Cancer Letters 357 (2015) 8–42

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Skin cancer and new treatment perspectives: A review

M.C.F. Simões [a,*], J.J.S. Sousa [a], A.A.C.C. Pais [b]

[a] Faculty of Pharmacy, University of Coimbra, Azinhaga de Santa Comba, Coimbra 3000-548, Portugal
[b] Department of Chemistry, University of Coimbra, Rua Larga, Coimbra 3004-535, Portugal

ARTICLE INFO

Article history:
Received 19 September 2014
Received in revised form 31 October 2014
Accepted 4 November 2014

Keywords:
Skin cancer
Nanoparticles
Polymers
Immunotherapy
Phototherapy

ABSTRACT

Skin cancers are by far the most common malignancy of humans, particularly in the white population. The growing incidence of cutaneous malignancies has heralded the need for multiple treatment options. Although surgical modalities remain the mainstay of treatment, new research and fresh innovation are still required to reduce morbidity and mortality. Approaches for skin cancer may pass through new technological methods instead of new molecules. The first part of this paper provides a review of the state of the art regarding skin cancer disease as well as epidemiology data. Then, it describes the gold standards of the current recommended therapies worldwide and the actual needs of these patients. This is the first paper that highlights the novel and future therapeutic perspectives for the treatment of skin malignancies, new therapeutic agents and promising technological approaches, from nanotechnology to immunotherapy.

Abbreviations: 5-ALA, 5-aminolevulinic acid; 5-FU, 5-fluorouracil; APC, antigen presenting cell; BCC, basal cell carcinoma; CAP, cold atmospheric plasma; CM, cutaneous malignant melanoma; CNP, cerium oxide nanoparticle; CNT, carbon nanotube; COX-2, cyclooxygenase II; CPP, cell-penetrating peptide; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; c-KIT, tyrosine-protein kinase kit; DC, dendritic cell; ECT, electrochemotherapy; EGF, epidermal growth factor; EPT, electroporation therapy; GNP, gold nanoparticle; HA, hyaluronic acid; HIFU, high intensity focused ultrasound; Hsp, heat shock protein; IFN, interferon; IL, interleukin; ISPI, *in situ* photoimmunotherapy; MAPK, mitogen-activated protein kinase; MBCSP, magnetic-based core–shell particle; MNP, magnetic nanoparticle; MNT, modular nanotransporter; MRI, magnetic resonance imaging; MRgFUS, magnetic resonance-guided focused ultrasound; MSC, mesenchymal stem cell; MSH, melanocyte-stimulating hormone; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NGO, nanoscale graphene oxide; NIR, near-infrared; NLC, nanostructured lipid carrier; NLS, nuclear localization sequence; NmPDT, nanomaterial-mediated photodynamic therapy; NmPTT, nanomaterial-mediated photothermal therapy; NMSC, nonmelanocytic skin cancer; NP, nanoparticle; NSC, neural stem cell; nsPEF, nanosecond pulsed electric fields; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; PDT, photodynamic therapy; PEG, polyethylene glycol; PEI, polyethylenimine; PI3K, phosphatidylinositol-3-kinases; PLGA, poly(D,L lactic-co-glycolic acid); PTD, protein transduction domain; PTT, photothermal therapy; QD, quantum dot; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; SCC, squamous cell carcinoma; SCF, SKP1-CUL1-F-box protein; SiNP, silica nanoparticle; SLN, solid lipid nanoparticle; TAA, tumor-associated antigen; TGF, tumor growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; T4N5, T4 endonuclease 5; US, ultrasound.

* Corresponding author. Tel.: +351 239 488 433; fax: +351 239 488 503.
E-mail address: martacfsimoes@gmail.com (M.C.F. Simões).

http://dx.doi.org/10.1016/j.canlet.2014.11.001
0304-3835/

State of the art for skin cancer

Skin cancer

The skin is the largest organ of the body, covering approximately 16% of body mass. Skin is organized into two primary layers, epidermis and dermis, which are composed of several components, as epithelial, mesenchymal, glandular and neurovascular. Epidermis, of ectodermal derivation, is the peripheral layer of skin that contacts with the environment, working as a physiochemical barrier against environmental stressors such as pathogens, chemicals and UV. This layer acts as body's armor [1–6]. The most abundant cells of the epidermis are keratinocytes, characterized by their expression of cytokeratins and tightly connected to each other by desmossomes and thigh junctions. Dermis, originated from the mesoderm, underlies the epidermis and anchorages cutaneous structures such as hair follicles, nerves, sebaceous glands and sweat glands. Dermis also contains abundant immune cells and fibroblasts, which actively participate in many physiological responses in the skin [1,7] (Fig. 1). Epidermal keratinocytes, as a result of cell division by keratinocyte stem cells in the *stratum basale* (basal layer), undergo a programmed differentiation as they migrate outward through the surface of the skin to eventually form corneocytes, which are tightly-linked dead but undamaged cells, constituting the principal barrier of the epidermal coating [7,8].

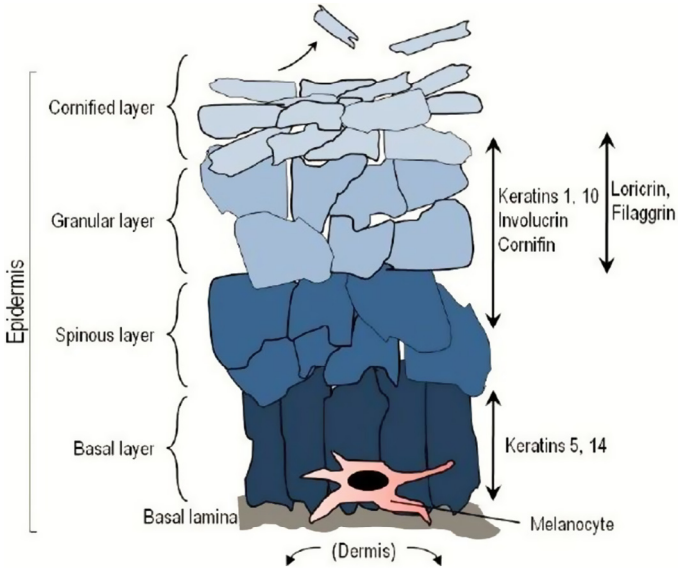

Fig. 1. Epidermal structure and keratinocyte differentiation. Keratinocytes are produced in the *stratum basale*, undergoing a programmed series of differentiation involving enucleation and accumulation of cytokeratins and tight junctions with each other. The basic coats are identified by the morphology and differentiation stage of keratinocytes as indicated through the expression of collected proteins. Reproduced from reference [1] with permission of MDPI AG. [Original citation: http://dx.doi.org/10.3390/ijms140612222, accessed in 2014.03.15].

Skin cancers are by far the most common malignancy of humans, particularly in the white population, with over a million cases detected each year [1,9,10]. Skin cancers are named according to the cell from which they arise and the clinical behavior. The three commonest types are basal cell carcinomas (BCCs), and squamous cell carcinomas (SCCs) (both also referred as nonmelanocytic skin cancer – NMSC) and cutaneous malignant melanomas (CMs) (also designed as malignant melanoma of the skin or melanoma) [1,11,12]. Identical to many other cancers which environmental etiologies (in this case UV) contributed to, skin cancer incidence increases significantly with age, presumably reflecting the long latency between carcinogen exposure and cancer establishment [1]. U.S. estimates consider that approximately 1 in 5 Americans will develop skin cancer [1,13,14]. They account for nearly 15 thousand deaths and more than three billion dollars *per year* in medical costs in the U.S.A. [1,15]. In 2010, an estimated 68,130 new cases of melanoma were diagnosed and approximately 8700 patients died of the disease in the U.S.A. However, these data for new cases, described in Table 1, may represent a substantial underestimation, because many cases of skin cancer are not reported and the incidence of these malignancies continues to increase dramatically [9,17].

Non-melanomatous skin cancers

NMSC greatly outnumber melanomas in incidence, but fortunately most are much easier to treat and have much better long-term prognosis. The two major forms, BCC and SCC, are both derived from epidermal keratinocytes. They are less deadly than melanoma mainly due to their tendency to remain confined to their primary site of disease, which makes their management much more straightforward. The devastating majority of keratinocyte malignancies progress in the areas of skin most exposed to UV, such as on the face and arms [1,12]. Additionally, these NMSCs represent the most common type of cancer in humans and they can result in significant disfigurement, leading to physical and emotional adverse consequences for the patients [12,16,19].

Reports from both the U.S.A. and Europe suggest that the incidence of NMSC is progressively and worryingly increasing [1,13]. It is estimated that 2–3 million cases of NMSCs occur worldwide each year [12,14]. The incidence varies, with very high rates in the Caucasian populations of the world [16]. The overall upward trend observed in most parts of Europe, Canada, U.S.A. and Australia shows an average increase between 3% and 8% a year [12]. The incidence of NMSCs is over 1.3 million cases each year in the U.S.A.; in fact, according to literature [19] this incidence rate is expected to double in the next 30 years. Although there is little information systematically collected and available regarding NMSC in the European population, it is known that NMSC mortality rates in Europe are higher in men and women in southern European countries (Greece, Spain, Portugal and Italy) and low in Nordic countries [20].

Approximately 30% of all newly diagnosed cancers in the U.S.A. are BCC, making it the most commonly diagnosed cancer in this country [12,22]. BCC, which accounts for 80–85% of all NMSCs, hardly metastasizes to other organs [16,24]. It is the most frequent malignancy in white people, with the worldwide incidence increasing by up to 10%, with highest rates in elderly men and increasing incidence in young women. Although mortality is low, this malignancy causes considerable morbidity and places a huge burden on healthcare systems worldwide [12,24]. SCC, which accounts for 15–20% of all NMSCs, is more likely to invade other tissues and can cause death [12,16]. In Europe, studies indicate that the rates of BCC have increased at a similar rate over the past four decades, on average increasing by 20/100,000 person-years every 15 years, a 5% increase *per year*. Concerning SCC, studies suggested an increasing

Table 1
Overview of epidemiological data of the three commonest types of skin cancer: BCC, SCC (both referred as NMSC) and CM.

Highlights of epidemiological data		
Basal cell carcinomas (BCCs)	Squamous cell carcinomas (SCCs)	Cutaneous malignant melanomas (CMs)
NMSCs represent the most common type of cancer in humans [12,16]. 2–3 million cases of NMSCs occur worldwide each year [12,14]. 1.3 million cases each year in the U.S.A. [19] Europe, Canada, U.S.A. and Australia show an average increase between 3% and 8% a year [12]. NMSC mortality rates in Europe are higher in men and women in southern European countries and low in Nordic countries [20]. Rate is expected to double in the next 30 years [19].		In 2010, 68,130 new cases of CM were diagnosed and approximately 8700 patients died in the U.S.A., despite counting less than 5% of all skin cancers in the U.S.A. [17,18]. Over 20 thousand deaths from CM were estimated in Europe in 2008 [21].
30% of all newly diagnosed cancers in the U.S.A. are BCC [12,22].	SCC is more likely to invade other tissues and can cause death than BCC [12,16].	65 thousand people a year worldwide die [12,23].
Accounts for 80–85% of all NMSCs [16,24].	Accounts for 15–20% of all NMSCs [12,16].	132 thousand new cases occur worldwide each year [12,14].
The worldwide incidence is increasing by up to 10%, with highest rates in elderly men and increasing incidence in young women [12,24].	Increasing trend, although the rate of increase varies between countries [25].	Incidence rates are at least 16 times greater in Caucasians than African Americans and 10 times greater than Hispanics [12,26].

trend, although the rate of increase varied from country to country [25].

Malignant melanoma

Malignant melanoma of the skin or CM is the deadliest form of skin cancer. Assumed to arise from epidermal melanocytes, CM is often a treatment-refractory and metastasis-prone malignancy, whose incidence has amplified gradually and significantly over the last several decades [1,12]. Melanoma frequently is detected on the trunk of men and the lower legs of women, although it can be found on the head, neck, or elsewhere [12,26]. Early-detected melanomas can be "cured" by surgical excision alone. Nonetheless, CMs are fast to invade and metastasize, being the long-term survival poor for advanced disease. A lot of progress have been made in the treatment of CM with targeted therapy [27–31] and immunotherapy [32,33], but CM is still particularly problematic to treat once it has spread outside its original site [1].

The epidemiology of CM is more documented than NMSC. It is estimated that in Europe, in 2000, 37 thousand deaths were caused by melanoma [20]. Moreover, it is estimated that 132 thousand new cases of melanoma occur worldwide each year [12,14]. Incidence rates are at least 16 times greater in Caucasians than African Americans and 10 times higher than Hispanics [12,26]. The World Health Organization (WHO) also estimates that as many as 65 thousand people a year worldwide die from malignant skin cancer, approximately 48 thousand of whom are registered [12,23]. CM represents less than 5% of all skin cancers in the U.S.A., but accounts for the vast majority of all skin cancer deaths [18,23].

CM incidence rates across Europe have changed markedly during the past five decades. Recent analyses of European data have identified up to tenfold increases in melanoma incidence in Scandinavian countries in the decades since the 1950s, with lesser but still considerable increases in western European nations [34]. Over 20 thousand deaths from CM were estimated in Europe in 2008, with Central and Eastern Europe (CEE) having the largest share (35.5%) [21]. CM mortality rates in the mid-1990s were highest in Nordic countries and lowest in southern European populations, such as Greece, Spain and Portugal [20,34]. The highest incidence rates of melanoma are reported from (essentially European migrant populations in) Australia and New Zealand (non-Maori population) where the annual incidence is more than double the highest rates recorded in Europe [20,34].

Causative factors

It is not known why skin cancer incidence has grown so dramatically over the past decades, but the reason is likely to be multifactorial, with influences covering increased UV exposure, environment, hereditary risk factors and improved surveillance and earlier recognition [35–45]. A stronger awareness of the causative factors for skin cancer is essential in the respective prevention [43]. Recent studies suggest that epidermal cells develop into malignant tumors through more than one pathway, as evidenced by differing molecular profiles, anatomical distributions and risk factor profiles for subgroups of skin malignancies. This field is moving rapidly and it is likely that in the near future, a clearer understanding of the origins of skin cancer will be delivered [34,46].

Gender differences in melanoma prognosis have been reported in several studies indicating an increased survival proportion in females compared with men [42,46,47]. The major constitutional risk factors for melanoma include fair pigmentation, poor tanning ability, multiple nevi, clinically atypical or dysplastic nevi and freckling [1,37,39,46,48].

Still, one of the greatest risk factors for the development of skin cancer is ultraviolet radiation (UV) from sun exposure [43,48]. UV has harmful effects via direct and indirect mechanisms, such as gene mutations, formation of cyclobutane pyrimidine dimers, immunosuppression and oxidative stress [46]. In fact, a fair skin complexion, which has low levels of a UV-blocking dark pigment (eumelanin) in the epidermis, is one of the major causes for the development of cutaneous melanoma. Individuals with light skin pigmentation suffer more skin injuries from UV because it is fairly easy for UV to get through the epidermis and damage cells in the profounder layers of the skin (keratinocytes and melanocytes). As a result, fair-skinned individuals are exposed to increased doses of UV and UV-induced mutations, which contribute to melanoma and other forms of skin cancer directly, accumulating over time [1,46]. In addition, ozone depletion, the level of UV light, elevation, latitude, altitude and weather conditions influence the emission of UV attaining the earth's surface [27,43,45,49,50].

Additional risk factors for skin cancer have been studied. Organ transplant receivers and HIV patients have an increased frequency of skin cancers. Some treatments, including radiation therapy, phototherapy, psoralen and long-wave ultraviolet radiation (PUVA), can also predispose to skin malignancies. Viral infections such as the human papilloma virus (HPV) can cause SCC. Patients with familial genetic patterns are vulnerable to particular types of skin cancers. Ionizing radiation, pollutants, chemicals and occupational exposures have also been linked with skin cancers. Diet, smoking, exposure to artificial UV emission (as tanning beds), skin color and aging are also attributable risk factors. Furthermore, skin malignancies have also been found in dermatoses and various types of keratoses, chronically injured wounds and scars [27,34,43,45,46,48–51]. Additional lifestyle aspects and iatrogenic exposures, such as immunosuppressive therapeutics and nonsteroidal anti-inflammatory drugs are being considered and recent studies revealed a cumulative and dose-dependent protective effect of NSAIDs. Also amiodarone may be associated with an increased risk of incident cancer, especially in males, with a dose-dependent effect [52]. Lately, a curious relationship of melanoma with Parkinson disease has been noted, with a higher than expected frequency of melanoma in patients with Parkinson's disease and *vice versa*. In contrast, NMSC is considered associated with a reduced risk of Parkinson's disease [46,51,53].

New information has been shared recently which has direct significance to the current understanding of the interplay of causal factors of this type of cancer. Genetic epidemiologists characterized two major groups of genes associated with skin cancer: *high* and *low-risk genes*. They have identified a region on the short arm of chromosome 9 associated with melanoma which was also absent in cancer cell lines. The deleted locus was later identified as harboring the *CDKN2A* gene. Genetic CDKN2A mutations have been demonstrated in 25%–50% of families with heritable melanoma [34,46]. Two other genes have been recognized within the same locus, one of which, *P14ARF*, overlaps CDKN2A and shares some coding regions, even though in a different reading frame. The other high-risk gene, *CDKN2B*, lies very close to CDKN2A and has a similar mechanism of action. The three proteins encoded by these genes ($p16^{INK4a}$, $p14^{ARF}$ and $p15^{INK4b}$) are potential tumor suppressors and each plays a role in cell-cycle arrest [54]. Another, very rare, high-penetrance familial melanoma gene, *CDK4*, encodes the primary target of $p16^{INK4a}$ [55]. Currently, it is considered that these genes may play a role in melanoma growth, although the evidence favors mutations in CDKN2A as the most predominant event. Numerous other genes have been related with an increased risk of skin cancer, as part of a cancer syndrome. As an example, patients with *XP* have one of several very particular mutations which make them incapable to repair UV-damaged DNA. These patients develop CM at more than thousand times the rate of the normal population. Cowden disease is another autosomal dominant disease which is caused by mutation in the *PTEN* gene. Affected patients develop breast and thyroid cancer mostly, but also melanoma [34,46].

Among low-risk genes, the typical candidates were genes associated with pigmentation or which encode DNA repair enzymes [34]. One of the most important alleles that influences skin cancer risk is the melanocortin-1-receptor (*MC1R*), whose role is crucial to the adaptive pigmentation (tanning) response. The MC1R gene encodes a receptor for the melanocyte-stimulating hormone (MSH), being this complex responsible for the synthesis of melanin by melanocytes. Moreover, MC1R exercises a potent influence on the melanocytes' aptitude to regenerate UV-induced DNA damage, throughout the nucleotide excision DNA-repair pathway [1,34,46]. The hypothesis of a relationship between the MC1R gene and skin cancer was tested by numerous studies and a very modest increment was found. Therefore, it is suggested that the association of MC1R with melanoma is mediated almost exclusively through the effects of this gene on skin-color and not through other pathways [34,46]. Other low-risk candidate genes that have been explored for possible associations with skin malignancies include polymorphisms in various DNA repair genes and apoptosis (from the XP gene family (*XPC* [56–59], *XPD* [59–61]) *BrCa2* [62], *TERT/CLPTM1L, TIPARP* (formerly PARP-1), *ATM, CASP8*), but also in pigmentation (*ASIP, TYR*) and nevus proliferation (*PLA2G6, MTAP, IRF4*). Recently, a rare functional nonsynonymous variant (E318K) within the *MITF* gene, that alters the gene's transcriptional activity, was recognized and related with melanoma [46]. At least one study has also described the risks related with polymorphism of the Vitamin D receptor [63].

Apoptosis is generally regarded as a critical regulatory event in the development of malignancies. Nonetheless, rather than just focusing on a single cell survival protein or proliferation marker, it is important to examine the overall balance of all life and death determinants. The focus is being confined to a few important cell survival pathways mediated by NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), Bcl-2, p53, TRAIL (TNF-related apoptosis-inducing ligand), ubiquitin ligases, COX-2 overexpression, MAPK/ERK (mitogen-activated protein kinase) pathway and the Sonic Hedgehog (Hh) signaling system [64].

Several cell survival pathways are regulated by NF-κB, which control cell growth through activation and/or deactivation of cell cycle arrest and apoptosis. However, clinical studies have shown that the net effect of disrupting NF-κB signaling in the epidermis is not only to impact terminal differentiation but also to promote the appearance of SCC. Immunohistochemical-based investigations of cell survival profiles in skin cancer have also revealed overexpression of Bcl-2 and Bcl-x (anti-apoptotic oncoproteins of the Bcl-2 protein family) in BCCs and SCC, respectively, as well as in melanoma [64,65]. As an example, Oblimersen is an 18-base antisense agent that downregulates the Bcl-2 protein. A phase I/II study of patients with advanced melanoma suggested that oblimersen can increase tumor cell apoptosis and sensitize melanoma cells to chemotherapy [66].

The p53 gene is the most commonly mutated gene identified in human cancers. It is a pro-apoptotic protein, a member of the Bcl-2 protein family, acting as a "guardian of the genome" and blocking proliferative expansion of damaged cells. UV-induced mutation in the p53 gene has been implicated as an important factor for developing skin cancer, as a reduced susceptibility to apoptosis would favor clonal expansion of mutated keratinocytes. The majority of NMSC contains p53 mutations (more than 90% for SCC and 50% for BCC), unlike melanoma, which exhibits a very low frequency of p53 mutations. Another downstream effector pathway regulated by p53 involves Fas/FasL (Fas receptor upon binding to the FasL induces apoptosis), and hence alteration in p53 may confer a death defying phenotype by deregulating the Fas/FasL apoptotic pathway as observed in several types of skin cancer including SCC and CM. Not only can skin tumor cells fail to express Fas, but they also can concomitantly express FasL, and thereby kill infiltrating antitumor T cells that express Fas [64,65,67–69]. It was also observed that premalignant keratinocytes in actinic keratoses and malignant cells in SCC reduce their expression of the death receptor for TRAIL (a ligand that induces apoptosis from TNF family) [70]. Moreover, it appears that the response of normal keratinocytes *in vivo* to UV irradiation is to reduce levels of TRAIL and its death receptors [64].

Receptor tyrosine kinases (RTKs) are widely dysregulated in cancers. In CM, alterations in the EGF receptor (EGFR), Met RTK (c-MET) and Kit receptor tyrosine kinase (c-KIT) result in changes to the associated signaling cascades [65]. Activating mutations and/or gene amplification of KIT have been found in 28% of melanomas that arise in chronically sun-damaged skin [71]. Regarding EGFR, immunohistochemically specific binding was detected in approximately 50% of BCC and about 100% of cutaneous SCC, being overexpressed in 73% of SCC cases. In SCC, this overexpression seems also to lead to the acquisition of a more aggressive phenotype [67]. The EGFR can be activated by EGF, TGF (tumor growth factor), amphiregulin and heparin-binding EGF. Following ligand binding, tyrosine-phosphorylated EGFR initiates the activation of downstream pathways, which results in cell proliferation, migration, adhesion, anti-apoptosis, angiogenesis and metastasis [72,73]. Downstream pathways include MAPK (also known as RAS/RAF/MEK/ERK signal transduction cascade) and Phosphatidylinositol-3-kinase (PI3K) signaling cascades (two major pathways that originate from the RTKs) and then, dysregulation in these signaling pathways may result in aberrant cell proliferation and/or apoptosis, and eventual tumor development [65,66,73]. Dysregulation of this pathway may also involve membrane receptors, RAF and RAS proteins and genes involved in other pathways such as PI3K, PTEN, Akt, which are also involved in regulating RAF activity. Furthermore, many studies have revealed that the RAF/MEK/ERK pathway also influences chemotherapeutic drug resistance. Many human cancers show abnormal activation of this pathway and B-RAF and N-RAS represent the most important identified mutations. N-RAS mutations are found in 33% of primary and 26% of metastatic melanoma tumors and are correlated with sun exposure and nodular lesions, resulting in its constitutive activation even in the absence of activation. In addition, melanoma cancers exhibit B-RAF mutations in up to 70% of cases that are responsible, in large part, for the constitutive hyperactivation of survival/antiapoptotic pathways such as the MAPK, NF-κB, and PI3K/AKT [65]. The K-RAS mutation occurs in low percentage (10%) of SCC, although some researchers have found a high rate of mutation in H-RAS [67]. Among the substrates of the MAPK/ERK pathway are the members of the p90 ribosomal S6 kinase (RSK). The p90 comprises a family of serine/threonine kinases that lies at the terminus of the ERK pathway. In mammals, four RSK genes (RSK1–RSK4) have been identified [74]. RSK is thought to stimulate cell cycle progression, survival, proliferation and cell transformation, through the response to many growth factors, hormones, neurotransmitters, and environmental stresses such as UV [75,76]. Recently, it has been found that RSK2 plays a key role in human skin cancer development. Activation of RSK2 by EGF and UV through the extracellular-activated protein kinase signaling pathway induces cell cycle progression, proliferation and transformation [76,77].

Therapeutically, it is important to point that Cetuximab and Panitumumab are approved anti-EGFR, being cetuximab used in advanced SCC. Panitumumab is the first anti-EGFR agent that has been approved for pretesting for the presence of K-RAS (wild-type) as a biomarker for response to therapy [73]. Imatinib and Nilotinib inhibit c-KIT. Sorafenib is an oral multi-kinase inhibitor and is one of the first clinically used. It indiscriminately binds several molecular targets including B-RAF, C-RAF and any RTKs such as the VEGF and PDGF. Sorafenib efficacy in melanoma patients has been observed when associated with chemotherapy [65]. Vemurafenib is a B-RAF inhibitor and Bevacizumab is a monoclonal immunoglobulin G antibody directed against VEGF [66]. MEK kinases are immediately downstream B-RAF effector and, to date, there are many ongoing trials

because it is considered another important point of intervention in the MAPK pathway of the B-RAF and N-RAS melanoma mutants. PI3K/AKT/mTOR is also being studied as a target for therapy because preclinical study results have demonstrated that, in the resistant cells to conventional therapy, this pathway appears constitutively activated or activated in a residual manner. Between mTOR targets there are Temsirolimus and Everolimus that are considered the most advanced agents to the PI3K pathway [65].

The relationship between COX-2 over-expression and NMSC development and progression has been very well documented. COX-2 overexpression could be a consequence of UV-B radiation exposure and the subsequent activation of different signaling pathways, like MAPK, PI3K and NF-κB. It was described that up-regulation of COX-2 expression ranges between 50 and 91% in SCC, 56 and 80% in BCC. Topical and oral COX-2 inhibitors, as celecoxib, have been used in the treatment of NMSC. Cetuximab has also been used in combination with COX-2 inhibitors for the treatment of advanced cutaneous SCC [73,78].

Ubiquitin E3 ligases are a diverse family of protein complexes that mediate the ubiquitination and subsequent proteolytic turnover of proteins in a highly specific manner. Among all E3 ubiquitin ligases, the SCF (SKP1-CUL1-F-box protein) E3 ligases are the largest family. Aberrant regulation of SCF E3 ligases is associated with various human diseases, including skin cancer, and thus are attractive therapeutic targets for skin cancer. SCF E3 ligases regulate cell proliferation, apoptosis, vasculogenesis, and tumorigenesis by targeting the degradation of many critical cellular regulators, like caspases. Overall, some components of SCF E3 ligases are overexpressed to activate SCF E3 ligases during skin carcinogenesis. As a matter of fact, MLN4924, a small molecule inhibitor of NEDD8 activating enzyme that inhibits SCF E3 ligases, is currently in clinical trials for the treatment of melanoma [79–81].

Finally, it is important to point out another signaling pathway which is relevant for the development of BCC, namely the Sonic hedgehog signal transduction pathway. The Hh-signaling comprises three main components: Ptch1, Smo and Shh. Ptch1 is a transmembrane protein that together with Smoothened forms a receptor complex for Shh. Binding of Shh to Ptch1 (physiological activation) or mutational inactivation of Ptch1 (pathological activation) suspends the inhibition of Smoothened, which results in the activation of the hedgehog pathway. Loss-of-function mutations in Patched (Ptch1 gene) are implicated in constitutive activation of the Sonic hedgehog pathway in BCC, and inherited Ptch1 mutations underlie basal cell nevus syndrome in which a typical feature is multiple BCC. Around 50–60% sporadic BCCs contain point mutations in the Ptch1 gene. Smoothened-activating mutations have also been found in 21% of sporadic BCCs. However, SCCs neither present mutations on these genes, indicating that they are not expressed similarly in the stem cells responsible for BCC and SCC development [64,69,82,83].

Thus, tumor cells in skin utilize many strategies to avoid apoptosis. It should be noted that the overexposure of the referenced molecules did not by itself result in skin cancer, but only following various oncogenic stimuli. In addition, many molecular determinants remain unknown, as do the interrelationships among various pathways [64]. Nonetheless, this is a fast moving area and it is likely that very soon genome association studies will provide new data, turning obsolete the above one. All identified associations require formal testing in properly designed validation studies [34,46].

Treatment of patients with skin cancer

The growing incidence of cutaneous malignancies has heralded the need for multiple treatment options. Choice of treatment depends on the location of tumor, progression degree, margins and dimensions [84]. Surgical modalities remain the mainstay for the treatment of skin cancer [85–87]. The treatment has 3 central goals: complete eradication of the cancer, preservation of normal function and cosmesis [84].

Gold standards for treatment

The gold standard for treatment of skin malignancies is excision biopsy. As highlighted in the literature [86], for NMSC not easily treated with elliptical excision, treatment options include curettage and diathermy, liquid nitrogen, imiquimod or 5-fluorouracil (5-FU), radiotherapy or excision and flap repair/graft. Nonetheless, it is recommended that the only therapeutic options that should be used on the face are excision or radiotherapy.

Electrodesiccation and curettage or diathermy may be advantageous for superficial BCCs and Bowen disease on the body and members. Liquid nitrogen is useful for superficial lesions (on body and members). Topical 5-FU is appropriate for the management of Bowen disease and imiquimod for the treatment of superficial BCCs, where surgery and other treatments are not suitable. These topical agents can produce an intense local inflammatory response, which may require an amendment in the posology. Although radiotherapy is usually reserved for the elderly, it can have an extraordinary cure rate and is mostly useful for margin control or for treating very large or awkwardly placed lesions. Nonetheless, where possible, lesions should be removed by excision or Moh's micrographic surgery (suitable for problematic tumors such as those poorly defined, recurrent or anatomically challenging). Last but not least, Photodynamic Therapy (PDT) is used for superficial BCCs and involves the application of a photosensitizing cream (e.g., methyl aminolevulinate) followed by exposure to an intense light. Its principle is based on optical activation of a photosensitive agent and conversion of local oxygen into harmful radicals [84,86,88,89]. Although surgery is 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, as imiquimod, interferon (IFN) and 5-FU. In addition, PDT has also been shown to be effective alone or in combination with topical immunomodulators for the treatment of NMSC and premalignant lesions [88].

The treatment of CM *in situ* has been a controversial topic in the literature for over a decade. Surgical excision with 5 mm margins is the standard of care in the U.S.A.; however, margins larger than 5 mm may be required when treating larger or indistinct lesions. For clinically ill-defined melanomas arising on UV-damaged skin, especially in regions of aesthetic concern, some forms of Mohs surgery may provide the highest cure rate and create the smallest surgical defect. Radiation is much less frequently used, being a useful second-line therapy if surgery is not indicated. Topical imiquimod therapy appears to provide relatively low cure rates for CM, and because some of these lesions contain an unrecognized invasive component, should be used with extreme caution [90]. A cooperation of specialists from the European Dermatology Forum (EDF), the European Association of Dermato-Oncology (EADO) and the European Organization of Research and Treatment of Cancer (EORTC) was formed to create recommendations on melanoma identification and treatment, based on literature reviews and clinical experience. It is stated that CMs should be excised with one to two centimeter safety margins. Sentinel lymph node dissection is usually offered as a staging procedure in patients with tumors more than 1 mm in thickness, although there is no clear survival benefit for this approach yet. IFN-α treatment may be employed in patients with stage II and III melanoma as an adjuvant therapy, in spite of being associated with substantial toxicity. In the absence of surgical options, systemic treatment is indicated. For some metastatic patients, systemic chemotherapy (dacarbazine, temozolomide, or carboplatin/

paclitaxel) continues to play an important role. B-RAF inhibitors like vemurafenib for B-RAF mutated patients, as well as the CTLA-4 antibody ipilimumab (recombinant, fully human IgG1 monoclonal antibody against cytotoxic T lymphocyte-associated antigen 4) offer fresh therapeutic prospects apart from conventional chemotherapy. Nevertheless, therapeutic decisions in stage IV patients are controversial and should be primarily made by an interdisciplinary oncology team [71,91–99].

Taking into account the growing incidence of skin malignancies and the clinical influence of a premature detection and treatment, there has been increasing attention paid to the consensus guidelines for the management of skin cancer. The guidelines established by these groups are essentially based on current evidence and expert panel consensus when evidence is lacking. However, there have been a plethora of guidelines issued from groups of different disciplines, with conflicting recommendations. Consistent recommendations are established when proven evidence (staging, excisional margins, sentinel lymph node biopsy) is available. On the other hand, controversial recommendations are set where there is a deficiency of level-one evidence in the literature (screening, adjuvant therapy, follow-up intensity). As an example, effective strategies for advanced CM remain poor, resulting in regional discrepancies in treatment recommendations [100,101]. Notable guidelines of CM and NMSC are set and updated by National Comprehensive Cancer Network (NCCN, U.S.A. [102].), Cancer Care Ontario (CCO, Canada [103]) and Canadian Medical Association (CMA, Canada [104]), European Society for Medical Oncology (ESMO, Europe [105]), Australian Cancer Network (ACN, Australia and New Zealand [106]), National Health Service (NHS, United Kingdom [107–109]) and National Institute for Health and Clinical Excellence (NICE, United Kingdom [110]). Nonetheless, literature studies [111] suggested that compliance with guidelines is suboptimal. Disparity in the treatment of skin cancer occurs despite efforts to systematize care. This may lead to erroneous staging, morbidity and reduced outcomes, besides being also cost ineffective.

In summary, the standard treatment methods are superficial ablative techniques (electrodesiccation, curettage and cryotherapy) used primarily for low-risk tumors and full-thickness techniques (Mohs micrographic surgery, excisional surgery, and radiotherapy) used to treat high-risk tumors. Additional adjuvant chemo and immunotherapy must also be considered. Deletion of the whole tumor is critical to limit and prevent tumor relapse [84]. As more information is obtained, the hope is that all patients with skin cancer will be treated according to the same worldwide set of systematic guidelines, providing patients with the best care and chance for long-term survival [100].

Demanding innovation

Along with therapy innovation, many questions arise that remain to be answered. Therefore, new research and fresh innovation are still required. The major necessity of skin cancer patients is still to reduce morbidity and mortality, mainly caused by CM. In fact, cancers characterized by local invasiveness, proximity to vital structures or metastasis are still considered practically incurable [1,91].

First of all, which patients are appropriate for a particular treatment? Clinical decision trees exist, nonetheless, given the heterogeneity of the patients as tumor location and dimension and comorbidities, each patient has to be evaluated on an individual basis, combining multidisciplinary consultation (medical oncology, radiation oncology and surgical specialties). In addition, patients have different tolerance levels for adverse effects. Second, can the response degree be increased when combined with other therapeutic options, as other chemotherapies or radiation? Can recurrence rates be reduced when associating other treatments? Clinical trials are warranted to answer these questions. Third, what is the influence of the treatment on progression free survival or overall survival? Given the disease infrequency, multicenter registry studies are needed. Fourth, what is the influence of the treatment on quality of life and psychosocial outcomes? Definitely, it is linked to the efficacy of the therapy, as well as the tolerability of the side effects, an important issue concerning this kind of treatment. Instruments based on the patient's response should be developed to better assess these endpoints, preferably in a previous manner. Fifth, which are the clinical predictors for the treatment response? Prospective studies are warranted to assess this information. Sixth, are there tumor biomarkers that assist the response prediction of the treatment? Given the strength of many malignancies, the earlier decision of which treatment to choose could be life-saving. Current studies are ongoing to assess for mutations, gene expression changes or protein levels within signaling pathways that could be associated with response or lack of response, with the ultimate objective of supporting clinical choice [112,113].

Limited progress has been made in the treatment of skin cancer over the past 4 decades, through the use of immunotherapy, chemotherapy, radiotherapy and combinations. In addition, new therapies have yielded reduced response rates and low median survival, associated with significant toxic profiles [114–122]. As an example, ipilimumab shows ability as a potentially effective treatment in metastatic melanoma. However, major limitations include an impossibility to predict the responders and serious adverse reactions, as numerous immune-mediated toxicities [114]. The upgraded efficacy of treatment arises with the potential expense of toxicity. The side effects vary, depending on the specific agents used as adjuvants, as well as on the dose used, the extent of treatment and the route of administration [123].

Recently, highly targeted therapies and immunotherapies based on pathogenesis knowledge have become available either commercially or through clinical trials [27–33,112]. In addition, diagnostic tests that preview the likelihood of response to these medicinal products are under evaluation. Localized therapy allows for increased substance accumulation in the tumor without toxicity to the rest of the body, but does not permit the targeting of metastases [124]. Nonetheless, many additional issues remain to be clarified with regard to the use, efficacy and adverse effects of skin cancer therapy. There are still gaps in the knowledge of signaling pathways involved in cancer. Other challenges include expanding the envelope of druggability for less tractable targets, understanding and overcoming drug resistance, and designing intelligent and effective drug combinations [113]. However, many treatment solutions for skin cancer may pass throughout new *technological* approaches instead of new molecules, as they may overpass concerns as molecules' systemic toxicity. With limited disease free-survival rates and drug resistance following current treatments, additional therapy options are essential [114].

Investigational approaches: new therapeutic agents

The discovery and development of molecule cancer therapeutics have been revolutionized over the last decade. Notably, a lot of progress has been made from a one-size-fits-all perspective, that emphasized cytotoxic chemotherapy, to a personalized therapeutics approach, converging on the discovery of targeted drugs that reaches the specific genetic vulnerabilities, addictions and dependencies of cancer cells [113,114].

Since UV radiation is involved in the development of skin cancer, photoprotection is essential. Further measures include identifying high-risk patients for early detection along with using substances, such as retinoids, that are effective in reducing the risk of premalignant cells turning into carcinomas [88]. Fresher therapeutics under evaluation include α-difluoromethylornithine [125–127], T4 endonuclease 5 (T4N5) [128–130], monoterpene perillyl alcohol (POH)

[131–134] and DL-α-tocopherol [135,136]. Nonetheless, α-difluoromethylornithine and DL-α-tocopherol recently failed to demonstrate any relevant protective effect [126,136].

Recent studies in the progress of novel therapies for metastatic melanoma, such as anti-CTLA-4, programmed cell death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1) pathway blocking antibodies, as well as association methodologies of cytotoxic chemotherapy and angiogenesis inhibitors, have returned encouraging results. Signaling via co-inhibitory receptors, or *checkpoint molecules*, such as CTLA-4 and PD-1, contribute to the down-modulation of $CD8^+$ and $CD4^+$ effector T-cell function, making these receptors logical targets for drugs such as ipilimumab and tremelimumab (anti-CTLA-4 monoclonal antibodies), and nivolumab and pembrolizumab (anti-PD-1 monoclonal antibodies) [137,138].

Targeted therapy refers to the treatment of cancer cells, leaving healthy cells intact. This therapy aims to eradicate all the cancer cells before killing too many healthy cells. This is problematic with ordinary chemotherapies which are toxic to both [139]. Efforts are being taken to find targeted therapies within the MAPK pathway that could be used alone or in combination with B-RAF inhibitors (vemurafenib, already approved). There has been significant successful investigation into MEK inhibition [114,140–142], as with trametinib [143] and dabrafenib [144] (both already approved both by EMA and FDA), with confident results. Another option being explored for targeted therapy in CM is the receptor tyrosine kinase, c-KIT (or CD117), as imatinib, but is still controversial [114,141,145–147]. Nonetheless, there are numerous tyrosine kinase inhibitors and trials involving other KIT-directed therapies ongoing in patients with melanomas harboring c-KIT mutations [114,141,148]. Attempts have been made to target RAS indirectly by blocking important post-translation modification. Farnesyl transferase inhibitors, such as lonafarnib, block RAS by inhibiting its farnesylation and blocking translocation of RAS to the plasma membrane [100,141,149]. A single clinical trial tried to treat CM by inhibiting RAS via farnesyl transferase suppression, but significant toxicity and lack of efficacy were obtained [114]. In addition, members of the subfamily of BH3-only proteins (Bcl-2 family), which functions as proapoptotic triggers [150], as well as RAF inhibitors (RAF kinase family is involved in cell growth) [46,151] are being investigated for CM therapy.

The current *state* of the art, where survival-prolonging treatments are missing, suggests that *personalized cancer therapy* will be the future of skin cancer treatment. As stated in the literature [113], there can be no doubt that we have moved into the era of personalized or stratified medicine which ensures that the right drug is given to the right patient at the right time, thereby ensuring fast progression through clinical trials and maximum therapeutic benefit to patients [100,113,152,153].

New technological perspectives for skin cancer treatment

In recent years it has become more and more obvious that the development of new therapies *per se* is not enough to verify progress in drug therapy. Experimental figures obtained *in vitro* are very often followed by unacceptable results *in vivo*. A hopeful strategy to overcome this problem includes the development of suitable drug carrier systems. The key advantage is that the *in vivo* fate of the drug is no longer mainly determined by the characteristics of the drug, but by the carrier system, which must permit a localized and controlled drug release [154]. Drug administration in the treatment of skin cancer has been historically achieved through topical and systemic delivery systems (intravenous, intramuscular, subcutaneous, intratumoral and gastrointestinal/oral). A lot of progress has been made regarding these approaches (through different administration routes) and several new studies are being published worldwide.

1. Nanocarriers

Colloidal carriers have attracted increasing attention during recent years. They are vesicular or particulate forms of nanometer size, required for effective carriage of loaded drug to the target. Investigational approaches include nanoparticles, nanoemulsions, nanosuspensions, liposomes, micelles, vesicles, soluble polymer–drug conjugates, and liquid crystal dispersions. The existence of diverse colloidal carrier systems raises the question as to which of them might be the most suitable for the desired purpose. Aspects to consider include drug loading capacity, possibility of drug targeting, *in vivo* target of the carrier (interaction with the biological surrounding, degradation rate, accumulation in organs), toxicity, scaling up production, storage stability and, of course, overall costs of the process [154,155].

Polymers and polymeric nanoparticles

Polymers from natural and synthetic sources have been used for carrying purposes [156,157]. These systems in the submicron dimensions comprise nanospheres, polymeric nanocapsules, polymersomes, dendrimers and water soluble polymer–drug conjugates. The main benefit of these systems is the wealth of chemical modifications. Nonetheless, problems of polymer based nanoparticles (NPs) arise from its toxicity, residual organic solvents (from the production process) and the scaling up for industrial production. Additionally, polymer hydrolysis during storage has to be taken in account and lyophilization is often required to prevent polymer degradation [154,158–161].

In this context, nanogels have to be highlighted. Nanogels are swollen nano-sized networks composed of hydrophilic or amphiphilic polymer chains, which can be non-ionic or ionic [162]. This new carrier may be used for drug delivery or to naturally absorb biological molecules, as they are able to connect through hydrogen bonds, salt bonds, or hydrophobic interactions. The loading capacity of nanogels is superior to most other drug carriers. Moreover, their affinity to aqueous environments, superior colloidal stability, reduced cell toxicity and the internal aqueous media make them the suitable candidates for uptake and delivery of proteins, peptides, and other biological compounds [147,162,163].

Several studies have been performed employing polymers and polymeric NPs in the treatment of skin malignancies. A study [164] describes the release and retention of quercetin (an herbal lipophilic drug with anticancer properties) from ethylcellulose NPs, given topically, intended for skin cancer; the authors concluded that the drug being lipophilic could be engaged in the skin for longer periods, reducing the dose and frequency of drug administration [164]. Genistein loaded-poly(DL-lactide) nanocapsules were also studied, with increased and promising skin penetration, as well as curcumin loaded chitin nanogels [165], proving specific advantages in the treatment of CM with effective transdermal penetration [166]. Poly(D,L lactic-co-glycolic acid) (PLGA) based NPs are being studied for topical delivery of Protoporphyrin IX in PDT [167], as well as chitosan NPs containing 5-aminolevulinic acid (5-ALA) [168], as described in Table 2. 5-FU-loaded chitosan micro and nanogels were also studied in 2013 [170,171]. In addition, several commercial anticancer drugs have also been successfully associated with dendrimers such as poly(amidoamine), either through physical interactions or chemical bonding [160]. Pegylated IFN (PEG-IFN), already available on the pharmaceutical market, is a form of recombinant human IFN that has been chemically modified by the covalent attachment of a branched metoxpolyethylene glycol moiety, with great results in the treatment of skin malignancies [271,272].

Liposomes

Liposomes are globular vesicles composed of one or more phospholipid bilayers. Hydrophilic substances are solubilized in the inner

aqueous core and lipophilic drugs may be incorporated into the lipid bilayers [273,274]. Drug release, biodistribution and *in vivo* stability depend on particle size, surface hydrophobicity, charge and membrane fluidity [275]. It is possible to avoid liposomes' fast reticuloendothelial uptake through the incorporation of natural compounds (as gangliosides), by the use of chemically modified polyethylene glycols (PEGs) or by the incorporation of specific antibodies. These are called *stealth liposomes* and effectively allow drug targeting [274,276]. Liposome based drug carriers also permit the intravenous injection of lipophilic drugs with very low water solubility, as amphotericin B (AmBisome®) [277]. The toxicity of the liposome system is 1/10 compared to the micelle-based amphotericin formulation. The main drawback of this system is related with stability difficulties that might lead to liposome aggregation and degradation during storage, which compromise its performance [154].

Examples, besides liposomal amphotericin B, are the encapsulation of the UV-DNA repair enzyme T4N5 for topical application in order to prevent skin cancer [174,175], aloe-emodin liposomes for the treatment of NMSC [176] or ultra-deformable liposomes containing bleomycin (Bleosome®) as SCC therapy [177]. The major evidence of medical utility and efficiency of this technique is the marketed liposomal doxorubicin (Caelyx®) [178], widely used in clinical practice.

In addition, liposomes delivering DNA, proteins, siRNA, asODNs and radioactive elements are promising new nanotechnology based therapies. For CM, and by means of liposomes, studies have been performed with Allovectin-7, Mart-1 mRNA, BAX mRNA, shRNA and siRNA (targeting PI3K/Akt and MAPK pathways) [124]. An interesting study [179] demonstrated that a c-Myc siRNA delivered by the liposomes effectively suppressed the production of c-Myc in the tumor and inhibited tumor progress in mouse models [179,278,279].

Nanosuspensions and nanoemulsions

Nanosuspensions are colloidal particles composed of only a drug and an emulsifier. Nanoemulsions (composed of oil-in-water (O/W) or water-in-oil (W/O)) are lipid droplets with a drug and an emulsifier. Fatty oils or middle chain triglycerides are used for the lipid phase, which amounts to typically 10–20% of the emulsion. Systems based on nanosuspensions/nanoemlusions are employed as drug carriers for lipophilic drugs and several formulations are commercialized so far. In fact, compared with solubilization-based formulations of the same drug, a decrease of side effects was found using these systems [274,280]. Nonetheless, the possibility of controlled drug release is limited due to the small dimension and the liquid state of the carrier. Therefore, for the majority of drugs, a quick release of the drug is observed [281]. Advantages of nanoemulsions comprise safety and an elevated content of the lipid phase, as well as the possibility of industrial scale production (through high pressure homogenization technique) [154].

Recently, new developments in PDT have been observed: skin-rejuvenating effects were found, new photosensitizers (as self-adhesive 5-ALA patch and nanoemulsion formulation of 5-ALA), pretreatments to enhance penetration of the photosensitizer and new formulations of photosensitizers, mainly through nanoemulsions, intend to intensify PDT [181,282]. In addition, nanoemulsions of foscan, a second-generation photosensitizer drug widely used in PDT, were also studied, showing promising results [182].

Lipid nanoparticles

Lipid NPs are pioneering carrier systems technologically developed as an alternative to traditional vehicles such as polymeric NPs, liposomes and emulsions. These traditional vehicles revealed relevant advantages such as site-specific targeting and modified release of the actives. Nonetheless, no irrelevant problems arose, such as cytotoxicity of the polymers and a complex scaling up process [155,283,284]. A lipid nanoparticle is described as a solid lipophilic matrix in which active substances can be incorporated. Dimensions are mostly between 150 and 300 nm, but smaller sizes, as <100 nm, or larger sizes, up to 1000 nm, can be obtained and employed for special needs [285]. They can be derived from oil-in-water nanoemulsions, where the liquid lipid of the oil droplets is replaced by a solid lipid, i.e. solid at body temperature. Therefore, lipid NPs remain solid after administration. This means that they may provide a matrix for modified release of the active substances. At the same time, chemically labile active molecules can be protected by the matrix. Two generations of lipid nanoparticles are distinguished: solid lipid nanoparticles (SLNs – first generation) and nanostructured lipid carriers (NLCs – second generation). SLNs are made from solid lipid only, but the NLCs form a blend of solid and liquid lipids, still the blend being solid at body temperature [284–288] (Fig. 2).

The main advantages comparing with other colloidal systems are high biocompatibility, good physical stability, possibility of modified release of drugs (achieved through adhesiveness and stickiness to the mucosa, in addition to the prolonged release from the nanoparticle), easy large scale production and economical raw materials. NLC is described as an unusual structure for improved drug accommodation in order to increase the payload and prevent drug expulsion during storage. Therefore, NLCs associate modified drug release features with advantages over SLN. NLCs have so far been studied for topical use, but they offer all the advantages and production aims of SLN [142,285–287,289,290].

Consequently, SLN and NLC contribute to the progress of a fresh and harmless strategy for skin drug delivery. These systems can be formulated to reach the desired release and to control important factors such as occlusion effect, skin hydration and percutaneous absorption of loaded drugs (Fig. 3) [142,154,283,286,291]. These new technological approaches have been shown to improve the efficacy and residence time of the cytotoxic drugs with concomitant reduction in side-effects. The salient characteristics of lipid NPs which make them the right carrier for antineoplastic agents are their ability to encapsulate drugs of different physiochemical properties, to improve drug stability, to reduce *in vitro* and *in vivo* toxicity and to enhance drug efficacy and pharmacokinetics [292,293].

An elevated loading capacity was not possible yet with lipid NPs. As a solution, LDC (lipid–drug conjugates) nanoparticles were developed. The aim of this technique is to transform the hydrophilic drug into a more lipophilic and insoluble molecule through conjugation with a lipidic structure. This conjugation may be performed by covalent linkage or simply by formation of a salt with a fatty acid. Taking into account the molecular weight of the two parts of the conjugate molecule, a drug loading of approximately 30–50% is attainable [287].

Several therapies have been studied concerning these new approaches. Specifically, studies have been performed with docetaxel [183] and topotecano [184], which have demonstrated efficient incorporation into NLC, entrapment efficacy and a sustained and continuous release pattern. In addition, idarubicin [185], doxorubicin [185,186], 5-FU [293], camptothecin [188], daunorubicin [187] and etoposide [189] have also been studied, with confident results [284]. SLNs were used as a carrier for resveratrol, a promising chemopreventive drug: the cytostatic effect of the conjugate was much more evident than that of resveratrol in solution [191]. Nitrosyl ruthenium complex-loaded lipid carriers were also developed and characterized to further explore its topical administration for skin cancer treatment [192]. In addition, recently, lipid NPs modified with cell-penetrating peptides (CPPs) were prepared for the delivery of siRNA into cells, demonstrating to improve the stability in serum and enhancing gene silencing [215]. Lipid-enveloped polymeric NPs for melanoma immunotherapy were also developed [193], as well as hyaluronan-coated lipid-based NPs loaded

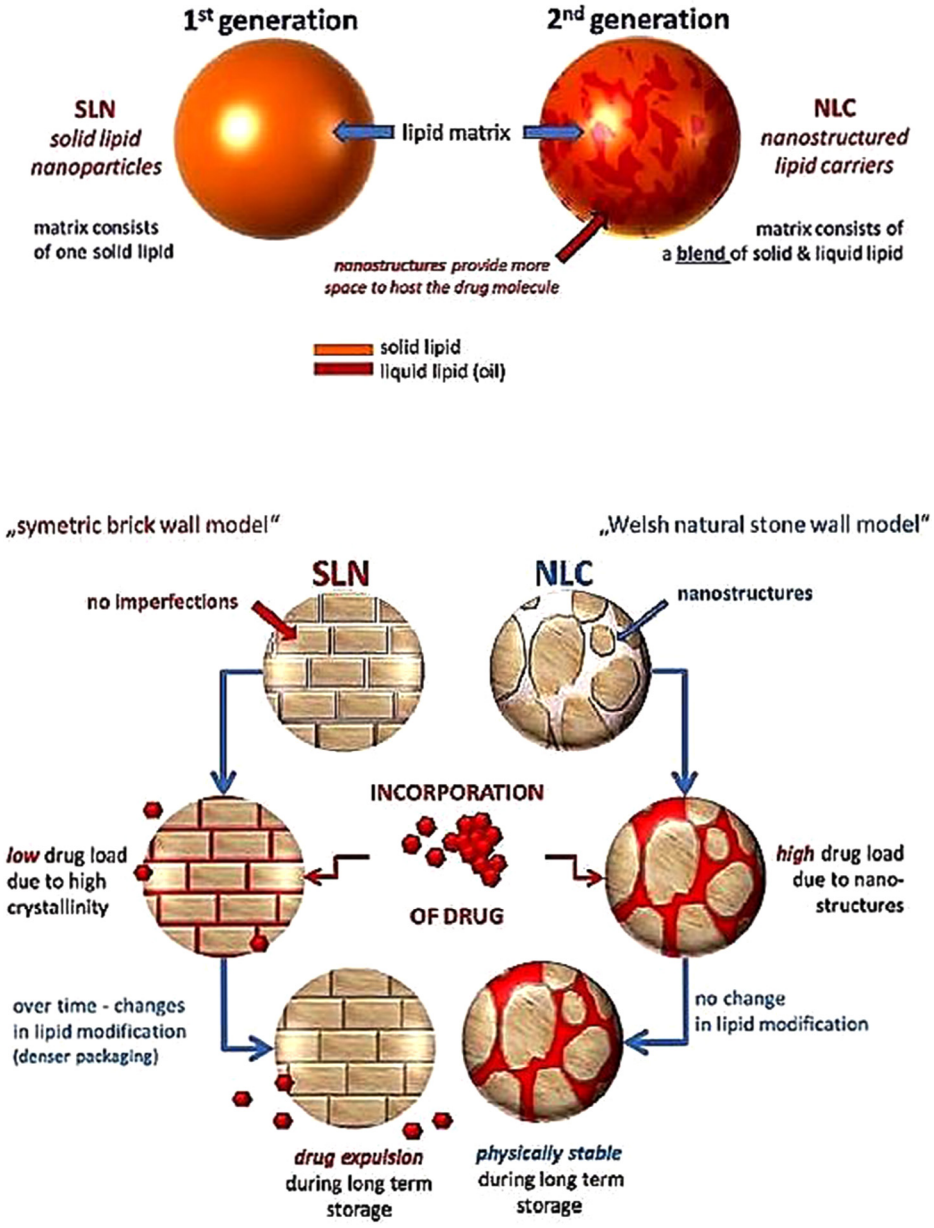

Fig. 2. Comparison between SLN and NLC structures. Reproduced from reference [285] with permission of Bentham Science Publishers. [Original citation: http://dx.doi.org/10.2174/157016311796799062, accessed in 2014.04.05].

with methotrexate, improving affinity toward B16F10 murine CM cells [190].

Carbon-based nanoparticles

Carbon-based materials like graphite, fullerenes, diamond, nanowires, nanotubes, nanoribbons and graphene have been used for various applications in optoelectronics, tissue and biomedical engineering, sensors, medical implants and medical devices [294]. Nanowires are a nanostructure characterized by cylindrical cross-sections of less than 100 nm but can be hundreds of microns long, and includes the well-described carbon nanotubes (CNTs). Several studies have been performed with CNTs (long carbon tubes that can be single or multi-walled and have the ability to act as biopersistent fibers [295]) but also with fullerenes (1-nm scale carbon spheres of 60 carbon atoms) [295].

One of the key benefits of CNTs is their aptitude to deliver active substances directly in cancer cells: there have been recently several studies *in vitro* and *in vivo* using antibody-functionalized CNTs loaded with antineoplastic agents. Another clinical application of CNTs is the ability to be used as an intravenous injection. However, one of the issues with injecting CNTs is the risk of blood vessel obstruction because of the size of this complex. Substances can be loaded inside the CNT or attached to the outer surface via functional groups, through either covalent or noncovalent bonding, including hydrophobic, π–π stacking, and electrostatic interactions [296]. *In vitro* studies have already been performed with gemcitabine, carboplatine and with zinc-phthalocyanine and zinc oxide (nanowires for PDT) [195,297–299]. Although drug delivery is the core application of CNTs for the treatment of cancer, it has also been found that gene delivery is also possible. CNT seems to represent a good nonviral carrier because it crosses the cell membrane by an endocytosis process, being transferred without any degradation, when functionalization of CNTs is used [296], as exemplified by a study [300] with siRNA. Concerning fullerenes, although they are hydrophobic, they may be

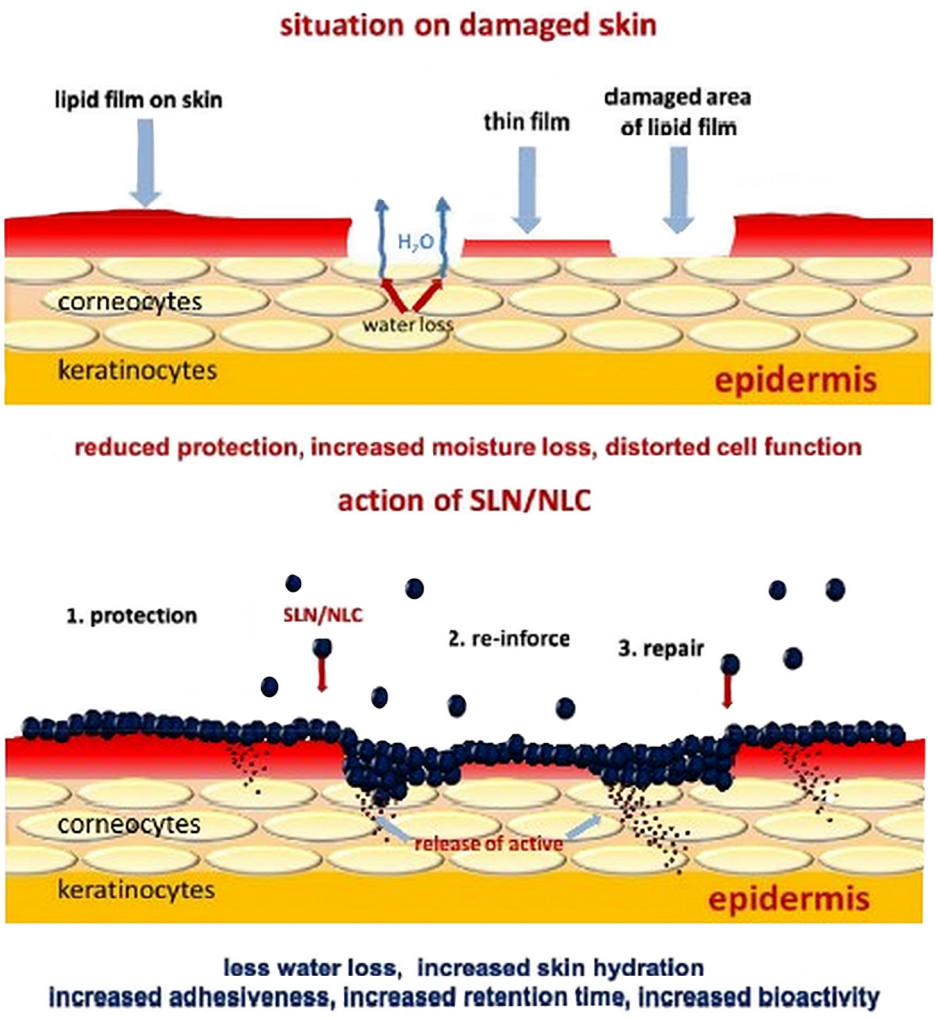

Fig. 3. Action of lipid nanoparticles (SLN/NLC) on damaged skin. Reproduced from reference [285] with permission of Bentham Science Publishers. [Original citation: http://dx.doi.org/10.2174/157016311796799062, accessed in 2014.04.05].

functionalized by hydrophilic moieties, becoming water-soluble and capable of carrying genes, proteins and other biomolecules for delivery purposes. Their small, spherical and hollow characteristics provide several therapeutic prospects. Even though fullerenes have been studied for topical use (as photosensitizers for PDT), a mass of new opportunities for their application in the future might be expected [301,302]. Nonetheless, CNTs and fullerenes still need further investigation for application in skin treatments [301].

Graphene is an important element of these carbon family materials due to its distinctive characteristics: each carbon atom is attached to other carbon atoms in the same plane with a strong bond. In addition, the interlayer binding by weak van der Waals forces turns it into a soft material, contrasting to diamond. As stated in the literature [294], the strong carbon–carbon bonding in the plane, aromatic structure, presence of free π electrons and reactive sites for surface reactions make graphene a unique material with exceptional mechanical, physicochemical, thermal, electronic, optical and biomedical properties, being considered as the skinniest and most resistant monolayer accomplished of free existence. High specific surface area, π–π stacking and electrostatic or hydrophobic interactions of graphene can be studied to attain high drug loading of poorly soluble drugs without compromising therapeutic efficacy. Use of functionalized graphene to generate drug release with external stimuli (such as immune stimuli, magnetic field, pH, or infrared radiations) is a fast developing issue as a novel delivery method. Likewise, skill to trigger endosomal escape of loaded molecule into cytosol has potential for efficient drug and gene delivery to the nucleus [294]. One of the initial works in this field was performed by Liu et al. [196]. They combined PEG-functionalized nanoscale graphene oxide (NGO) sheets loaded with a camptothecin analogue, SN38. The designed complex (NGO–PEG–SN38) showed acceptable water solubility, while retaining high efficiency of the active substance. This initial study led to a series of new ones performed by several research groups for investigation of graphene-based materials in drug delivery, as doxorubicin [303–306], rhodamine B [307], camptothecin [197–199], and 5-FU (with graphene NPs [308] and CNTs [200]). Graphene has also been explored for applications in gene delivery (plasmid DNA [198,201,309,310], siRNA [311]), gene–drug co-delivery [311] and protein [312]/enzyme [203] delivery (as ribonuclease A and kinase A). One of the new approaches for the use of graphene in gene delivery includes its functionalization with cationic polymer such as polyethylenimine (PEI) [294]. PEI has been broadly studied as a gene vector due to its robust electrostatic interactions with negatively charged phosphates of nucleic acids. In addition, it also offers easy chemical adaptation to accomplish increased transfection efficiency, cell selectivity and reduced cytotoxicity; nonetheless, high toxicity and reduced biocompatibility limit its clinical application [294,309–311].

The majority of these studies have highlighted the potential of carbon-based materials as drug and gene delivery methods *in vitro* to cancer therapy; however, there is a need to demonstrate their relevance *in vivo*, focusing particularly on safety, efficacy and body distribution. Despite many unanswered questions, this family embraces great potential in clinical practice. Further investigations into preclinical studies to elucidate mechanisms and signaling pathways will be essential in moving toward clinical use.

Magnetic nanoparticles (MNPs)

The potential of MNPs in nanotechnology has been strongly discussed. Magnetic/metallic NPs are small, which may be valuable for aspects of cell targeting [313]. Crystal symmetry breaking at the surface has profound ramifications. For example, in metallic compounds, the surface atoms oxidize rapidly, forming oxides that are typically ferromagnetic or antiferromagnetic [314]. Two features play an important role for the *in vivo* uses: size and surface functionality. Particles with diameters of 10–40 nm including ultra-small MNPs are important for prolonged blood circulation.

Biomedical applications *in vivo* may be separated in therapeutic (hyperthermia and drug-targeting) and diagnostic applications (Magnetic Resonance Imaging (MRI)) [315]. As stated in the literature [315], placing superparamagnetic iron oxide in altering current magnetic fields randomly flips the magnetization direction between the parallel and antiparallel orientations, allowing the transfer of magnetic energy to the particles in the form of heat, a property that can be used *in vivo* to increase the temperature of tumor tissues to destroy the pathological cells by hyperthermia. The benefit of magnetic hyperthermia is that it permits a restricted heating to the tumor area. In addition, the potential for application of iron oxide MNPs in drug targeting has recently increased. MNPs in combination with an external magnetic field and/or magnetizable implants allow the delivery of particles to the desired area, fixing them at the local site while the medication is released (magnetic drug targeting). This leads to a concentrated dose of the anticancer drugs and retain them locally for longer periods. The exteriors of these particles are generally changed with organic polymers and metals or oxides to make them biocompatible and suitable for further functionalization through the connection of various bioactive molecules. Protection against corrosion is still a challenge and suitable protection strategies are necessary, as coating with silica, surfactant/polymer or carbon, or embedding MNPs in a matrix [314–316].

MNPs composed of iron derivatives (magnetic, paramagnetic or superparamagnetic) have potential application for drug delivery to skin. A study [313] showed that the rigid MNPs smaller than 10 nm can pass through the skin, reaching the *stratum granulosum* [317]. In another study [206], a magnetic-based core–shell particle (MBCSP) drug delivery system was successfully developed to target skin cancer cells. The core, composed of PLGA–magnetite to load either hydrophilic or hydrophobic anticancer drugs, provides controlled release of drugs via polymer degradation, simultaneously reducing magnetite toxicity. In addition, the shell was made of thermo-responsive polymer for temperature-dependent release of anti-cancer drugs. The presence of iron leads to the accumulation of particles at the target site throughout the application of a magnetic field. Thus, MBCSP may provide combined therapies to cancer by both hyperthermia and drug release from a thermo-responsive polymer shell. In another study, albumin/5-FU loaded magnetic nanocomposite spheres were produced and tested on a skin cancer mouse model. The results clearly indicated that the magnetic targeted NPs exhibited significantly superior efficacy [207]. In addition, as an example of MNPs' possible complexity and cancer future treatments, a multifunctional micellar hybrid nanoparticle containing MNPs for MRI detection, quantum dots (QDs) for near infrared (NIR) fluorescent imaging, PEG to increase circulation times, tumor-specific F3 peptide for targeting and doxorubicin as a therapeutic payload has been developed, whose efficacy has been demonstrated *in vitro* and *in vivo* [205]. Functionalized superparamagnetic iron-oxide NPs were also developed, using magnetism for the targeted transdermal chemotherapy of skin tumors with epirubicin [208].

Gold nanoparticles (GNPs)

GNPs are being studied since the 19th century. Although common oxidation states of gold include +1 and +3, GNPs exist in a non-oxidized state (Au [0]) [318]. For clinical use, they are being studied as carriers for delivery of drugs, imaging molecules, genes and for the development of novel cancer therapy products [295,319,320]. These NPs possess several characteristics that are useful for cancer therapy. Besides being small, they can penetrate through the body, accumulating in tumors. In addition, GNPs exhibit single physicochemical properties, as surface plasmon resonance (SPR) and the ability to bind amine and thiol groups, tolerating surface modification [318]. Currently, NP functionalization is the subject of concentrated investigation [321]. *In vivo*, GNPs passively accumulate at tumor locations that have immature and leaky vasculature, with wider fenestrations than normal blood vessels (enhanced permeability and retention (EPR) effect). Nonetheless, due to the heterogeneity of tumor irrigation as well as particle uptake by the reticuloendothelial system, difficulties in EPR effect employed in tumor drug delivery exist [318]. One strategy is the association of EPR effect with longer circulation times (through PEGylation), in order to increase concentrations of drugs in tumors [322]. Tumor targeting can be also attained by binding tumor-specific recognition molecules such as monoclonal antibodies [323–325]. However, toxicity studies of GNPs have been conflicting: while some studies have shown no toxicity, others demonstrated reactive oxygen species (ROS) production, mitochondrial toxicity, cytokine release, apoptosis and necrosis [295,318,321].

A significant demonstration of the potential of multifunctional GNPs for drug delivery was the use of these particles (carriers) covalently bound to cetuximab (targeting agent) and gemcitabine (therapeutic drug) in pancreatic cancer [326]. In addition, another study proved that GNP–cetuximab–gemcitabine nanocomplex was superior to any of the agents alone or in combination [327]. Important and curious studies have also been performed for breast cancer therapy, demonstrating that the binding and activation of membrane receptors and subsequent protein expression strongly depend on particle size (40- and 50-nm NPs demonstrated the greatest effect) [328]. Additionally, literature [210] demonstrates that gold nanorods are effective as photothermal agents. Other gold nanostructures such as gold nanoshells [320,329], gold nanocages [330] and gold nanospheres [331] have also demonstrated effective photothermal kill of tumor cells (Fig. 4) [333,334]. GNPs are also being studied to enhance radiotherapy [319], with promising results.

Regarding skin cancer, a highly efficient drug vector for PDT drug delivery was developed through the synthesis of PEGylated GNPs, which act as a water-soluble and biocompatible "cage" that allows delivery of a hydrophobic drug to its local of action (Fig. 5). The process of drug delivery was highly efficient and passive targeting prefers the tumor site [211]. In addition, a study [213] showed that delivering doxorubicin by GNPs was very effective against a CM cell line. Doxorubicin was also loaded into DNA and aptamer stabilized GNPs, increasing selectivity toward cancer cells and reducing cell viability in CM cell lines [214]. Another group [336] also used GNPs to carry Zn[II]-phthalocyanine disulfide to test PDT efficacy in a mouse model of xenograft melanoma; more effective treatment outcomes were found with GNPs. Interestingly, crosslink-stabilized multilamellar lipid vesicles were also used as carriers to transport amphiphilic GNPs (embedded in the capsule walls) into CM cells for enhanced radiotherapy [212].

Most hyperthermia research has used particles with silica cores and a gold coating [337]. These nanoshells have been studied for

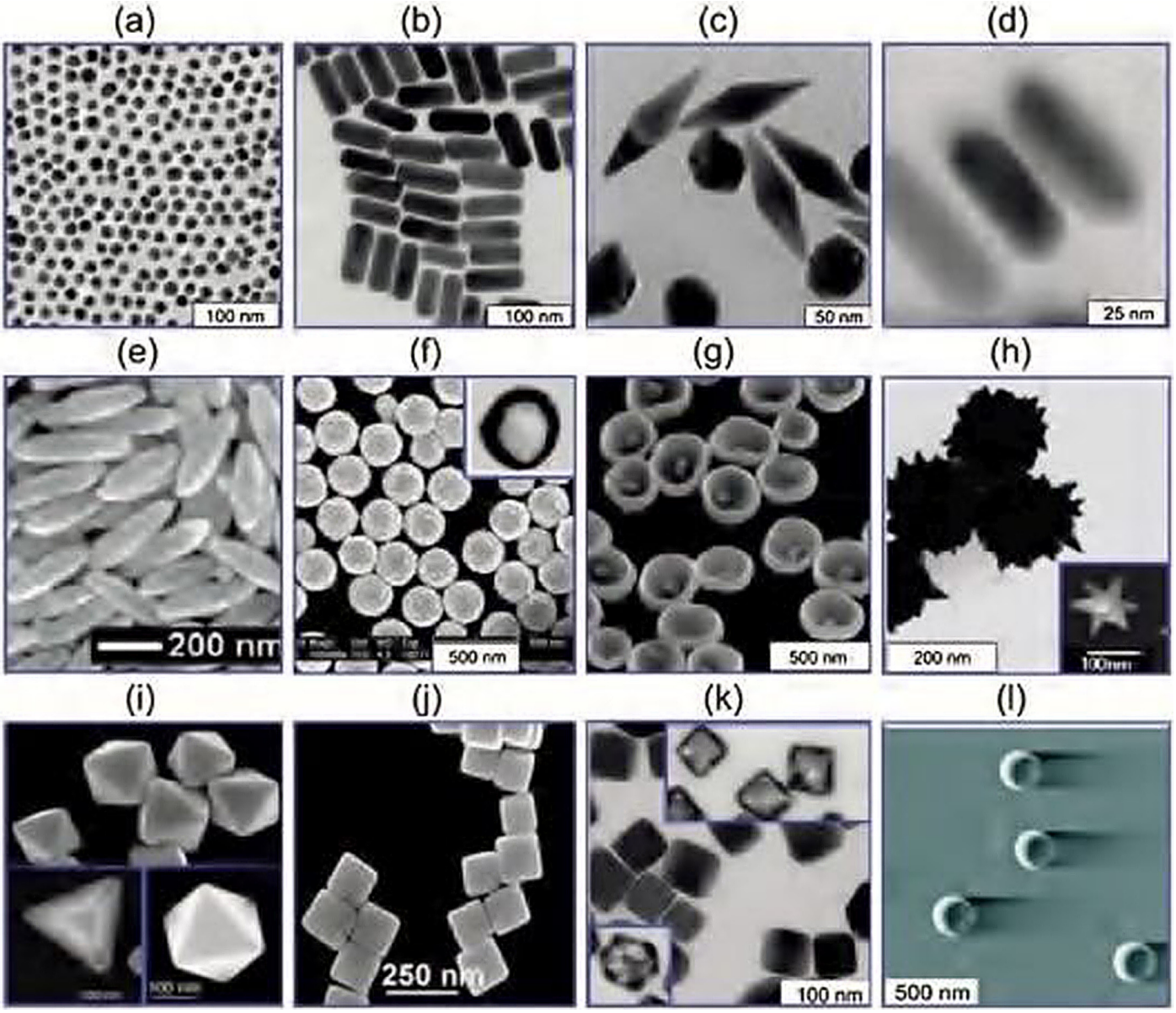

Fig. 4. Plasmonic gold nanostructures: a) nanospheres, b) nanorods, c) gold bipyramids, d) gold nanorods with silver shells, e) nanorice, f) SiO_2/Au nanoshells, g) nanobowls with bottom cores, h) spikey SiO_2/Au nanoshells (inset is a gold nanostar), i) gold tetrahedra, octahedral and cuboctahedra, j) gold nanocubes, k) silver nanocubes (insets are gold nanocages), l) gold nanocrescents. Reproduced from reference [332] with permission of The Royal Society of Chemistry [Original citation: http://dx.doi.org/10.1039/C1CS15166E, accessed in 2014.09.06].

the treatment of human breast and prostate cancer, following intravenous injection and laser therapy, with encouraging results [338,339]. GNP for the treatment of cancer is a hypothesis already being tested in clinical trials. The first to have reached clinical trials is CYT-6091, a citrate-coated GNP bound with thiolated PEG and TNF (Tumor necrosis factor)-α (Aurimmune). Intracellular GNPs were detectable in post-treatment tumor biopsies, including melanoma cells, but not in normal tissue. Studies of CYT-6091 bound with paclitaxel have demonstrated 10 times more paclitaxel uptake in solid tumors than paclitaxel alone [318,340].

Gold solutions are also used to produce nanoshells composed of gold and copper, or gold and silver as contrast agents in MRI, and gold–silica for photothermal ablation of tumor cells [295,338,341,342].

Modular nanotransporters (MNTs)

An MNT is a modular polypeptide that may contain four moieties: an internalizable ligand, an endosomolytic module, a nuclear localization sequence (NLS) and a carrier domain [227]. The first MNT module ligand has two functions: specific acknowledgment of a cancer target cell and penetration via receptor-mediated endocytosis. The endosomolytic module makes it possible to leave the endocytotic pathway before getting into lysosomes, in order to have time for contact with importins. Therefore, this second module is able to make defects in membranes only at the pH of endosomes. The third module (NLS) permits delivery into the cell nucleus, recognizing importins located in the hyaloplasm. Last but not least, the fourth module acts as a carrier for joining the transported drug. Depending on the type of ligand, MNT for different target cells may be produced. MNT modules may be

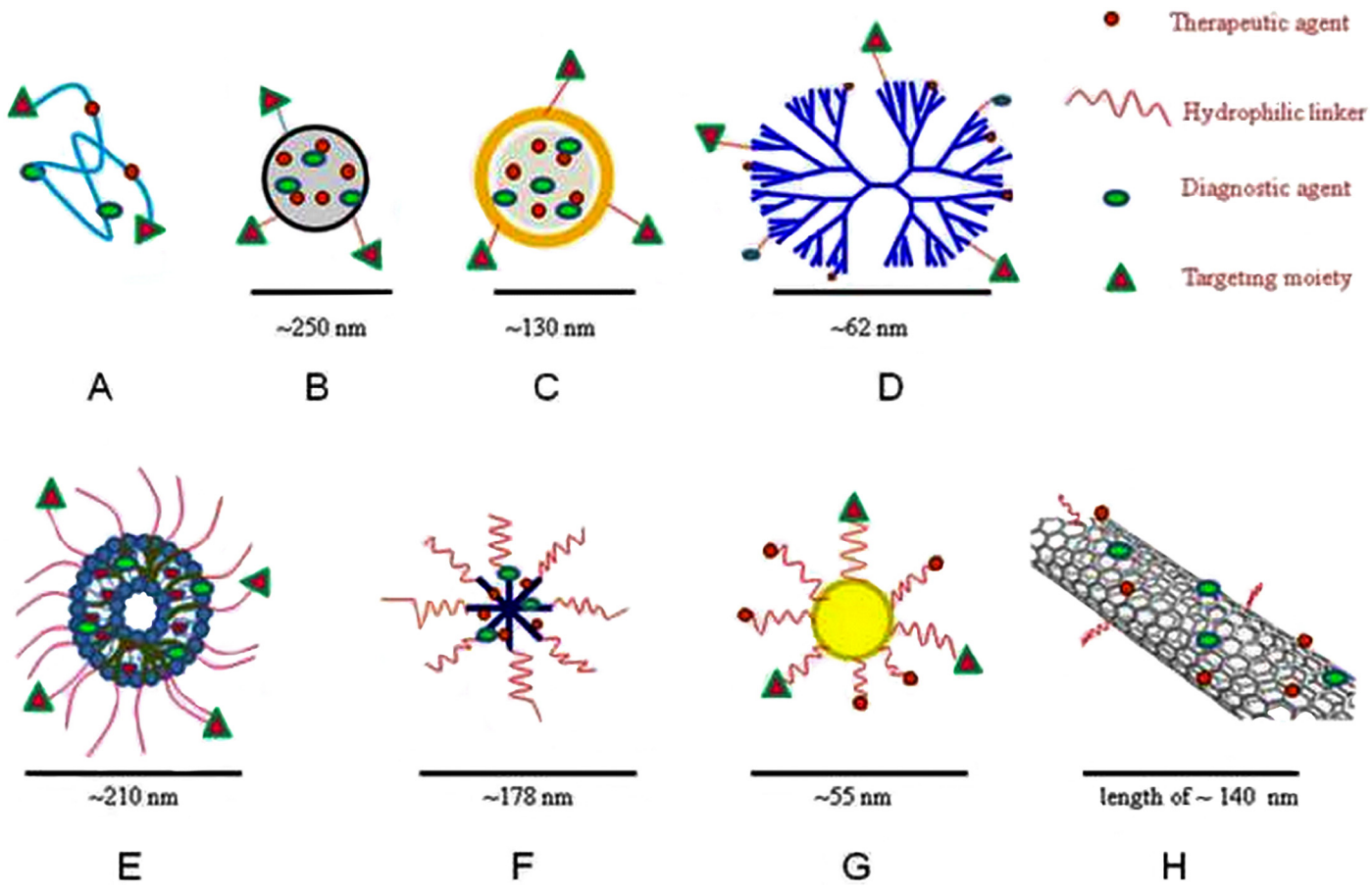

Fig. 5. Schematic diagram of different colloidal nanoparticles: a) polymer–drug conjugate, b) polymeric nanoparticle, c) solid lipid nanoparticle, d) dendrimer, e) liposome, f) micelle, g) gold nanoparticle, h) carbon nanotube. Reproduced from reference [335] with permission of Ivyspring International Publisher. [Original citation: http://dx.doi.org/10.7150/thno.8698, accessed in 2014.05.20].

transposed or replaced, making it possible to use for delivery of different drugs into diverse target cells [343].

Therefore, an MNT is a multifunctional transporting platform, i.e., a recombinant nanotransporter which artificially generate a detail of the cell transport machinery (or exploits the cell transport machinery), designed to get into a specific cellular compartment (the nucleus). This novel approach provides a new drug carrier that could, in principle, be applicable to any arrangement of cell surface receptor and drug (photosensitizers, oligonucleotides, radionuclides) which requires nuclear delivery to achieve maximum effectiveness. MNT has, in fact, most of the looked-for characteristics for an NP delivery system: small size, stability under physiological conditions, nontoxic ingredients and degradation products, moderate lifetime in the body and a mechanism for release into the hyaloplasm. Further, MNT owns the indispensable combination of functional modules, designed to provide specific delivery from the surface of the target cell to its nucleus. NLS, which is necessary to achieve entry into the interphase nucleus, has the ability to increase the size of the nuclear pore complex from 8–10 nm to 40 nm, which restricts the access of larger systems, including most liposomes and NPs. Besides, MNT has significant uniformity in size and composition [227,343].

Evidence is provided that MNT selectively accumulates in cancer cells, with the highest concentration in the nucleus. Significantly, MNT-mediated delivery of photosensitizers result in more than 90% tumor growth inhibition, prolonging survival compared with the free drug, while producing few side effects [227]. *In vitro* studies have shown that chlorin e6-DTox-HMP-NLS-EGF (functionalized with epidermal growth factor) had a 3000 times higher efficacy compared with free photosensitizer [228]; similarly, DTox-HMP-NLS-EGF labeled with a α-emitter ^{211}At was significantly more cytotoxic than free ^{211}At [344].

In vitro studies in CM cells have shown that connecting a MNT to bacteriochlorin *p* resulted in a more than 200-fold enhancement in cytotoxicity and 93% CM growth inhibition compared with free photosensitizer [229,345]. The first *in vivo* study (and unique, in accordance to literature research) was performed by Slastnikova et al. [227], which interestingly tested this hypothesis using two MNTs, one targeted to the α-melanocyte-stimulating hormone (α-MSH, receptor overexpressed on melanoma cancer cells) and the other to the EGF, receptor overexpressed on several other cancers, in B16-F1 melanoma-bearing mice. The *in vivo* therapeutic effects were assessed by PDT studies, which demonstrated preferential uptake in tumor tissue, particularly in the nucleus.

Research on the role of MNTs in cancer, although promising, is still preliminary. Further studies are required to apply this novel technological approach in skin cancer treatment.

Redox-active nanoparticles

The employment of cerium oxide nanoparticles (CNPs) is a novel and encouraging approach, as those particles *per se* appear to demonstrate an antineoplastic effect via their oxygen chemical reactivity. These NPs of spherical shape have unique antioxidant capacity due to alternating Ce(+3) and Ce(+4) oxidation states and crystal defects (defects in the crystal framework due to the presence of Ce(+3) play an important role in tuning the redox activity of CNPs) [346–348].

CNPs were found to be effective against pathologies linked to chronic oxidative stress and inflammation. CNPs are well tolerated, which makes these particles suitable for nanobiology and regenerative medicine [348]. Therefore, the effect of those NPs on human CM was investigated [236], *in vitro* and *in vivo*. Interestingly, concentrations of CNPs (polymer-coated) being innocuous for stromal cells showed a cytotoxic, proapoptotic, and anti-invasive capacity on melanoma. *In vivo* studies revealed a decrease of tumor weight and volume after treatment with CNPs [349]. The application of redox-active CNPs may soon constitute a new paradigm in the treatment and prevention of cancers.

Quantum dots (QDs)

Recently, nanocrystal semiconductor QDs have attracted the attention of many scientific groups owing to their potential for use in the management and treatment of cancer. QDs provide a nanoscale scaffold for designing multifunctional NPs with both imaging and therapeutic functions. The surface of QDs may be modified to improve specificity, sensitivity, solubility, and visualization of the target tissue. However, due to their composition of heavy metals and a few reports of cytotoxicity, QDs have been the subject of toxicological analysis and several groups have reported that the release of toxic metals might be limited with surface coatings, such as PEG or micelle encapsulation [295,302]. QDs preferentially are collected in the upper layers of the *stratum corneum* and in hair follicles, penetrating throughout the skin by getting through intracellular lipid lamellae along the edges of differentiated corneocytes. In addition, QD skin penetration and toxicity depend on physicochemical properties as particle size, shape, chemical structure of the core/shell and surface coating, charge and pH of the applied vehicle [317].

QDs have been studied for the treatment and monitoring of CM, through topic and intravenous administration [240,241,350], showing to accumulate in the tumor tissue compared with normal cells. For example, a polysaccharide-based hybrid nanogel that integrated optical pH-sensing, cancer cell imaging and controlled drug release into a single nanoparticle system (QD) was prepared [238], for *in vivo* testing (CM mouse models). The hybrid nanogel provides excellent stability as a drug carrier, which cannot only provide a high drug loading capacity, but also offer a pH-sustained release of the drug molecules. Recently, nanostructured lipid carriers with QDs, called QDNLCs, for integrating imaging and therapy were studied [239]. Results showed that camptothecin accumulation in melanomas increased by 6.4-fold after incorporation into QDNLCs. Likewise, multifunctional liposomes loaded with QDs and anticancer drugs were prepared for simultaneous bioimaging and drug delivery in an *in vivo* CM model, with encouraging results in both imaging and intratumoral drug accumulation [351]. Although further research is still required, derivatized QDs have improved tumor localization and may enhance skin cancer monitoring and chemotherapy.

Silica nanoparticles (SiNPs)

Lately silica-based NPs have been studied as a new method for drug and gene delivery due to their unique features, such as small and uniform pores, large surface area and pore volume, low toxicity and biocompatibility. The porous structure of SiNPs can not only act as a suitable carrier for hydrophobic photosensitizers, but also allow the oxygen and generated singlet oxygen permeability that is essential for PDT [243,244].

In vitro and *in vivo* studies demonstrated the active uptake of these NPs with photosensitizers into the cytosol of CM cells. Significant injuries to such impregnated tumor cells were observed upon irradiation. Thus, the potential of using ceramic-based NPs as drug carriers for PDT has been demonstrated [243–245]. Interestingly, HA degrading enzyme (Hyaluronidase) was immobilized on SiNPs and tested in a human CM model. At the end of the experiment, tumor volume reduction with SiNP-immobilized Hyaluronidase was significantly enhanced compared to non-immobilized Hyaluronidase [246]. Furthermore, versatile nanocomposite NPs were synthesized by decorating the surface of SiNPs with multiple magnetite nanocrystals. Integrating a multitude of magnetite nanocrystals on the silica surface led to the enhancement of MR signal due to the synergistic magnetism. Doxorubicin was loaded in the pores, inducing efficient *in vivo* cell death, which demonstrates that it was successfully delivered to the tumor sites and its anticancer activity was retained [352].

In addition, scientists believe that the surface modification of SiNPs with antibodies specific to CM cells will lead to improved diagnosis and targeted treatment of melanoma [244].

2. Protein-based therapies

Protein transduction technology

Numerous natural and synthetic cationic peptides have the ability to cross lipid bilayers and get into cells. These peptides have been classified as protein transduction domains (PTDs), also called cell-penetrating peptides (CPPs). Recombinant technology is used to modify the biophysical properties of proteins and peptides, particularly with respect to their cell permeability, using PTDs/CPPs. They have been used as transduction carriers for NPs, oligonucleotides, peptides and full-length proteins *in vitro* as well as *in vivo*. Skin is definitely a suitable target for this new carrier system. Nonetheless, little is known about the biological effects of PTD/CPP-containing proteins resulting from intradermal (i.d.) application. Among the PTDs, poly-arginine peptides, especially nona-arginine (R9), are transported efficiently with low cytotoxicity [353–357]. A study in the literature [353] demonstrated that i.d. application of R9-peptide itself did not have quantifiable results, but recombinant R9-PTD-containing fusion proteins (especially immunogenic) did stimulate inflammatory cell infiltration (lymphocyte, monocyte and neutrophil) at the injection area. In addition, R9-PTD-containing proteins persisted *in vivo* at the site of injection for a long period when administered i.d. and were transduced into local dermal cells, including cancer mass. These biological effects may be pertinent to the development of novel molecular-targeting strategies in the treatment of skin malignancies.

Therefore, *in vivo* administration of R9-PTD-containing fusion proteins to local skin lesions may be applicable as a novel molecular-targeting method in clinical applications. In other words, administration of PTD-containing fusion proteins might be an appropriate strategy that induces the overexpression of specific molecules. For example, the dominant-negative form of rR9-Smad3 may be transduced into eosinophils, resulting in the inhibition of the TGF-β mediated signal cascade. A lot of signaling pathways may be induced/suppressed throughout this novel approach, as inhibiting the *STAT3*-mediated signaling cascade [353,357]. Recent findings suggest that *ex vivo* and *in vivo* application of R9-PTD-containing fusion proteins, including immunogenic antigens, to cells and local skin lesions might be useful as a novel approach to cell transduction and molecule delivery, being applicable to patients in replacement of gene therapy [353]. Despite all the drawbacks, membrane-transduction technology could suggest a simple alternative for the control of biological processes *in vitro* and *in vivo* [152,353]. Can this technology deliver in clinical practice? The answer might not be so far: further investigation is certainly demanded.

Hsps chaperone-based therapies

Heat shock proteins (Hsps) were discovered as a group of proteins that are strongly induced by heat shock and other (chemical, physical) stresses. The Hsps have been afterwards characterized as molecular chaperones, proteins which share the property of modifying the structures and interactions of other proteins. Hsps are overexpressed in an extensive range of malignancies and are involved in cell proliferation, differentiation, metastasis, death and

recognition by the immune system. The mechanism that underlies the role of Hsp in tumor progression and resistance to treatment is the induction of protection from spontaneous apoptosis as well as from the apoptosis generated by therapy. Therefore, Hsp levels are valuable biomarkers for carcinogenesis in some tissues and a signal of the differentiation and aggressiveness degree of some cancers, as Hsp27, Hsp70 and Hsp90 as markers of keratinocyte differentiation of the skin. In addition, the circulating Hsp and anti-Hsp antibodies in cancer patients may be suitable for tumor diagnosis. Hsp overexpression may also predict the response to some treatments [358–362].

Implication of Hsps in cancer evolution and response to antineoplastic treatment has led to its effective targeting in therapy throughout 2 core strategies: a) modification of Hsp expression or molecular chaperone activity; b) use of Hsps in anticancer vaccines, using as adjuvants to present tumor antigens to the immune system [216,358,363]. In fact, gene gun vaccination proved to be a successful method of introducing antitumor molecules into mice. In particular, this method of vaccine administration has been used with established efficacy for the treatment of CM [364]. Promising data were obtained in studies in which the complex of Hsp70 with tumor peptides derived from melanoma was administered therapeutically [217]. Moreover, additional data show that the intratumoral delivery of Hsp70 can efficiently destroy CM [218,221]. A recent study [216] was performed to design a hydrogel-containing recombinant Hsp70 and apply it topically on CM. According to the results, Hsp70 diffused inside the tumor, through the skin. In addition, the gel reduced the tumor growth and prolonged the life of animals. Therefore, these data endorse the antineoplastic effect of Hsp70 and demonstrate the relevance of a new non-invasive technology of Hsp70-based therapy. In addition, another study [220] strategically incorporated a pathogen-derived, NF-κB-stimulating danger signal into the large stress chaperone Grp170 that was previously shown to facilitate antigen cross-presentation. The resultant chimeric molecule (Flagrp170) is proficient in tumor antigen transport and functional activation of dendritic cells (DCs). Intratumoral administration of Flagrp170 induces a superior antineoplastic response against CM but also distant lung metastasis, compared to unchanged molecules. Therefore, when the tumor microenvironment is targeted with this chimeric chaperone, mobilization and repair of antitumor immunity is achieved, supporting the clinical use of this new immune modulator in the treatment of metastatic malignancies.

Investigations on the role of Hsp in cancer, although encouraging, are still initial, and little information is available on how Hsp regulation could be disrupted in cancer. Further studies will be crucial in directing the investigation designed for targeting Hsps in skin cancer therapy.

3. Biological therapies

Immunotherapies

It is known that cancers are recognized by the immune system, and under some circumstances, the immune system may control or even eradicate tumors. Different immunotherapeutic approaches are currently being studied in many cancer types. Although studies have been most widely conducted in CM, they are providing valuable knowledge applicable to other cancers. Recent successes in the progress of novel therapies for metastatic CM, such as anti-CTLA-4, MAPK pathway inhibitors, PD-1/PD-L1 pathway blocking antibodies, as well as association methodologies of cytotoxic chemotherapy and angiogenesis inhibitors, have returned encouraging results [137,365]. Although these novel immunostimulatory approaches for skin cancer treatment have been already described in the present review, anti-CTLA-4 agents (in other words, antibodies that blocks the coinhibitory receptor CTLA-4) and PD-1/PD-L1 pathway blocking antibodies (or agents that target a second coinhibitory receptor, PD-1, or its ligand, PD-L1) deserve an additional reference, due to their current relevance in clinical research and practice.

Ipilimumab (approved by FDA in 2011) and tremelimumab (both anti-CTLA-4 monoclonal antibodies), nivolumab and pembrolizumab (anti-PD1 agents, the last one already approved by FDA during 2014) are also known as "checkpoint-blocking" antibodies. These new molecules contribute to the down-modulation of $CD8^+$ and $CD4^+$ effector T-cell function. Whereas CTLA-4 and PD-1 work as negative regulators, each plays a nonredundant role in modulating immune responses. CLTA-4, through engagement with its ligands, CD80 and CD86, plays an essential role in attenuating the early activation of naïve and memory T cells. On the other hand, PD-1 is primarily involved in modulating T cell activity in peripheral tissues via its interaction with PD-L1 and PD-L2. In addition, melanoma cells have been found to express high levels of the PD-L1 protein, a ligand for the PD-1 receptor [137,138] and PD-1 is highly expressed on lymphocytes infiltrating human tumors and circulating tumor-specific T cells, a phenotype that may be correlated with impaired T cell function. Together, these findings suggest that interrupting the PD-1:PD-L1/PD-L2 interaction could be an effective anticancer therapy by blunting inhibition of immune responses in the tumor microenvironment [138].

A significant survival advantage for "checkpoint-blocking" antibodies-treated patients was reported from clinical trials, highlighting the long-term survival benefit of receiving these new molecules [366,367]. Nonetheless, the clinical testing of the human CTLA-4-blocking antibodies ipilimumab and tremelimumab uncovered novel toxicities consisting of tissue-specific inflammation. Based on their presumed link to the activation of the immune system by CTLA-4 blockade, these toxicities have been referred to as irAE (immune-related adverse events). Tissues most often affected include the pituitary, and other endocrine glands (hypophysitis, hypothyroidism, thyroiditis, adrenal insufficiency), bowel (diarrhea, colitis), skin (rash, pruritus, vitiligo), and liver (hepatitis, elevated liver enzymes) [33,138,367,368]. In comparison with CTLA-4 blockade, the rates of previously defined irAE of anti-PD-1/PD-L1 antibodies appeared to be less frequent. However, a new and potentially serious irAE, pneumonitis, was rarely observed [138,369]. As an example, despite the mild to severe irAEs observed with ipilimumab in about 60% of patients, overall survival averaged 22–25% at 3–5 years. Similarly, pembrolizumab showed a response rate of almost 40%, although 79% of the patients presented with irAEs. Therefore, we may state that immunotherapy has come with a price: unsettled immunoregulation provoking immune dysfunction, which has been leading to autoimmune disorders [370].

Moreover, as CTLA-4 and PD-1 regulate immune responses in different ways, combined blockade of both pathways may achieve superior antitumor efficacy. However, similarities in the toxicity profile for CTLA-4-blocking and PD-1/PD-L1-blocking agents highlight the importance of evaluating toxicity of combined therapy because it may bring a different spectrum of opportunistic disorders. Nevertheless, irAEs may not be unreasonably additive. The first clinical trial in humans with the concomitant administration of ipilimumab with nivolumab is currently ongoing (NCT01024231) [138,370,371]. Based on previous experience, while the spectrum of irAEs was similar to monotherapy, overall survival was higher at 24 weeks [370].

Evidence supports the concept of an immunological management of melanoma growth. Active immunotherapy (vaccination) and adoptive immunotherapy trials (T cell therapies) are being conducted in metastatic CM patients. Following the tumor antigen characterization, the application of tumor rejection antigens with adjuvants will become available as tumor vaccines. Recently, the idea of DC based-vaccines has been developed, as they have shown efficiency in priming and improving T cell responses in clinical

studies [137,139,372]. Monoclonal antibodies under analysis include those targeting the receptors CD40, CD137 (4-1BB), CD134 (OX40), glucocorticoid-induced TNF receptor, KIR (Killer-cell Immunoglobulin-like Receptors) and TGF-β. The interaction of CD40 with its ligand is essential in the communication between T cells and DCs and between helper T and B cells during humoral immune responses. A clinical trial of the anti-CD40 monoclonal antibody CP-870,893 combined with tremelimumab in patients with CM is still ongoing (NCT01103635) [137,139].

Recent advances in genetics and immune responses to tumors have increased interest in gene-based treatments for skin cancer. Understanding the molecular mechanisms underlying the interaction of nucleic acids with the immune system has increased interest in their application in tumor immunotherapy. Numerous basic strategies have emerged: a) reinforcement of the immune response throughout genetic modification of some target cell populations using immunostimulatory genes (as cytokines); b) genetic immunization with genes for melanoma-associated antigens recognized by cytotoxic T cells; c) interference with signaling pathways; d) suicide gene strategies [373,374].

The skin is a suitable target because not only resident skin DCs but also keratinocytes express functional Toll-like receptors (TLR) 3 and 9. The regulation of TLRs during skin tumor development is currently under investigation. Topical application of the TLR7-agonist imiquimod is approved for immunological treatment of actinic keratoses, which represent a frequently observed carcinoma *in situ*. Likewise, several TLR agonists are being considered to enhance adjuvant melanoma vaccines which intend to eliminate micrometastases: TLR agonists mimic a local viral infection, activate DCs and, then, natural killer and T cells; following recruitment of lymphocytes into tumor, cytotoxic activity and immunoregulation occur, which contributes to the immunological clearance of malignant cells [375,376]. In addition, CM cells were found to have a considerably higher quantity of TLR-4 levels. It was also [222] found that the coadministration of paclitaxel and icariside II enhanced apoptosis and decreased the levels of IL-8 and VEGF in human melanoma, through the inhibition of the TLR-4/MyD88 pathway [377].

Immunostimulatory ODNs (oligodeoxynucleotides) are being successfully combined with other forms of immunotherapy (as cytokines) or with chemotherapy. For example, CpG-ODN (which activates TLR 9-expressing cells) exhibited synergistic effects with recombinant IL-2 or IL-18 for the treatment of CM [376,378,379]. Of particular interest has been the concept of combining different TLR-agonists for additive effects on DC activation and stimulation of T cells, being currently under evaluation [375]. In addition, CpG-ODNs have been combined with DC-based vaccines for CM [223,378]. The discovery of RNA interference has led to great interest in the use of siRNA to abolish the expression of genes involved in neoplastic transformation. siRNA can be designed in order to attack molecular pathways involved in transformation and, in addition, are able to stimulate the immune system [375]. The use of siRNA preparations for the treatment of skin malignancies depends on the efficiency of transfection and the level of silencing the targeted genes. Currently, preclinical and clinical studies are being performed in order to assess the effectiveness and safety of these formulations [380]. An interesting study was also completed [179] which demonstrated that a c-Myc siRNA effectively suppressed the production of c-Myc in the tumor and inhibited tumor progress in mouse models.

Cytosolic pattern recognition receptors were recently identified, which are able to detect nucleic acids inside the cells. It could be shown that RNA is also detected by helicases RIG-I and MDA-5 in the cytosol [381]. Notably, TLR-independent immunostimulatory effects mediated by cytosolic pathogen recognition receptors such as RIG-I or MDA-5 can be observed in many cell types including skin tumor cells because helicases are ubiquitously expressed. Therefore, TLR-independent effects may mediate some of the direct effects of immunostimulatory nucleic acids on skin malignancies, which may involve activation of the type I IFN system and induction of cell cycle arrest, inhibition of protein synthesis and apoptosis [375,382]. Further research is still required to constitute a new CM treatment modality.

Stem cells

Stem cells have been recently studied for use as drug carriers. This system aims to explore the tumor homing properties of stem cells, in order to actively deliver the drug or imaging agent. Comparing with targeting achieved with NPs, stem cells are able to home to the tumor, while NPs increase the chances of being internalized by malignant cells. Different types of stem cells have been studied: MSCs (mesenchymal stem cells) and NSCs (neural stem cells). In fact, both may be laden with NPs without modifying regular cellular function. In order to stimulate the targeting of NPs to a larger portion of cancer cells, stem cells and NPs may be associated [139].

Several signaling factors in the tumor microenvironment aids MSC recruitment, as TGF-β and IL-6 [383]. Surprisingly, although MSCs stimulate carcinogenesis, they can be also used as targeted drug carriers [384]. Systemically-injected MSCs expressing IFN-β or cytokine are efficient in the reduction of tumor growth, throughout the inducement of local immune response [385–387]. Additionally, MSCs have been successfully used to deliver TNF-related apoptosis-inducing ligand (TRAIL) to tumors, in order to induce apoptosis in cancer cells [388–391]. MSCs are able to infiltrate into the tumor, being possible that a higher proportion of cells is exposed to the therapy. In addition, MSCs should be an effective vehicle for targeted delivery of theranostics, which allows drug delivery and simultaneous monitoring. In fact, a variety of NPs have been loaded into MSCs for targeted delivery to cancer cells [392–394].

NSCs can also be applied for tumor NP drug delivery. NSCs exist in the CNS and show wide tropism to experimental glioma [392,395]. Nonetheless, accumulating evidence suggests that CM might be a syndrome of stem cells [396]. An interesting study of magnetic NPs loaded into NSCs was performed, in order to verify the inhibition of CM growth through hyperthermia by an alternating magnetic field in a mouse model, resulting in a significant regression of the tumors [230]. Additionally, stem cell-based PDT [231] and NSC-based enzyme/prodrug therapeutic approach for CM are also being studied *in vitro* [232], with promising results. However, the methodological challenges involved in the isolation of NSCs remain the major obstacle in their extensive use and development as carriers for NP delivery [139].

4. Other technological approaches: miscellaneous

Hyaluronic acid (HA) as a carrier and a ligand

In recent years, HA has been discovered as a promising candidate for cellular delivery of several therapeutic agents because of its ability to recognize specific receptors that overexpressed on cancer cells [397]. In addition, the skin is an organ with a high level of CD44, a receptor that binds HA. HA has been used as a carrier and a ligand on liposomes or NPs (polymeric or lipid constitution) to target drugs to CD44 over-expressing cells. Drugs can be merged to HA via the carboxylate on the glucuronic acid residue, the hydroxyl on the *N*-acetylglucosamine, or the reducing end which is located on a disaccharide. Drugs delivered through HA-modified liposomes and NPs exhibited good anti-tumor activity. The HA environment is also a potential target for anti-cancer therapies [398,399].

Ultrasound methodologies

Methodologies using external forces may also improve penetration of NPs into cancers. This provides another resource to affect

a larger proportion of cells without the need of a ligand [139]. The ability to non-invasively target a specific position without increasing systemic exposure leads to the employment of ultrasound (US) techniques as a novel strategy using physical energy to enhance drug delivery, through sonophoresis, pulsed US, high intensity focused ultrasound (HIFU), MR-guided focused US (MRgFUS). US may be also used as hyperthermia for cancer therapy [400].

Hence, pulsed US enhances the penetration of NPs into tumors *in vitro* [139]. For cancer therapy, US may induce effects not only through heating, but also through nonthermal mechanisms including ultrasonic cavitation, gas body activation, mechanical stress or other undetermined nonthermal procedures [400]. Concentration of particles in the tumor core, intermediate layers and near the surface is determined by particle size, charge and the applied US field. In fact, US reduces the packing density of cells, which is a major blockade to the penetration of drugs into malignancies, through the cavitation of NPs, which produce significant mechanical impacts on cells and extracellular matrix [139,401]. The mechanical effects produced by acoustic cavitation have been exploited to enhance direct intracellular drug delivery via sonoporation (formation of temporary pores in the cell membrane induced by US) [402,403]. For example, US has been shown to enhance radionuclide uptake in pancreatic tumor cells *in vitro* [404] and, in pre-clinical studies, US use has been shown to increase the efficacy of adriamycin in ovarian cancer and enhance the uptake of radiolabeled monoclonal antibody to human epidermoid tumor [400,401].

For transdermal drug delivery, the *stratum corneum* forms a barrier to drug diffusion for molecules which have a weight greater than 500 Da [405]. Low-frequency US (sonophoresis) has the ability to increase permeability of this *stratum*. This permeabilization methodology may be useful in order to avoid the multiple use of needles [400,406]. It is one of the most talented and easy novel drug delivery systems and has been shown to improve the skin permeation and release of drugs that have poor absorption/permeation profiles through the *stratum corneum*. Although the transdermal route is suitable for lipophilic drugs, US-mediated delivery is better for hydrophilic drugs. Several formulations have been used in sonophoretic studies: gels, solutions, creams, ointments, microspheres, solid lipid microparticles, and liposomes [407]. For example, in an *in vitro* study, low-frequency US selectively sensitizes skin cancer cells against quercetin, inducing cytotoxicity, while having minimal effect in normal cell lines [235].

Microbubble-based strategies are under study for US directed and targeted therapy [400]. Microbubbles have low density and their stabilization by lipid coatings creates low-density particles with uncommon properties for imaging and drug delivery. Perfluorocarbon (PFC) gases captured within lipid coatings turn these NPs stable for blood circulation. Microbubbles can be cavitated with US energy for site-specific local delivery of bioactive materials [408]. In this strategy, the external US exposure activates microbubbles in the circulation, which may also act as drug carriers at a desired site of treatment. The therapeutic molecules may be attached to the outer surface of bubbles, integrated in the bubble shell or loaded into the interior of these particles; therefore, drugs may be released in the vascular compartment through US-induced particle disruption [409]. One of the advantages of this novel drug delivery approach is that the dose of agent is lowered, with a consequent minimization of undesirable effects away from the treatment site [234,400]. In addition, US-mediated gene delivery with microbubbles has emerged as an attractive carrier system for site-specific and noninvasive gene therapy. Several preclinical studies have reported successful gene delivery into solid tumors with significant therapeutic effects using this novel approach [410].

Cancer therapy through US induced hyperthermia involves heating a tumor to about 42 °C for about 1 hour, which appears to be effective in reducing tumor growth; nonetheless, a modest efficacy has been reported in clinical trials. Scientific research suggests that hyperthermia can be valuable for drug delivery using NPs. However, progress in hyperthermia cancer treatment has shifted to the use of HIFU. HIFU was initially studied clinically for thermal ablation, as it is able to produce very high intensities (between 50° and 80 °C) at the focal spot. The position of this spot must be carefully controlled and moved in order to ablate larger volumes of tissue [400,411]. In fact, some multi-center studies have established the use of HIFU as a viable option for solid cancers [411–413]. In addition, US based guidance and monitoring offers the possibility of incorporating both treatment and imaging modality in one compact system: specialized clinical systems have US therapy sub-systems integrated into MR-imagers (MRgFUS). FUS in these applications is directed within the first 2–20 mm of the skin and subcutaneous tissue, as very small lesions of 1 mm^3 up to several 10 s of cm^3 may be produced [400]. For decades HIFU has promised to permit truly non-invasive tumor ablation. Only now, however, with recent improvements in imaging, has this objective finally emerged as a real therapeutic possibility for skin cancer [414]. Although very promising, further investigation is required [233,415].

Significant progress is being achieved in the area of non-invasive therapeutic applications. Therefore, US may be used not only as a synergistic utensil to increase the penetration and concentration of diagnostic and therapeutic NPs into skin cancer, but also solitary, in the form of hyperthermia/HIFU [401]. With further studies focused on *in vivo* testing, these techniques may provide novel treatment options for skin cancer.

Electroporation (EPT), electrochemotherapy (ECT), iontophoresis and nanosecond pulsed electric fields (nsPEF)

EPT involves the application of short [1] and high-voltage (50–500 V) pulses to the skin to cause disorganization of the lipid structure of the *stratum corneum* and thereby enhance drug delivery. The combination with US not only reduces the threshold voltage for electroporation, but also increases transdermal transport, showing a synergistic effect that may be clinically explored in the near future [407]. Therefore, EPT is a new cancer treatment strategy in which a locally applied electrical field enhances cell permeability, permitting intracellular accumulation of an agent. The combination of brief permeabilizing electric pulses with a low-toxicity anticancer drug is known as ECT [416]. On the other hand, the administration of ionic therapeutic agents through the skin by a low-level electric current is denominated iontophoresis and is applied mainly to oligonucleotides and peptides. It appears that this may not be an appropriate method for the systemic delivery of larger peptides. The combined use of iontophoresis and electroporation should be effective in the delivery of oligonucleotides, genes, peptides and proteins [417].

The clinical application of EPT/ECT/iontophoresis in skin cancer (and its metastasis) is already being verified in humans, attesting to be a promising new treatment that should be evaluated as an alternative to surgery [416,418,419]. Recently, responses of lesions treated with ECT with bleomycin in CM patients were analyzed, with favorable outcomes and demonstrating that ECT is a reliable and effective technique [248–250,420]. Similar results were obtained with cisplatin [252]. The combination of iontophoresis with doxorubicin-loaded SLNs was also explored, increasing doxorubicin cytotoxicity against CM cells by 50% [251]. In addition, recent findings propose that the inflammatory responses resulting from local cytotoxic treatments, such as ECT, may enhance the activity of immunotherapeutic agents, suggesting that the combination with immunotherapy may be a novel strategy to stimulate durable effects and improve survival [421,422]. Studies suggested that delivery of IL-15 or IL-12 plasmids by EPT resulted in increased expression within the tumor compared to the control injection, which resulted in tumor regression, long-term survival and greater protection against tumor

recurrence [255,423]. Two immunotherapies have progressed to CM clinical trials. Delivery of a plasmid DNA encoding IL-12 or IL-2 using EPT has been demonstrated to be safe in humans [254,424,425]. Interestingly, immunotherapy plasmid DNA delivered via EPT to B16F10 melanoma tumor model was used to achieve effective suppression of metastases [253].

One of the approaches to skin cancer treatment is irreversible EPT, resulting from permanent permeabilization using microsecond domain pulses with larger amplitudes. Nonetheless, if the electric pulses are shortened into the nanosecond domain, they may independently induce apoptosis within the tumor cells themselves, causing the tumor to slowly self-destruct without requiring toxic drugs or EPT: nsPEF. These pulses generate small and long-lasting nanopores in the membrane of tumor cells, resulting in increased permeability to small molecules and ions, as well as an increase in intracellular Ca^{2+}, DNA fragmentation, disruption of the tumor blood supply and the initiation of apoptosis. The two main reasons to be so effective are that they are fast enough to penetrate into the cell and all organelles, and short enough to cause small pore formation in membranes without significant heating. The application of electrical pulses to murine melanomas *in vivo* triggers both necrosis and apoptosis, resulting in tumor remission within an average of 47 days in all the animals treated. In addition, none of the CMs recurred during a 4-month period after the initial CM had disappeared. A single human subject applied nanoelectropulse therapy to BCC and had a complete response [256,257,426]. Nonetheless, further research is still required.

Microneedles

Recently micro-scale needles have been developed, which painlessly penetrate the skin, increasing the absorption of drugs (hydrophilic, low potent, high molecular weight) through the bypass of the *stratum corneum*. Microneedles have shown to be safe and easy-to-use for drug administration, a substitute to hypodermic injections and a possible controlled release method. Several studies have demonstrated that such devices increase transdermal delivery of large molecules and may be employed alone or with other methods such as EPT and iontophoresis, as well as with different drug carriers, as NPs [427]. The combination of NPs and microneedle technologies for special applications is being analyzed [163,428]. For example, a microparticulate melanoma cancer vaccine was formulated and delivered using microneedle; after vaccination, the animals presented immuno protection [258]. Further research is required to recognize the behavior of NPs with novel delivery devices such as microneedles [428].

Gene gun therapy

Originally, particle-mediated gene transfer based on gunpowder acceleration was developed to deliver genes to plant cells. Although helium had replaced gunpowder as the propellant for most devices, currently hand-held instruments are used, based on a ballistic particle-mediated delivery system. These devices are available commercially as Helios® gene gun (Bio-Rad, Hercules, CA). The gene gun accelerates DNA-coated gold particles into target tissues, penetrating through the cell membrane. At this point, the DNA disassociates from the gold particle and can be expressed [429]. Gene-based immunization with transgenic DNA vectors expressing cytokines, chemokines or tumor-associated antigens (TAA) represents a promising approach to increase cytotoxic T cell immunity against cancer diseases. Besides particle-mediated vehicles, gene gun technology has been developed as a nonviral method for gene transfer. This approach has been shown to induce both humoral and cell-mediated immune responses in animals. A wide range of cell types has been successfully transfected *ex vivo* and *in vitro* by gene gun technology, including CM cells [259,260,430–435]. In fact, many of the preclinical and clinical studies have focused on CM as this cancer type has been considered an immunoresponsive tumor for which several TAAs have been identified [436]. For example, in an interesting study [261], the introduction of fused-gene DNA vaccine by gene gun reduced the size of established tumors (CM models) and prolonged the lifespan of tumor-bearing mice. Still in rudimentary stages, further research is required.

Phototherapies

Immune conjugates of GNPs or iron oxide particles have been tested as light absorbers. As they are able to absorb light and release absorbed energy in the form of temperature, cell damage is attained by heat [279]. Photothermal therapy (PTT) has been using GNPs to inhibit tumor development in rats with SCC. The technique involves the use of high power pulsed laser irradiation and photosensitizing agents with an especially short lifetime in electronically excited states. This method causes less surrounding tissue damage, making this treatment feasible [437,438]. It is achieved by conjugating NPs to monoclonal antibodies or hormones. Lately, low weight gold nanospheres conjugated to MSH analogues were successfully developed to evaluate their potential for selective photothermal ablation in murine CM [266]. In addition, a biopolymer (HA) had helped graphene oxide NPs penetrate skin and accumulate inside CM cells, where laser light heat up the material to kill cancer cells. The NIR irradiation resulted in complete ablation of tumor cells with no recurrence [265]. CdTe and CdSe fluorescent QDs, coated with a silica shell, have also shown great potential in the treatment of mouse melanoma tumors by PTT [264]. In this context, PEG chain was optimized in order to stabilize gold nanorods in the blood circulation, used successfully for *in vivo* PTT [263].

In situ photoimmunotherapy (ISPI) is another promising method for the treatment of metastatic CM that combines local, selective PTT with immunological stimulation using immunoadjuvants. Tumor cells swell and are disrupted due to temperature, releasing TAAs and thermally inducing Hsps. Antigen presenting cells (APCs), particularly DCs, can capture these antigens and present the antigens to T cells that can induce effective immune responses against tumors. Evidence from clinical studies with imiquimod has demonstrated ISPI to be a useful method to treat metastatic CM [262].

Although its use is limited due to costs, patient adhesion and pain, PDT is also another promising treatment strategy for skin cancer. Passive carriers (for photosensitive agents) are preferred due to sustained liberation. This method may also counteract the side effects of photosensitivity, as they are able to accumulate in target cells saving the surrounding cells from the undesired effects. Preferential accumulation of NPs in target tumor tissue also improves its efficacy [279]. Recent studies have shown the efficacy of NPs in combination, as chemo and PDT. For example, chemotherapy and PDT using doxorubicin and methylene blue have had significant therapeutic effects against drug resistant tumors [439].

Interestingly, recent studies have shown that the nanomaterial-mediated photothermal effects (NmPTT) of gold nanoshells not only are able to absorb NIR light, but can also emit fluorescence, exerting photodynamic therapeutic (NmPDT) complete destruction of solid tumors. The modes of NmPDT and NmPTT can be controlled and switched by changing the excitation wavelength. The combination of both effects on the destruction of solid tumors is far better than pure NmPTT, demonstrating that gold nanoshells are able to serve as multi-functional theranostic agents (imaging, NmPDT and NmPTT) upon single NIR light excitation under ultra-low laser doses [267,440].

Cold atmospheric plasma (CAP)

CAP is an ionized gas that has recently been extensively studied as a possible new therapy in oncology, as it has been demonstrated to induce apoptosis, necrosis, cell detachment and senescence

Table 2
New technological perspectives for skin cancer treatment: overview of recent published studies.

Technology	Molecules/drug	Methodology	Highlights	Ref.
Polymers and polymeric nanoparticles	Quercetin	NPs prepared by nanoprecipitation technique using ethylcellulose as polymer	– Lipophilic drug/molecule; – Topical use; – Sustained and controlled release, as ethylcellulose acts as a reservoir in skin.	[164]
	Genistein	Polymeric nanocapsules based on poly(DL-lactide) prepared by nanoprecipitation technique	– Lipophilic and unstable drug/molecule; – Incorporation in a semi-solid gel formulation; – Increased skin penetration.	[165]
	Curcumin	Chitin nanogels	– Drug and polymer are insoluble in water; – Higher release at acidic pH; – Specific toxicity on CM cell lines; – Nanogels showed a 4-fold increase in steady state transdermal flux of curcumin compared to control curcumin solution.	[166]
	Protoporphyrin IX	PLGA based NPs	– Sustained and controlled release; – Localized effect in the epidermis and dermis, the site of action for topical PDT.	[167]
	Aminolevulinic acid derivatives (5-ALA and its ester derivative 8-ALA)	Chitosan NPs	– Localized effect for topical PDT; – Control and improve the permeation of photosensitizers and prodrugs through the skin, improving the efficiency of PDT.	[168]
		Dendrimers with aminolevulinic acid residues attached via ester linkages to an aromatic core	– Well-defined structure capable of incorporating a high drug payload; – Good correlation with cellular phototoxicity following light exposure (PDT), together with minimal dark toxicity.	[169]
	5-FU	Pectin-coated chitosan microgels prepared through a superhydrophobic surface-based encapsulation technology	– Oral and topical chemotherapy; – Drug-loaded chitosan dispersions are cross-linked and coated with chitosan or pectin; – Growth inhibition of cancer cells by 5-FU is greater when incorporated to chitosan microgels.	[170]
		Chitin nanogels	– pH responsive swelling and drug release; – Specific toxicity on CM cell lines; – Retention in the deeper layers of skin was 4–5 times more from chitin nanogels than from control 5-FU.	[171]
	Cisplatin	PAMAM (poly(amidoamine)) dendrimers	– Cisplatin–dendrimer complexes accumulate preferentially at the tumor site *in vivo* (mice); – Slower release, higher accumulation in solid tumors, and lower toxicity than free cisplatin.	[172]
Liposomes	Sorafenib	Pegylated nanoliposomal ceramide combined with sorafenib	– Pegylated nanoliposomes contain 30 mol% ceramide; – Nanoliposomal ceramide enhances effectiveness of sorafenib causing synergistic inhibition *in vitro* and *in vivo*.	[173]
	T4N5	Encapsulated in liposomes, composed of amphiphilic phospholipids, under mild conditions	– New approach for topical application of DNA repair enzymes to human skin in order to prevent skin cancer; – The enzyme entrapped in liposomes maintains thermal stability.	[174]
		T4N5 liposome lotion	– DNA repair enzymes to sun-damaged skin of patients lowered the rate of development of skin cancer.	[175]
	Aloe-emodin	Liposomes	– Liposomal formulation accelerated death of A431 and SCC25 cells (skin cancer models) and enhanced transdermal delivery of aloe-emodin.	[176]
	Bleomycin	Ultra-deformable liposomes (Bleosome®)	– Bleosome® facilitated entrapment of high concentrations of active bleomycin; – *In vitro* data revealed that the LD_{50} of bleomycin encapsulated in Bleosome® was approximately three-fold higher than free bleomycin solution.	[177]
	Doxorubicin	Pegylated liposomes	– Limited clinical efficacy in CM; – Whether the efficacy of liposomal doxorubicin can be improved by combinations needs further studies.	[178]
	c-Myc siRNA	Targeted liposome-polycation-DNA (LPD) NPs containing cationic liposomes	– Formulation targeted with anisamide, which binds with the sigma 1 receptor of melanoma cells; – Cationic lipid N,N-distearyl-N-methyl-N-2-(N′-arginyl) aminoethyl ammonium chloride (DSAA), which contains an arginine residue as the head group, is a critical element for the success of the nanoparticle; – Expression of Bcl-2 is downregulated in B16F10 melanoma cells after the treatment, rendering the cancer cell more sensitive to cancer therapy.	[179]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Nanosuspensions/ nanoemulsions	5-ALA	Self-adhesive 5-ALA patch	– Thin self-adhesive patch containing 5-ALA to facilitate PDT; phase III studies have already been finished: NCT00308854.	[180]
		Encapsulation in a nanoemulsion, by a spontaneous emulsification process	– Polymeric-based nanoemulsion produced from polylactide-polyglycol and egg-phosphatidylcholine lipids (50:50), prepared at 55 °C; – Oil-in-water (o/w) polymeric nanoemulsion.	[181]
	Foscan (photosensitizer)	Nanoemulsion as a colloidal vehicle of the oil/water (o/w) type for topical administration	– Second-generation photosensitizer drug for PDT; – Formulation stabilized based on the mixture of two phases, an aqueous solution and an organic medium consisting of nonionic surfactants and oil; – Foscan diffusion flux through skin layers increase when incorporated into the nanoemulsion.	[182]
Lipid nanoparticles	Docetaxel	NLC prepared by the modified film ultrasonication–dispersion method	– Effective inhibition of tumor growth and lower toxicity in murine CM treatment.	[183]
	Topotecan	NLC and SLN prepared by microemulsion technique	– High drug loading capacity for topotecan; – Nanoencapsulation sustained topotecan release and improved its chemical stability and cytotoxicity; – No significant differences between the NLCs and SLNs, both are potential carriers for topotecan.	[184]
	Doxorubicin/ idarubicin	Solid lipospheres	– Lipospheres composed of stearic acid and egg lecithin; – High drug loading capacity of drug.	[185]
	Doxorubicin	Cationic SLN, composed of used stearic acid or a 1:2 mixture of stearic acid and glyceryl behenate for topical chemotherapy	– The ratio lipid:water phase and the ratio surfactant (poloxamer):co-surfactant (cetylpyridinium chloride) affect the zeta potential values; – High entrapment efficiency in formulations with higher amounts of stearic acid (cationic charges on doxorubicin interact with the negative charges in stearic acid); – Encapsulation of doxorubicin significantly increases cytotoxicity.	[186]
	Daunorubicin	Cholesterol-rich nanoemulsion formulation prepared by high-pressure homogenization of lipid mixtures	– Improved tumor growth inhibition and survival rates with pronouncedly less toxicity in treated mice.	[187]
	Camptothecin	SLN coated with poloxamer 188, produced by high pressure homogenization, intended for peroral route	– High entrapment efficiency and sustained release; – *In vitro* drug release achieved up to a week; – *In vivo*, the area under curve (AUC) and mean residence time (MRT) of SLN increased significantly compared with control solution.	[188]
	Etoposide	SLN of various triglycerides, prepared by hot homogenization technique	– Trimysristin exhibited suitability for fabrication of NPs; – SLN enhanced the ratio of the drug that reaches to the highly perfused organs (metastasized tumors).	[189]
	Methotrexate	Hyaluronan-coated lipid based- NPs	– Half-life of 13.75 days and improved therapeutic outcome in a murine B16F10 melanoma; – Hyaluronan is a selective and safe active cellular targeting moiety.	[190]
	Resveratrol	SLNs	– Chemopreventive drug; – Resveratrol solubility, stability and intracellular delivery increased by loading into SLN; – Increased cytostatic effect of SLN–resveratrol: potential benefits for prevention of skin cancer.	[191]
	Nitrosyl ruthenium complex	SLNs and NLCs prepared via the microemulsification method	– Sustained release, nitric oxide (NO) donor, with CM cell culture toxicity; – Lyophilization improve the complex stability; – Approximately twice NO release from the SLN than from control solution.	[192]
	Antigenic peptides	Lipid-coated poly(D,L-lactide-co-glycolide) NPs by the double emulsion method	– Loading efficiency of hydrophilic peptides improved when lipids were introduced to formulate lipid-coated NPs; – Combinational delivery of lipid-coated NPs carrying different peptides significantly suppressed growth of inoculated B16 melanoma cells.	[193]
	OxBu (guanosine-analog phosphonate)	SLNs	– High entrapment efficiency; – OxBu-loaded-SLN efficacy was superior to equimolar 5-FU solution.	[194]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Carbon-based nanoparticles	Carboplatin	CNTs / carbon nanofibers	– CNTs act as a drug depot, constantly releasing up to 68% within 14 days; – Carboplatin released by CNTs exhibits a higher anticancer activity than free carboplatin.	[195]
	Camptothecin analogue (SN38)	PEGylated NGO	– SN38 is water insoluble; – Resulting complex exhibits excellent water solubility while maintaining its high cancer cell killing potency; – Graphene is a promising material for *in vivo* cancer treatment with various aromatic, low-solubility drugs, as SN38.	[196]
	Camptothecin	Thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM)-grafted graphene sheets	– Consist of about 50% polymer, which endows good solubility and stability in physiological solutions; – High loading capacity; – High potency for killing cancer cells *in vitro*.	[197]
		Chitosan-functionalized NGO sheets	– Chitosan-grafted NGO sheets consist of about 64 wt.% chitosan, which provides a good aqueous solubility and biocompatibility; – High loading capacity and high cytotoxicity.	[198]
		Multiwalled CNTs/graphene oxide, functionalized by poly(vinyl alcohol) (PVA)	– Functionalized by a highly hydrophilic and biocompatible moiety: PVA; – Camptothecin loaded through π–π interactions; – High cytotoxicity.	[199]
	5-FU	Graphene nanosheet–CNT–iron oxide nanoparticle hybrid	– Hybrid with superparamagnetic properties; – High loading capacity and pH-activated release profile; – Promising candidate for anti-cancer drug delivery systems.	[200]
	Plasmid DNA (pDNA)	Chitosan-functionalized NGO	– Good aqueous solubility and biocompatibility; – Reasonable transfection efficiency.	[198]
		Graphene oxide–PEI nanoconstruct	– Conjugation of low-molecular weight branched PEI to NGO improves DNA binding, condensation and transfection efficiency; – Covalent linking of PEI to NGO via an amide bond; – Promising candidate for efficient gene delivery.	[201]
	siRNA (B-raf)	Single-walled CNTs, functionalized non-covalently with succinated polyethyleimine (PEI-SA)	– Gene silencing (B-raf) induced by siRNA complexes achieved *in vitro* in B16-F10 cells; – *In vivo* delivery was topically applied; – Attenuation of tumor growth.	[202]
	Ribonuclease A (RNase A)	PEGylated NGO (functionalized with amine-terminated 6-armed PEG molecules)	– Physiological stability and biocompatibility, and high payload capacity; – Efficient delivery of RNase A to cytoplasm, protecting them from enzymatic hydrolysis and leading to cell death.	[203]
	Antigens	CNT-polymer composite for immunotherapy	– CNT-polymer composite acts as an artificial antigen-presenting cell to expand the number of T cells; – Antigens attached onto CNTs and combined with polymer NPs containing magnetite and IL-2; – Tumor growth delayed in a murine model for CM.	[204]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Magnetic nanoparticles	Doxorubicin	Micellar hybrid NPs that contain MNPs and QDs within a single PEG-phospholipid micelle	– Long-circulating NPs composed of spherical oleic-acid coated MNPs and QDs, encapsulated simultaneously within a micelle composed of a PEG-modified phospholipid; – Hydrophobic chains of the PEG-phospholipids interact strongly with hydrophobic chains attached to the MNPs and QDs, providing high dispersibility/stability; – Simultaneous targeted drug delivery and imaging of diseased tissue *in vitro* and *in vivo*.	[205]
	Curcumin/ doxorubicin	MBCSPs (curcumin in the core and doxorubicin in the shell)	– Thermo-responsive shell of poly(N-isopropylacrylamide-acrylamide-allylamine) and a core of PLGA embedded with MNPs; – MNPs conjugate with peptides that specifically bind to the α [5]β [3] receptors of CM cells; – Dual drug release mechanisms (a sustained release of drugs through degradation of PLGA core and a controlled release in response to changes in temperature via thermo-responsive polymer shell); – Dual targeting mechanisms (magnetic localization and receptor-mediated targeting).	[206]
	Albumin/5-FU	Magnetic nanocomposite spheres fabricated using an oil-in-oil emulsion/ solvent evaporation method	– Nanocomposite consists of human serum albumin, PLGA, 5-FU, MNPs; – Magnetic targeted drug delivery system exhibited significantly superior therapeutic effects in skin cancer.	[207]
	Epirubicin	Functionalized superparamagnetic iron-oxide NPs for transdermal administration	– Magnetism is used for the targeted transdermal chemotherapy of skin tumors; – MNPs loaded with epirubicin inhibit CM proliferation, in a dose-dependent ratio.	[208]
	–	Polymeric NPs with targeting moieties containing MNPs	– Poly(D,L-lactide-co-glycolide)-b-poly(ethylene glycol)-based nanocarrier containing iron oxide NPs, and human epithelial growth factor receptor on the outer shell;} – Decrease of tumor size correlated with an increase in both NP concentration and local temperature.	[209]
Gold nanoparticles	–	PTT using gold nanorods, functionalized with anti-EGF receptor monoclonal antibodies	– NIR region of the radiation spectrum is preferred to minimize the light extinction by intrinsic chromophores in native tissue; – Gold nanorods with suitable aspect ratios (length divided by width) can absorb and scatter strongly in the NIR region (650–900 nm). – The antibody-conjugated nanorods bind specifically to the surface of the malignant-type cells, requiring about half the laser energy to be photothermally destroyed than the nonmalignant cells.	[210]
	Phthalocyanines (photosensitizing agent)	PEGylated GNPs conjugates, administered *in vivo* by injection for PDT	– Water-soluble and biocompatible “cage” that allows delivery of a hydrophobic drug to its site for PDT; – Time for the maximum drug accumulation to the target tumor reduced to only <2 h, compared to 2 days for the free drug.	[211]
	–	Interbilayer-crosslinked multilamellar lipid vesicles as carriers to amphiphilic GNPs, embedded in the capsule walls	– Gold core surrounded by an amphiphilic mixed organic ligand shell; – Greater intracellular spread in CM cells; – Enhanced radiotherapeutic killing of CM cells due to the membrane-penetrating properties of these materials.	[212]
	Doxorubicin	GNPs	– GNPs of doxorubicin are more effective than QDs against the doxorubicin-resistant CM, with more than 60% of the conjugate reaching the cell nucleus.	[213]
		Functionalized GNPs with DNA and aptamer	– DNA and aptamer AS1411 increase the selectivity toward cancer cells; – Reduction of cell viability in CM cell lines.	[214]
Protein transduction technology	siRNA	Lipid NPs modified with CPP	– Improve the stability of the siRNA in serum; – Modification of lipid NPs with protamine-derived CPP is effective to facilitate internalization of siRNA in the cytoplasm and thereby to enhance gene silencing.	[215]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Hsps chaperone-based therapies	Hsp70	Hydrogel (topical application)	– Hydrogel composed of carbopol (1%), glycerol (1%), and dimethylsulfoxide (10%); – Hsp70 diffuses specifically through the skin layer inside the B16 tumor; – Almost two-fold reduction of B16 tumor growth caused by intratumorally delivered pure Hsp70.	[216]
		Vaccine	– Hsp70 induces an infiltration of T cells into the tumor site as well as the expression of IFN-gamma and IL-2, and delay metastases; – Effective antitumor immunity.	[217]
	Hsp70/MNPs	Combined therapy of recombinant Hsp70 and hyperthermia caused by MNPs	– Hyperthermia conducted using magnetite cationic liposomes, which have a positive surface charge and generate heat in an alternating magnetic field; – Following injection of Hsp70 and MNPs in CM nodules, magnetic field generates 43 °C, strongly inhibiting tumor growth and attaining complete regression of tumors in 20% of mice; – Great potential in skin cancer treatment.	[218]
	Hsp70/Hsp90 inhibitors	Combined Hsp90 inhibitor and Hsp70 inhibitor with ferromagnetic particle-mediated hyperthermia; administration through subcutaneous injections	– Hyperthermia produced using thermosensitive ferromagnetic particles; – When exposed to a magnetic field, the temperature of tissues containing ferromagnetic particles increased and stabilized; – Increased antitumor effects with important implications in skin cancer treatment.	[219]
	Flagrp170	Chimeric chaperone administered through adenoviruses (expressing Flagrp170)	– NF-κB-stimulating signal incorporated into the large stress chaperone Grp170, result in Flagrp170; – Flagrp170 is capable of transporting tumor antigens and inducing functional activation of DCs; – Superior *in vivo* antitumor response against CM and its distant metastasis compared with unmodified chaperone.	[220]
Immunotherapies	Hsp70/CD40L	Potent immunological memory induced by intradermal injections of Hsp70 and plasmid expressing CD40L	– Hsp70 acts as a potent immune adjuvant through TLR-4 signaling and local induction of TNF-alpha; – Plasmid expressing CD40L increased therapeutic efficacy and increased both the frequency and activity of T cells activated against tumor cells.	[221]
	Paclitaxel/icariside II	Immunotherapy by inhibition of TLR-4 signaling pathway	– Enhancement of apoptosis through the regulation of apoptotic proteins; – TLR-4 signaling is a novel target for reversing chemoresistance to paclitaxel: icariside treatment can effectively inhibit this paclitaxel-induced activation of TLR-4.	[222]
	CpG-ODN (immunoadjuvant)	Immunoadjuvant CpG in combination with DC immunotherapy (vaccination)	– Enhanced expression of maturation markers and secretion of IL-12p70 and IL-10; – Enhanced stimulation of antigen specific $CD4^+$ and $CD8^+$ T cells; – Tumor regression and long-term protection achieved in a murine B16 melanoma model.	[223]
	c-Myc siRNA	Anisamide-targeted NPs administered by injection	– NPs can systemically deliver siRNA into the cytoplasm of B16F10 murine CM cells; – siRNA delivered by the targeted NPs effectively suppress c-Myc expression in the tumor and inhibited tumor growth; – More significant tumor growth inhibition observed with NPs composed of N,N-distearyl-N-methyl-N-2-(N′-arginyl) aminoethyl ammonium chloride (DSAA), a guanidinium-containing cationic lipid, which induces ROS, triggering apoptosis.	[179]
	Poly-IC and blockade of the PD-1/PD-L1 pathway	Combinatorial cancer immunotherapy	– Repeated co-administration of poly-IC and blocking antibodies targeting the programmed cell death-1 (PD-1) pathway; – Tumor-reactive $CD8^+$ T cells mediate the antitumor effects, leading to high inhibition of tumor development.	[224]
	HspX (immunoadjuvant)	DCs-based immunotherapy	– The HspX protein induces DCs maturation and proinflammatory cytokine production (TNF-α, IL-1β, IL-6, and IFN-β) through TLR-4 binding; – Metastatic capacity of B16-BL6 melanoma cancer cells attenuated in mice that received HspX-stimulated DCs.	[225]
	Imiquimod (TLR-7 agonist)/paclitaxel	Micro-dispersions of drugs and poly (γ-glutamic acid) (γ-PGA) using a co-solvent (administered by intra-tumoral injection)	– Water insoluble drugs; – Significant tumor killing effect *in vitro*; – In a mouse CM tumor model, the treatment exemplified drastic inhibition of tumor growth leading to 70% survival.	[226]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Modular nanotransporters	Chlorin e (photosensitizer)	MNTs synthetized through expression in an *E. coli* strain carrying plasmid pREP4	– Two types of MNTs: one targeted to the α-MSH receptor and the other to the EGF receptor; – Increased cytotoxicity by a factor of more than 3000 for the EGFR-expressing A431 human epidermoid carcinoma cell line compared with free chlorin e; – More than 90% tumor growth inhibition and significantly prolonged survival compared with the free drug.	[227]
	Chlorin e/bacteriochlorin *p* (photosensitizers)	MNTs synthetized through expression in an *E. coli* strain carrying plasmid pREP4	– Targeted for cancer cells overexpressing ErbB1 (EGF) receptors; – Photosensitizers–transporter conjugates have more than 3000 times greater efficacy than free photosensitizers for target cells.	[228]
	Bacteriochlorin p (photosensitizer)	MNTs synthetized through expression in an *E. coli* strain carrying plasmid pREP4	– Ligand module, α-MSH, overexpressed on the surface of CM cells; – Increased accumulation in the skin and 93% CM growth inhibition of photosensitizer conjugated to the MNT compared to the free drug.	[229]
Stem cells	–	Neural progenitor cells as cell delivery vehicles for MNPs, for localized magnetic hyperthermia	– Core/shell iron/iron oxide MNP, functionalized with aminosiloxane-porphyrin; – MNPs travel to subcutaneous melanomas, and after magnetic field exposure, resulted in a measurable regression of the tumors.	[230]
	5-ALA	NSC	– NSC transfected with a plasmid expressing gaussia luciferase (gLuc); – Treatment comprised of 5-ALA as a prodrug, gaussia luciferase, and its substrate coelenterazine; – Retardation of tumor growth *in vivo* was observed after coelenterazine-mediated PDT.	[231]
	Irinotecan/ carboxylesterase	NSC expressing carboxylesterase (prodrug-activating enzyme)	– Carboxylesterase converts irinotecan into SN-38, a potent topoisomerase 1 inhibitor; – Robust migration of NSC to CM cells and increased tumor cell-killing by approximately 100-fold when compared to irinotecan alone.	[232]
Ultrasound methodologies	–	HIFU	– Higher survival rate and enhanced cytotoxic T lymphocytes activity in a murine melanoma (B16-F10) model, in the HIFU treatment groups.	[233]
	Paclitaxel	Microbubbles (acoustically active lipospheres)	– Vehicles are microbubbles surrounded by a shell of oil and lipid; microbubbles are deflected by radiation force to a vessel wall, leading to fragmentation; – Greater antiproliferative effect after insonation than free paclitaxel.	[234]
	Quercetin	Low-frequency US	– Pretreatment of cells with US selectively induced cytotoxicity in skin; – US reduced the LC_{50} of quercetin for skin cancer cells by almost 80-fold, while showing no effect on nonmalignant skin cells.	[235]
Redox-active nanoparticles	–	Polymer (dextran)-coated cerium-oxide NPs	– Concentrations of polymer-coated CNPs being nontoxic for stromal cells show a cytotoxic, proapoptotic, and anti-invasive capacity on CM cells; – *In vivo* show a decrease of tumor weight and volume after treatment with coated cerium-oxide NPs.	[236]
	Doxorubicin	Cerium-oxide NPs	– Cerium-oxide NPs enhance the antitumor activity of doxorubicin in human CM cells; – Synergistic effects on cytotoxicity, reactive oxygen species generation, and oxidative damage in tumor cells; – NPs do not cause DNA damage and even protect human dermal fibroblasts from doxorubicin-induced cytotoxicity.	[237]
Quantum dots	Temozolomide	Polysaccharide-based hybrid nanogels, prepared by *in situ* immobilization of CdSe QDs in the interior of polymer networks	– Hydroxypropylcellulose–poly(acrylic acid) (HPC-PAA) semi-interpenetrating polymer; – pH-sensing, cancer cell imaging, and controlled drug release into a single NP system; – HPC-PAA is pH and temperature dual responsive; – High drug loading capacity for temozolomide, and pH-triggered sustained-release.	[238]
	Camptothecin	NLCs with QDs	– High drug loading capacity; – Cytotoxicity of the NPs against CM cells was superior to that of free camptothecin.	[239]
	–	PEG-lipid coated QDs encapsulated into the aqueous core of liposome vesicles, by intravenous administration	– Rapid blood clearance was observed following intravenous administration of the cationic hybrid vesicles, while incorporation of PEG dramatically prolonged their blood circulation; – Rapid accumulation and prolonged retention within the tumor (CM).	[240]
	–	QDs concealed within hydrogel (poly-N-isopropylacrylamide) NPs	– Hydrogel encapsulated QDs are more readily taken up by CM cells; – 16-fold greater intratumoral uptake compared to non-derivatized QDs.	[241]
	Artificial APCs (immunotherapy)	Artificial APCs based on biocompatible iron-dextran paramagnetic particles and avidin-coated QD nanocrystals	– *In vivo*, both iron-dextran particles and QD nanocrystals enhanced tumor rejection in a mouse CM model; – First description of nanoscale artificial APCs that lead to effective T cell stimulation and inhibition of tumor growth.	[242]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Silica nanoparticles	2-Devinyl-2-(1-hexyloxyethyl) pyropheophorbide (photosensitizer)	Ultrafine organically modified SiNPs, synthesized in the nonpolar core of micelles by hydrolysis of triethoxyvinylsilane	– Stable aqueous dispersion of hydrophobic photosensitizers; – Suitable wavelength irradiation of the drug entrapped in NPs results in efficient generation of singlet oxygen, being possible by the inherent porosity of the NPs; – *In vitro* studies have demonstrated the active uptake into tumor cells, with significant damage upon irradiation.	[243]
	Silicon phthalocyanine 4 (Pc4) (photosensitizer)	SiNPs	– Pc4 aggregates in aqueous solutions, which dramatically reduces its PDT efficacy; – SiNPs improve the aqueous solubility, stability, and delivery of the drug but also increase its photodynamic efficacy, being more phototoxic to CM cells than free drug.	[244]
	Protoporphyrin IX (photosensitizer)	Phospholipid-capped, protoporphyrin IX loaded mesoporous SiNPs	– Cellular uptake of the nanoPDT system is greater than that of free Protoporphyrin IX; – nanoPDT system mitigates nearly 65% of CM growth *in vivo*.	[245]
	Hyaluronidase	SiNPs injected peritumorally	– CM tumor models indicate large overexpression of hyaluronan; – Tumor volume reduction with SiNP: immobilized hyaluronidase was significantly enhanced compared to non-immobilized hyaluronidase.	[246]
	Rose bengal (photosensitizer)	Modified mesoporous SiNPs	– Able to reduce cell proliferation in one of the most aggressive skin cancer types (SK-MEL-28) after green-light irradiation.	[247]
Electroporation, electrochemotherapy, iontophoresis and nanosecond pulsed electric fields	Bleomycin (intralesional)	ECT	– 72% of patients showed a complete response; – 5% showed a partial response.	[248]
	Bleomycin (intravenous injection)	ECT	– Complete response observed in 48.4% of the patients and a partial response in 38.3%; – 44.8% of complete responders experience a long-lasting response after one session, being disease-free after a mean duration of 27.5 months.	[249]
		ECT	– Bleomycin solution administered 8 minutes before the application of electric pulses, generated by a Cliniporator®; – Response rate of 100% and, twenty-four months later, local tumor control rate of 72%.	[250]
	Doxorubicin	Iontophoresis for topical delivery of SLN	– Iontophoresis of doxorubicin-SLN increased drug delivery to the viable epidermis, with 56% of all doxorubicin penetrating this skin layer; – Increased doxorubicin cytotoxicity against CM cells by 50%.	[251]
	Cisplatin	ECT	– Increased cellular accumulation and cytotoxicity in murine CM cell lines with low and high metastatic potentials (B16-F1 and B16-F10); – Significant dose-dependent tumor growth delay in the two tumor models used *in vivo*.	[252]
	pDNA (granulocyte macrophage colony-stimulating factor)	Immunotherapy-based gene electrotransfer (EPT)	– Combination of regulatory T cells depletion and immunotherapy pDNA delivered into the B16F10 melanoma tumor model via electroporation; – Not effective in increasing survival but effective in the suppression of metastases.	[253]
	Plasmid IL-12	EPT (DNA electroporation for immunotherapy)	– Patients received electroporation immediately after DNA injection; – 10% of patients showed complete regression of all metastases, whereas 42% showed disease stabilization or partial response.	[254]
	Plasmid IL-15	EPT (DNA electroporation for immunotherapy)	– Plasmid IL-15 was delivered three times over the course of a week in a CM mouse model; – Increased IL-15 expression within the tumor compared to the injection only control; – Tumor regression, long-term survival and greater protection against recurrence.	[255]
	–	nsPEF	– The application of high-voltage, ultrashort electrical pulses to murine melanomas *in vivo* results in complete tumor remission within an average of 47 days in the 17 animals treated; – None of these CMs recurred during a 4-month period after the initial CM had disappeared; – nsPEF leads to apoptosis and reduction of blood flow to the tumor.	[256]
	–	nsPEF	– nsPEF with intensity of 20 kV/cm and duration of 300 ns leads to apoptosis and angiogenesis inhibition.	[257]
Microneedles	Vaccine	Microparticulate melanoma cancer vaccine administered via the transdermal route using microneedle-based Dermaroller®	– Microparticles taken up by the APCs which demonstrated a strong IgG titer level; – Incorporation in an albumin matrix which acts as a synthetic adjuvant; – Animals showed protection after transdermal vaccination.	[258]

(*continued on next page*)

Table 2 (*continued*)

Technology	Molecules/drug	Methodology	Highlights	Ref.
Gene gun therapy	pWRG-neu (DNA vaccine)	Helios gene gun system (gene gun-mediated pWRG-neu immunization)	– DNA vaccine pWRG-neu immunized to mice in the abdominal skin 3 times at two-weekly interval; – Growth and metastasis of neu-overexpressed CM reduced dramatically.	[259]
	Tyrosinase-related protein 2 gene (TRP2)	DNA-coated gold beads administered by helios gene gun system (DNA vaccine)	– Biolistic DNA vaccination with plasmids encoding the TRP2 gene, in a DNA-coated gold beads; – Induction of TRP2-specific T-cells and antibodies against B16 melanoma cells.	[260]
	Fused-gene DNA vaccine (Her-2/neu and p53)	DNA-coated gold particles administered by helios gene gun system (DNA vaccine)	– Reduce the size of established tumors and prolong the lifespan of tumor-bearing mice; – Enhance the antigen-specific cellular and humoral immune responses.	[261]
Phototherapies	Imiquimod/ indocyanine green (photosensitizer)	ISPI	– One or multiple 6-week treatment cycles applied to a 200-cm^2 treatment site; – Complete response observed in 6 of 11 patients; – Probability of 12-month overall survival was 70%.	[262]
	–	Hyperthermia by poly(ethylene glycol)-modified gold nanorods	– PEG chain was optimized in order to stabilize the gold nanorods in the blood circulation after intravenous injection; – Significant tissue damage and suppression of tumor growth observed only in the presence of gold nanorods and laser irradiation.	[263]
	–	PTT with fluorescent CdTe and CdSe QDs, coated with a silica shell	– Growth of mouse CM tumors significantly inhibited after laser irradiation; – CdTe and CdSe QDs have great potential in the treatment of cancer using PTT.	[264]
	–	NGO–HA conjugate for PTT	– Remarkable transdermal delivery to tumor tissues in the skin of mice; – NIR irradiation resulted in complete ablation of tumor tissues with no recurrence of tumorigenesis.	[265]
	–	CM-targeted hollow gold nanospheres for PTT, administered by intravenous injection	– Gold nanospheres stabilized with PEG coating and attached with α-MSH analogue (whose receptor is overexpressed in CM); – Thin gold wall with hollow interior, which displays strong and tunable resonance absorption in the NIR region; – NPs were specifically taken up by CM cells and selective PTT of B16/F10 melanoma was obtained.	[266]
	–	PTT/PDT through gold nanoshells	– Gold nanoshells not only are able to absorb NIR light, but can also emit fluorescence, sensitize formation of singlet oxygen and exert photodynamic destruction of solid tumors in mice; – PDT/PTT can be controlled and switched from one to the other by simply changing the excitation wavelength; – Combination of PDT and PTT effects on destruction of solid tumors is far better than pure PTT effect and also than doxorubicin.	[267]
	5-ALA	PDT	– Metastatic skin cancer cells treated by 5-ALA and then irradiated by 90-femtosecond (fs) laser with different pulse powers for different durations; – 635 nm, 45 mW pulse energy at 90 fs laser pulse applications for 60 sec to 1 mM 5-ALA exposed cells decreased the cell proliferation by 30%.	[268]
Cold atmospheric plasma	–	Air plasma with GNPs conjugated to antibodies (anti-FAK)	– Human CM cells were placed 2 mm from the plasma source and exposed to 40 s treatment; – Non-thermal plasmas can stimulate GNPs located inside cells to cause cell death; – Air plasma is coupled with FAK-GNPs, resulting in a 5 times increase in cell death over the case with the plasma alone.	[269]
	–	Plasma jet (helium)	– *In vitro* and *in vivo* studies revealed that CAP selectively kills CM cells, whose mechanism is dependent on the CAP device and doses; – Beyond the direct external influence of the jet, CAP may induce living cells to produce their own oxidant species.	[270]
	–	Hand-held and battery-operated CAP device using the surface micro discharge (SMD) technology for air plasma production	– Irreversible cell inactivation with 2 min of CAP treatment, as it strongly induces apoptosis.	[242]

in tumor cells. Several different gases can be used to produce CAP such as Helium, Argon, Nitrogen, Heliox and air [441]. A few *in vitro* and *in vivo* studies have been published regarding successful CAP use in skin cancer [269,270,442–444].

Concluding remarks and future prospects

Skin cancer affects millions of people yearly. Although vast research has increased our knowledge, the disease remains fatal. Limitations in the current therapies are signal for the requirement of novel therapies. Among several treatment approaches, nanotechnology provides an exceptional opportunity on the molecular scale through specific interaction with cancer cells and inhibition of their function. Several formulations have already made their way into the medical practice and have become the standard of care. Most exciting are theranostic agents, which combine treatment with imaging into a unique formulation. Although expensive, theranostic properties may lead to fast development, as pharmacokinetic information and treatment efficacy data can be obtained at the same time.

Despite the noteworthy developments recently made, nanotechnology is still a young subject and little is known about the long-term exposure to these materials. Besides, due to the wide diversity of nanomaterials available, their toxicity may range from inert to highly toxic, which may be difficult in clinical development. In order to rapidly progress, focus must be retained on the safety of these materials. Even though there are several reports stating that a specific NP is biologically inert, capping agents and ligands may change the toxicity of the particle.

Therefore, for a successful translation of NPs to clinical practice, several features need to be considered: biodistribution, pharmacokinetics, metabolism, long-term toxicity and degradation. Although more research is necessary, nanotechnology may play an important role in individualized medicine. Since they show fundamentally new properties at the nano and micro scales, novel molecular architectures may be made-up with a high degree of precision and flexibility.

Conflict of interest

The authors declare no conflict of interest.

References

[1] J. D'Orazio, S. Jarrett, A. Amaro-Ortiz, T. Scott, UV radiation and the skin, Int. J. Mol. Sci. 14 (6) (2013) 12222–12248, doi:10.3390/ijms140612222.
[2] J.M. Elwood, J. Jopson, Melanoma and sun exposure: an overview of published studies, Int. J. Cancer 73 (2) (1997) 198–203.
[3] N.J. Lowe, An overview of ultraviolet radiation, sunscreens, and photo-induced dermatoses, Dermatol. Clin. 24 (1) (2006) 9–17, doi:10.1016/j.det.2005.08.001.
[4] A. Slominski, J. Wortsman, Neuroendocrinology of the skin, Endocr. Rev. 21 (5) (2000) 457–487, doi:10.1210/edrv.21.5.0410.
[5] E. Fuchs, Scratching the surface of skin development, Nature 445 (7130) (2007) 834–842, doi:10.1038/nature05659.
[6] A.T. Slominski, M.A. Zmijewski, C. Skobowiat, B. Zbytek, R.M. Slominski, J.D. Steketee, Sensing the environment: regulation of local and global homeostasis by the skin neuroendocrine system, Adv. Anat. Embryol. Cell Biol. 212 (2012) 1–115, v, vii.
[7] E. Proksch, J.M. Brandner, J.M. Jensen, The skin: an indispensable barrier, Exp. Dermatol. 17 (12) (2008) 1063–1072.
[8] K. Madison, Barrier function of the skin: "La Raison d'être" of the epidermis, J. Invest. Dermatol 121 (3) (2003) 231–241.
[9] H.W. Rogers, M.A. Weinstock, A.R. Harris, M.R. Hinckley, S.R. Feldman, A.B. Fleischer, et al., Incidence estimate of nonmelanoma skin cancer in the United States, 2006, Arch. Dermatol. 146 (3) (2010) 283–287, doi:10.1001/archdermatol.2010.19.
[10] A.C. Geller, G.D. Annas, Epidemiology of melanoma and nonmelanoma skin cancer, Semin. Oncol. Nurs. 19 (1) (2003) 2–11.
[11] B.K. Armstrong, A. Kricker, The epidemiology of UV induced skin cancer, J. Photochem. Photobiol. B. 63 (1–3) (2001) 8–18.
[12] D.L. Narayanan, R.N. Saladi, J.L. Fox, Ultraviolet radiation and skin cancer, Int. J. Dermatol. 49 (9) (2010) 978–986, doi:10.1111/j.1365-4632.2010.04474.x.
[13] M.R. Donaldson, B.M. Coldiron, No end in sight: the skin cancer epidemic continues, Semin. Cutan. Med. Surg. 30 (1) (2011) 3–5, doi:10.1016/j.sder.2011.01.002.
[14] WHO, Ultraviolet radiation and the INTERSUN Programme – Skin Cancers. World Health Organization. Available from: <http://www.who.int/uv/faq/skincancer/en/index1.html>, 2011 (accessed 14.03.16).
[15] F.R. de Gruijl, Skin cancer and solar UV radiation, Eur. J. Cancer 35 (14) (1999) 2003–2009.
[16] B. Suarez, G. Lopez-Abente, C. Martinez, C. Navarro, M.J. Tormo, S. Rosso, et al., Occupation and skin cancer: the results of the HELIOS-I multicenter case-control study, BMC Public Health 7 (2007) 180, doi:10.1186/1471-2458-7-180.
[17] D.G. Coit, R. Andtbacka, C.J. Anker, C.K. Bichakjian, W.E. Carson 3rd, A. Daud, et al., Melanoma, J. Natl. Compr. Canc. Netw. 10 (3) (2012) 366–400.
[18] P. David, S.Q. Wang, Skin cancer facts the skin cancer foundation. [updated October 9, 2013; Available from: <http://www.skincancer.org/skin-cancer-information/skin-cancer-facts> (accessed 14.03.16.).
[19] J.S. Rhee, B.A. Matthews, M. Neuburg, B.R. Logan, M. Burzynski, A.B. Nattinger, The skin cancer index: clinical responsiveness and predictors of quality of life, Laryngoscope 117 (3) (2007) 399–405, doi:10.1097/MLG.0b013e31802e2d88.
[20] P. Boyle, J.F. Dore, P. Autier, U. Ringborg, Cancer of the skin: a forgotten problem in Europe, Ann. Oncol. 15 (1) (2004) 5–6.
[21] A.M. Forsea, V. Del Marmol, E. de Vries, E.E. Bailey, A.C. Geller, Melanoma incidence and mortality in Europe: new estimates, persistent disparities, Br. J. Dermatol. 167 (5) (2012) 1124–1130, doi:10.1111/j.1365-2133.2012.11125.x.
[22] L. Rittie, S. Kansra, S.W. Stoll, Y. Li, J.E. Gudjonsson, Y. Shao, et al., Differential ErbB1 signaling in squamous cell versus basal cell carcinoma of the skin, Am. J. Pathol. 170 (6) (2007) 2089–2099, doi:10.2353/ajpath.2007.060537.
[23] AAD, Skin cancer – stats and facts American Academy of Dermatology. Available from: <http://www.aad.org/media-resources/stats-and-facts/conditions/skin-cancer>, 2011 (accessed 14.03.16.).
[24] L. O'Driscoll, J. McMorrow, P. Doolan, E. McKiernan, J.P. Mehta, E. Ryan, et al., Investigation of the molecular profile of basal cell carcinoma using whole genome microarrays, Mol. Cancer 5 (2006) 74, doi:10.1186/1476-4598-5-74.
[25] A. Lomas, J. Leonardi-Bee, F. Bath-Hextall, A systematic review of worldwide incidence of nonmelanoma skin cancer, Br. J. Dermatol. 166 (5) (2012) 1069–1080, doi:10.1111/j.1365-2133.2012.10830.x.
[26] H.M. Gloster Jr., K. Neal, Skin cancer in skin of color, J. Am. Acad. Dermatol. 55 (5) (2006) 741–760, quiz 61-4, doi:10.1016/j.jaad.2005.08.063.
[27] K.T. Flaherty, I. Puzanov, K.B. Kim, A. Ribas, G.A. McArthur, J.A. Sosman, et al., Inhibition of mutated, activated BRAF in metastatic melanoma, N. Engl. J. Med. 363 (9) (2010) 809–819, doi:10.1056/NEJMoa1002011.
[28] J.A. Sosman, K.B. Kim, L. Schuchter, R. Gonzalez, A.C. Pavlick, J.S. Weber, et al., Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib, N. Engl. J. Med. 366 (8) (2012) 707–714, doi:10.1056/NEJMoa1112302.
[29] V.A. Nikolaou, A.J. Stratigos, K.T. Flaherty, H. Tsao, Melanoma: new insights and new therapies, J. Invest. Dermatol. 132 (3 Pt 2) (2012) 854–863, doi:10.1038/jid.2011.421.
[30] K.T. Flaherty, Targeting metastatic melanoma, Annu. Rev. Med. 63 (2012) 171–183, doi:10.1146/annurev-med-050410-105655.
[31] Z. Ji, K.T. Flaherty, H. Tsao, Targeting the RAS pathway in melanoma, Trends Mol. Med. 18 (1) (2012) 27–35, doi:10.1016/j.molmed.2011.08.001.
[32] F.S. Hodi, D.A. Oble, J. Drappatz, E.F. Velazquez, N. Ramaiya, N. Ramakrishna, et al., CTLA-4 blockade with ipilimumab induces significant clinical benefit in a female with melanoma metastases to the CNS, Nat. Clin. Pract. Oncol. 5 (9) (2008) 557–561, doi:10.1038/ncponc1183.
[33] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, et al., Improved survival with ipilimumab in patients with metastatic melanoma, N. Engl. J. Med. 363 (8) (2010) 711–723, doi:10.1056/NEJMoa1003466.
[34] D. Whiteman, A. Green, Epidemiology of malignant melanoma, in: R. Dummer, M.R. Pittelkow, K. Iwatsuki, A. Green, N.M. Elwan (Eds.), Skin Cancer – a World-wide Perspective, Springer Berlin Heidelberg, 2011.
[35] D.S. Rigel, Epidemiology of melanoma, Semin. Cutan. Med. Surg. 29 (4) (2010) 204–209, doi:10.1016/j.sder.2010.10.005.
[36] S. Franceschi, M. Cristofolini, Cutaneous malignant melanoma: epidemiological considerations, Semin. Surg. Oncol. 8 (6) (1992) 345–352.
[37] J.K. Rivers, The detection and management of dysplastic nevi and early melanoma, World J. Surg. 16 (2) (1992) 166–172.
[38] C.F. Garland, F.C. Garland, E.D. Gorham, Rising trends in melanoma. An hypothesis concerning sunscreen effectiveness, Ann. Epidemiol. 3 (1) (1993) 103–110.
[39] S.N. Markovic, L.A. Erickson, R.D. Rao, R.H. Weenig, B.A. Pockaj, A. Bardia, et al., Malignant melanoma in the 21st century, part 1: epidemiology, risk factors, screening, prevention, and diagnosis, Mayo Clin. Proc. 82 (3) (2007) 364–380, doi:10.4065/82.3.364.
[40] L. Brochez, J.M. Naeyaert, Understanding the trends in melanoma incidence and mortality: where do we stand?, Eur. J. Dermatol. 10 (1) (2000) 71–75.
[41] T.L. Diepgen, V. Mahler, The epidemiology of skin cancer, Br. J. Dermatol. 146 (Suppl. 61) (2002) 1–6.
[42] J.S. Mulliken, J.E. Russak, D.S. Rigel, The effect of sunscreen on melanoma risk, Dermatol. Clin. 30 (3) (2012) 369–376, doi:10.1016/j.det.2012.04.002.
[43] R.N. Saladi, A.N. Persaud, The causes of skin cancer: a comprehensive review, Drugs Today (Barc.) 41 (1) (2005) 37–53, doi:10.1358/dot.2005.41.1.875777.

[44] U. Leiter, C. Garbe, Epidemiology of melanoma and nonmelanoma skin cancer – the role of sunlight, Adv. Exp. Med. Biol. 624 (2008) 89–103, doi:10.1007/978-0-387-77574-6_8.

[45] M. Norval, R.M. Lucas, A.P. Cullen, F.R. de Gruijl, J. Longstreth, Y. Takizawa, et al., The human health effects of ozone depletion and interactions with climate change, Photochem. Photobiol. Sci. 10 (2) (2011) 199–225, doi:10.1039/C0PP90044C.

[46] V. Nikolaou, A.J. Stratigos, Emerging trends in the epidemiology of melanoma, Br. J. Dermatol. 170 (1) (2014) 11–19, doi:10.1111/bjd.12492.

[47] M.A. Weinstock, Cutaneous melanoma: public health approach to early detection, Dermatol. Ther. 19 (1) (2006) 26–31, doi:10.1111/j.1529-8019.2005.00053.x.

[48] R. Gordon, Skin cancer: an overview of epidemiology and risk factors, Semin. Oncol. Nurs. 29 (3) (2013) 160–169, doi:10.1016/j.soncn.2013.06.002.

[49] W.B. Grant, J. Moan, J. Reichrath, Comment on "The effects on human health from stratospheric ozone depletion and its interactions with climate change" by M. Norval, A. P. Cullen, F. R. de Gruijl, J. Longstreth, Y. Takizawa, R. M. Lucas, F. P. Noonan and J. C. van der Leun, Photochem. Photobiol. Sci., 2007, 6, 232, Photochem. Photobiol. Sci. 6 (8) (2007) 912–915, doi:10.1039/B705482C.

[50] M. Norval, A.P. Cullen, F.R. de Gruijl, J. Longstreth, Y. Takizawa, R.M. Lucas, et al., The effects on human health from stratospheric ozone depletion and its interactions with climate change, Photochem. Photobiol. Sci. 6 (3) (2007) 232–251, doi:10.1039/B700018A.

[51] K. Togsverd-Bo, S.S. Sorensen, M. Haedersdal, Organ transplant recipients need intensive control and treatment of skin cancer, Ugeskr. Laeger 175 (20) (2013) 1408–1411.

[52] V.Y. Su, Y.W. Hu, K.T. Chou, S.M. Ou, Y.C. Lee, E.Y. Lin, et al., Amiodarone and the risk of cancer: a nationwide population-based study, Cancer 119 (9) (2013) 1699–1705, doi:10.1002/cncr.27881.

[53] F.P. Thinnes, Nonmelanoma skin cancer is associated with reduced Alzheimer disease risk, Neurology 81 (23) (2013) 2056, doi:10.1212/01.wnl.0000436924.94798.59.

[54] G. Peters, Tumor suppression for ARFicionados: the relative contributions of p16INK4a and p14ARF in melanoma, J. Natl. Cancer Inst. 100 (11) (2008) 757–759, doi:10.1093/jnci/djn156.

[55] L. Zuo, J. Weger, Q. Yang, A.M. Goldstein, M.A. Tucker, G.J. Walker, et al., Germline mutations in the p16INK4a binding domain of CDK4 in familial melanoma, Nat. Genet. 12 (1) (1996) 97–99, doi:10.1038/ng0196-97.

[56] D. Zhang, C. Chen, X. Fu, S. Gu, Y. Mao, Y. Xie, et al., A meta-analysis of DNA repair gene XPC polymorphisms and cancer risk, J. Hum. Genet. 53 (1) (2008) 18–33, doi:10.1007/s10038-007-0215-5.

[57] S. Blankenburg, I.R. Konig, R. Moessner, P. Laspe, K.M. Thoms, U. Krueger, et al., Assessment of 3 xeroderma pigmentosum group C gene polymorphisms and risk of cutaneous melanoma: a case-control study, Carcinogenesis 26 (6) (2005) 1085–1090, doi:10.1093/carcin/bgi055.

[58] S. Blankenburg, I.R. Konig, R. Moessner, P. Laspe, K.M. Thoms, U. Krueger, et al., No association between three xeroderma pigmentosum group C and one group G gene polymorphisms and risk of cutaneous melanoma, Eur. J. Hum. Genet. 13 (2) (2005) 253–255, doi:10.1038/sj.ejhg.5201296.

[59] R.C. Millikan, A. Hummer, C. Begg, J. Player, A.R. de Cotret, S. Winkel, et al., Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study, Carcinogenesis 27 (3) (2006) 610–618, doi:10.1093/carcin/bgi252.

[60] J. Han, G.A. Colditz, J.S. Liu, D.J. Hunter, Genetic variation in XPD, sun exposure, and risk of skin cancer, Cancer Epidemiol. Biomarkers Prev. 14 (6) (2005) 1539–1544, doi:10.1158/1055-9965.EPI-04-0846.

[61] A. Baccarelli, D. Calista, P. Minghetti, B. Marinelli, B. Albetti, T. Tseng, et al., XPD gene polymorphism and host characteristics in the association with cutaneous malignant melanoma risk, Br. J. Cancer 90 (2) (2004) 497–502, doi:10.1038/sj.bjc.6601385.

[62] T. Debniak, R.J. Scott, B. Gorski, C. Cybulski, T. van de Wetering, P. Serrano-Fernandez, et al., Common variants of DNA repair genes and malignant melanoma, Eur. J. Cancer 44 (1) (2008) 110–114, doi:10.1016/j.ejca.2007.10.006.

[63] C. Li, Z. Liu, L.E. Wang, J.E. Gershenwald, J.E. Lee, V.G. Prieto, et al., Haplotype and genotypes of the VDR gene and cutaneous melanoma risk in non-Hispanic whites in Texas: a case-control study, Int. J. Cancer 122 (9) (2008) 2077–2084, doi:10.1002/ijc.23357.

[64] B.J. Nickoloff, J.Z. Qin, V. Chaturvedi, P. Bacon, J. Panella, M.F. Denning, Life and death signaling pathways contributing to skin cancer, J. Investig. Dermatol. Symp. Proc. 7 (1) (2002) 27–35, doi:10.1046/j.1523-1747.2002.19633.x.

[65] F. Maira, A. Catania, S. Candido, A.E. Russo, J.A. McCubrey, M. Libra, et al., Molecular targeted therapy in melanoma: a way to reverse resistance to conventional drugs, Curr. Drug Deliv. 9 (1) (2012) 17–29.

[66] V. Nikolaou, A. Stratigos, D. Bafaloukos, A. Katsambas, Antiangiogenic and antiapoptotic treatment in advanced melanoma, Clin. Dermatol. 31 (3) (2013) 257–263, doi:10.1016/j.clindermatol.2012.08.018.

[67] P. Uribe, S. Gonzalez, Epidermal growth factor receptor (EGFR) and squamous cell carcinoma of the skin: molecular bases for EGFR-targeted therapy, Pathol. Res. Pract. 207 (6) (2011) 337–342, doi:10.1016/j.prp.2011.03.002.

[68] G. Ling, A. Ahmadian, A. Persson, A.B. Unden, G. Afink, C. Williams, et al., PATCHED and p53 gene alterations in sporadic and hereditary basal cell cancer, Oncogene 20 (53) (2001) 7770–7778, doi:10.1038/sj.onc.1204946.

[69] J. Reichrath, Molecular Mechanisms of Basal Cell and Squamous Cell Carcinomas, Springer Science + Business Media, Inc., New York. 2006. 137 p.

[70] R.W. Johnstone, A.J. Frew, M.J. Smyth, The TRAIL apoptotic pathway in cancer onset, progression and therapy, Nat. Rev. Cancer 8 (10) (2008) 782–798.

[71] A.M. Eggermont, Advances in systemic treatment of melanoma, Ann. Oncol. 21 (Suppl. 7) (2010) vii339–vii344, doi:10.1093/annonc/mdq364.

[72] J. Wu, F. Zuo, J. Du, P.F. Wong, H. Qin, J. Xu, Icariside II induces apoptosis via inhibition of the EGFR pathways in A431 human epidermoid carcinoma cells, Mol. Med. Rep. 8 (2) (2013) 597–602, doi:10.3892/mmr.2013.1557.

[73] M.H. Khan, M. Alam, S. Yoo, Epidermal growth factor receptor inhibitors in the treatment of nonmelanoma skin cancers, Dermatol. Surg. 37 (9) (2011) 1199–1209, doi:10.1111/j.1524-4725.2011.02038.x.

[74] P.P. Roux, S.A. Richards, J. Blenis, Phosphorylation of p90 ribosomal S6 kinase (RSK) regulates extracellular signal-regulated kinase docking and RSK activity, Mol. Cell. Biol. 23 (14) (2003) 4796–4804.

[75] C. Hauge, M. Frodin, RSK and MSK in MAP kinase signalling, J. Cell Sci. 119 (Pt 15) (2006) 3021–3023, doi:10.1242/jcs.02950.

[76] N. Arul, Y.Y. Cho, A rising cancer prevention target of RSK2 in human skin cancer, Front. Oncol. 3 (2013) 201, doi:10.3389/fonc.2013.00201.

[77] Y.Y. Cho, M.H. Lee, C.J. Lee, K. Yao, H.S. Lee, A.M. Bode, et al., RSK2 as a key regulator in human skin cancer, Carcinogenesis 33 (12) (2012) 2529–2537, doi:10.1093/carcin/bgs271.

[78] D.H.G. Maglio, M.L. Paz, E.M. Cela, J. Leoni, Cyclooxygenase-2 overexpression in non-melanoma skin cancer: molecular pathways involved as targets for prevention and treatment. 2011. InTech. 272 p.

[79] C.M. Xie, W. Wei, Y. Sun, Role of SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligases in skin cancer, J. Genet. Genomics. 40 (3) (2013) 97–106, doi:10.1016/j.jgg.2013.02.001.

[80] L. Jia, Y. Sun, SCF E3 ubiquitin ligases as anticancer targets, Curr. Cancer Drug Targets 11 (3) (2011) 347–356.

[81] D. Wei, Y. Sun, R.I.N.G. Small, Finger proteins RBX1 and RBX2 of SCF E3 ubiquitin ligases: the role in cancer and as cancer targets, Genes Cancer 1 (7) (2010) 700–707, doi:10.1177/1947601910382776.

[82] M. Mancuso, S. Pazzaglia, M. Tanori, H. Hahn, P. Merola, S. Rebessi, et al., Basal cell carcinoma and its development: insights from radiation-induced tumors in Ptch1-deficient mice, Cancer Res. 64 (3) (2004) 934–941.

[83] A. Sarasin, The molecular pathways of ultraviolet-induced carcinogenesis, Mutat. Res. 428 (1–2) (1999) 5–10.

[84] J.C. Martinez, C.C. Otley, The management of melanoma and nonmelanoma skin cancer: a review for the primary care physician, Mayo Clin. Proc. 76 (12) (2001) 1253–1265, doi:10.4065/76.12.1253.

[85] E.M. Galiczynski, A.T. Vidimos, Nonsurgical treatment of nonmelanoma skin cancer, Dermatol. Clin. 29 (2) (2011) 297–309, x, doi:10.1016/j.det.2011.01.011.

[86] P. Clarke, Nonmelanoma skin cancers – treatment options, Aust. Fam. Physician 41 (7) (2012) 476–480.

[87] V. Lazareth, Management of non-melanoma skin cancer, Semin. Oncol. Nurs. 29 (3) (2013) 182–194, doi:10.1016/j.soncn.2013.06.004.

[88] S. Amini, M.H. Viera, W. Valins, B. Berman, Nonsurgical innovations in the treatment of nonmelanoma skin cancer, J. Clin. Aesthet. Dermatol. 3 (6) (2010) 20–34.

[89] T.H. Nguyen, D.Q. Ho, Nonmelanoma skin cancer, Curr. Treat. Options Oncol. 3 (3) (2002) 193–203.

[90] C. Erickson, S.J. Miller, Treatment options in melanoma in situ: topical and radiation therapy, excision and Mohs surgery, Int. J. Dermatol. 49 (5) (2010) 482–491, doi:10.1111/j.1365-4632.2010.04423.x.

[91] C. Garbe, K. Peris, A. Hauschild, P. Saiag, M. Middleton, A. Spatz, et al., Diagnosis and treatment of melanoma. European consensus-based interdisciplinary guideline–Update 2012, Eur. J. Cancer 48 (15) (2012) 2375–2390, doi:10.1016/j.ejca.2012.06.013.

[92] M. Sanlorenzo, I. Vujic, C. Posch, A. Dajee, A. Yen, S. Kim, et al., Melanoma immunotherapy, Cancer Biol. Ther. 15 (6) (2014) 665–674, doi:10.4161/cbt.28555.

[93] P. Ascierto, V. Chiarion-Sileni, A. Muggiano, M. Mandalà, N. Pimpinelli, M. Del Vecchio, et al., Interferon alpha for the adjuvant treatment of melanoma: review of international literature and practical recommendations from an expert panel on the use of interferon, J. Chemother. (2013) 1973947813Y0000000154.

[94] S. Bhatia, S.S. Tykodi, J.A. Thompson, Treatment of metastatic melanoma: an overview, Oncology (Williston Park) 23 (6) (2009) 488–496.

[95] H. Tsao, A.J. Sober, Melanoma treatment update, Dermatol. Clin. 23 (2) (2005) 323–333, doi:10.1016/j.det.2004.09.005.

[96] T.K. Eigentler, C. Garbe, New landscape in the treatment of melanoma: a 2012 update, Oncologie 15 (2) (2013) 71–77, doi:10.1007/s10269-013-2258-x.

[97] A.M. Leung, D.M. Hari, D.L. Morton, Surgery for distant melanoma metastasis, Cancer J. 18 (2) (2012) 176–184, doi:10.1097/PPO.0b013e31824bc981.

[98] M.E. Dawn, A.G. Dawn, S.J. Miller, Mohs surgery for the treatment of melanoma in situ: a review, Dermatol. Surg. 33 (4) (2007) 395–402, doi:10.1111/j.1524-4725.2007.33085.x.

[99] A. Testori, P. Rutkowski, J. Marsden, L. Bastholt, V. Chiarion-Sileni, A. Hauschild, et al., Surgery and radiotherapy in the treatment of cutaneous melanoma, Ann. Oncol. 20 (Suppl. 6) (2009) vi22–vi29, doi:10.1093/annonc/mdp257.

[100] Z.V. Fong, K.K. Tanabe, Comparison of melanoma guidelines in the U.S.A., Canada, Europe, Australia and New Zealand: a critical appraisal and comprehensive review, Br. J. Dermatol. 170 (1) (2014) 20–30, doi:10.1111/bjd.12687.

[101] J.R. Marsden, J.A. Newton-Bishop, L. Burrows, M. Cook, P.G. Corrie, N.H. Cox, et al., Revised UK guidelines for the management of cutaneous melanoma 2010, J. Plast. Reconstr. Aesthet. Surg. 63 (9) (2010) 1401–1419, doi:10.1016/j.bjps.2010.07.006.

[102] National Comprehensive Cancer Network Inc., NCCN Guidelines® Fort Washington. Available from: <http://www.nccn.org/professionals/physician_gls/f_guidelines.asp>, 2014 (accessed 14.03.29.).
[103] Cancer Care Ontario, Melanoma Evidence-based Series (EBS) and Practice Guidelines (PG) Ontario. Available from: <https://www.cancercare.on.ca/toolbox/qualityguidelines/diseasesite/melanoma-ebs/>, 2013 (accessed 14.03.29.).
[104] Canadian Medical Association, CMA Infobase: Clinical Practice Guidelines Database (CPGs). Available from: <http://www.cma.ca/>, 2014 (accessed 14.03.29.).
[105] R. Dummer, A. Hauschild, M. Guggenheim, U. Keilholz, G. Pentheroudakis, E.G.W. Group, Cutaneous melanoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 23 (Suppl. 7) (2012) vii86–vii91, doi:10.1093/annonc/mds229.
[106] Australian Cancer Network Melanoma Guidelines Revision Working Party, Clinical practice guidelines for the management of melanoma in Australia and New Zealand. Wellington: Cancer Council Australia and Australian Cancer Network, Sydney and New Zealand Guidelines Group. Available from: <https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/cp111.pdf>, 2008 (accessed 14.03.29).
[107] N.R. Telfer, G.B. Colver, C.A. Morton, British Association of D, Guidelines for the management of basal cell carcinoma, Br. J. Dermatol. 159 (1) (2008) 35–48, doi:10.1111/j.1365-2133.2008.08666.x.
[108] D.L. Roberts, A.V. Anstey, R.J. Barlow, N.H. Cox, J.A. Newton Bishop, P.G. Corrie, et al., U.K. guidelines for the management of cutaneous melanoma, Br. J. Dermatol. 146 (1) (2002) 7–17.
[109] R. Motley, P. Kersey, C. Lawrence, British Association of D, British Association of Plastic S, Royal College of Radiologists FoCO, Multiprofessional guidelines for the management of the patient with primary cutaneous squamous cell carcinoma, Br. J. Dermatol. 146 (1) (2002) 18–25.
[110] National Collaborating Centre for Cancer, Guidance on Cancer Services. Improving Outcomes for People with Skin Tumours including Melanoma. The Manual. National Institute for Health and Clinical Excellence, United Kingdom, 2006. Available from: <http://www.nice.org.uk/nicemedia/live/10901/28906/28906.pdf> (accessed14.03.29.).
[111] J.L. Erickson, J.M. Velasco, T.J. Hieken, Compliance with melanoma treatment guidelines in a community teaching hospital: time trends and other variables, Ann. Surg. Oncol. 15 (4) (2008) 1211–1217, doi:10.1245/s10434-007-9789-6.
[112] S.V. Mohan, A.L. Chang, Advanced basal cell carcinoma: epidemiology and therapeutic innovations, Curr. Dermatol. Rep. 3 (2014) 40–45, doi:10.1007/s13671-014-0069-y.
[113] S. Hoelder, P.A. Clarke, P. Workman, Discovery of small molecule cancer drugs: successes, challenges and opportunities, Mol. Oncol. 6 (2) (2012) 155–176, doi:10.1016/j.molonc.2012.02.004.
[114] R. Chakraborty, C.N. Wieland, N.I. Comfere, Molecular targeted therapies in metastatic melanoma, Pharmacogenomics Per. Med. 6 (2013) 49–56, doi:10.2147/PGPM.S44800.
[115] C.H. Heath, N.L. Deep, L. Nabell, W.R. Carroll, R. Desmond, L. Clemons, et al., Phase 1 study of erlotinib plus radiation therapy in patients with advanced cutaneous squamous cell carcinoma, Int. J. Radiat. Oncol. Biol. Phys. 85 (5) (2013) 1275–1281, doi:10.1016/j.ijrobp.2012.09.030.
[116] J. Ulrich, J.T. Hartmann, W. Dorr, S. Ugurel, Skin toxicity of anti-cancer therapy, J. Dtsch. Dermatol. Ges. 6 (11) (2008) 959–977, doi:10.1111/j.1610-0387.2008.06831.x.
[117] B.A. Jereczek-Fossa, A. Zarowski, F. Milani, R. Orecchia, Radiotherapy-induced ear toxicity, Cancer Treat. Rev. 29 (5) (2003) 417–430, doi:10.1016/s0305-7372(03)00066-5.
[118] J.D. Bahner, J.S. Bordeaux, Non-melanoma skin cancers: photodynamic therapy, cryotherapy, 5-fluorouracil, imiquimod, diclofenac, or what? Facts and controversies, Clin. Dermatol. 31 (6) (2013) 792–798, doi:10.1016/j.clindermatol.2013.08.020.
[119] K. Plichta, H.B. Mackley, Radiotherapy for cutaneous malignancies of the head and neck, Operat. Tech. Otolaryngol. Head Neck Surg. 24 (1) (2013) 59–62, doi:10.1016/j.otot.2012.12.004.
[120] M. Mandala, D. Massi, V. De Giorgi, Cutaneous toxicities of BRAF inhibitors: clinical and pathological challenges and call to action, Crit. Rev. Oncol. Hematol. 88 (2) (2013) 318–337, doi:10.1016/j.critrevonc.2013.06.002.
[121] A. Kawczyk-Krupka, A.M. Bugaj, W. Latos, K. Zaremba, A. Sieron, Photodynamic therapy in treatment of cutaneous and choroidal melanoma, Photodiagnosis Photodyn. Ther. 10 (4) (2013) 503–509, doi:10.1016/j.pdpdt.2013.05.006.
[122] M.E. Lacouture, S.A. Boerner, P.M. Lorusso, Non-rash skin toxicities associated with novel targeted therapies, Clin. Lung Cancer 8 (Suppl. 1) (2006) S36–S42.
[123] A.H. Partridge, H.J. Burstein, E.P. Winer, Side effects of chemotherapy and combined chemohormonal therapy in women with early-stage breast cancer, J. Natl. Cancer Inst. Monogr. 30 (30) (2001) 135–142.
[124] M.A. Tran, R.J. Watts, G.P. Robertson, Use of liposomes as drug delivery vehicles for treatment of melanoma, Pigment Cell Melanoma Res. 22 (4) (2009) 388–399, doi:10.1111/j.1755-148X.2009.00581.x.
[125] H.L. Gensler, Prevention by alpha-difluoromethylornithine of skin carcinogenesis and immunosuppression induced by ultraviolet irradiation, J. Cancer Res. Clin. Oncol. 117 (4) (1991) 345–350.
[126] S.M. Kreul, T. Havighurst, K. Kim, E.A. Mendonca, G.S. Wood, S. Snow, et al., A phase III skin cancer chemoprevention study of DFMO: long-term follow-up of skin cancer events and toxicity, Cancer Prev. Res. 5 (12) (2012) 1368–1374, doi:10.1158/1940-6207.CAPR-12-0233.
[127] H.H. Bailey, K. Kim, A.K. Verma, K. Sielaff, P.O. Larson, S. Snow, et al., A randomized, double-blind, placebo-controlled phase 3 skin cancer prevention study of {alpha}-difluoromethylornithine in subjects with previous history of skin cancer, Cancer Prev. Res. 3 (1) (2010) 35–47, doi:10.1158/1940-6207.CAPR-09-0096.
[128] D.B. Yarosh, DNA repair, immunosuppression, and skin cancer, Cutis 74 (5 Suppl.) (2004) 10–13.
[129] E. Hacker, H.K. Muller, N. Hayward, P. Fahey, G. Walker, Enhancement of DNA repair using topical T4 endonuclease V does not inhibit melanoma formation in Cdk4(R24C/R24C)/Tyr-Nras(Q61K) mice following neonatal UVR, Pigment Cell Melanoma Res. 23 (1) (2010) 121–128, doi:10.1111/j.1755-148X.2009.00643.x.
[130] J.A. Cafardi, R. Shafi, M. Athar, C.A. Elmets, Prospects for skin cancer treatment and prevention: the potential contribution of an engineered virus, J. Invest. Dermatol. 131 (3) (2011) 559–561, doi:10.1038/jid.2010.394.
[131] S.P. Stratton, K.L. Saboda, P.B. Myrdal, A. Gupta, N.E. McKenzie, C. Brooks, et al., Phase 1 study of topical perillyl alcohol cream for chemoprevention of skin cancer, Nutr. Cancer 60 (3) (2008) 325–330, doi:10.1080/01635580701840391.
[132] S.P. Stratton, D.S. Alberts, J.G. Einspahr, P.M. Sagerman, J.A. Warneke, C. Curiel-Lewandrowski, et al., A phase 2a study of topical perillyl alcohol cream for chemoprevention of skin cancer, Cancer Prev. Res. 3 (2) (2010) 160–169, doi:10.1158/1940-6207.CAPR-09-0183.
[133] M. Barthelman, W. Chen, H.L. Gensler, C. Huang, Z. Dong, G.T. Bowden, Inhibitory effects of perillyl alcohol on UVB-induced murine skin cancer and AP-1 transactivation, Cancer Res. 58 (4) (1998) 711–716.
[134] J.T. Belanger, Perillyl alcohol: applications in oncology, Altern. Med. Rev. 3 (6) (1998) 448–457.
[135] H.L. Gensler, M. Aickin, Y.M. Peng, M. Xu, Importance of the form of topical vitamin E for prevention of photocarcinogenesis, Nutr. Cancer 26 (2) (1996) 183–191, doi:10.1080/01635589609514474.
[136] J.A. Foote, J.R. Ranger-Moore, J.G. Einspahr, K. Saboda, J. Kenyon, J. Warneke, et al., Chemoprevention of human actinic keratoses by topical DL-alpha-tocopherol, Cancer Prev. Res. 2 (4) (2009) 394–400, doi:10.1158/1940-6207.CAPR-08-0210.
[137] D.J. Shah, R.S. Dronca, Latest advances in chemotherapeutic, targeted, and immune approaches in the treatment of metastatic melanoma, Mayo Clin. Proc. 89 (4) (2014) 504–519, doi:10.1016/j.mayocp.2014.02.002.
[138] M.K. Callahan, J.D. Wolchok, At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy, J. Leukoc. Biol. 94 (1) (2013) 41–53, doi:10.1189/jlb.1212631.
[139] F.M. Kievit, M. Zhang, Cancer nanotheranostics: improving imaging and therapy by targeted delivery across biological barriers, Adv. Mater. 23 (36) (2011) H217–H247, doi:10.1002/adma.201102313.
[140] K.G. Finegan, C. Tournier, The mitogen-activated protein kinase kinase 4 has a pro-oncogenic role in skin cancer, Cancer Res. 70 (14) (2010) 5797–5806, doi:10.1158/0008-5472.CAN-09-3669.
[141] P. Giovanni, P. Giovanni, T. Aldo, L. Pietro, L. Gabriele, G. Fabio, et al., Molecular targeted approaches for advanced BRAF V600, N-RAS, c-KIT, and GNAQ melanomas, Dis. Markers 2014 (2014) 671283, doi:10.1155/2014/671283.
[142] M.A. Iqbal, S. Md, J.K. Sahni, S. Baboota, S. Dang, J. Ali, Nanostructured lipid carriers system: recent advances in drug delivery, J. Drug Target. 20 (10) (2012) 813–830, doi:10.3109/1061186X.2012.716845.
[143] M.V. Blagosklonny, Selective anti-cancer agents as anti-aging drugs, Cancer Biol. Ther. 14 (12) (2013) 1092–1097, doi:10.4161/cbt.27350.
[144] R. Kainthla, K.B. Kim, G.S. Falchook, Dabrafenib for treatment of BRAF-mutant melanoma, Pharmacogenomics Per. Med. 7 (2014) 21–29, doi:10.2147/PGPM.S37220.
[145] I. Satzger, T. Schaefer, U. Kuettler, V. Broecker, B. Voelker, H. Ostertag, et al., Analysis of c-KIT expression and KIT gene mutation in human mucosal melanomas, Br. J. Cancer 99 (12) (2008) 2065–2069, doi:10.1038/sj.bjc.6604791.
[146] S. Ugurel, R. Hildenbrand, A. Zimpfer, P. La Rosee, P. Paschka, A. Sucker, et al., Lack of clinical efficacy of imatinib in metastatic melanoma, Br. J. Cancer 92 (8) (2005) 1398–1405, doi:10.1038/sj.bjc.6602529.
[147] S. Rigogliuso, M.A. Sabatino, G. Adamo, N. Grimaldi, C. Dispenza, G. Ghersi, Polymeric nanogels: nanocarriers for drug delivery application, Chem. Engin. Trans. 27 (2012) 247–252, doi:10.3303/cet1227042.
[148] S.J. Lee, T.H. Jung, H. Kim, D. Jeong, G. Choi, W.K. Park, et al., Inhibition of c-Kit signaling by diosmetin isolated from Chrysanthemum morifolium, Arch. Pharm. Res. 37 (2) (2014) 175–185, doi:10.1007/s12272-013-0158-7.
[149] A.T. Baines, D. Xu, C.J. Der, Inhibition of Ras for cancer treatment: the search continues, Future Med. Chem. 3 (14) (2011) 1787–1808, doi:10.4155/fmc.11.121.
[150] M. Plotz, J. Eberle, BH3-only proteins – possible proapoptotic triggers for melanoma therapy, Exp. Dermatol. 23 (6) (2014) 375–378, doi:10.1111/exd.12399.
[151] A. Berger, S.A. Quast, M. Plotz, N.F. Kuhn, U. Trefzer, J. Eberle, RAF inhibition overcomes resistance to TRAIL-induced apoptosis in melanoma cells, J. Invest. Dermatol. 134 (2) (2014) 430–440, doi:10.1038/jid.2013.347.
[152] T.A. Yap, P. Workman, Exploiting the cancer genome: strategies for the discovery and clinical development of targeted molecular therapeutics, Annu. Rev. Pharmacol. Toxicol. 52 (2012) 549–573, doi:10.1146/annurev-pharmtox-010611-134532.
[153] J.S. de Bono, A. Ashworth, Translating cancer research into targeted therapeutics, Nature 467 (7315) (2010) 543–549, doi:10.1038/nature09339.

[154] W. Mehnert, K. Mäder, Solid lipid nanoparticles, Adv. Drug Deliv. Rev. 64 (2012) 83–101, doi:10.1016/j.addr.2012.09.021.
[155] C. Puglia, F. Bonina, Lipid nanoparticles as novel delivery systems for cosmetics and dermal pharmaceuticals, Expert Opin. Drug Deliv. 9 (4) (2012) 429–441, doi:10.1517/17425247.2012.666967.
[156] B.G. Müller, H. Leuenberger, T. Kissel, Albumin nanospheres as carriers for passive drug targeting: an optimized manufacturing technique, Pharm. Res. 13 (1) (1996) 32–37.
[157] Y. Hoshino, H. Koide, K. Furuya, W.W. Haberaecker 3rd, S.H. Lee, T. Kodama, et al., The rational design of a synthetic polymer nanoparticle that neutralizes a toxic peptide in vivo, Proc. Natl. Acad. Sci. U.S.A. 109 (1) (2012) 33–38, doi:10.1073/pnas.1112828109.
[158] C.E. Mora-Huertas, H. Fessi, A. Elaissari, Polymer-based nanocapsules for drug delivery, Int. J. Pharm. 385 (1–2) (2010) 113–142, doi:10.1016/j.ijpharm.2009.10.018.
[159] X. Chen, S. Wong, Polymeric Nanocapsule and Polymersome. Cancer Theranostics, Elsevier Inc., San Diego. 2014. p. 544.
[160] S. Svenson, Dendrimers as versatile platform in drug delivery applications, Eur. J. Pharm. Biopharm. 71 (3) (2009) 445–462, doi:10.1016/j.ejpb.2008.09.023.
[161] B. Klajnert, M. Bryszewska, Dendrimers: properties and applications, Acta Biochim. Pol. 48 (1) (2001) 199–208.
[162] A.V. Kabanov, S.V. Vinogradov, Nanogels as pharmaceutical carriers: finite networks of infinite capabilities, Angew. Chem. Int. Ed. Engl. 48 (30) (2009) 5418–5429, doi:10.1002/anie.200900441.
[163] Z. Zhang, P.C. Tsai, T. Ramezanli, B.B. Michniak-Kohn, Polymeric nanoparticles-based topical delivery systems for the treatment of dermatological diseases, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 5 (3) (2013) 205–218, doi:10.1002/wnan.1211.
[164] S. Sahu, S. Saraf, C.D. Kaur, S. Saraf, Biocompatible nanoparticles for sustained topical delivery of anticancer phytoconstituent quercetin, Pak. J. Biol. Sci. 16 (13) (2013) 601–609, doi:10.3903/pjbs.2013.601.609.
[165] A.L. Zampieri, F.S. Ferreira, E.C. Resende, M.P. Gaeti, D.G. Diniz, S.F. Taveira, et al., Biodegradable polymeric nanocapsules based on poly(DL-lactide) for genistein topical delivery: obtention, characterization and skin permeation studies, J. Biomed. Nanotechnol. 9 (3) (2013) 527–534.
[166] S. Mangalathillam, N.S. Rejinold, A. Nair, V.K. Lakshmanan, S.V. Nair, R. Jayakumar, Curcumin loaded chitin nanogels for skin cancer treatment via the transdermal route, Nanoscale 4 (1) (2012) 239–250, doi:10.1039/c1nr11271f.
[167] C.L. da Silva, J.O. Del Ciampo, F.C. Rossetti, M.V. Bentley, M.B. Pierre, PLGA nanoparticles as delivery systems for protoporphyrin IX in topical PDT: cutaneous penetration of photosensitizer observed by fluorescence microscopy, J. Nanosci. Nanotechnol. 13 (10) (2013) 6533–6540.
[168] D.M. Ferreira, Y.Y. Saga, E. Aluicio-Sarduy, A.C. Tedesco, Chitosan nanoparticles for melanoma cancer treatment by photodynamic therapy and electrochemotherapy using aminolevulinic acid derivatives, Curr. Med. Chem. 20 (14) (2013) 1904–1911.
[169] S. Battah, S. Balaratnam, A. Casas, S. O'Neill, C. Edwards, A. Batlle, et al., Macromolecular delivery of 5-aminolaevulinic acid for photodynamic therapy using dendrimer conjugates, Mol. Cancer Ther. 6 (3) (2007) 876–885, doi:10.1158/1535-7163.MCT-06-0359.
[170] A.M. Puga, A.C. Lima, J.F. Mano, A. Concheiro, C. Alvarez-Lorenzo, Pectin-coated chitosan microgels crosslinked on superhydrophobic surfaces for 5-fluorouracil encapsulation, Carbohydr. Poly. 98 (1) (2013) 331–340, doi:10.1016/j.carbpol.2013.05.091.
[171] M. Sabitha, N. Sanoj Rejinold, A. Nair, V.K. Lakshmanan, S.V. Nair, R. Jayakumar, Development and evaluation of 5-fluorouracil loaded chitin nanogels for treatment of skin cancer, Carbohydr. Poly. 91 (1) (2013) 48–57, doi:10.1016/j.carbpol.2012.07.060.
[172] N. Malik, E.G. Evagorou, R. Duncan, Dendrimer-platinate: a novel approach to cancer chemotherapy, Anticancer Drugs 10 (8) (1999) 767–776.
[173] M.A. Tran, C.D. Smith, M. Kester, G.P. Robertson, Combining nanoliposomal ceramide with sorafenib synergistically inhibits melanoma and breast cancer cell survival to decrease tumor development, Clin. Cancer Res. 14 (11) (2008) 3571–3581, doi:10.1158/1078-0432.ccr-07-4881.
[174] J. Ceccoli, N. Rosales, J. Tsimis, D.B. Yarosh, Encapsulation of the UV-DNA repair enzyme T4 endonuclease V in liposomes and delivery to human cells, J. Invest. Dermatol. 93 (2) (1989) 190–194.
[175] D. Yarosh, J. Klein, A. O'Connor, J. Hawk, E. Rafal, P. Wolf, Effect of topically applied T4 endonuclease V in liposomes on skin cancer in xeroderma pigmentosum: a randomised study. Xeroderma Pigmentosum Study Group, Lancet 357 (9260) (2001) 926–929.
[176] T.H. Chou, C.H. Liang, The molecular effects of aloe-emodin (AE)/liposome-AE on human nonmelanoma skin cancer cells and skin permeation, Chem. Res. Toxicol. 22 (12) (2009) 2017–2028, doi:10.1021/tx900318a.
[177] K.G. Lau, Y. Hattori, S. Chopra, E.A. O'Toole, A. Storey, T. Nagai, et al., Ultradeformable liposomes containing bleomycin: in vitro stability and toxicity on human cutaneous keratinocyte cell lines, Int. J. Pharm. 300 (1–2) (2005) 4–12, doi:10.1016/j.ijpharm.2005.04.019.
[178] W. Fink, C. Zimpfer-Rechner, A. Thoelke, R. Figl, M. Kaatz, S. Ugurel, et al., Clinical phase II study of pegylated liposomal doxorubicin as second-line treatment in disseminated melanoma, Onkologie 27 (6) (2004) 540–544.
[179] Y. Chen, S.R. Bathula, Q. Yang, L. Huang, Targeted nanoparticles deliver siRNA to melanoma, J. Invest. Dermatol. 130 (12) (2010) 2790–2798, doi:10.1038/jid.2010.222.
[180] A. Hauschild, E. Stockfleth, G. Popp, F. Borrosch, H. Bruning, R. Dominicus, et al., Optimization of photodynamic therapy with a novel self-adhesive 5-aminolaevulinic acid patch: results of two randomized controlled phase III studies, Br. J. Dermatol. 160 (5) (2009) 1066–1074, doi:10.1111/j.1365-2133.2009.09040.x.
[181] S.K. Passos, P.E. de Souza, P.K. Soares, D.R. Eid, F.L. Primo, A.C. Tedesco, et al., Quantitative approach to skin field cancerization using a nanoencapsulated photodynamic therapy agent: a pilot study, Clin. Cosmet. Investig. Dermatol. 6 (2013) 51–59, doi:10.2147/CCID.S36758.
[182] F.L. Primo, M.V. Bentley, A.C. Tedesco, Photophysical studies and in vitro skin permeation/retention of foscan/nanoemulsion (NE) applicable to photodynamic therapy skin cancer treatment, J. Nanosci. Nanotechnol. 8 (1) (2008) 340–347.
[183] D. Liu, Z. Liu, L. Wang, C. Zhang, N. Zhang, Nanostructured lipid carriers as novel carrier for parenteral delivery of docetaxel, Colloids Surf. B. Biointerfaces 85 (2011) 262–269.
[184] L.G. Souza, E.J. Silva, A.L. Martins, M.F. Mota, R.C. Braga, E.M. Lima, et al., Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release, Eur. J. Pharm. Biopharm. 79 (1) (2011) 189–196, doi:10.1016/j.ejpb.2011.02.012.
[185] R. Cavalli, O. Caputo, M.R. Gasco, Solid lipospheres of doxorubicin and idarubicin, Int. J. Pharm. 89 (1) (1993) R9–R12, doi:10.1016/0378-5173(93)90313-5.
[186] S.F. Taveira, L.M. Araujo, D.C. de Santana, A. Nomizo, L.A. de Freitas, R.F. Lopez, Development of cationic solid lipid nanoparticles with factorial design-based studies for topical administration of doxorubicin, J. Biomed. Nanotechnol. 8 (2) (2012) 219–228.
[187] T.C. Contente, I.F. Kretzer, F.B. Filippin-Monteiro, D.A. Maria, R.C. Maranhao, Association of daunorubicin to a lipid nanoemulsion that binds to low-density lipoprotein receptors enhances the antitumour action and decreases the toxicity of the drug in melanoma-bearing mice, J. Pharm. Pharmacol. (2014) doi:10.1111/jphp.12296.
[188] S. Yang, J. Zhu, Y. Lu, B. Liang, C. Yang, Body distribution of camptothecin solid lipid nanoparticles after oral administration, Pharm. Res. 16 (5) (1999) 751–757.
[189] R.B. Athawale, D.S. Jain, K.K. Singh, R.P. Gude, Etoposide loaded solid lipid nanoparticles for curtailing B16F10 melanoma colonization in lung, Biomed. Pharmacother. 68 (2) (2014) 231–240, doi:10.1016/j.biopha.2014.01.004.
[190] S. Mizrahy, M. Goldsmith, S. Leviatan-Ben-Arye, E. Kisin-Finfer, O. Redy, S. Srinivasan, et al., Tumor targeting profiling of hyaluronan-coated lipid based-nanoparticles, Nanoscale 6 (7) (2014) 3742–3752, doi:10.1039/c3nr06102g.
[191] K. Teskac, J. Kristl, The evidence for solid lipid nanoparticles mediated cell uptake of resveratrol, Int. J. Pharm. 390 (1) (2010) 61–69, doi:10.1016/j.ijpharm.2009.10.011.
[192] F. Marquele-Oliveira, D.C. Santana, S.F. Taveira, D.M. Vermeulen, A.R. de Oliveira, R.S. da Silva, et al., Development of nitrosyl ruthenium complex-loaded lipid carriers for topical administration: improvement in skin stability and in nitric oxide release by visible light irradiation, J. Pharm. Biomed. Anal. 53 (4) (2010) 843–851, doi:10.1016/j.jpba.2010.06.007.
[193] S. Tan, T. Sasada, A. Bershteyn, K. Yang, T. Ioji, Z. Zhang, Combinational delivery of lipid-enveloped polymeric nanoparticles carrying different peptides for anti-tumor immunotherapy, Nanomedicine (London, England) 9 (5) (2014) 635–647, doi:10.2217/nnm.13.67.
[194] C. Ali-von Laue, C. Zoschke, N. Do, D. Lehnen, S. Kuchler, W. Mehnert, et al., Improving topical non-melanoma skin cancer treatment: in vitro efficacy of a novel guanosine-analog phosphonate, Skin Pharmacol. Physiol. 27 (4) (2014) 173, doi:10.1159/000354118.
[195] M. Arlt, D. Haase, S. Hampel, S. Oswald, A. Bachmatiuk, R. Klingeler, et al., Delivery of carboplatin by carbon-based nanocontainers mediates increased cancer cell death, Nanotechnology 21 (33) (2010) 335101, doi:10.1088/0957-4484/21/33/335101.
[196] Z. Liu, J.T. Robinson, X. Sun, H. Dai, PEGylated nanographene oxide for delivery of water-insoluble cancer drugs, J. Am. Chem. Soc. 130 (33) (2008) 10876–10877, doi:10.1021/ja803688x.
[197] Y. Pan, H. Bao, N.G. Sahoo, T. Wu, L. Li, Water-soluble poly(N-isopropylacrylamide)-graphene sheets synthesized via click chemistry for drug delivery, Adv. Funct. Mater. 21 (14) (2011) 2754–2763, doi:10.1002/adfm.201100078.
[198] H. Bao, Y. Pan, Y. Ping, N.G. Sahoo, T. Wu, L. Li, et al., Chitosan-functionalized graphene oxide as a nanocarrier for drug and gene delivery, Small 7 (11) (2011) 1569–1578, doi:10.1002/smll.201100191.
[199] N.G. Sahoo, H. Bao, Y. Pan, M. Pal, M. Kakran, H.K. Cheng, et al., Functionalized carbon nanomaterials as nanocarriers for loading and delivery of a poorly water-soluble anticancer drug: a comparative study, Chem. Commun. 47 (18) (2011) 5235–5237, doi:10.1039/c1cc00075f.
[200] X. Fan, G. Jiao, L. Gao, P. Jin, X. Li, The preparation and drug delivery of a graphene–carbon nanotube–Fe_3O_4 nanoparticle hybrid, J. Mater. Chem. B 1 (20) (2013) 2658, doi:10.1039/c3tb00493g.
[201] B. Chen, M. Liu, L. Zhang, J. Huang, J. Yao, Z. Zhang, Polyethylenimine-functionalized graphene oxide as an efficient gene delivery vector, J. Mater. Chem. 21 (21) (2011) 7736, doi:10.1039/c1jm10341e.
[202] K.S. Siu, D. Chen, X. Zheng, X. Zhang, N. Johnston, Y. Liu, et al., Non-covalently functionalized single-walled carbon nanotube for topical siRNA delivery into

melanoma, Biomaterials 35 (10) (2014) 3435–3442, doi:10.1016/j.biomaterials.2013.12.079.
[203] H. Shen, M. Liu, H. He, L. Zhang, J. Huang, Y. Chong, et al., PEGylated graphene oxide-mediated protein delivery for cell function regulation, ACS Appl. Mater. Interfaces 4 (11) (2012) 6317–6323, doi:10.1021/am3019367.
[204] T.R. Fadel, F.A. Sharp, N. Vudattu, R. Ragheb, J. Garyu, D. Kim, et al., A carbon nanotube-polymer composite for T-cell therapy, Nat. Nanotechnol. 9 (8) (2014) 639–647, doi:10.1038/nnano.2014.154.
[205] J.H. Park, G. von Maltzahn, E. Ruoslahti, S.N. Bhatia, M.J. Sailor, Micellar hybrid nanoparticles for simultaneous magnetofluorescent imaging and drug delivery, Angew. Chem. Int. Ed. Engl. 47 (38) (2008) 7284–7288, doi:10.1002/anie.200801810.
[206] A.S. Wadajkar, Z. Bhavsar, C.Y. Ko, B. Koppolu, W. Cui, L. Tang, et al., Multifunctional particles for melanoma-targeted drug delivery, Acta Biomater. 8 (8) (2012) 2996–3004, doi:10.1016/j.actbio.2012.04.042.
[207] H. Misak, N. Zacharias, Z. Song, S. Hwang, K.P. Man, R. Asmatulu, et al., Skin cancer treatment by albumin/5-Fu loaded magnetic nanocomposite spheres in a mouse model, J. Biotechnol. 164 (1) (2013) 130–136, doi:10.1016/j.jbiotec.2013.01.003.
[208] Y.F. Rao, W. Chen, X.G. Liang, Y.Z. Huang, J. Miao, L. Liu, et al., Epirubicin-loaded superparamagnetic iron-oxide nanoparticles for transdermal delivery: cancer therapy by circumventing the skin barrier, Small (2014) doi:10.1002/smll.201400775.
[209] G. Baldi, C. Ravagli, F. Mazzantini, G. Loudos, J. Adan, M. Masa, et al., In vivo anticancer evaluation of the hyperthermic efficacy of anti-human epidermal growth factor receptor-targeted PEG-based nanocarrier containing magnetic nanoparticles, Int. J. Nanomedicine 9 (2014) 3037–3056, doi:10.2147/IJN.S61273.
[210] X. Huang, I.H. El-Sayed, W. Qian, M.A. El-Sayed, Cancer cell imaging and photothermal therapy in the near-infrared region by using gold nanorods, J. Am. Chem. Soc. 128 (6) (2006) 2115–2120, doi:10.1021/ja057254a.
[211] Y. Cheng, A. C Samia, J.D. Meyers, I. Panagopoulos, B. Fei, C. Burda, Highly efficient drug delivery with gold nanoparticle vectors for in vivo photodynamic therapy of cancer, J. Am. Chem. Soc. 130 (32) (2008) 10643–10647, doi:10.1021/ja801631c.
[212] Y.S. Yang, R.P. Carney, F. Stellacci, D.J. Irvine, Enhancing radiotherapy by lipid nanocapsule-mediated delivery of amphiphilic gold nanoparticles to intracellular membranes, ACS Nano. 8 (9) (2014) 8992–9002, doi:10.1021/nn502146r.
[213] X. Zhang, H. Chibli, D. Kong, J. Nadeau, Comparative cytotoxicity of gold-doxorubicin and InP-doxorubicin conjugates, Nanotechnology 23 (27) (2012) 275103, doi:10.1088/0957-4484/23/27/275103.
[214] A. Latorre, C. Posch, Y. Garcimartin, A. Celli, M. Sanlorenzo, I. Vujic, et al., DNA and aptamer stabilized gold nanoparticles for targeted delivery of anticancer therapeutics, Nanoscale 6 (13) (2014) 7436–7442, doi:10.1039/c4nr00019f.
[215] T. Asai, T. Tsuzuku, S. Takahashi, A. Okamoto, T. Dewa, M. Nango, et al., Cell-penetrating peptide-conjugated lipid nanoparticles for siRNA delivery, Biochem. Biophys. Res. Commun. 444 (4) (2014) 599–604, doi:10.1016/j.bbrc.2014.01.107.
[216] S.V. Abkin, K.M. Pankratova, E.Y. Komarova, I.V. Guzhova, B.A. Margulis, Hsp70 chaperone-based gel composition as a novel immunotherapeutic anti-tumor tool, Cell Stress Chaperones 18 (3) (2013) 391–396, doi:10.1007/s12192-012-0391-x.
[217] H. Geng, G.M. Zhang, H. Xiao, Y. Yuan, D. Li, H. Zhang, et al., HSP70 vaccine in combination with gene therapy with plasmid DNA encoding sPD-1 overcomes immune resistance and suppresses the progression of pulmonary metastatic melanoma, Int. J. Cancer 118 (11) (2006) 2657–2664, doi:10.1002/ijc.21795.
[218] A. Ito, F. Matsuoka, H. Honda, T. Kobayashi, Antitumor effects of combined therapy of recombinant heat shock protein 70 and hyperthermia using magnetic nanoparticles in an experimental subcutaneous murine melanoma, Cancer Immunol. Immunother. 53 (1) (2004) 26–32.
[219] T. Miyagawa, H. Saito, Y. Minamiya, K. Mitobe, S. Takashima, N. Takahashi, et al., Inhibition of Hsp90 and 70 sensitizes melanoma cells to hyperthermia using ferromagnetic particles with a low Curie temperature, Int. J. Clin. Oncol. 19 (4) (2014) 722–730, doi:10.1007/s10147-013-0606-x.
[220] X. Yu, C. Guo, H. Yi, J. Qian, P.B. Fisher, J.R. Subjeck, et al., A multifunctional chimeric chaperone serves as a novel immune modulator inducing therapeutic antitumor immunity, Cancer Res. 73 (7) (2013) 2093–2103, doi:10.1158/0008-5472.CAN-12-1740.
[221] L. Sanchez-Perez, T. Kottke, G.A. Daniels, R.M. Diaz, J. Thompson, J. Pulido, et al., Killing of normal melanocytes, combined with heat shock protein 70 and CD40L expression, cures large established melanomas, J. Immunol. 177 (6) (2006) 4168–4177.
[222] J. Wu, M. Guan, P.F. Wong, H. Yu, J. Dong, J. Xu, Icariside II potentiates paclitaxel-induced apoptosis in human melanoma A375 cells by inhibiting TLR4 signaling pathway, Food Chem. Toxicol. 50 (9) (2012) 3019–3024, doi:10.1016/j.fct.2012.06.027.
[223] S. Pilon-Thomas, W. Li, J.J. Briggs, J. Djeu, J.J. Mulé, A.I. Riker, Immunostimulatory effects of CpG-ODN upon dendritic cell-based immunotherapy in a murine melanoma model, J. Immunother. 29 (4) (2006) 381–387.
[224] T. Nagato, E. Celis, A novel combinatorial cancer immunotherapy: poly-IC and blockade of the PD-1/PD-L1 pathway, Oncoimmunology 3 (2014) e28440, doi:10.4161/onci.28440.
[225] I.D. Jung, S.J. Shin, M.G. Lee, T.H. Kang, H.D. Han, S.J. Lee, et al., Enhancement of tumor-specific T cell-mediated immunity in dendritic cell-based vaccines by Mycobacterium tuberculosis heat shock protein X, J. Immunol. 193 (3) (2014) 1233–1245, doi:10.4049/jimmunol.1400656.
[226] A. Seth, M.B. Heo, Y.T. Lim, Poly (gamma-glutamic acid) based combination of water-insoluble paclitaxel and TLR7 agonist for chemo-immunotherapy, Biomaterials 35 (27) (2014) 7992–8001, doi:10.1016/j.biomaterials.2014.05.076.
[227] T.A. Slastnikova, A.A. Rosenkranz, P.V. Gulak, R.M. Schiffelers, T.N. Lupanova, Y.V. Khramtsov, et al., Modular nanotransporters: a multipurpose in vivo working platform for targeted drug delivery, Int. J. Nanomedicine 7 (2012) 467–482, doi:10.2147/IJN.S28249.
[228] D.G. Gilyazova, A.A. Rosenkranz, P.V. Gulak, V.G. Lunin, O.V. Sergienko, Y.V. Khramtsov, et al., Targeting cancer cells by novel engineered modular transporters, Cancer Res. 66 (21) (2006) 10534–10540, doi:10.1158/0008-5472.CAN-06-2393.
[229] T.A. Slastnikova, A.A. Rosenkranz, T.N. Lupanova, P.V. Gulak, N.V. Gnuchev, A.S. Sobolev, Study of efficiency of the modular nanotransporter for targeted delivery of photosensitizers to melanoma cell nuclei in vivo, Dokl. Biochem. Biophys. 446 (2012) 235–237, doi:10.1134/S1607672912050146.
[230] R.S. Rachakatla, S. Balivada, G.M. Seo, C.B. Myers, H. Wang, T.N. Samarakoon, et al., Attenuation of mouse melanoma by A/C magnetic field after delivery of bi-magnetic nanoparticles by neural progenitor cells, ACS Nano. 4 (12) (2010) 7093–7104, doi:10.1021/nn100870z.
[231] T.B. Shrestha, G.M. Seo, M.T. Basel, M. Kalita, H. Wang, D. Villanueva, et al., Stem cell-based photodynamic therapy, Photochem. Photobiol. Sci. 11 (7) (2012) 1251–1258, doi:10.1039/c2pp05417e.
[232] M. Gutova, J. Najbauer, M.Y. Chen, P.M. Potter, S.U. Kim, K.S. Aboody, Therapeutic targeting of melanoma cells using neural stem cells expressing carboxylesterase, a CPT-11 activating enzyme, Curr. Stem Cell Res. Ther. 5 (3) (2010) 273–276.
[233] Y. Xing, X. Lu, E.C. Pua, P. Zhong, The effect of high intensity focused ultrasound treatment on metastases in a murine melanoma model, Biochem. Biophys. Res. Commun. 375 (4) (2008) 645–650, doi:10.1016/j.bbrc.2008.08.072.
[234] M.S. Tartis, J. McCallan, A.F. Lum, R. LaBell, S.M. Stieger, T.O. Matsunaga, et al., Therapeutic effects of paclitaxel-containing ultrasound contrast agents, Ultrasound Med. Biol. 32 (11) (2006) 1771–1780, doi:10.1016/j.ultrasmedbio.2006.03.017.
[235] S. Paliwal, J. Sundaram, S. Mitragotri, Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound, Br. J. Cancer 92 (3) (2005) 499–502, doi:10.1038/sj.bjc.6602364.
[236] L. Alili, M. Sack, C. von Montfort, S. Giri, S. Das, K.S. Carroll, et al., Downregulation of tumor growth and invasion by redox-active nanoparticles, Antioxid. Redox Signal. 19 (8) (2013) 765–778, doi:10.1089/ars.2012.4831.
[237] M. Sack, L. Alili, E. Karaman, S. Das, A. Gupta, S. Seal, et al., Combination of conventional chemotherapeutics with redox-active cerium oxide nanoparticles–a novel aspect in cancer therapy, Mol. Cancer Ther. 13 (7) (2014) 1740–1749, doi:10.1158/1535-7163.MCT-13-0950.
[238] W. Wu, M. Aiello, T. Zhou, A. Berliner, P. Banerjee, S. Zhou, In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery, Biomaterials 31 (11) (2010) 3023–3031, doi:10.1016/j.biomaterials.2010.01.011.
[239] S.H. Hsu, C.J. Wen, S.A. Al-Suwayeh, Y.J. Huang, J.Y. Fang, Formulation design and evaluation of quantum dot-loaded nanostructured lipid carriers for integrating bioimaging and anticancer therapy, Nanomedicine (London, England) 8 (8) (2013) 1253–1269, doi:10.2217/nnm.12.170.
[240] W.T. Al-Jamal, K.T. Al-Jamal, B. Tian, A. Cakebread, J.M. Halket, K. Kostarelos, Tumor targeting of functionalized quantum dot-liposome hybrids by intravenous administration, Mol. Pharm. 6 (2) (2009) 520–530, doi:10.1021/mp800187d.
[241] A. Nair, J. Shen, P. Thevenot, L. Zou, T. Cai, Z. Hu, et al., Enhanced intratumoral uptake of quantum dots concealed within hydrogel nanoparticles, Nanotechnology 19 (48) (2008) 485102, doi:10.1088/0957-4484/19/48/485102.
[242] K. Perica, A. De Leon Medero, M. Durai, Y.L. Chiu, J.G. Bieler, L. Sibener, et al., Nanoscale artificial antigen presenting cells for T cell immunotherapy, Nanomedicine 10 (1) (2014) 119–129, doi:10.1016/j.nano.2013.06.015.
[243] I. Roy, T.Y. Ohulchanskyy, H.E. Pudavar, E.J. Bergey, A.R. Oseroff, J. Morgan, et al., Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug-carrier system for photodynamic therapy, J. Am. Chem. Soc. 125 (26) (2003) 7860–7865, doi:10.1021/ja0343095.
[244] B. Zhao, J.J. Yin, P.J. Bilski, C.F. Chignell, J.E. Roberts, Y.Y. He, Enhanced photodynamic efficacy towards melanoma cells by encapsulation of Pc4 in silica nanoparticles, Toxicol. Appl. Pharmacol. 241 (2) (2009) 163–172, doi:10.1016/j.taap.2009.08.010.
[245] I.T. Teng, Y.J. Chang, L.S. Wang, H.Y. Lu, L.C. Wu, C.M. Yang, et al., Phospholipid-functionalized mesoporous silica nanocarriers for selective photodynamic therapy of cancer, Biomaterials 34 (30) (2013) 7462–7470, doi:10.1016/j.biomaterials.2013.06.001.
[246] P. Scodeller, P.N. Catalano, N. Salguero, H. Duran, A. Wolosiuk, G.J. Soler-Illia, Hyaluronan degrading silica nanoparticles for skin cancer therapy, Nanoscale 5 (20) (2013) 9690–9698, doi:10.1039/c3nr02787b.
[247] E. Gianotti, B. Martins Estevao, F. Cucinotta, N. Hioka, M. Rizzi, F. Reno, et al., An efficient rose bengal based nanoplatform for photodynamic therapy, Chemistry 20 (35) (2014) 10921–10925, doi:10.1002/chem.201404296.

[248] C.M. Byrne, J.F. Thompson, H. Johnston, P. Hersey, M.J. Quinn, T. Michael Hughes, et al., Treatment of metastatic melanoma using electroporation therapy with bleomycin (electrochemotherapy), Melanoma Res. 15 (1) (2005) 45–51.
[249] C. Caraco, N. Mozzillo, U. Marone, E. Simeone, L. Benedetto, G. Di Monta, et al., Long-lasting response to electrochemotherapy in melanoma patients with cutaneous metastasis, BMC Cancer 13 (2013) 564, doi:10.1186/1471-2407-13-564.
[250] F. Ricotti, K. Giuliodori, I. Cataldi, A. Campanati, G. Ganzetti, G. Ricotti, et al., Electrochemotherapy: an effective local treatment of cutaneous and subcutaneous melanoma metastases, Dermatol. Ther. 27 (3) (2014) 148–152, doi:10.1111/dth.12098.
[251] S.F. Taveira, D.C. De Santana, L.M. Araujo, F. Marquele-Oliveira, A. Nomizo, R.F. Lopez, Effect of iontophoresis on topical delivery of doxorubicin-loaded solid lipid nanoparticles, J. Biomed. Nanotechnol. 10 (7) (2014) 1382–1390.
[252] R. Hudej, D. Miklavcic, M. Cemazar, V. Todorovic, G. Sersa, A. Bergamo, et al., Modulation of activity of known cytotoxic ruthenium(III) compound (KP418) with hampered transmembrane transport in electrochemotherapy in vitro and in vivo, J. Membr. Biol. (2014) doi:10.1007/s00232-014-9696-2.
[253] P.F. Forde, M. Sadadcharam, L.J. Hall, T.R. O'Donovan, M. de Kruijf, W.L. Byrne, et al., Enhancement of electroporation facilitated immunogene therapy via T-reg depletion, Cancer Gene Ther. 21 (8) (2014) 349–354, doi:10.1038/cgt.2014.35.
[254] A.I. Daud, R.C. DeConti, S. Andrews, P. Urbas, A.I. Riker, V.K. Sondak, et al., Phase I trial of interleukin-12 plasmid electroporation in patients with metastatic melanoma, J. Clin. Oncol. 26 (36) (2008) 5896–5903, doi:10.1200/JCO.2007.15.6794.
[255] B. Marrero, S. Shirley, R. Heller, Delivery of interleukin-15 to B16 melanoma by electroporation leads to tumor regression and long-term survival, Technol. Cancer Res. Treat. 13 (6) (2014) 551–560, doi:10.7785/tcrtexpress.2013.600252.
[256] R. Nuccitelli, X. Chen, A.G. Pakhomov, W.H. Baldwin, S. Sheikh, J.L. Pomicter, et al., A new pulsed electric field therapy for melanoma disrupts the tumor's blood supply and causes complete remission without recurrence, Int. J. Cancer 125 (2) (2009) 438–445, doi:10.1002/ijc.24345.
[257] F. Guo, C. Yao, C. Li, Y. Mi, Q. Peng, J. Tang, In vivo evidences of nanosecond pulsed electric fields for melanoma malignancy treatment on tumor-bearing BALB/c nude mice, Technol. Cancer Res. Treat. 13 (4) (2014) 337–344, doi:10.7785/tcrt.2012.500385.
[258] T. Bhowmik, B. D'Souza, R. Shashidharamurthy, C. Oettinger, P. Selvaraj, M.J. D'Souza, A novel microparticulate vaccine for melanoma cancer using transdermal delivery, J. Microencapsul. 28 (4) (2011) 294–300, doi:10.3109/02652048.2011.559287.
[259] Y.Y. Zha, C. Lin, S.R. Zhang, X. Liang, X.Y. Zhang, M. Fu, et al., Antitumor effect of gene gun-mediated DNA vaccine pWRG-neu immunization in C57BL/6 mice, Zhonghua Zhong Liu Za Zhi 25 (4) (2003) 320–324.
[260] J. Steitz, T. Tuting, Biolistic DNA vaccination against melanoma, Methods Mol. Biol. 940 (2013) 317–337, doi:10.1007/978-1-62703-110-3_24.
[261] W. Sun, H. Qian, X. Zhang, C. Zhou, X. Liang, D. Wang, et al., Induction of protective and therapeutic antitumour immunity using a novel tumour-associated antigen-specific DNA vaccine, Immunol. Cell Biol. 84 (5) (2006) 440–447, doi:10.1111/j.1440-1711.2006.01453.x.
[262] X. Li, M.F. Naylor, H. Le, R.E. Nordquist, T.K. Teague, C.A. Howard, et al., Clinical effects of in situ photoimmunotherapy on late-stage melanoma patients: a preliminary study, Cancer Biol. Ther. 10 (11) (2010) 1081–1087, doi:10.4161/cbt.10.11.13434.
[263] T. Niidome, Y. Akiyama, M. Yamagata, T. Kawano, T. Mori, Y. Niidome, et al., Poly(ethylene glycol)-modified gold nanorods as a photothermal nanodevice for hyperthermia, J. Biomater. Sci. Polymer, Edn 20 (9) (2009) 1203–1215, doi:10.1163/156856209X452953.
[264] M. Chu, X. Pan, D. Zhang, Q. Wu, J. Peng, W. Hai, The therapeutic efficacy of CdTe and CdSe quantum dots for photothermal cancer therapy, Biomaterials 33 (29) (2012) 7071–7083, doi:10.1016/j.biomaterials.2012.06.062.
[265] H.S. Jung, W.H. Kong, D.K. Sung, M.Y. Lee, S.E. Beack, H. do Keum, et al., Nanographene oxide-hyaluronic acid conjugate for photothermal ablation therapy of skin cancer, ACS Nano. 8 (1) (2014) 260–268, doi:10.1021/nn405383a.
[266] W. Lu, C. Xiong, G. Zhang, Q. Huang, R. Zhang, J.Z. Zhang, et al., Targeted photothermal ablation of murine melanomas with melanocyte-stimulating hormone analog-conjugated hollow gold nanospheres, Clin. Cancer Res. 15 (3) (2009) 876–886, doi:10.1158/1078-0432.CCR-08-1480.
[267] R. Vankayala, C.C. Lin, P. Kalluru, C.S. Chiang, K.C. Hwang, Gold nanoshells-mediated bimodal photodynamic and photothermal cancer treatment using ultra-low doses of near infra-red light, Biomaterials 35 (21) (2014) 5527–5538, doi:10.1016/j.biomaterials.2014.03.065.
[268] M.D. Kars, R. Kara, Y. Gundogdu, A. Kepceoglu, H.S. Kilic, Femtosecond laser induced photodynamic therapy on 5-ALA treated SKMEL-30 cells: an efficient theranostic strategy to combat melanoma, Biomed. Pharmacother. 68 (5) (2014) 657–662, doi:10.1016/j.biopha.2014.04.001.
[269] G.C. Kim, G.J. Kim, S.R. Park, S.M. Jeon, H.J. Seo, F. Iza, et al., Air plasma coupled with antibody-conjugated nanoparticles: a new weapon against cancer, J. Phys. D Appl. Phys. 42 (3) (2009) 032005.
[270] M. Keidar, R. Walk, A. Shashurin, P. Srinivasan, A. Sandler, S. Dasgupta, et al., Cold plasma selectivity and the possibility of a paradigm shift in cancer therapy, Br. J. Cancer 105 (9) (2011) 1295–1301, doi:10.1038/bjc.2011.386.
[271] H. Pehamberger, Perspectives of pegylated interferon use in dermatological oncology, Recent Results Cancer Res. 160 (2002) 158–164.
[272] R. Di Trolio, E. Simeone, G. Di Lorenzo, A.M. Grimaldi, A. Romano, F. Ayala, et al., Update on PEG-interferon α-2b as adjuvant therapy in melanoma, Anticancer Res. 32 (9) (2012) 3901–3909.
[273] A. Sharma, U.S. Sharma, Liposomes in drug delivery: progress and limitations, Int. J. Pharm. 154 (1997) 123–140.
[274] R. Parhi, P. Suresh, Preparation and characterization of solid lipid nanoparticles-a review, Curr. Drug Discov. Technol. 9 (1) (2012) 2–16.
[275] J.H. Senior, Fate and behavior of liposomes in vivo: a review of controlling factors, Crit. Rev. Ther. Drug Carrier Syst. 3 (2) (1987) 123–193.
[276] M.C. Woodle, Sterically stabilized liposome therapeutics, Adv. Drug Deliv. Rev. 16 (1995) 249–265.
[277] R. Janknegt, S. de Marie, I.A. Bakker-Woudenberg, D.J. Crommelin, Liposomal and lipid formulations of amphotericin B. Clinical pharmacokinetics, Clin. Pharmacokinet. 23 (4) (1992) 279–291, doi:10.2165/00003088-199223040-00004.
[278] M.B. Weiss, A.E. Aplin, Paying "particle" attention to novel melanoma treatment strategies, J. Invest. Dermatol. 130 (12) (2010) 2699–2701, doi:10.1038/jid.2010.293.
[279] J.R. Antonio, C.R. Antonio, I.L. Cardeal, J.M. Ballavenuto, J.R. Oliveira, Nanotechnology in dermatology, An. Bras. Dermatol. 89 (1) (2014) 126–136, doi:10.1590/abd1806-4841.20142228.
[280] M. Jumaa, B.W. Müller, Lipid emulsions as a novel system to reduce the hemolytic activity of lytic agents: mechanism of the protective effect, Eur. J. Pharm. Sci. 9 (3) (2000) 285–290.
[281] B. Magenheim, M.Y. Levy, S. Benita, A new in vitro technique for the evaluation of drug release profile from colloidal carriers – ultrafiltration technique at low pressure, Int. J. Pharm. 94 (1–3) (1993) 115–123, doi:10.1016/0378-5173(93)90015-8.
[282] E. Kohl, S. Karrer, New developments in photodynamic therapy, Hautarzt 64 (5) (2013) 363–369, doi:10.1007/s00105-012-2513-x.
[283] M. Uner, Preparation, characterization and physico-chemical properties of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC): their benefits as colloidal drug carrier systems, Pharmazie 61 (5) (2006) 375–386.
[284] P. Severino, T. Andreani, A.S. Macedo, J.F. Fangueiro, M.H. Santana, A.M. Silva, et al., Current state-of-art and new trends on lipid nanoparticles (SLN and NLC) for oral drug delivery, J. Drug Deliv. 2012 (2012) 750891, doi:10.1155/2012/750891.
[285] R.H. Muller, R. Shegokar, C.M. Keck, 20 years of lipid nanoparticles (SLN and NLC): present state of development and industrial applications, Curr. Drug Discov. Technol. 8 (3) (2011) 207–227.
[286] R.H. Muller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv. Drug Deliv. Rev. 54 (Suppl. 1) (2002) S131–S155.
[287] M. Muchow, P. Maincent, R.H. Muller, Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery, Drug Dev. Ind. Pharm. 34 (12) (2008) 1394–1405, doi:10.1080/03639040802130061.
[288] R.H. Muller, R.D. Petersen, A. Hommoss, J. Pardeike, Nanostructured lipid carriers (NLC) in cosmetic dermal products, Adv. Drug Deliv. Rev. 59 (6) (2007) 522–530, doi:10.1016/j.addr.2007.04.012.
[289] M. Uner, G. Yener, Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives, Int. J. Nanomedicine 2 (3) (2007) 289–300.
[290] S. Selvamuthukumar, R. Velmurugan, Nanostructured lipid carriers: a potential drug carrier for cancer chemotherapy, Lipids Health Dis. 11 (2012) 159, doi:10.1186/1476-511X-11-159.
[291] J. Pardeike, A. Hommoss, R.H. Muller, Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products, Int. J. Pharm. 366 (1–2) (2009) 170–184, doi:10.1016/j.ijpharm.2008.10.003.
[292] M.D. Joshi, R.H. Muller, Lipid nanoparticles for parenteral delivery of actives, Eur. J. Pharm. Biopharm. 71 (2) (2009) 161–172, doi:10.1016/j.ejpb.2008.09.003.
[293] A.E. Yassin, M.K. Anwer, H.A. Mowafy, I.M. El-Bagory, M.A. Bayomi, I.A. Alsarra, Optimization of 5-flurouracil solid-lipid nanoparticles: a preliminary study to treat colon cancer, Int. J. Med. Sci. 7 (6) (2010) 398–408.
[294] S. Goenka, V. Sant, S. Sant, Graphene-based nanomaterials for drug delivery and tissue engineering, J. Control. Release 173 (2014) 75–88, doi:10.1016/j.jconrel.2013.10.017.
[295] W.H. De Jong, P.J. Borm, Drug delivery and nanoparticles: applications and hazards, Int. J. Nanomedicine 3 (2) (2008) 133–149.
[296] S.Y. Madani, N. Naderi, O. Dissanayake, A. Tan, A.M. Seifalian, A new era of cancer treatment: carbon nanotubes as drug delivery tools, Int. J. Nanomedicine 6 (2011) 2963–2979, doi:10.2147/IJN.S16923.
[297] N.W. Kam, H. Dai, Carbon nanotubes as intracellular protein transporters: generality and biological functionality, J. Am. Chem. Soc. 127 (16) (2005) 6021–6026.
[298] J.U. Menon, P. Jadeja, P. Tambe, K. Vu, B. Yuan, K.T. Nguyen, Nanomaterials for photo-based diagnostic and therapeutic applications, Theranostics 3 (3) (2013) 152–166, doi:10.7150/thno.5327.
[299] M. Fakhar-e-Alam, S. Kishwar, M. Willander, Photodynamic effects of zinc oxide nanowires in skin cancer and fibroblast, Lasers Med. Sci. 29 (3) (2014) 1189–1194, doi:10.1007/s10103-013-1501-4.
[300] J.E. Podesta, K.T. Al-Jamal, M.A. Herrero, B. Tian, H. Ali-Boucetta, V. Hegde, et al., Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model, Small 5 (10) (2009) 1176–1185, doi:10.1002/smll.200801572.

[301] S. Gupta, R. Bansal, S. Gupta, N. Jindal, A. Jindal, Nanocarriers and nanoparticles for skin care and dermatological treatments, Indian Dermatol. Online J. 4 (4) (2013) 267–272, doi:10.4103/2229-5178.120635.
[302] I.A. Siddiqui, V.M. Adhami, J.C. Chamcheu, H. Mukhtar, Impact of nanotechnology in cancer: emphasis on nanochemoprevention, Int. J. Nanomedicine 7 (2012) 591–605, doi:10.2147/IJN.S26026.
[303] X. Sun, Z. Liu, K. Welsher, J.T. Robinson, A. Goodwin, S. Zaric, et al., Nano-graphene oxide for cellular imaging and drug delivery, Nano Res. 1 (3) (2008) 203–212, doi:10.1007/s12274-008-8021-8.
[304] U. Dembereldorj, M. Kim, S. Kim, E.-O. Ganbold, S.Y. Lee, S.-W. Joo, A spatiotemporal anticancer drug release platform of PEGylated graphene oxide triggered by glutathione in vitro and in vivo, J. Mater. Chem. 22 (45) (2012) 23845, doi:10.1039/c2jm34853e.
[305] K. Liu, J.-J. Zhang, F.-F. Cheng, T.-T. Zheng, C. Wang, J.-J. Zhu, Green and facile synthesis of highly biocompatible graphene nanosheets and its application for cellular imaging and drug delivery, J. Mater. Chem. 21 (32) (2011) 12034, doi:10.1039/c1jm10749f.
[306] L. Zhang, J. Xia, Q. Zhao, L. Liu, Z. Zhang, Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs, Small 6 (4) (2010) 537–544, doi:10.1002/smll.200901680.
[307] X. Li, X. Huang, D. Liu, X. Wang, S. Song, L. Zhou, et al., Synthesis of 3D hierarchical Fe_3O_4/graphene composites with high lithium storage capacity and for controlled drug delivery, J. Phys. Chem. C 115 (44) (2011) 21567–21573, doi:10.1021/jp204502n.
[308] V.K. Rana, M.-C. Choi, J.-Y. Kong, G.Y. Kim, M.J. Kim, S.-H. Kim, et al., Synthesis and drug-delivery behavior of chitosan-functionalized graphene oxide hybrid nanosheets, Macromol. Mater. Eng. 296 (2) (2011) 131–140, doi:10.1002/mame.201000307.
[309] L. Feng, S. Zhang, Z. Liu, Graphene based gene transfection, Nanoscale 3 (3) (2011) 1252–1257, doi:10.1039/c0nr00680g.
[310] H. Kim, R. Namgung, K. Singha, I.K. Oh, W.J. Kim, Graphene oxide-polyethylenimine nanoconstruct as a gene delivery vector and bioimaging tool, Bioconjug. Chem. 22 (12) (2011) 2558–2567, doi:10.1021/bc200397j.
[311] L. Zhang, Z. Lu, Q. Zhao, J. Huang, H. Shen, Z. Zhang, Enhanced chemotherapy efficacy by sequential delivery of siRNA and anticancer drugs using PEI-grafted graphene oxide, Small 7 (4) (2011) 460–464, doi:10.1002/smll.201001522.
[312] W.G. La, S. Park, H.H. Yoon, G.J. Jeong, T.J. Lee, S.H. Bhang, et al., Delivery of a therapeutic protein for bone regeneration from a substrate coated with graphene oxide, Small 9 (23) (2013) 4051–4060, doi:10.1002/smll.201300571.
[313] B. Baroli, M.G. Ennas, F. Loffredo, M. Isola, R. Pinna, M.A. Lopez-Quintela, Penetration of metallic nanoparticles in human full-thickness skin, J. Invest. Dermatol. 127 (7) (2007) 1701–1712, doi:10.1038/sj.jid.5700733.
[314] M.A. Willard, L.K. Kurihara, E.E. Carpenter, S. Calvin, V.G. Harris, Chemically prepared magnetic nanoparticles, Int. Mater. Rev. 49 (3–4) (2004) 125–170, doi:10.1179/095066004225021882.
[315] A. Akbarzadeh, M. Samiei, S. Davaran, Magnetic nanoparticles: preparation, physical properties, and applications in biomedicine, Nanoscale Res. Lett. 7 (1) (2012) 144, doi:10.1186/1556-276X-7-144.
[316] A.H. Lu, E.L. Salabas, F. Schuth, Magnetic nanoparticles: synthesis, protection, functionalization, and application, Angew. Chem. Int. Ed. Engl. 46 (8) (2007) 1222–1244, doi:10.1002/anie.200602866.
[317] P. Desai, R.R. Patlolla, M. Singh, Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery, Mol. Membr. Biol. 27 (7) (2010) 247–259, doi:10.3109/09687688.2010.522203.
[318] S. Jain, D.G. Hirst, J.M. O'Sullivan, Gold nanoparticles as novel agents for cancer therapy, Br. J. Radiol. 85 (1010) (2012) 101–113, doi:10.1259/bjr/59448833.
[319] J.F. Hainfeld, D.N. Slatkin, H.M. Smilowitz, The use of gold nanoparticles to enhance radiotherapy in mice, Phys. Med. Biol. 49 (18) (2004) N309–N315.
[320] C. Loo, A. Lin, L. Hirsch, M.H. Lee, J. Barton, N. Halas, et al., Nanoshell-enabled photonics-based imaging and therapy of cancer, Technol. Cancer Res. Treat. 3 (1) (2004) 33–40.
[321] A. Nel, T. Xia, L. Mädler, N. Li, Toxic potential of materials at the nanolevel, Science 311 (5761) (2006) 622–627.
[322] G. Kaul, M. Amiji, Long-circulating poly(ethylene glycol)-modified gelatin nanoparticles for intracellular delivery, Pharm. Res. 19 (7) (2002) 1061–1067.
[323] M. Eghtedari, A.V. Liopo, J.A. Copland, A.A. Oraevsky, M. Motamedi, Engineering of hetero-functional gold nanorods for the in vivo molecular targeting of breast cancer cells, Nano Lett. 9 (1) (2009) 287–291, doi:10.1021/nl802915q.
[324] B.D. Chithrani, A.A. Ghazani, W.C. Chan, Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells, Nano Lett. 6 (4) (2006) 662–668, doi:10.1021/nl052396o.
[325] I.H. El-Sayed, X. Huang, M.A. El-Sayed, Surface plasmon resonance scattering and absorption of anti-EGFR antibody conjugated gold nanoparticles in cancer diagnostics: applications in oral cancer, Nano Lett. 5 (5) (2005) 829–834, doi:10.1021/nl050074e.
[326] C.R. Patra, R. Bhattacharya, D. Mukhopadhyay, P. Mukherjee, Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer, Adv. Drug Deliv. Rev. 62 (3) (2010) 346–361, doi:10.1016/j.addr.2009.11.007.
[327] C.R. Patra, R. Bhattacharya, E. Wang, A. Katarya, J.S. Lau, S. Dutta, et al., Targeted delivery of gemcitabine to pancreatic adenocarcinoma using cetuximab as a targeting agent, Cancer Res. 68 (6) (2008) 1970–1978, doi:10.1158/0008-5472.CAN-07-6102.
[328] W. Jiang, B.Y. Kim, J.T. Rutka, W.C. Chan, Nanoparticle-mediated cellular response is size-dependent, Nat. Nanotechnol. 3 (3) (2008) 145–150, doi:10.1038/nnano.2008.30.
[329] C. Loo, A. Lowery, N. Halas, J. West, R. Drezek, Immunotargeted nanoshells for integrated cancer imaging and therapy, Nano Lett. 5 (4) (2005) 709–711, doi:10.1021/nl050127s.
[330] J. Chen, D. Wang, J. Xi, L. Au, A. Siekkinen, A. Warsen, et al., Immuno gold nanocages with tailored optical properties for targeted photothermal destruction of cancer cells, Nano Lett. 7 (5) (2007) 1318–1322, doi:10.1021/nl070345g.
[331] X. Huang, P.K. Jain, I.H. El-Sayed, M.A. El-Sayed, Plasmonic photothermal therapy (PPTT) using gold nanoparticles, Lasers Med. Sci. 23 (3) (2008) 217–228.
[332] L. Dykman, N. Khlebtsov, Gold nanoparticles in biomedical applications: recent advances and perspectives, Chem. Soc. Rev. 41 (6) (2012) 2256–2282, doi:10.1039/c1cs15166e.
[333] P.C. Chen, S.C. Mwakwari, A.K. Oyelere, Gold nanoparticles: from nanomedicine to nanosensing, Nanotech. Sci. Appl. 1 (2008) 45–65.
[334] S. Jelveh, D.B. Chithrani, Gold nanostructures as a platform for combinational therapy in future cancer therapeutics, Cancers 3 (1) (2011) 1081–1110, doi:10.3390/cancers3011081.
[335] M.S. Muthu, D.T. Leong, L. Mei, S.S. Feng, Nanotheranostics – application and further development of nanomedicine strategies for advanced theranostics, Theranostics 4 (6) (2014) 660–677, doi:10.7150/thno.8698.
[336] M. Camerin, M. Magaraggia, M. Soncin, G. Jori, M. Moreno, I. Chambrier, et al., The in vivo efficacy of phthalocyanine-nanoparticle conjugates for the photodynamic therapy of amelanotic melanoma, Eur. J. Cancer 46 (10) (2010) 1910–1918, doi:10.1016/j.ejca.2010.02.037.
[337] S. Lal, S.E. Clare, N.J. Halas, Nanoshell-enabled photothermal cancer therapy: impending clinical impact, Acc. Chem. Res. 41 (12) (2008) 1842–1851, doi:10.1021/ar800150g.
[338] J.M. Stern, J. Stanfield, Y. Lotan, S. Park, J.T. Hsieh, J.A. Cadeddu, Efficacy of laser-activated gold nanoshells in ablating prostate cancer cells in vitro, J. Endourol. 21 (8) (2007) 939–943, doi:10.1089/end.2007.0437.
[339] L.R. Hirsch, R.J. Stafford, J.A. Bankson, S.R. Sershen, B. Rivera, R.E. Price, et al., Nanoshell-mediated near-infrared thermal therapy of tumors under magnetic resonance guidance, Proc. Natl. Acad. Sci. U.S.A. 100 (23) (2003) 13549–13554, doi:10.1073/pnas.2232479100.
[340] I.H. El-Sayed, Nanotechnology in head and neck cancer: the race is on, Curr. Oncol. Rep. 12 (2) (2010) 121–128, doi:10.1007/s11912-010-0087-2.
[341] C.H. Su, H.S. Sheu, C.Y. Lin, C.C. Huang, Y.W. Lo, Y.C. Pu, et al., Nanoshell magnetic resonance imaging contrast agents, J. Am. Chem. Soc. 129 (7) (2007) 2139–2146, doi:10.1021/ja0672066.
[342] R.J. Bernardi, A.R. Lowery, P.A. Thompson, S.M. Blaney, J.L. West, Immunonanoshells for targeted photothermal ablation in medulloblastoma and glioma: an in vitro evaluation using human cell lines, J. Neurooncol. 86 (2) (2008) 165–172.
[343] A.S. Sobolev, Modular nanotransporters of anticancer drugs conferring cell specificity and higher efficiency, Biochemistry (Moscow) 74 (13) (2010) 1567–1574, doi:10.1134/s0006297909130094.
[344] A.A. Rosenkranz, G. Vaidyanathan, O.R. Pozzi, V.G. Lunin, M.R. Zalutsky, A.S. Sobolev, Engineered modular recombinant transporters: application of new platform for targeted radiotherapeutic agents to alpha-particle emitting 211 At, Int. J. Radiat. Oncol. Biol. Phys. 72 (1) (2008) 193–200, doi:10.1016/j.ijrobp.2008.05.055.
[345] A.A. Rosenkranz, V.G. Lunin, P.V. Gulak, O.V. Sergienko, M.A. Shumiantseva, O.L. Voronina, et al., Recombinant modular transporters for cell-specific nuclear delivery of locally acting drugs enhance photosensitizer activity, FASEB J. 17 (9) (2003) 1121–1123, doi:10.1096/fj.02-0888fje.
[346] A. Arya, N.K. Sethy, M. Das, S.K. Singh, A. Das, S.K. Ujjain, et al., Cerium oxide nanoparticles prevent apoptosis in primary cortical culture by stabilizing mitochondrial membrane potential, Free Radic. Res. 48 (7) (2014) 784–793, doi:10.3109/10715762.2014.906593.
[347] T. Naganuma, E. Traversa, The effect of cerium valence states at cerium oxide nanoparticle surfaces on cell proliferation, Biomaterials 35 (15) (2014) 4441–4453, doi:10.1016/j.biomaterials.2014.01.074.
[348] S. Das, J.M. Dowding, K.E. Klump, J.F. McGinnis, W. Self, S. Seal, Cerium oxide nanoparticles: applications and prospects in nanomedicine, Nanomedicine (London, England) 8 (9) (2013) 1483–1508, doi:10.2217/nnm.13.133.
[349] S. Babu, J.H. Cho, J.M. Dowding, E. Heckert, C. Komanski, S. Das, et al., Multicolored redox active upconverter cerium oxide nanoparticle for bio-imaging and therapeutics, Chem. Commun. 46 (37) (2010) 6915–6917, doi:10.1039/c0cc01832e.
[350] H. Zheng, G. Chen, F. Song, L.A. DeLouise, Z. Lou, The cytotoxicity of OPA-modified CdSe/ZnS core/shell quantum dots and its modulation by silibinin in human skin cells, J. Biomed. Nanotechnol. 7 (5) (2011) 648–658.
[351] C.J. Wen, C.T. Sung, I.A. Aljuffali, Y.J. Huang, J.Y. Fang, Nanocomposite liposomes containing quantum dots and anticancer drugs for bioimaging and therapeutic delivery: a comparison of cationic, PEGylated and deformable liposomes, Nanotechnology 24 (32) (2013) 325101, doi:10.1088/0957-4484/24/32/325101.
[352] J.E. Lee, N. Lee, H. Kim, J. Kim, S.H. Choi, J.H. Kim, et al., Uniform mesoporous dye-doped silica nanoparticles decorated with multiple magnetite nanocrystals for simultaneous enhanced magnetic resonance imaging, fluorescence imaging, and drug delivery, J. Am. Chem. Soc. 132 (2) (2010) 552–557, doi:10.1021/ja905793q.
[353] N. Shibagaki, T. Okamoto, H. Mitsui, T. Inozume, M. Kanzaki, S. Shimada, Novel immunotherapeutic approaches to skin cancer treatments using protein

transduction technology, J. Dermatol. Sci. 61 (3) (2011) 153–161, doi:10.1016/j.jdermsci.2010.12.003.
[354] J.S. Wadia, S.F. Dowdy, Protein transduction technology, Curr. Opin. Biotechnol. 13 (1) (2002) 52–56.
[355] H. Noguchi, S. Matsumoto, Protein transduction technology: a novel therapeutic perspective, Acta Med. Okayama 60 (1) (2006) 1–11.
[356] K.G. Ford, B.E. Souberbielle, D. Darling, F. Farzaneh, Protein transduction: an alternative to genetic intervention?, Gene Ther. 8 (1) (2001) 1–4, doi:10.1038/sj.gt.3301383.
[357] P.S. Kabouridis, Biological applications of protein transduction technology, Trends Biotechnol. 21 (11) (2003) 498–503, doi:10.1016/j.tibtech.2003.09.008.
[358] D.R. Ciocca, S.K. Calderwood, Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications, Cell Stress Chaperones 10 (2) (2005) 86–103, doi:10.1379/CSC-99r.1.
[359] M.L. Verschoor, R. Ungard, A. Harbottle, J.P. Jakupciak, R.L. Parr, G. Singh, Mitochondria and cancer: past, present, and future, BioMed Res. Int. 2013 (2013) 612369, doi:10.1155/2013/612369.
[360] S.K. Calderwood, Molecular cochaperones: tumor growth and cancer treatment, Scientifica 2013 (2013) 217513, doi:10.1155/2013/217513.
[361] A. Kalogeraki, F. Garbagnati, K. Darivianaki, G.S. Delides, M. Santinami, E.N. Stathopoulos, et al., HSP-70, C-myc and HLA-DR expression in patients with cutaneous malignant melanoma metastatic in lymph nodes, Anticancer Res. 26 (5A) (2006) 3551–3554.
[362] M.P. Protti, S. Heltai, M. Bellone, M. Ferrarini, A.A. Manfredi, C. Rugarli, Constitutive expression of the heat shock protein 72 kDa in human melanoma cells, Cancer Lett. 85 (2) (1994) 211–216.
[363] C.J. Denman, J. McCracken, V. Hariharan, J. Klarquist, K. Oyarbide-Valencia, J.A. Guevara-Patino, et al., HSP70i accelerates depigmentation in a mouse model of autoimmune vitiligo, J. Invest. Dermatol. 128 (8) (2008) 2041–2048, doi:10.1038/jid.2008.45.
[364] J. Steitz, J. Bruck, K. Steinbrink, A. Enk, J. Knop, T. Tuting, Genetic immunization of mice with human tyrosinase-related protein 2: implications for the immunotherapy of melanoma, Int. J. Cancer 86 (1) (2000) 89–94.
[365] A.J. Olszanski, Current and future roles of targeted therapy and immunotherapy in advanced melanoma, J. Manag. Care Pharm. 20 (4) (2014) 346–356.
[366] A. Ribas, R. Kefford, M.A. Marshall, C.J. Punt, J.B. Haanen, M. Marmol, et al., Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma, J. Clin. Oncol. 31 (5) (2013) 616–622, doi:10.1200/jco.2012.44.6112.
[367] J.D. Wolchok, F.S. Hodi, J.S. Weber, J.P. Allison, W.J. Urba, C. Robert, et al., Development of ipilimumab: a novel immunotherapeutic approach for the treatment of advanced melanoma, Ann. N. Y. Acad. Sci. 1291 (2013) 1–13, doi:10.1111/nyas.12180.
[368] Y.C. Kong, W.Z. Wei, Y. Tomer, Opportunistic autoimmune disorders: from immunotherapy to immune dysregulation, Ann. N. Y. Acad. Sci. 1183 (2010) 222–236, doi:10.1111/j.1749-6632.2009.05138.x.
[369] D.S. Chen, B.A. Irving, F.S. Hodi, Molecular pathways: next-generation immunotherapy – inhibiting programmed death-ligand 1 and programmed death-1, Clin. Cancer Res. 18 (24) (2012) 6580–6587, doi:10.1158/1078-0432.ccr-12-1362.
[370] Y.C. Kong, J.C. Flynn, Opportunistic autoimmune disorders potentiated by immune-checkpoint inhibitors Anti-CTLA-4 and Anti-PD-1, Front. Immunol. 5 (2014) 206, doi:10.3389/fimmu.2014.00206.
[371] J.D. Wolchok, H. Kluger, M.K. Callahan, M.A. Postow, N.A. Rizvi, A.M. Lesokhin, et al., Nivolumab plus ipilimumab in advanced melanoma, N. Engl. J. Med. 369 (2) (2013) 122–133, doi:10.1056/NEJMoa1302369.
[372] F. Ghiringhelli, L. Zitvogel, Vaccine strategies against melanoma, Med. Sci. (Paris) 22 (2) (2006) 183–187, doi:10.1051/medsci/2006222183.
[373] D. Schadendorf, Gene-based therapy of malignant melanoma, Semin. Oncol. 29 (5) (2002) 503–512.
[374] Y. Sun, D. Schadendorf, Gene-based immunotherapy of skin cancers, Recent Results Cancer Res. 160 (2002) 170–184.
[375] J. Wenzel, D. Tormo, T. Tuting, Toll-like receptor-agonists in the treatment of skin cancer: history, current developments and future prospects, Handb. Exp. Pharmacol. 183 (2008) 201–220, doi:10.1007/978-3-540-72167-3_10.
[376] A.O. Huen, A.H. Rook, Toll receptor agonist therapy of skin cancer and cutaneous T-cell lymphoma, Curr. Opin. Oncol. 26 (2) (2014) 237–244, doi:10.1097/CCO.0000000000000048.
[377] C.W. Mai, Y.B. Kang, M.R. Pichika, Should a Toll-like receptor 4 (TLR-4) agonist or antagonist be designed to treat cancer? TLR-4: its expression and effects in the ten most common cancers, OncoTargets Ther. 6 (2013) 1573–1587, doi:10.2147/OTT.S50838.
[378] B. Jahrsdorfer, G.J. Weiner, CpG oligodeoxynucleotides as immunotherapy in cancer, Update Cancer Ther. 3 (1) (2008) 27–32, doi:10.1016/j.uct.2007.11.003.
[379] G. Mignot, E. Ullrich, M. Bonmort, C. Menard, L. Apetoh, J. Taieb, et al., The critical role of IL-15 in the antitumor effects mediated by the combination therapy imatinib and IL-2, J. Immunol. 180 (10) (2008) 6477–6483, doi:10.4049/jimmunol.180.10.6477.
[380] J. Boguslawska, M. Malecki, siRNA preparations in gene therapy of melanoma, Med. Wieku Rozwoj. 17 (3) (2013) 196–201.
[381] H. Kato, O. Takeuchi, S. Sato, M. Yoneyama, M. Yamamoto, K. Matsui, et al., Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses, Nature 441 (7089) (2006) 101–105, doi:10.1038/nature04734.
[382] M.P. Schon, B.G. Wienrich, C. Drewniok, A.B. Bong, J. Eberle, C.C. Geilen, et al., Death receptor-independent apoptosis in malignant melanoma induced by the small-molecule immune response modifier imiquimod, J. Invest. Dermatol. 122 (5) (2004) 1266–1276, doi:10.1111/j.0022-202X.2004.22528.x.
[383] C.P. El-Haibi, A.E. Karnoub, Mesenchymal stem cells in the pathogenesis and therapy of breast cancer, J. Mammary Gland Biol. Neoplasia 15 (4) (2010) 399–409, doi:10.1007/s10911-010-9196-7.
[384] Y.L. Hu, Y.H. Fu, Y. Tabata, J.Q. Gao, Mesenchymal stem cells: a promising targeted-delivery vehicle in cancer gene therapy, J. Control. Release 147 (2) (2010) 154–162, doi:10.1016/j.jconrel.2010.05.015.
[385] X. Chen, X. Lin, J. Zhao, W. Shi, H. Zhang, Y. Wang, et al., A tumor-selective biotherapy with prolonged impact on established metastases based on cytokine gene-engineered MSCs, Mol. Ther. 16 (4) (2008) 749–756, doi:10.1038/mt.2008.3.
[386] A. Nakamizo, F. Marini, T. Amano, A. Khan, M. Studeny, J. Gumin, et al., Human bone marrow-derived mesenchymal stem cells in the treatment of gliomas, Cancer Res. 65 (8) (2005) 3307–3318, doi:10.1158/0008-5472.CAN-04-1874.
[387] M. Studeny, F.C. Marini, R.E. Champlin, C. Zompetta, I.J. Fidler, M. Andreeff, Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors, Cancer Res. 62 (13) (2002) 3603–3608.
[388] G. Grisendi, R. Bussolari, L. Cafarelli, I. Petak, V. Rasini, E. Veronesi, et al., Adipose-derived mesenchymal stem cells as stable source of tumor necrosis factor-related apoptosis-inducing ligand delivery for cancer therapy, Cancer Res. 70 (9) (2010) 3718–3729, doi:10.1158/0008-5472.CAN-09-1865.
[389] L.S. Sasportas, R. Kasmieh, H. Wakimoto, S. Hingtgen, J.A. van de Water, G. Mohapatra, et al., Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy, Proc. Natl. Acad. Sci. U.S.A. 106 (12) (2009) 4822–4827, doi:10.1073/pnas.0806647106.
[390] A. Mohr, S.M. Albarenque, L. Deedigan, R. Yu, M. Reidy, S. Fulda, et al., Targeting of XIAP combined with systemic mesenchymal stem cell-mediated delivery of sTRAIL ligand inhibits metastatic growth of pancreatic carcinoma cells, Stem Cells 28 (11) (2010) 2109–2120, doi:10.1002/stem.533.
[391] M.R. Loebinger, E.K. Sage, D. Davies, S.M. Janes, TRAIL-expressing mesenchymal stem cells kill the putative cancer stem cell population, Br. J. Cancer 103 (11) (2010) 1692–1697, doi:10.1038/sj.bjc.6605952.
[392] M. Roger, A. Clavreul, M.C. Venier-Julienne, C. Passirani, C. Montero-Menei, P. Menei, The potential of combinations of drug-loaded nanoparticle systems and adult stem cells for glioma therapy, Biomaterials 32 (8) (2011) 2106–2116, doi:10.1016/j.biomaterials.2010.11.056.
[393] M. Roger, A. Clavreul, M.C. Venier-Julienne, C. Passirani, L. Sindji, P. Schiller, et al., Mesenchymal stem cells as cellular vehicles for delivery of nanoparticles to brain tumors, Biomaterials 31 (32) (2010) 8393–8401, doi:10.1016/j.biomaterials.2010.07.048.
[394] C. Tang, P.J. Russell, R. Martiniello-Wilks, J.E. Rasko, A. Khatri, Concise review: nanoparticles and cellular carriers-allies in cancer imaging and cellular gene therapy?, Stem Cells 28 (9) (2010) 1686–1702, doi:10.1002/stem.473.
[395] K.S. Aboody, A. Brown, N.G. Rainov, K.A. Bower, S. Liu, W. Yang, et al., Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas, Proc. Natl. Acad. Sci. U.S.A. 97 (23) (2000) 12846–12851, doi:10.1073/pnas.97.23.12846.
[396] O. Shakhova, Neural crest stem cells in melanoma development, Curr. Opin. Oncol. 26 (2) (2014) 215–221, doi:10.1097/CCO.0000000000000046.
[397] K.Y. Choi, G. Saravanakumar, J.H. Park, K. Park, Hyaluronic acid-based nanocarriers for intracellular targeting: interfacial interactions with proteins in cancer, Colloids Surf. B. Biointerfaces 99 (2012) 82–94, doi:10.1016/j.colsurfb.2011.10.029.
[398] V.M. Platt, F.C. Szoka Jr., Anticancer therapeutics: targeting macromolecules and nanocarriers to hyaluronan or CD44, a hyaluronan receptor, Mol. Pharm. 5 (4) (2008) 474–486, doi:10.1021/mp800024g.
[399] H. Sun, R.V. Benjaminsen, K. Almdal, T.L. Andresen, Hyaluronic acid immobilized polyacrylamide nanoparticle sensors for CD44 receptor targeting and pH measurement in cells, Bioconjug. Chem. 23 (11) (2012) 2247–2255, doi:10.1021/bc300349n.
[400] D.L. Miller, N.B. Smith, M.R. Bailey, G.J. Czarnota, K. Hynynen, I.R. Makin, et al., Overview of therapeutic ultrasound applications and safety considerations, J. Ultrasound Med. 31 (4) (2012) 623–634.
[401] S.J. Grainger, J.V. Serna, S. Sunny, Y. Zhou, C.X. Deng, M.E. El-Sayed, Pulsed ultrasound enhances nanoparticle penetration into breast cancer spheroids, Mol. Pharm. 7 (6) (2010) 2006–2019, doi:10.1021/mp100280b.
[402] H.R. Guzman, A.J. McNamara, D.X. Nguyen, M.R. Prausnitz, Bioeffects caused by changes in acoustic cavitation bubble density and cell concentration: a unified explanation based on cell-to-bubble ratio and blast radius, Ultrasound Med. Biol. 29 (8) (2003) 1211–1222.
[403] C.X. Deng, F. Sieling, H. Pan, J. Cui, Ultrasound-induced cell membrane porosity, Ultrasound Med. Biol. 30 (4) (2004) 519–526, doi:10.1016/j.ultrasmedbio.2004.01.005.
[404] A. van Wamel, A. Bouakaz, B. Bernard, F. ten Cate, N. de Jong, Radionuclide tumour therapy with ultrasound contrast microbubbles, Ultrasonics 42 (1–9) (2004) 903–906, doi:10.1016/j.ultras.2003.11.013.
[405] I. Lavon, J. Kost, Ultrasound and transdermal drug delivery, Drug Discov. Today 9 (15) (2004) 670–676, doi:10.1016/S1359-6446(04)03170-8.
[406] N.B. Smith, Applications of ultrasonic skin permeation in transdermal drug delivery, Expert Opin. Drug Deliv. 5 (10) (2008) 1107–1120, doi:10.1517/17425247.5.10.1107.
[407] R. Rao, S. Nanda, Sonophoresis: recent advancements and future trends, J. Pharm. Pharmacol. 61 (6) (2009) 689–705, doi:10.1211/jpp.61.06.0001.

[408] E.C. Unger, T. Porter, W. Culp, R. Labell, T. Matsunaga, R. Zutshi, Therapeutic applications of lipid-coated microbubbles, Adv. Drug Deliv. Rev. 56 (9) (2004) 1291–1314, doi:10.1016/j.addr.2003.12.006.
[409] K. Ferrara, R. Pollard, M. Borden, Ultrasound microbubble contrast agents: fundamentals and application to gene and drug delivery, Annu. Rev. Biomed. Eng. 9 (2007) 415–447, doi:10.1146/annurev.bioeng.8.061505.095852.
[410] C.M. Panje, D.S. Wang, J.K. Willmann, Ultrasound and microbubble-mediated gene delivery in cancer: progress and perspectives, Invest. Radiol. 48 (11) (2013) 755–769, doi:10.1097/RLI.0b013e3182982cc1.
[411] A. Kamaya, S. Machtaler, S. Safari Sanjani, A. Nikoozadeh, F. Graham Sommer, B.T. Pierre Khuri-Yakub, et al., New technologies in clinical ultrasound, Semin. Roentgenol. 48 (3) (2013) 214–223, doi:10.1053/j.ro.2013.03.009.
[412] A. Gelet, J.Y. Chapelon, R. Bouvier, O. Rouviere, Y. Lasne, D. Lyonnet, et al., Transrectal high-intensity focused ultrasound: minimally invasive therapy of localized prostate cancer, J. Endourol. 14 (6) (2000) 519–528.
[413] S. Thuroff, C. Chaussy, G. Vallancien, W. Wieland, H.J. Kiel, A. Le Duc, et al., High-intensity focused ultrasound and localized prostate cancer: efficacy results from the European multicentric study, J. Endourol. 17 (8) (2003) 673–677, doi:10.1089/089277903322518699.
[414] J.E. Kennedy, High-intensity focused ultrasound in the treatment of solid tumours, Nat. Rev. Cancer 5 (4) (2005) 321–327, doi:10.1038/nrc1591.
[415] R. Braakman, P. van der Valk, J.L. van Delft, D. de Wolff-Rouendaal, J.A. Oosterhuis, The effects of ultrasonically induced hyperthermia on experimental tumors in the rabbit eye, Invest. Ophthalmol. Vis. Sci. 30 (5) (1989) 835–844.
[416] F.J. Landstrom, C.O. Nilsson, S. Crafoord, J.A. Reizenstein, G.B. Adamsson, L.A. Lofgren, Electroporation therapy of skin cancer in the head and neck area, Dermatol. Surg. 36 (8) (2010) 1245–1250, doi:10.1111/j.1524-4725.2010.01617.x.
[417] N. Kanikkannan, Iontophoresis-based transdermal delivery systems, Biodrugs 16 (5) (2002) 339–347.
[418] L.G. Campana, A. Testori, N. Mozzillo, C.R. Rossi, Treatment of metastatic melanoma with electrochemotherapy, J. Surg. Oncol. 109 (4) (2014) 301–307.
[419] B. Mali, T. Jarm, M. Snoj, G. Sersa, D. Miklavcic, Antitumor effectiveness of electrochemotherapy: a systematic review and meta-analysis, Eur. J. Surg. Oncol. 39 (1) (2013) 4–16, doi:10.1016/j.ejso.2012.08.016.
[420] E. Kis, I. Szegesdi, H. Ocsai, R. Gyulai, L. Kemeny, J. Olah, Electrochemotherapy of melanoma cutaneous metastases, Orv. Hetil. 151 (3) (2010) 99–101, doi:10.1556/OH.2010.28781.
[421] P. Queirolo, F. Marincola, F. Spagnolo, Electrochemotherapy for the management of melanoma skin metastasis: a review of the literature and possible combinations with immunotherapy, Arch. Dermatol. Res. 306 (6) (2014) 521–526, doi:10.1007/s00403-014-1462-x.
[422] A. Testori, C.R. Rossi, G. Tosti, Utility of electrochemotherapy in melanoma treatment, Curr. Opin. Oncol. 24 (2) (2012) 155–161, doi:10.1097/CCO.0b013e32834fcaa8.
[423] E. Cha, A. Daud, Plasmid IL-12 electroporation in melanoma, Hum. Vaccin. Immunother. 8 (11) (2012) 1734–1738, doi:10.4161/hv.22573.
[424] L.C. Heller, R. Heller, Electroporation gene therapy preclinical and clinical trials for melanoma, Curr. Gene Ther. 10 (4) (2010) 312–317.
[425] H.M. Horton, P.A. Lalor, A.P. Rolland, IL-2 plasmid electroporation: from preclinical studies to phase I clinical trial, Methods Mol. Biol. 423 (2008) 361–372, doi:10.1007/978-1-59745-194-9_28.
[426] E.B. Garon, D. Sawcer, P.T. Vernier, T. Tang, Y. Sun, L. Marcu, et al., In vitro and in vivo evaluation and a case report of intense nanosecond pulsed electric field as a local therapy for human malignancies, Int. J. Cancer 121 (3) (2007) 675–682, doi:10.1002/ijc.22723.
[427] M.G. Nava-Arzaluz, I. Calderon-Lojero, D. Quintanar-Guerrero, R. Villalobos-Garcia, A. Ganem-Quintanar, Microneedles as transdermal delivery systems: combination with other enhancing strategies, Curr. Drug Deliv. 9 (1) (2012) 57–73.
[428] S.A. Coulman, A. Anstey, C. Gateley, A. Morrissey, P. McLoughlin, C. Allender, et al., Microneedle mediated delivery of nanoparticles into human skin, Int. J. Pharm. 366 (1–2) (2009) 190–200, doi:10.1016/j.ijpharm.2008.08.040.
[429] M.T. Lin, L. Pulkkinen, J. Uitto, K. Yoon, The gene gun: current applications in cutaneous gene therapy, Int. J. Dermatol. 39 (3) (2000) 161–170.
[430] K. Aravindaram, N.S. Yang, Gene gun delivery systems for cancer vaccine approaches, Methods Mol. Biol. 542 (2009) 167–178, doi:10.1007/978-1-59745-561-9_9.
[431] A. Dietrich, K. Kraus, U. Brinckmann, C. Stockmar, A. Muller, U.G. Liebert, et al., Antitumoral and antimetastatic effects of continuous particle-mediated cytokine gene therapy, Recent Results Cancer Res. 162 (2003) 157–168.
[432] A. Dietrich, K. Kraus, U. Brinckmann, T. Friedrich, A. Muller, U.G. Liebert, et al., Complex cancer gene therapy in mice melanoma, Langenbecks Arch. Surg. 387 (3–4) (2002) 177–182, doi:10.1007/s00423-002-0299-5.
[433] Y. Zha, C. Lin, X. Liang, [The use of gene gun in cancer gene therapy], Zhonghua Yi Xue Za Zhi 80 (7) (2000) 522–525.
[434] C.F. Rochlitz, Gene therapy of cancer, Swiss Med. Wkly 131 (1–2) (2001) 4–9, doi:2001/01/smw-09649.
[435] K. Tanigawa, H. Yu, R. Sun, B.J. Nickoloff, A.E. Chang, Gene gun application in the generation of effector T cells for adoptive immunotherapy, Cancer Immunol. Immunother. 48 (11) (2000) 635–643.
[436] A.E. Chang, K. Tanigawa, J.G. Turner, E.C. Chang, H. Yu, Use of gene gun for genetic immunotherapy : in vitro and in vivo methods, Methods Mol. Med. 61 (2001) 223–240, doi:10.1385/1-59259-145-0:223.
[437] E.B. Dickerson, E.C. Dreaden, X. Huang, I.H. El-Sayed, H. Chu, S. Pushpanketh, et al., Gold nanorod assisted near-infrared plasmonic photothermal therapy (PPTT) of squamous cell carcinoma in mice, Cancer Lett. 269 (1) (2008) 57–66, doi:10.1016/j.canlet.2008.04.026.
[438] M. Camerin, S. Rello-Varona, A. Villanueva, M.A. Rodgers, G. Jori, Metallo-naphthalocyanines as photothermal sensitisers for experimental tumours: in vitro and in vivo studies, Lasers Surg. Med. 41 (9) (2009) 665–673, doi:10.1002/lsm.20846.
[439] A. Khdair, D. Chen, Y. Patil, L. Ma, Q.P. Dou, M.P. Shekhar, et al., Nanoparticle-mediated combination chemotherapy and photodynamic therapy overcomes tumor drug resistance, J. Control. Release 141 (2) (2010) 137–144, doi:10.1016/j.jconrel.2009.09.004.
[440] R. Vankayala, Y.K. Huang, P. Kalluru, C.S. Chiang, K.C. Hwang, First demonstration of gold nanorods-mediated photodynamic therapeutic destruction of tumors via near infra-red light activation, Small 10 (8) (2014) 1612–1622, doi:10.1002/smll.201302719.
[441] C. Hoffmann, C. Berganza, J. Zhang, Cold atmospheric plasma: methods of production and application in dentistry and oncology, Med. Gas Res. 3 (1) (2013) 21, doi:10.1186/2045-9912-3-21.
[442] S. Arndt, E. Wacker, Y.F. Li, T. Shimizu, H.M. Thomas, G.E. Morfill, et al., Cold atmospheric plasma, a new strategy to induce senescence in melanoma cells, Exp. Dermatol. 22 (4) (2013) 284–289, doi:10.1111/exd.12127.
[443] G. Fridman, A. Shereshevsky, M.M. Jost, A.D. Brooks, A. Fridman, A. Gutsol, et al., Floating electrode dielectric barrier discharge plasma in air promoting apoptotic behavior in melanoma skin cancer cell lines, Plasma Chem. Plasma Process. 27 (2) (2007) 163–176, doi:10.1007/s11090-007-9048-4.
[444] J.Y. Kim, Y. Wei, J. Li, P. Foy, T. Hawkins, J. Ballato, et al., Single-cell-level microplasma cancer therapy, Small 7 (16) (2011) 2291–2295, doi:10.1002/smll.201100456.