study to evaluate the safety and efficacy of topical difluoromethylornithine (DFMO) with and without a topical corticosteroid cream (triamcinolone 0.1%) in the therapy of actinic keratoses (AK) on the forearms. ClinicalTrials.gov Identifier: NCT00021294. http://clinicaltrials.gov/ct2/show/NCT00021294. Accessed on: March 4, 2010
64. Carbone PP. Chemoprevention of skin cancers with DFMO: a controlled, randomized clinical trial. ClinicalTrials.gov Identifier: NCT00005884. http://clinicaltrials.gov/ct2/show/NCT00005884. Accessed on: March 4, 2010
65. Edwards L, Levine N, Weidner M, et al. Effect of intralesional interferon in actinic keratoses. *Arch Derm*. 1986;122:779–782.
66. Edwards L, Levine N, Smiles KA. The effect of topical interferon alpha 2b on actinic keratoses. *J Dermatol Surg Oncol*. 1990;16(5):446–449.
67. Telfer NR, Colver GB, Bowers PW. Guidelines for the management of basal cell carcinoma. British Association of Dermatologists. *Br J Dermatol.* 1999;141(3):415–423.
68. Tucker SB, Polasek JW, Perri AJ, Goldsmith EA. Long-term follow-up of basal cell carcinomas treated with perilesional interferon alfa 2b as monotherapy. *J Am Acad Dermatol*. 2006;54(6):1033–1038.
69. Cornell RC, Greenway HT, Tucker SB, et al. Intralesional interferon therapy for basal cell carcinoma. *J Am Acad Dermatol*. 1990;23:694–700.
70. Chimenti S, Peris K, Di Cristofaro S, et al. Use of recombinant interferon alfa-2b in the treatment of basal cell carcinoma. *Dermatology*. 1995;190:214–217.
71. Greenway HT, Cornell RC, Tanner DJ, et al. Treatment of basal cell carcinoma with intralesional interferon. *J Am Acad Derm*. 1986; 15:437–443.
72. Stenquist B, Wennberg AM, Gisslen H, et al. Treatment of aggressive basal cell carcinoma with intralesional interferon: evaluation of efficacy by Mohs surgery. *J Am Acad Derm*. 1992; 27:65–69.
73. Bostanci S, Kocyigit P, Alp A, et al. Treatment of basal cell carcinoma located in the head and neck region with intralesional interferon alpha-2a: evaluation of long-term follow-up results. *Clin Drug Investig*. 2005;25(10):661–667.
74. Ikic D, Padovan I, Pipic N, et al. Interferon therapy for basal cell carcinoma and squamous cell carcinoma. *Int J Clin Pharm Ther Toxicol*. 1991;29:342–346.
75. Edwards L, Berman B, Rapini RP, et al. Treatment of cutaneous squamous cell carcinoma by intralesional interferon-alp 2b therapy. *Arch Derm*. 1992;128:1486–1489.
76. Buechner S. Intralesional interferon-alpha 2b in the treatment of basal cell carcinoma. *J Am Acad Derm*. 1991;24:731–734.
77. Anasagasti-Angulo L, Garcia-Vega Y, Barcelona-Perez S, et al. Treatment of advanced, recurrent, resistant to previous treatments basal and squamous cell skin carcinomas with a synergistic formulation of interferons. Open, prospective study. *BMC Cancer*. 2009;9:262.
78. Ladoyanni E, Nambi R. Psoriasis exacerbated by interferon-alpha in a patient with chronic myeloid leukemia. *J Drugs Dermatol*. 2005;4(2):221–222.
79. Egberts F, Lischner S, Russo P, et al. Diagnostic and therapeutic procedures for management of melanoma during pregnancy: risk for the fetus? *J Dtsch Dermatol Ges*. 2006;4:717–720.
80. Berman B, Villa AM, Ramirez CC. Mechanisms of action of new treatment modalities for actinic keratosis. *J Drugs Dermatol*. 2006;5(2):167–173.
81. Chakrabarty A, Geisse JK. Medical therapies for non-melanoma skin cancer. *Clin Dermatol*. 2004;22(3):183–188.
82. Reymann F. Treatment of basal cell carcinoma of the skin with 5-fluorouracil ointment. A 10-year follow-up study. *Dermatologica*. 1979;158(5):368–372.
83. Epstein E. Fluorouracil paste treatment of thin basal cell carcinomas. *Arch Dermatol*. 1985;121(2):207–213.
84. Mohs FE, Jones DL, Bloom RF. Tendency of fluorouracil to conceal deep foci of invasive basal cell carcinoma. *Arch Dermatol*. 1978;114(7):1021–1022.
85. Bargman H, Hochman J. Topical treatment of Bowen's disease with 5-fluorouracil. *J Cutan Med Surg*. 2003;7(2):101–105.
86. Salim A, Leman JA, McColl JH, et al. Randomized comparison of photodynamic therapy with topical 5-fluorouracil in Bowen's disease. *Br J Dermatol*. 2003;148(3):539–543.
87. Perrett CM, McGregor JM, Warwick J, et al. Treatment of post-transplant premalignant skin disease: a randomized intrapatient comparative study of 5-fluorouracil cream and topical photodynamic therapy. *Br J Dermatol*. 2007;156(2):320–328.
88. Hamouda B, Jamila Z, Najet R, et al. Topical 5-fluorouracil to treat multiple or unresectable facial squamous cell carcinomas in xeroderma pigmentosum. *J Am Acad Dermatol*. 2001;44(6):1054.
89. Cartei G, Cartei F, Interlandi G, et al. Oral 5-fluorouracil in squamous cell carcinoma of the skin in the aged. *Am J Clin Oncol*. 2000;23(2):181–184.
90. Yentzer B, Hick J, Williams L, et al. Adherence to a topical regimen of 5-fluorouracil, 0.5%, cream for the treatment of actinic keratoses. *Arch Dermatol*. 2009;145(2):203–205.
91. Weiss J, Menter A, Hevia O, et al. Effective treatment of actinic keratosis with 0.5% fluorouracil cream for 1, 2, or 4 weeks. *Cutis*. 2002;70(2 Suppl):22–29.
92. Loven K, Stein L, Furst K, et al. Evaluation of the efficacy and tolerability of 0.5% fluorouracil cream and 5% fluorouracil cream applied to each side of the face in patients with actinic keratosis. *Clin Ther*. 2002;24(6):990–1000.
93. Berman B, Bienstock L, Kuritzky L, et al; Primary Care Education Consortium; Texas Academy of Family Physicians. Actinic keratoses: sequelae and treatments. Recommendations from a consensus panel. *J Fam Pract*. 2006;55(5):suppl 1–8.
94. Pearlman DL. Weekly pulse dosing: effective and comfortable topical 5-fluorouracil treatment of multiple facial actinic keratoses. *J Am Acad Dermatol*. 1991;25(4):665–667.
95. Lane DP. Cancer. p53, guardian of the genome. *Nature*. 1992;358(6381):15–16.
96. Fu W, Cockerell C. The actinic (solar) keratosis: a 21st-century perspective. *Arch Dermatol*. 2003;139(1):66–70.
97. Price NM. The treatment of actinic keratoses with a combination of 5-fluorouracil and imiquimod creams. *J Drugs Dermatol*. 2007;6(8):778–781.
98. The VA Keratinocyte Carcinoma Chemoprevention Trial (VAKCCT). ClinicalTrials.gov Identifier: NCT00847912. http://www.clinicaltrials.gov/ct2/show/NCT00847912. Accessed on: March 4, 2010
99. FDA Approval for Imiquimod. National Cancer Institute. http://www.cancer.gov/cancertopics/druginfo/fda-imiquimod Accessed on: March 4, 2010
100. Schön MP, Schön M. Imiquimod: mode of action. *Br J Dermatol*. 2007;157 Suppl 2:8–13.
101. Yamamoto Y, Uede K, Otani T, et al. Different apoptotic pat-

terns observed in tissues damaged by phenol and TCA peels. *J Dermatol Sci*. 2006; 2(suppl):75–81.
102. Stuzin JM. Phenol peeling and the history of phenol peeling. *Clin Plast Surg*. 1998;25:1–19.
103. Kaminaka C, Yamamoto Y, Yonei N, et al. Phenol peels as a novel therapeutic approach for actinic keratosis and Bowen disease: prospective pilot trial with assessment of clinical, histologic, and immunohistochemical correlations. *J Am Acad Dermatol*. 2009;60(4):615–625.
104. Imbertson LM, Beaurline JM, Couture AM, et al. Cytokine induction in hairless mouse and rat skin after topical application of the immune response modifiers imiquimod and S-28463. *J Invest Dermatol*. 1998;110:734–739.
105. Wagner TL, Ahonen CL, Couture AM, et al. Modulation of TH1 and TH2 cytokine production with the immune response modifiers, R-848 and imiquimod. *Cell Immunol*. 1999;191:10–19.
106. Hemmi H, Kaisho T, Takeuchi O, et al. Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway. *Nat Immunol*. 2002;3:196–200.
107. Clifford JL, Walch E, Yang X, et al. Suppression of type I interferon signaling proteins is an early event in squamous skin carcinogenesis. *Clin Cancer Res*. 2002;8:2067–2072.
108. Schön M, Bong AB, Drewniok C, et al. Tumor-selective induction of apoptosis and the small-molecule immune response modifier imiquimod. *J Natl Cancer Inst*. 2003;95(15):1138–1149.
109. Schön MP, Wienrich BG, Drewniok C, et al. Death receptor-independent apoptosis in malignant melanoma induced by the small-molecule immune response modifier imiquimod. *J Invest Dermatol*. 2004;122(5):1266–1276.
110. Cryns V, Yuan J. Proteases to die for. *Genes Dev*. 1998;12(11):1551–1570.
111. Tyring S, Conant M, Marini M, et al. Imiquimod: an international update on therapeutic uses in dermatology. *Int J Dermatol*. 2002;41(11):810–816.
112. Ooi T, Barnetson RS, Zhuang L, et al. Imiquimod-induced regression of actinic keratosis is associated with infiltration by T lymphocytes and dendritic cells: a randomized controlled trial. *Br J Dermatol*. 2006;154(1):72–78.
113. Stockfleth E, Meyer T, Benninghoff B, et al. A randomized, double-blind, vehicle-controlled study to assess 5% imiquimod cream for the treatment of multiple actinic keratoses. *Arch Dermatol*. 2002;138(11):1498–1502.
114. Salasche SJ, Levine N, Morrison L. Cycle therapy of actinic keratoses of the face and scalp with 5% topical imiquimod cream: an open-label trial. *J Am Acad Dermatol*. 2002;47(4):571–577.
115. Lebwohl M, Dinehart S, Whiting D, et al. Imiquimod 5% cream for the treatment of actinic keratosis: results from two phase III, randomized, double-blind, parallel group, vehicle-controlled trials. *J Am Acad Dermatol*. 2004;50(5):714–721.
116. Szeimies RM, Gerritsen MJ, Gupta G, et al. Imiquimod 5% cream for the treatment of actinic keratosis: results from a phase III, randomized, double-blind, vehicle-controlled, clinical trial with histology. *J Am Acad Dermatol*. 2004;51(4):547–555.
117. Gupta AK, Davey V, Mcphail H. Evaluation of the effectiveness of imiquimod and 5-fluorouracil for the treatment of actinic keratosis: Critical review and meta-analysis of efficacy studies. *J Cutan Med Surg*. 2005;9(5):209–214.
118. Stockfleth E, Sterry W, Carey-Yard M, et al. Multicentre, open-label study using imiquimod 5% cream in one or two 4-week courses of treatment for multiple actinic keratoses on the head. *Br J Dermatol*. 2007;157 Suppl 2:41–46.
119. Alomar A, Bichel J, McRae S. Vehicle-controlled, randomized, double-blind study to assess safety and efficacy of imiquimod 5% cream applied once daily 3 days per week in one or two courses of treatment of actinic keratoses on the head. *Br J Dermatol*. 2007;157(1): 133–141.
120. Korman N, Moy R, Ling M, et al. Dosing with 5% imiquimod cream 3 times per week for the treatment of actinic keratosis: results of two phase 3, randomized, double-blind, parallel-group, vehicle-controlled trials. *Arch Dermatol*. 2005;141(4):467–473.
121. Zeichner JA, Stern DW, Uliasz A, et al. Placebo-controlled, double-blind, randomized pilot study of imiquimod 5% cream applied once per week for 6 months for the treatment of actinic keratoses. *J Am Acad Dermatol*. 2009;60(1):59–62.
122. Tan JK, Thomas DR, Poulin Y, et al. Efficacy of imiquimod as an adjunct to cryotherapy for actinic keratoses. *J Cutan Med Surg*. 2007;11(6):195–201.
123. Ulrich C, Bichel J, Euvrard S, et al. Topical immunomodulation under systemic immunosuppression: results of a multicentre, randomized, placebo-controlled safety and efficacy study of imiquimod 5% cream for the treatment of actinic keratoses in kidney, heart, and liver transplant patients. *Br J Dermatol*. 2007;157 Suppl 2:25–31.
124. Swanson N, Rosen T Berman B, et al. Optimizing imiquimod for treating actinic keratosis of the full face or balding scalp: imiquimod 2.5% and 3.75% applied daily for two 2-week or 3-week cycles. Presented at: The 12th World Congress on Cancers of the Skin; May 3–6, 2009; Tel-Aviv, Israel.
125. Swanson N, Hanke CW, Berman B, et al. Twelve month sustained clearance of actinic keratoses of the full face or balding scalp after imiquimod 2.5% and 3.75% applied daily for two 2-week or 3-week cycles. Presented at: The Hawaii Dermatology Seminar; February 2010; Waikaloa, Hawaii.
126. Patel GK, Goodwin R, Chawla M, et al. Imiquimod 5% cream monotherapy for cutaneous squamous cell carcinoma in situ (Bowen's disease): a randomized, double-blind, placebo-controlled trial. *J Am Acad Dermatol*. 2006;54(6):1025–1032.
127. Rosen T, Harting M, Gibson M. Treatment of Bowen's disease with topical 5% imiquimod cream: retrospective study. *Dermatol Surg*. 2007;33(4):427–431.
128. Peris K, Micantonio T, Fargnoli MC, et al. Imiquimod 5% cream in the treatment of Bowen's disease and invasive squamous cell carcinoma. *J Am Acad Dermatol*. 2006;55(2): 324–327.
129. Schroeder TL, Sengelmann RD. Squamous cell carcinoma *in situ* of the penis successfully treated with imiquimod 5% cream. *J Am Acad Dermatol*. 2002;46(4):545–548.
130. Orengo I, Rosen T, Guill CK. Treatment of squamous cell carcinoma *in situ* of the penis with 5% imiquimod cream: a case report. *J Am Acad Dermatol*. 2002;47(4 Suppl):S225–S228.
131. van Egmond S, Hoedemaker C, Sinclair R. Successful treatment of perianal Bowen's disease with imiquimod. I*nt J Dermatol*. 2007;46(3):318–319.
132. Thai KE, Sinclair RD. Treatment of Bowen's disease of the penis with imiquimod. *J Am Acad Dermatol*. 2002;46(3): 470–471.
133. Taliaferro SJ, Cohen GF. Bowen's disease of the penis treated with topical imiquimod 5% cream. *J Drugs Dermatol*. 2008;7(5):483–485.
134. Pehoushek J, Smith KJ. Imiquimod and 5% fluorouracil therapy for anal and perianal squamous cell carcinoma *in situ* in an HIV-1-positive man. *Arch Dermatol*. 2001;137(1):14–16.
135. Brannan PA, Anderson HK, Kersten RC, et al. Bowen disease

of the eyelid successfully treated with imiquimod. *Ophthal Plast Reconstr Surg*. 2005;21(4):321–322.
136. Kossard S. Treatment of large facial Bowen's disease: case report. *Clin Exp Dermatol*. 2003;28 Suppl 1:13–15.
137. Prabhu S, Rao R, Sripathi H, et al. Successful use of imiquimod 5% cream in Bowen's disease. *Indian J Dermatol Venereol Leprol*. 2007;73(6):423–425.
138. Smith KJ, Germain M, Skelton H. Squamous cell carcinoma *in situ* (Bowen's disease) in renal transplant patients treated with 5% imiquimod and 5% 5-fluorouracil therapy. *Dermatol Surg*. 2001;27(6):561–564.
139. Murua AA, González LC, García-Río I, et al. Coexisting perianal squamous cell carcinoma, Bowen's disease, and condylomata acuminata treated with topical imiquimod 5%. *Int J Dermatol*. 2008;47(12):1334–1336.
140. Konstantopoulou M, Lord MG, Macfarlane AW. Treatment of invasive squamous cell carcinoma with 5-percent imiquimod cream. *Dermatol Online J*. 2006;12(3):10.
141. Nouri K, O'Connell C, Rivas MP. Imiquimod for the treatment of Bowen's disease and invasive squamous cell carcinoma. *J Drugs Dermatol*. 2003;2(6):669–673.
142. Martin-Garcia RF. Imiquimod: an effective alternative for the treatment of invasive cutaneous squamous cell carcinoma. *Dermatol Surg*. 2005;31(3):371–374.
143. Oster-Schmidt C. Two cases of squamous cell carcinoma treated with topical imiquimod 5%. *J Eur Acad Dermatol Venereol*. 2004;18(1):93–95.
144. Hengge UR, Schaller J. Successful treatment of invasive squamous cell carcinoma using topical imiquimod. *Arch Dermatol*. 2004;140:404–406.
145. Flórez Á, Feal C, de la Torre C, Cruces M. Invasive squamous cell carcinoma treated with imiquimod 5% cream. *Acta Derm Venereol*. 2004;84:227–228.
146. Oster-Schmidt C, Dirschka T. Therapy of cutaneous cell carcinoma in two retirement home residents. *J Dtsch Dermatol*. 2005;3(9):705–708.
147. Geisse J, Caro I, Lindholm J, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from two phase III, randomized, vehicle-controlled studies. *J Am Acad Dermatol*. 2004;50(5):722–733.
148. Beutner KR, Geisse JK, Helman D, et al. Therapeutic response of basal cell carcinoma to the immune response modifier imiquimod 5% cream. *J Am Acad Dermatol*. 1999;41(6):1002–1007.
149. Geisse JK, Rich P, Pandya A, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: a double-blind, randomized, vehicle-controlled study. *J Am Acad Dermatol*. 2002;47(3):390–398.
150. Schulze HJ, Cribier B, Requena L, et al. Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. *Br J Dermatol*. 2005;152(5):939–947.
151. Shumack S, Robinson J, Kossard S, et al. Efficacy of topical 5% imiquimod cream for the treatment of nodular basal cell carcinoma: comparison of dosing regimens. *Arch Dermatol*. 2002;138(9): 1165–1171.
152. Sterry W, Ruzicka T, Herrera E, et al. Imiquimod 5% cream for the treatment of superficial and nodular basal cell carcinoma: randomized studies comparing low-frequency dosing with and without occlusion. *Br J Dermatol*. 2002;147(6): 1227–1236.
153. Love WE, Bernhard JD, Bordeaux JS. Topical imiquimod or fluorouracil therapy for basal and squamous cell carcinoma: a systematic review. *Arch Dermatol*. 2009;145(12):1431–1438.
154. Testerman TL, Gerster JF, Imbertson LM, et al. Cytokine induction by the immunomodulators imiquimod and S-27609. *J Leukoc Biol*. 1995;58:365–372.
155. Tomai MA, Gibson SJ, Imbertson LM, et al. Immunomodulating and antiviral activities of the imidazoquinoline S-28463. *Antiviral Res*. 1995;28:253–264.
156. Jones T. Resiquimod 3M. *Curr Opin Investig Drugs*. 2003;4:214–218.
157. Szeimies RM, Bichel J, Ortonne JP, et al. A phase II dose-ranging study of topical resiquimod to treat actinic keratosis. *Br J Dermatol*. 2008;159(1):205–210.
158. Ogbourne SM, Suhrbier A, Jones B, et al. Antitumor activity of 3-ingenyl angelate: plasma membrane and mitochondrial disruption and necrotic cell death. *Cancer Res*. 2004;64(8):2833–2839.
159. Siller G, Gebauer K, Welburn P, et al. PEP005 (ingenol mebutate) gel, a novel agent for the treatment of actinic keratosis: results of a randomized, double-blind, vehicle-controlled, multicentre, phase IIa study. *Australas J Dermatol*. 2009;50(1):16–22.
160. Weedon D, Chick J. Home treatment of basal cell carcinoma. *Med J Aust*. 1976;1:928.
161. Green AC, Beardmore GL. Home treatment of skin cancer and solar keratoses. *Australas J Dermatol*. 1988;29:127–130.
162. Ivanov VN, Bhoumik A, Ronai Z. Death receptors and melanoma resistance to apoptosis. *Oncogene*. 2003;22:3152–3161.
163. Johnstone RW, Ruefli AA, Lowe SW. Apoptosis: a link between cancer genetics and chemotherapy. *Cell*. 2002;108:153–164.
164. Challacombe JM, Suhrbier A, Parsons PG, et al. Neutrophils are a key component of the antitumor efficacy of topical chemotherapy with ingenol-3-angelate. *J Immunol*. 2006;177(11):8123–8132.
165. Anderson L, Schmieder GJ, Werschler WP, et al. Randomized, double-blind, double-dummy, vehicle-controlled study of ingenol mebutate gel 0.025% and 0.05% for actinic keratosis. *J Am Acad Dermatol*. 2009;60(6):934–943.
166. A multi-center, randomized, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of PEP005 (Ingenol Mebutate) gel, 0.015% in patients with actinic keratoses on the head (face or scalp) (region-IIa). ClinicalTrials.gov Identifier: NCT00916006. http://www.clinicaltrials.gov/ct2/show/NCT00916006. Accessed on: March 4, 2010.
167. A multi-center, randomized, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of PEP005 (Ingenol Mebutate) gel, 0.015% in patients with actinic keratoses on the head (face or scalp) (region-IIb). ClinicalTrials.gov Identifier: NCT00915551. http://www.clinicaltrials.gov/ct2/show/NCT00915551. Accessed on: March 4, 2010.
168. A multi-center, open-label study to evaluate the safety and efficacy of PEP005 (Ingenol Mebutate) gel, 0.05% in patients with actinic keratoses on non-head locations (trunk and extremities). ClinicalTrials.gov Identifier: NCT00917306. http://www.clinicaltrials.gov/ct2/show/NCT00917306. Accessed on: March 4, 2010.
169. A multi-center, randomized, parallel group, double-blind, vehicle-controlled study to evaluate the efficacy and safety of PEP005 (Ingenol Mebutate) gel, 0.05%, in patients with actinic keratoses on n-head locations (region-Ib). ClinicalTrials.gov Identifier: NCT00942604. http:// www.clinicaltrials.gov/ct2/show/NCT00942604. Accessed on: March 4,

2010.
170. Marks F, Fürstenberger G, Müller-Decker K. Metabolic targets of cancer chemoprevention: interruption of tumor development by inhibitors of arachidonic acid metabolism. *Recent Results Cancer Res*. 1999;151:45–67.
171. Fosslien E. Molecular pathology of cyclooxygenase-2 in neoplasia. *Ann Clin Lab Sci*. 2000;30(1):3–21.
172. Kundu N, Smyth MJ, Samsel L, Fulton AM. Cyclooxygenase inhibitors block cell growth, increase ceramide and inhibit cell cycle. *Breast Cancer Res Treat*. 2002;76(1):57–64.
173. An KP, Athar M, Tang X, et al. Cyclooxygenase-2 expression in murine and human nonmelanoma skin cancers: implications for therapeutic approaches. *Photochem Photobiol*. 2002;76(1):73–80.
174. Rivers JK, McLean DI. An open study to assess the efficacy and safety of topical 3% diclofenac in a 2.5% hyaluronic acid gel for the treatment of actinic keratoses. *Arch Dermatol*. 1997;133(10):1239–1242.
175. Wolf JE Jr, Taylor JR, Tschen E, Kang S. Topical 3.0% diclofenac in 2.5% hyaluronan gel in the treatment of actinic keratoses. *Int J Dermatol*. 2001;40(11):709–713.
176. McEwan LE, Smith JG. Topical diclofenac/hyaluronic acid gel in the treatment of solar keratoses. *Australas J Dermatol*. 1997;38(4):187–189.
177. Smith SR, Morhenn VB, Piacquadio DJ. Bilateral comparison of the efficacy and tolerability of 3% diclofenac sodium gel and 5% 5-fluorouracil cream in the treatment of actinic keratoses of the face and scalp. *J Drugs Dermatol*. 2006;5(2):156–159.
178. Kose O, Koc E, Erbil AH, et al. Comparison of the efficacy and tolerability of 3% diclofenac sodium gel and 5% imiquimod cream in the treatment of actinic keratosis. *J Dermatolog Treat*. 2008;19(3):159–163.
179. Rivers JK. Topical 3% diclofenac in 2.5% hyaluronan gel for the treatment ofactinic keratoses. *Skin Therapy Lett*. 2004;9(1):1–3.
180. Berlin JM, Rigel DS. Diclofenac sodium 3% gel in the treatment of actinic keratoses postcryosurgery. *J Drugs Dermatol*. 2008;7(7):669–673.
181. Rivers JK, Arlette J, Shear N, et al. Topical treatment of actinic keratoses with 3.0% diclofenac in 2.5% hyaluronan gel. *Br J Dermatol*. 2002;146(1):94–100.
182. Clouser MC, Roe DJ, Foote JA, et al. Effect of non-steroidal anti-inflammatory drugs on non-melanoma skin cancer incidence in the SKICAP-AK trial. *Pharmacoepidemiol Drug Saf*. 2009;18(4):276–283.
183. Dawe SA, Salisbury JR, Higgins E. Two cases of Bowen's disease successfully treated topically with 3% diclofenac in 2.5% hyaluronan gel. *Clin Exp Dermatol*. 2005;30(6):712–713.
184. Patel MJ, Stockfleth E. Does progression from actinic keratosis and Bowen's disease end with treatment: diclofenac 3% gel, an old drug in a new environment? *Br J Dermatol*. 2007;156 Suppl 3:53–56.
185. Neubert T, Lehmann P. Bowen's disease—a review of newer treatment options. *Ther Clin Risk Manag*. 2008;4(5):1085–1095.
186. Kismet K, Akay MT, Abbasoglu O, Ercan A. Celecoxib: a potent cyclooxygenase-2 inhibitor in cancer prevention. *Cancer Detect Prev*. 2004;28(2):127–142.
187. A phase II/III randomized, double-blind, placebo-controlled clinical trial of celecoxib in subjects with actinic keratoses. ClinicalTrials.gov Identifier: NCT00027976. http://clinicaltrials.gov/ct2/show/NCT00027976. Accessed on: March 4, 2010.
188. A phase II randomized, double-blind, placebo-controlled clinical trial of Celecoxib in subjects with basal cell nevus syndrome. ClinicalTrials.gov Identifier: NCT00023621. http://clinicaltrials.gov/ct2/show/NCT00023621. Accessed on: March 4, 2010.
189. Jäger S, Laszczyk MN, Scheffler A. A preliminary pharmacokinetic study of betulin, the main pentacyclic triterpene from extract of outer bark of birch (Betulae alba cortex). *Molecules*. 2008;13(12): 3224–3235.
190. Krasutsky PA. Birch bark research and development. *Nat Prod Rep*. 2006;23(6):919–942.
191. Huyke C, Reuter J, Rödig M, et al. Treatment of actinic keratoses with a novel betulin-based oleogel. A prospective, randomized, comparative pilot study. *J Dtsch Dermatol Ges*. 2009;7(2):128–133.
192. Incardona JP, Gaffield W, Kapur RP, Roelink H. The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. *Development*. 1998;125(18): 3553–3562.
193. Miller SJ, Yu TC. Cyclopamine as a potential therapeutic agent for treatment of tumors related to hedgehog pathway mutations. *Dermatol Surg*. 2002;28(2):187.
194. McMahon AP. More surprises in the hedgehog signaling pathway. *Cell*. 2000;100(2):185–188.
195. Lupi O. Correlations between the Sonic hedgehog pathway and basal cell carcinoma. *Int J Dermatol*. 2007;46(11): 1113–1117.
196. Rudin CM. Beyond the scalpel: targeting hedgehog in skin cancer prevention. *Cancer Prev Res* (Phila Pa). 2010;3(1): 1–3.
197. Epstein EH. Basal cell carcinomas: attack of the hedgehog. *Nat Rev Cancer*. 2008;8(10):743–754.
198. Johnson RL, Rothman AL, Xie J, et al. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. *Science*. 1996;272(5268):1668–1671.
199. Von Hoff DD, LoRusso PM, Rudin CM, et al. Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. *N Engl J Med*. 2009;361(12):1164–1172.
200. Dlugosz AA, Talpaz M. Following the hedgehog to new cancer therapies. *N Engl J Med*. 2009;361(12):1202–1205.
201. Rudin CM, Hann CL, Laterra J, et al. Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. *N Engl J Med*. 2009;361(12):1173–1178.
202. A randomized, phase II multicenter trial evaluating the efficacy and safety of a systemic hedgehog pathway antagonist (GDC-0449) in patients with basal cell nevus syndrome (BCNS). ClinicalTrials.gov Identifier: NCT00957229. http://www.clinicaltrials.gov/ct2/show/ NCT00957229. Accessed on: March 4, 2010
203. An open-label, multicenter extension study of GDC-0449 (hedgehog pathway inhibitor) in patients treated with GDC-0449 in a previous Genentech-sponsored phase I or phase II cancer study. ClinicalTrials.gov Identifier: NCT00959647. http://www.clinicaltrials.gov/ct2/show/NCT00959647. Accessed on: March 4, 2010
204. Buggiani G, Troiano M, Rossi R, et al. Photodynamic therapy: off-label and alternative use in dermatological practice. *Photodiagnosis Photodyn Ther*. 2008;5(2):134–138.
205. Wang XL, Wang HW, Guo MX et al. Treatment of skin cancer and pre-cancer using topical ALA-PDT—a single hospital experience. *Photodiagnosis Photodyn Ther*. 2008;5(2): 127–133.
206. Szeimies RM, Karrer S, Radakovic-Fijan S, et al. Photodynamic therapy using topical methyl 5-aminolevulinate compared with cryotherapy for actinic keratosis: a

prospective, randomized study. *J Am Acad Dermatol*. 2002;47(2):258–262.
207. Touma D, Yaar M, Whitehead S, Konnikov N, et al. A trial of short incubation, broad-area photodynamic therapy for facial actinic keratoses and diffuse photodamage. *Arch Dermatol*. 2004;140(1):33–40.
208. Braathen LR, Szeimies RM, Basset-Seguin N, et al. Guidelines on the use of photodynamic therapy for nonmelanoma skin cancer: an international consensus. International Society for Photodynamic Therapy in Dermatology, 2005. *J Am Acad Dermatol*. 2007;56(1):125–143.
209. Piacquadio DJ, Chen DM, Farber HF, et al. Photodynamic therapy with aminolevulinic acid topical solution and visible blue light in the treatment of multiple actinic keratoses of the face and scalp: investigator-blinded, phase 3, multicenter trials. *Arch Dermatol*. 2004;140(1):41–46.
210. Apalla Z, Sotiriou E, Chovarda E, et al. Skin cancer: preventive photodynamic therapy in patients with face and scalp cancerization. A randomized placebo controlled study. *Br J Dermatol*. 2010;162(1):171–175. Epub 2009 Oct 26.
211. Pariser DM, Lowe NJ, Stewart DM, et al. Photodynamic therapy with topical methyl aminolevulinate for actinic keratosis: results of a prospective randomized multicenter trial. *J Am Acad Dermatol*. 2003;48(2):227–232.
212. Tarstedt M, Rosdahl I, Berne B, et al. A randomized multicenter study to compare two treatment regimens of topical methyl aminolevulinate (Metvix)-PDT in actinic keratosis of the face and scalp. *Acta Derm Venereol*. 2005;85(5): 424–428.
213. Freeman M, Vinciullo C, Francis D, et al. A comparison of photodynamic therapy using topical methyl aminolevulinate (Metvix) with single cycle cryotherapy in patients with actinic keratosis: a prospective, randomized study. *J Dermatolog Treat*. 2003;14(2): 99–106.
214. Smith S, Piacquadio D, Morhenn V, Atkin D, Fitzpatrick R. Short incubation PDT versus 5-FU in treating actinic keratoses. *J Drugs Dermatol*. 2003;2(6):629–635.
215. Sotiriou E, Apalla Z, Maliamani F, et al. Intraindividual, right-left comparison of topical 5-aminolevulinic acid photodynamic therapy vs. 5% imiquimod cream for actinic keratoses on the upper extremities. *J Eur Acad Dermatol Venereol*. 2009;23(9):1061–1065. Epub 2009 Apr 8.
216. Shaffelburg M. Treatment of actinic keratoses with sequential use of photodynamic therapy; and imiquimod 5% cream. *J Drugs Dermatol*. 2009;8(1):35–39.
217. de Haas ER, de Vijlder HC, Sterenborg HJ, et al. Fractionated aminolevulinic acid-photodynamic therapy provides additional evidence for the use of PDT for non-melanoma skin cancer. *J Eur Acad Dermatol Venereol*. 2008;22(4): 426–430.
218. Attili SK, Lesar A, McNeill A, et al. An open pilot study of ambulatory photodynamic therapy using a wearable low-irradiance organic light-emitting diode light source in the treatment of nonmelanoma skin cancer. *Br J Dermatol*. 2009;161(1):170–173.
219. Marcus SL. A randomized, evaluator-blinded, parallel group comparison of PDT with Levulan topical solution plus blue light versus Levulan Topical Solution Vehicle plus blue light for the treatment of AK and reduction of new NMSC in organ transplant recipients. ClinicalTrials.gov Identifier: NCT00865878.http://clinicaltrials.gov/ct2/show/NCT00865878.Accessed on: March 4, 2010.
220. Hantash BM, Stewart DB, Cooper ZA, et al. Facial resurfacing for non-melanoma skin cancer prophylaxis. *Arch Dermatol*. 2006;142(8):976–982.
221. Trimas SJ, Ellis DA, Metz RD. The carbon dioxide laser. An alternative for the treatment of actinically damaged skin. *Dermatol Surg*. 1997;23(10):885–889.
222. Massey RA, Eliezri YD. A case report of laser resurfacing as a skin cancer prophylaxis. *Dermatol Surg*. 1999;25(6): 513–516.
223. Iyer S, Friedli A, Bowes L, et al. Full face laser resurfacing: therapy and prophylaxis for actinic keratoses and non-melanoma skin cancer. *Lasers Surg Med*. 2004;34(2): 114–119.
224. Ostertag JU, Quaedvlieg PJ, Neumann MH, et al. Recurrence rates and long-term follow-up after laser resurfacing as a treatment for widespread actinic keratoses on the face and scalp. *Dermatol Surg*. 2006;32(2):261–267.
225. Halachmi S, Lapidoth M. Lasers in skin cancer prophylaxis. *Expert Rev Anticancer Ther*. 2008 ;8(11):1713–1717.
226. Covadonga Martínez-González M, del Pozo J, Paradela S, et al. Bowen's disease treated by carbon dioxide laser. A series of 44 patients. *J Dermatolog Treat*. 2008;19(5):293–299.
227. Moskalik K, Kozlov A, Demin E, et al. The efficacy of facial skin cancer treatment with high-energy pulsed neodymium and Nd:YAG lasers. *Photomed Laser Surg*. 2009;27(2): 345–349.
228. Barnetson RS, Ooi TK, Zhuang L, et al. [Nle4-D-Phe7]-alpha-melanocyte-stimulating hormone significantly increased pigmentation and decreased UV damage in fair-skinned Caucasian volunteers. *J Invest Dermatol*. 2006;126(8): 1869–1878.
229. Sawyer TK, Sanfilippo PJ, Hruby VJ, et al. 4-Norleucine, 7-D-phenylalanine-alpha-melanocyte-stimulating hormone: a highly potent alpha-melanotropin with ultralong biological activity. *Proc Natl Acad Sci U S A*. 1980;77(10):5754—578.
230. CT-2) A multicentre, randomised, double-blind, placebo controlled, phase II study to evaluate the safety and efficacy of subcutaneous bioresorbable Implants of Afamelanotide (CUV1647) for the Prophylactic Treatment of Pre-Cancerous Skin Lesions of the Head, Forearms and Hands in Immune Compromised, Organ Transplant Patients. ClinicalTrials.gov Identifier: NCT00829192. http:// www.clinicaltrials.gov/ct2/show/NCT00829192. Accessed on : March 4, 2010.
231. Toll A, Salgado R, Yébenes M, et al. Epidermal growth factor receptor gene numerical aberrations are frequent events in actinic keratoses and invasive cutaneous squamous cell carcinomas. *Exp Dermato*l. 2010;19(2):151–153.
232. Seiverling EV, Fernanadez EM, Adams D. Epidermal growth factor receptor (EGFR) inhibitor associated skin eruption. *J Drugs Dermatol*. 2006;5(4):368–369.
233. Kris MG, Natale RB, Herbst RS, et al. Efficacy of gefitinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, in symptomatic patients with non-small cell lung cancer: a randomized trial. *JAMA*. 2003 Oct 22;290(16): 2149–2158.
234. A phase II study of ZD1839 (Iressa), epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor, in treatment of recurrent or metastatic squamous cell carcinoma of the skin. ClinicalTrials.gov Identifier: NCT00054691.http://www.clinicaltrials.gov/ct2/show/NCT00054691. Accessed on: March 4, 2010.
235. A phase III trial of Erlotinib and radiotherapy in patients with stage III cutaneous squamous cell carcinomas. ClinicalTrials.gov Identifier: NCT00369512. http://www.clinicaltrials.gov/ct2/show/NCT00369512. Accessed on: March 4, 2010.

236. Multicenter randomized phase II study of Erlotinib, Cisplatin and radiotherapy versus Cisplatin and radiotherapy in patients with stage III and IV squamous cell carcinoma of the head and neck. ClinicalTrials.gov Identifier: NCT01009489. http://www.clinicaltrials.gov/ct2/show/NCT01009489. Accessed on: March 4, 2010.
237. Randomized study of Bevacizumab/Tarceva and Tarceva/Sulindac in squamous cell carcinoma of the head and neck. ClinicalTrials.gov Identifier: NCT00392665. http://www.clinicaltrials.gov/ct2/show/NCT00392665. Accessed on: March 4, 2010.
238. Phase I/II study of secondary primary tumor prevention with epidermal growth factor receptor (EGFR), tyrosine kinase inhibitor Erlotinib (OSI-774, Tarceva™), and cyclooxygenase-2 (COX-2) Inhibitor (Celecoxib) in early stage (stage I/II) squamous cell carcinoma of head and neck. ClinicalTrials.gov Identifier: NCT00400374. http://www.clinicaltrials.gov/ct2/show/NCT00400374. Accessed on: March 4, 2010.
239. Phase II study of Cetuximab as monotherapy and first line Treatment in patients with locally advanced or metastatic squamous cell carcinoma of the skin expressing EGFR. ClinicalTrials.gov Identifier: NCT00240682. http://www.clinicaltrials.gov/ct2/show/NCT00240682. Accessed on: March 4, 2010.
240. Phase 2, single-arm, open-label, multi-center trial of second-line panitumumab monotherapy in patients with metastatic or recurrent squamous cell carcinoma of the head and neck. ClinicalTrials.gov Identifier: NCT00446446. http://www.clinicaltrials.gov/ct2/show/NCT00446446. Accessed on: March 4, 2010.
241. A randomized, open-label, controlled, phase II trial of combination chemotherapy with or without panitumumab as first-line treatment of subjects with metastatic or recurrent head and neck cancer, and cross-over second-line panitumumab monotherapy of subjects who fail the combination chemotherapy. ClinicalTrials.gov Identifier: NCT00454779. http://www.clinicaltrials.gov/ct2/show/ NCT00454779. Accessed on: March 4, 2010.
242. Phase I study panitumumab plus chemoradiotherapy and induction chemotherapy in patients with locally advanced squamous cell cancer of the head and neck. ClinicalTrials.gov Identifier: NCT00513383. http://www.clinicaltrials.gov/ct2/show/NCT00513383. Accessed on: March 4, 2010.
243. A phase 2 randomized trial of radiotherapy plus panitumumab compared to chemoradiotherapy with unresected, locally advanced squamous cell carcinoma of the head and neck. ClinicalTrials.gov Identifier: NCT00547157. http://www.clinicaltrials.gov/ct2/show/ NCT00547157. Accessed on: March 4, 2010.
244. A phase II trial of postoperative radiation, cisplatin, and panitumumab in locally advanced head and neck cancer. ClinicalTrials.gov Identifier: NCT00798655. http://www.clinicaltrials.gov/ct2/show/NCT00798655. Accessed on: March 4, 2010.
245. Jirakulaporn T, Mathew J, Lindgren BR, et al. Efficacy of capecitabine in secondary prevention of skin cancer in solid organ-transplanted recipients (OTR). *J Clin Oncol*. 2009;27(15S):1519.