

# Pentacyclic Triterpenes of the Lupane, Oleanane and Ursane Group as Tools in Cancer Therapy

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## Key words

- pentacyclic triterpenes
- multifunctional agent
- cancer treatment

## Abstract

Today cancer treatment is not only a question of eliminating cancer cells by induction of cell death. New therapeutic strategies also include targeting the tumour microenvironment, avoiding angiogenesis, modulating the immune response or the chronic inflammation that is often associated with cancer. Furthermore, the induction of redifferentiation of dedifferentiated cancer cells is an interesting aspect in developing new therapy strategies. Plants provide a broad spectrum of potential drug substances for cancer therapy with multifaceted effects and targets. Pentacyclic triterpenes are one group of promising secondary plant metabolites. This review summarizes the potential of triterpenes belonging to the lupane, oleanane or ursane group, to treat cancer by different modes of action. Since Pisha et al. reported in 1995 that betulinic acid is a highly promising anticancer drug after inducing apoptosis in melanoma cell lines *in vitro* and *in vivo*, experimental work focused on the apoptosis inducing mechanisms of betulinic acid and other triterpenes. The antitumour effects were subsequently confirmed in a series of cancer cell lines from other origins, for example breast, colon, lung and neuroblastoma. In addition, in the last decade many studies have shown further effects that justify the expectation that triterpenes are useful to treat cancer by several modes of action. Thus, triterpene acids are known mainly for their antiangiogenic effects as well as their differentiation inducing effects. In particular, lupane-type triterpenes, such as betulin, betulinic acid and lupeol, display anti-inflammatory activities which often accompany immune modulation. Triterpene acids as well as triterpene monoalcohols and diols also show an antioxidative potential. The pharmacological potential of triterpenes of the lupane, oleanane or ursane type for cancer treatment seems high; although up to now no clinical trial has been pub-

lished using these triterpenes in cancer therapy. They provide a multitarget potential for coping with new cancer strategies. Whether this is an effective approach for cancer treatment has to be proven. Because various triterpenes are an increasingly promising group of plant metabolites, the utilisation of different plants as their sources is of interest. Parts of plants, for example birch bark, rosemary leaves, apple peel and mistletoe shoots are rich in triterpenes and provide different triterpene compositions.

## Abbreviations

|         |  |
|---------|--|
| ACC:    | antigen-dependent complement-mediated cytotoxicity |
| ADCC:   | antibody-dependent cell mediated cytotoxicity      |
| APN:    | aminopeptidase N                                   |
| ARE:    | antioxidant response element                       |
| BAEC:   | bovine aortic endothelial cell                     |
| CAM:    | chick embryo chorioallantoic membrane              |
| CAT:    | catalase   |
| COX-2:  | cyclooxygenase-2                                   |
| DMBA:   | 7,12-dimethylbenz(a)anthracene                     |
| DMSO:   | dimethylsulfoxide                                  |
| ECM:    | extracellular matrix                               |
| FGF:    | fibroblast growth factor                           |
| GM-CSF: | granulocyte-macrophage colony-stimulating factor   |
| GSH:    | glutathione  |
| HUVEC:  | human umbilical vein endothelial cell              |
| ID:     | inhibition dose                                    |
| IL:     | interleukin  |
| iNOS:   | inducible NO-synthase                              |
| LD:     | lethal dose  |
| NADP*:  | nicotinamide adenine dinucleotide phosphate        |
| NF-κB:  | nuclear factor-kappaB                              |
| NO:     | nitric oxide                                       |

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Nrf2: nuclear factor E2-related factor 2  
 PCNA: proliferating cell nuclear antigen  
 PGE<sub>2</sub>: prostaglandin E<sub>2</sub>  
 PLA<sub>2</sub>: phospholipase A<sub>2</sub>  
 PPAR: peroxisome proliferator activated receptor  
 ROS: reactive oxygen species  
 SOD: superoxide dismutase

Sp: specificity protein  
 TE: triterpene dry extract  
 TNF: tumour necrosis factor  
 TPA: 12-O-tetradecanoylphorbol-13-acetate  
 TRPC6: transient receptor potential channel 6  
 VEGF: vascular endothelial growth factor

## Introduction

Today solid tumours are no longer considered as a mere accumulation of abnormal, malignant cancer cells. The tumour environment, the tumour stroma, is becoming more and more important. Therefore, treatment strategies have to be changed. Most therapies now try to eliminate cancer cells by inducing apoptosis or necrosis. New therapy strategies include the treatment of the tumour environment, avoiding angiogenesis and modulating the immune response or the chronic inflammation that is often associated with cancer promotion and progression. Another approach is to redifferentiate proliferating tumour cells.

In addition, chemoprevention is important to avoid cancer promotion. Cancer results from a multistage carcinogenesis process: initiation, promotion and progression. Because reducing the initiation phase to a zero level is impossible, the most efficient intervention would be at the promotion phase to eliminate premalignant cells before they become malignant [1]. Therefore, the concept of delaying or preventing this transformation is worth testing in future studies [2].

Pentacyclic triterpenes are secondary plant metabolites which arise from cyclization of squalene [3]. This article focused on triterpenes of the lupeol, oleanane and ursane type. They are found in different plant organs, e.g., in bark, cork, or in the wax covering leaves or peel. Low amounts (<0.1% of the dry weight of a plant organ) are ubiquitously present in plants. However, there are a few species that display a high amount of these pentacyclic triterpenes (> 1% of the dry weight of the plant organ). The highest triterpene amount has been found in the outer bark of white birch. The white outer bark contains up to 34% (w/w) betulin [4]. Beside

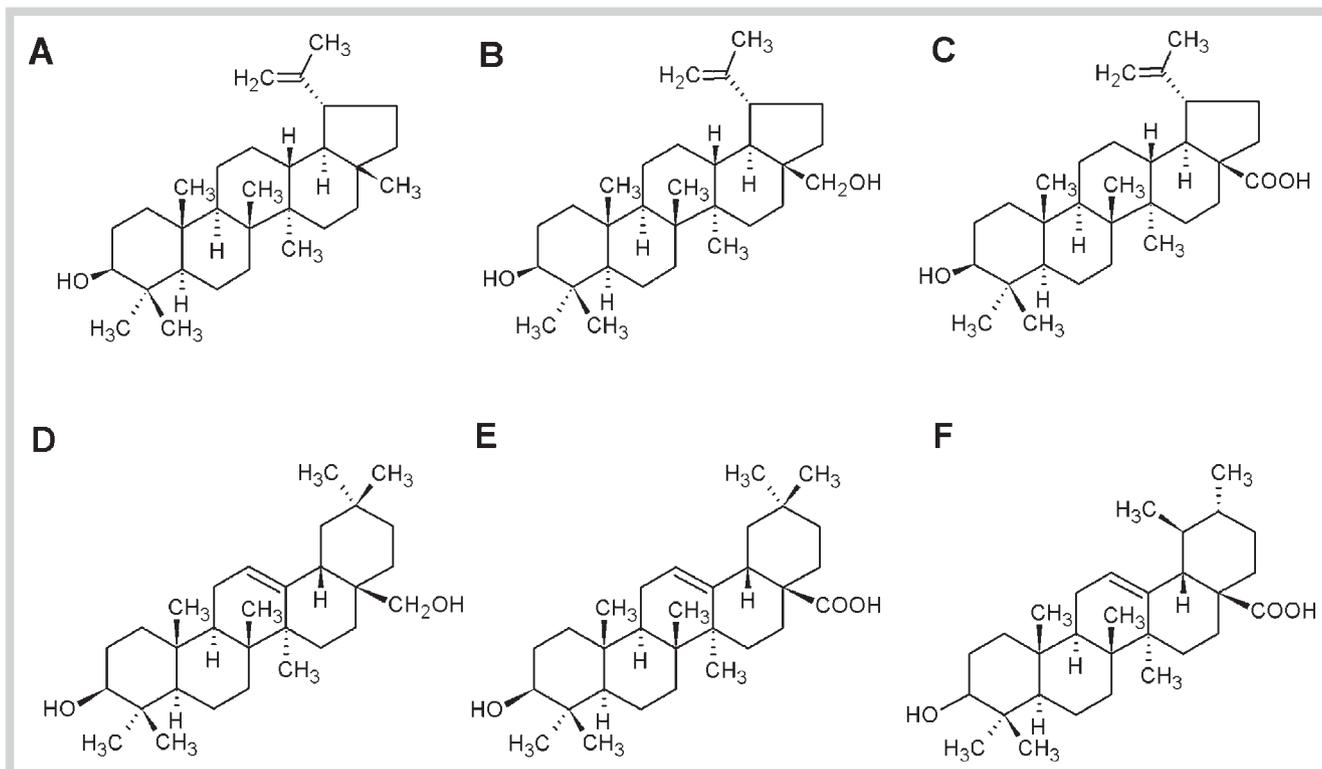
the outer bark of birch, leaves of rosemary and olive, mistletoe as well as plane tree bark and apple peelings contain more than 1% (w/w) of these pentacyclic triterpenes (● Table 1). These plants can be used to obtain triterpene dry extracts consisting of 50–90% (w/w) triterpenes [5]. Depending on the plant material, lupeol, betulin, betulinic acid, oleanolic acid, ursolic acid or an equal mixture of these substances are the main components of such dry extracts [6] (● Fig. 1). This kind of triterpene extract may be used as starting material for further pharmaceutical development.

In the last 15 years hundreds of publications have highlighted the broad spectrum of biological activities of lupane, oleanane and ursane triterpenes. The literature search for this review is based on an actual PubMed search focused on the last two years. On account of the low water solubility of triterpenes, special attention was given to the concentrations used in *in vitro* experiments. Concentrations above 100 μM often bias the results, because of an insoluble fraction. In case of *in vivo* data, we included effects that are described by almost all different work groups.

Because of their cytotoxicity against various cancer cell lines the group of lupane, oleanane and ursane triterpenes are considered as promising anticancer drugs. Nevertheless, due to their various pharmacological activities including antiangiogenic, anti-inflammatory as well as antioxidant effects and the ability to enhance cell differentiation, they are more than a simple cytotoxic anticancer drug and are suitable for modern cancer strategies (● Fig. 2). Moreover, they are regarded as essential parts of human nutrition because of their chemopreventive potential to fend off cancer promotion [7,8].

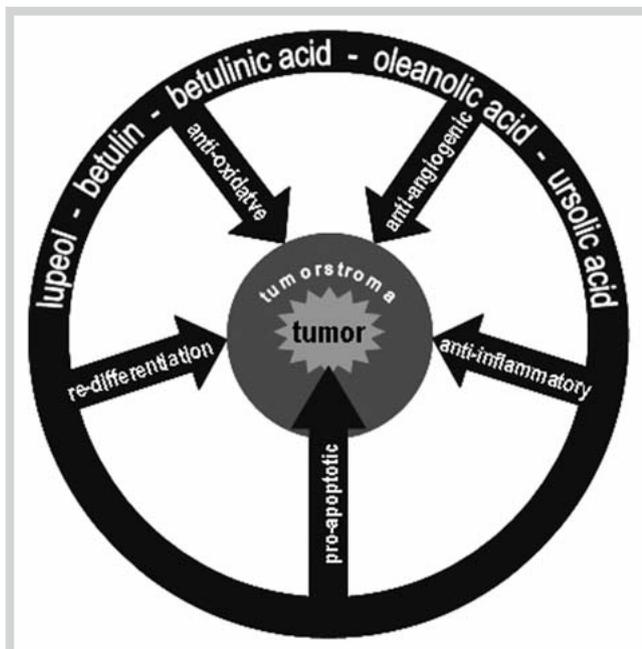
| Plant  | Part          | Triterpene     | Amount   | Reference |
|--|---------------|----------------|----------|-----------|
| <i>Betula alba</i> L., Betulaceae            | bark          | lupeol         | 1–2%     | [5]       |
|  |               | betulin        | 10–34%   | [4, 5]    |
|  |               | betulinic acid | 0.5–1.5% | [5]       |
|  |               | oleanolic acid | 0–1.5%   | [5]       |
| <i>Rosmarinus officinalis</i> L., Lamiaceae  | leaves        | betulinic acid | 1.5%     | [6]       |
|  |               | oleanolic acid | 1.2%     | [6]       |
|  |               | ursolic acid   | 3.0%     | [6]       |
| <i>Malus domestica</i> Mill., Rosaceae       | fruit<br>peel | ursolic acid   | 2.0%     | [6]       |
|  |               |                |          |           |
| <i>Platanus</i> L., Platanaceae              | bark          | betulinic acid | 2.4%     | [6]       |
| <i>Viscum album</i> L., Viscaceae            | sapling       | oleanolic acid | 1.0%     | [6]       |
| <i>Olea europaea</i> L., Oleaceae            | leaves        | oleanolic acid | 3.1%     | [6]       |
| <i>Nerium oleander</i> L., Apocynaceae       | leaves        | ursolic acid   | 1.2%     | [6]       |
| <i>Arctostaphylos uva-ursi</i> L., Ericaceae | leaves        | ursolic acid   | 1.2%     | [6]       |
| <i>Coffea</i> L., Rubiaceae                  | leaves        | ursolic acid   | 1.8%     | [6]       |
| <i>Eucalyptus</i> L'Hér., Myrtaceae          | leaves        | ursolic acid   | 1.2%     | [6]       |
| <i>Lavandula angustifolia</i> L., Lamiaceae  | leaves        | ursolic acid   | 1.6%     | [6]       |
| <i>Salvia officinalis</i> L., Lamiaceae      | leaves        | ursolic acid   | 1.8%     | [6]       |
| <i>Syzygium aromaticum</i> L., Myrtaceae     | flowers       | oleanolic acid | 1.6%     | [6]       |
| <i>Thymus vulgaris</i> L., Lamiaceae         | leaves        | ursolic acid   | 1.0%     | [6]       |

**Table 1** Plants which display a high amount of pentacyclic triterpenes.



**Fig. 1** Chemical structures of (A) lupeol, (B) betulin, (C) betulinic acid, (D) erythrodiol, (E) oleanolic acid and (F) ursolic acid. Lupeol, betulin and betulinic acid belong to the lupan type pentacyclic triterpenes consisting of four six-rings and one five-ring, whereas erythrodiol and oleanolic acid exhibit an

oleanan structure with five six-rings. This is quite similar to the ursan group, which is represented by ursolic acid. The difference between the oleanan and the ursan type is the methyl-group localization of the E-ring.



**Fig. 2** Pentacyclic triterpenes such as lupeol, betulin, betulinic acid, erythrodiol, oleanolic acid and ursolic acid exhibit various pharmacological activities. Besides targeting tumour cells by induction of apoptosis, they also modulate the tumour environment displaying antiangiogenic, anti-inflammatory as well as antioxidant effects and enhancing cell differentiation. The efficacy of each single compound might differ with respect to the various activities, so the combination of these substances might have a benefit in order to treat cancer from different from different angles in modern cancer strategies.

### Apoptosis

Induction of apoptosis by pro-apoptotic agents is one important part of cancer therapy. But apoptosis in cancer cells is often impaired or even blocked by mutated genes regulating the cell cycle or an imbalanced ratio of pro- and antiapoptotic proteins. Therefore it is necessary to target different steps of the apoptotic process to bypass such blocks with respect to the type of cancer. This review highlights only a few aspects of the knowledge about triterpenes and apoptosis. But it should give an impression of the diversity of mechanisms triggered by these triterpenes and with it the chance to overcome apoptosis resistance in cancer cells.

Triterpenes trigger apoptosis by different modes of action, as extensively described in a series of reviews, especially for betulinic acid [7–9]. First it was assumed that betulinic acid is a selective cytotoxic compound against melanoma cells. However, up to now a large panel of cancer cell lines have proven to be sensitive to betulinic acid and other pentacyclic triterpenes. It is also assumed by some authors that there is a selective sensitivity against malignant cells. Nevertheless, cytotoxicity against primary cells such as fibroblasts, melanocytes, keratinocytes, neuronal cells and peripheral blood lymphocytes is reported, but they seem to tolerate higher triterpene concentrations than cancer cells of the same origin [10–14]. Whether this may result in a positive effect *in vivo*, when cancer cells are in a united cell structure is questionable. But in the case of triterpene acids another possibility to enhance their activity in cancer tissue was observed. *In vitro* the activity of betulinic acid was increased by decreasing the pH [15]. And interestingly in athymic mice carrying human melanoma xenografts, its highest concentration after in-

traperitoneal injection (500 mg/kg) compared with other tissues like liver, lung, and kidney was found in the tumour tissue, which often exhibits a lower pH, caused by a changed metabolism. This could be an explanation of the triterpene acid accumulation in the melanoma tissue. Up to now, only a few investigations include pH variations [15, 16], but this fact could be an important factor for increasing the activity of triterpene acids in cancer treatment and should be investigated in more detail.

The apoptosis mechanism of betulinic acid has been investigated quite well and was reviewed in 2009 by Fulda [9]. In short, betulinic acid induces apoptosis via the intrinsic pathway by affecting the mitochondrial membrane potential [17] and initiates reactive oxygen species (ROS) generation linked to an activation of pro-apoptotic p38 MAPK and SAP/JNK kinases [18, 19]. A similar increase of ROS was also observed for oleanolic acid (25  $\mu$ M) in astrocytoma cell lines [20]. While recently published data reported Bax/Bak-independent apoptosis induction by betulinic acid in various cancer cell lines [21], a number of publications show a modulation of anti- and pro-apoptotic proteins of the Bcl-2 family [13, 22–25]. The modulation of pro- and antiapoptotic factors is complex and probably cell-type-dependent. It is likely that context dependency also plays a role with respect to nuclear factor kappa-B (NF- $\kappa$ B) modulation. While NF- $\kappa$ B is activated by betulinic acid (20  $\mu$ M) in a variety of cancer cell lines resulting in induction of apoptosis [26], NF- $\kappa$ B inhibition is observed in chemoresistant androgen-refractory prostate cancer cells exhibiting constitutive Rel/NF- $\kappa$ B activation [27]. Similar effects of triterpenes on NF- $\kappa$ B related to inflammation have been observed and are discussed later. One important detail to overcome some types of apoptosis resistance is the independence of betulinic acid induced apoptosis of p53 that is frequently mutated in cancer cells [13, 22, 24].

While apoptosis induced by betulinic acid seems to be independent of the Fas receptor [22], lupeol targets this receptor and consequently activates the extrinsic pathway via caspase 8. For example, lupeol (20  $\mu$ M) significantly increased the expression of the FADD protein and the Fas receptor in androgen sensitive prostate cancer cells [28]. Furthermore, lupeol sensitises chemoresistant human pancreatic cancer cells (PaC), to undergo apoptosis by recombinant TRAIL via suppression of cFLIP [29]. Besides, various targets of lupeol are reported to overcome apoptosis resistance by inhibition of oncogenes and activation of tumour suppressor genes. At a concentration of 30  $\mu$ M, lupeol reduces the expression of commonly overexpressed Ras oncoprotein resulting in the inhibition of the PI3K/Akt pathway that is known for promoting cell growth [30]. Coincidentally, the expression of phospho-p38 MAPK, which triggers an antiapoptotic response to tumour cells, was decreased together with NF- $\kappa$ B occurrence. These modulations were accompanied by induction of apoptosis in the otherwise resistant pancreatic cells [30].

Also ursolic acid and oleanolic acid exhibit pro-apoptotic activity, as reviewed by Ovesna and colleagues in 2004 [8]. Recent results indicated a modulation of the Bcl-2 protein family due to a suppression of NF- $\kappa$ B by ursolic acid (50  $\mu$ M) in B16.F10 mouse melanoma cells. Induction of apoptosis was accompanied by activation of p53 and caspase-3 gene expression [31]. Oleanolic acid (80  $\mu$ M) showed apoptosis induction in leukaemia cells (HL60) via activation of caspase-9 and caspase-3 accompanied by the cleavage of poly(ADP-ribose) polymerase (PARP) [32].

Betulin has often been found to be inactive or weakly active against several cancer cell lines such as melanoma (MEL-2), epidermoid carcinoma (KB) [33], leukaemia (HL60, U937, K562) or

neuroblastoma (GOTO, NB-1) [34]. However, in nonmalignant, immortalised HaCaT keratinocytes induction of caspase-dependent apoptosis has been observed [5] and recently, Pyo et al. revealed anticancer activity of betulin (20  $\mu$ M) against a human lung cancer cell line (A549) by induction of apoptosis [35]. Erythrodiol, the closely related diol of the oleanane group, has not been investigated very thoroughly either, but in 2008 apoptotic activity in HT-29 human adenocarcinoma cells at concentrations of 50–150  $\mu$ M was reported [36]. In the case of betulin and erythrodiol, it is difficult to evaluate their pro-apoptotic potential compared to betulinic acid, because of the low amount of published data.

Sometimes only moderate pro-apoptotic effects of triterpenes are observed *in vitro*, as reported for immortalised HaCaT keratinocytes or human epidermoid carcinoma cells (A431) treated with a triterpene dry extract from birch bark containing 80% betulin and up to 4% betulinic acid and smaller amounts of lupeol and oleanolic acid [5]. It was only able to induce a twofold higher apoptosis rate in HaCaT keratinocytes. At first these results seem to have no relevance for therapeutic treatment. Nevertheless the triterpene extract was successful *in vivo* treating actinic keratosis [37].

In summary, the apoptotic pathway for betulinic acid is well known. Triggering the intrinsic pathway via destruction of the mitochondrial membrane potential and including MAP kinase and PI3K/Akt pathways, seems to be the mode of action. In respect of the antioxidative activity discussed later, the induction of ROS species in the case of apoptosis is highly interesting. Potentially the concentration is the critical parameter causing apoptosis or an antioxidative effect. Oleanolic acid may act in a way similar to betulinic acid by activating caspase-9.

Unlike triterpene acids, lupeol triggers the extrinsic pathway via the Fas-receptor. There is still a lack of data for the diols betulin and erythrodiol, thus a prediction of their mechanism is not yet possible.

Due to the different mechanisms triggered, the use of different triterpenes, also in a mixture may increase the chance of overcoming the chemoresistance of tumour cells. At the moment, this is the conclusion drawn only from the results of various researches carried out independently. Only well organised widespread analysis of a cancer cell panel treated with different triterpenes under standardised parameters could generate really comparable data for determining which triterpene or triterpene mixtures exhibit the best chance of being active against a particular cancer cell. But this could be an opportunity for tapping the full potential of triterpenes that induce apoptosis.

### Antiangiogenic Effects

▼  
Angiogenesis is a key process for the outgrowth of cancer cells and their spread into other tissues. Therefore, suppressing this process is one important pillar of cancer treatment. There are four key steps in angiogenesis which are potential therapeutic targets: degradation of extracellular matrix, migration and proliferation of aortic endothelial cells and the formation of new blood vessels. The initial results in 1995 of Sohn and colleagues provided an indication that ursolic acid and oleanolic acid have antiangiogenic effects on bovine aortic endothelial cells (BAEC) in the CAM (chick embryo chorioallantoic membrane) assay [38]. Here, ursolic acid (ID<sub>50</sub>: 4  $\mu$ M) was more effective than oleanolic acid (ID<sub>50</sub>: 40  $\mu$ M). However, the key steps of angiogenesis targeted by triterpene acids had not been identified at this time. Further investigations focussing on the different key steps disclosed ef-

fects on the angiogenic process but these proved controversial. In serum free cultures of human umbilical vein endothelial cells (HUVECs), ursolic acid (10–100  $\mu\text{M}$ ) increases expression of adhesion molecules that support angiogenesis, such as ICAM-1 and CD31, and the expression of angiogenic growth factors, particularly vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2) [39]. This suggests a possible support of the migration step and the structure formation process. Furthermore, 4–20  $\mu\text{M}$  ursolic acid failed to produce a significant inhibition of the invasion capability of BAEC through matrigel. In addition, the degradation of extracellular matrix (ECM) by ECM degradation proteins as MMP-2 and urokinase was assumed to be stimulated by ursolic acid (4  $\mu\text{M}$ ) due to an increased expression shown by gelatinase and urokinase zymography of these enzymes in BAEC [40]. But this should be investigated in more detail, considering that two years later Jedinak found a strong inhibition of urokinase activity by ursolic acid in a cell free system [41]. Despite the enhancement of pro-angiogenic factors, in 2004 Cardenas confirmed Sohn's (1995) observation of the anti-angiogenic effect of ursolic acid in the CAM assay. Furthermore, ursolic acid treatment of HUVECs and rat aortic rings that were stimulated by cultivation in medium supplemented with serum in contrast to serum free medium surprisingly caused inhibition of the angiogenic phenotype, including the formation of a capillary network-like structure by HUVECs and a greater extent of endothelial sprouting in rat aortic rings [39]. Due to the sometimes contradictory effects of ursolic acid on different steps of angiogenesis, one must be cautious concerning its antiangiogenic potential. The generation of *in vivo* data is necessary in order to include the influence of the tissue milieu and thus appropriately evaluate its effects.

For betulinic acid, older studies revealed that it inhibits enzymatic activity of aminopeptidase N (APN) in a cell free system [42]. APN is a widely distributed, membrane-bound, zinc-dependent metalloproteinase that is known to play an important role in tumour-vasculogenesis and is essential for the endothelial cell tube formation [43,44]. Betulinic acid (2  $\mu\text{M}$ ) potently inhibits basic fibroblast growth factor (bFGF)-induced invasion and tube formation of BAECs [45]. Initially it was assumed that APN could be the target of betulinic acid. Kwon et al. confirmed that betulinic acid strongly inhibits enzymatic activity of APN in a cell free system, but not when enzymatic activity was measured in betulinic acid treated endothelial cells. He provided evidence that the antiangiogenic activity of betulinic acid was accompanied by modulation of the mitochondrial membrane function by decreasing the mitochondrial redox potential. This effect could be blocked by different mitochondrial permeability transition inhibitors such as cyclosporine A or bongkreic acid. In view of the effects of betulinic acid on the mitochondrial membrane, this seems to be a target structure worth considering. But in this context, the modulation of the mitochondrial membrane does not cause the release of apoptogenic factors that directly trigger cell death. Betulinic acid at up to 9  $\mu\text{M}$  did not affect the endothelial cell viability in the formed tubes. Kwon hypothesised that betulinic acid had a specific effect on the angiogenic differentiation of endothelial cells, rather than an antiproliferative activity [45]. It is known, that a modulation of the mitochondrial oxidative phosphorylation can enhance angiogenic differentiation of endothelial cells, stopping their proliferative activity [46]. However, in a human prostate cancer cell line (LNCaP) and *in vivo*, betulinic acid acts via decreasing expression of VEGF [47]. Thus there might be also an antiproliferative effect. The mechanism might

be due to activation of selective proteasome-dependent degradation of the transcription factors specificity protein 1 (Sp1), Sp3, and Sp4 which regulate the VEGF expression and that are mostly overexpressed in tumours, as was shown by Chintharlapalli and colleagues [47]. The concentration-dependent effect on the transcription factors and the expression of VEGF by betulinic acid could be fully blocked by using a proteasome inhibitor.

Only one publication reports an antiangiogenic effect of lupeol. You and colleagues found that lupeol also inhibits HUVEC tube formation [48].

Based on the current literature, primarily triterpene acids seem to have an antiangiogenic effect. However, to clarify the exact mechanisms by which they exert this effect more experimental work is still needed, even if there are some doubts in the case of ursolic acid because of the upregulation of pro-angiogenic factors such as MMP-2 or VEGF *in vitro*. It is necessary to interpret these pro-angiogenic data very carefully. There are four key steps in angiogenesis. These include degradation of the extracellular matrix, migration and proliferation of aortic endothelial cells and the formation of new blood vessels. All four steps are necessary for successful angiogenesis. Most *in vitro* experiments focus only on parts of the process. This does not enable a prediction of full angiogenesis. Instead, *in vivo* models, such as the CAM assay, consider the whole process. Thus they provide information about the efficacy of the substances on the end result, namely forming new blood vessels, i.e., not just on one essential factor in a complex network, such as VEGF.

Nevertheless, the *in vitro* data give hints for understanding the mechanism. In respect of the differentiation inducing activity of triterpenes discussed later, the most interesting result is that besides the regulation of endothelial cell proliferation by modulation of growth factors such as VEGF, the aspect of inducing differentiation to stop proliferation may also play a role in the angiogenic efficacy of triterpenes.

### Anti-inflammatory Effects



Recent studies have revealed a clear role for inflammation in the development and progression of cancer and in the immune response against it by orchestrating the tumour supporting environment [49].

Lupanes, oleananes and ursanes applied orally or topically exhibit significant anti-inflammatory activity *in vivo*. This was demonstrated in 12-O-tetradecanoylphorbol-13-acetate (TPA), carrageenan, serotonin or croton oil induced paw/ear oedema tests, as well as in arthritic animal models [50–56]. Efforts to work out the underlying mechanism *in vitro* are in progress (reviewed in [7,8,57–59]) and several potential targets have been discovered. Besides direct effects on the morphology or the activity of immune cells, such as macrophages, dendritic cells, T cells or other leukocytes, which may suppress the immune response [60–63], an influence on pro-inflammatory cytokines, e.g., TNF- $\alpha$ , INF $\gamma$ , IL-1 $\beta$ , IL-6, IL-2, IL-4, IL-5, IL-8, or IL-13 [31,50,60,64–66] has been reported.

The expression of these cytokines is regulated by the transcription factor NF- $\kappa$ B, which is therefore a pivotal target. Furthermore, NF- $\kappa$ B is commonly overexpressed in cancer cells. On the one hand this may support the maintenance of a chronically inflamed microenvironment and on the other hand it often suppresses apoptosis of the tumour cells [67]. In the last few years, several groups have published controversial data concerning the

activity of betulinic acid and ursolic acid on NF- $\kappa$ B [26,68]. Kasperczyk et al. [26] postulated an NF- $\kappa$ B activating effect of betulinic acid (13–22  $\mu$ M) on various cancer cell lines (neuroblastoma, melanoma, glioblastoma). In contrast, in 2003 Takada and Aggarwal described an inhibition of NF- $\kappa$ B regulated cyclooxygenase-2 (COX-2) expression and determined a maximal suppressive effect of betulinic acid at a concentration of 30  $\mu$ M on NF- $\kappa$ B in colon carcinoma cells [68]. Similarly NF- $\kappa$ B in melanoma cells was inhibited by ursolic acid (50  $\mu$ M) accompanied by downregulation of pro-inflammatory cytokines such as TNF- $\alpha$ ; IL-1 $\beta$ , IL-6, and GM-CSF and apoptosis occurred after 48 h [31]. Also, carcinogen-induced NF- $\kappa$ B expression is decreased by ursolic acid [69]. However, in contrast to this, in resting macrophages ursolic acid and also oleanolic acid activate NF- $\kappa$ B causing increased expression of pro-inflammatory mediators such as TNF- $\alpha$  at concentrations of 5  $\mu$ M and 4  $\mu$ M, respectively [70,71].

At first these results seem to be contradictory, but the different observations could be based on concentration-dependent effects, as observed for oleanolic acid with respect to the TNF- $\alpha$  production of human mononuclear cells [65] or for betulinic acid concerning TNF- $\alpha$  and IL-1 $\beta$  production in non-stimulated RAW264.7 macrophages [63]. Another conceivable reason is the influence of the milieu or the cell status that may crucially modulate triterpene effects. Their investigation on TNF- $\alpha$  or nitric oxide (NO) production via inducible NO-synthase (iNOS) indicate that [63,71,72]. Using a stimulated cell system (e.g., activated macrophages) it was possible to observe an inhibition of pro-inflammatory mediators by triterpene treatment [63,72]. However, treatment of non-stimulated cells, such as resting macrophages, with triterpenes led to an increase of pro-inflammatory factors such as TNF- $\alpha$  or IL-1 $\beta$  [64,71]. Certainly, these are *in vitro* data and up to now it is not clear whether these findings are meaningful for *in vivo* models or in therapeutic use. But triterpene-induced effects seem to be critically affected by environmental conditions.

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) provides substrate for cyclooxygenase and 5-lipoxygenase. These pathways are major pathways of the inflammation process. Betulin and betulinic acid [73], as well as oleanolic acid [74], can inhibit PLA<sub>2</sub>. Downstream, COX-2 and its product prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are also repressed by lupeol, betulin, betulinic, and ursolic acid [61,63,68,75,76]. Again COX-2 expression is regulated by NF- $\kappa$ B, suggesting an inhibitory effect of triterpenes on this transcription factor [68,77].

Unfortunately, the use of different cell systems, with a diverse metabolic background, plus the usage of different triterpenes, in various concentrations, precludes suggesting an exact mode of action for the anti-inflammatory effect of these substances. However, the abundance of data, and especially the *in vivo* observations, evidenced the anti-inflammatory potential of the listed pentacyclic triterpenes. A promising target for the triterpenes presented seems to be NF- $\kappa$ B. A number of proteins modulated by triterpenes, such as TNF- $\alpha$ , IL-8 or COX-2 are under control of this transcription factor. However, the milieu, such as the cell status, has to be considered because it seems to have a strong influence on the triterpene effects and should receive special attention.

### Antioxidative Effects

ROS are well recognised as playing a dual role as both deleterious and beneficial species. ROS are normally generated by tightly regulated enzymes to maintain moderate concentrations, provid-

ing beneficial physiological effects, for example in cellular responses to noxia, or in the regulation of immune responses. Overproduction results in oxidative stress that can be an important mediator of damage to cell structures [78]. Initially increased levels of ROS disrupt cell membrane integrity by oxidation of unsaturated membrane lipids. Lipid peroxidation is commonly related to cardiovascular diseases [79], autoimmune diseases or chronic inflammation [80]. Furthermore, free radicals cause DNA damage which may result in tumour initiation and promotion [81]. Thus regulation of the ROS level may be an important preventive measure and may also support the anticancer therapies, by avoiding oxidative stress.

The organism uses two antioxidative mechanisms to regulate the level of free radicals, first an enzymatic and second a non-enzymatic system [82,83]. The enzymatic system concerns enzymes such as superoxide dismutase (SOD) or catalase (CAT) that are oxidised and reduced within a cascade to eliminate the free radicals. The non-enzymatic system deals with antioxidants. One of the body's own antioxidants is glutathione (GSH). It exists as a monomeric tripeptide (GSH); when oxidised a GSSG dimer is generated. In order to use the reducing power of glutathione to catalyze disulfide reductions in the presence of NADPH and glutathione reductase, enzymes such as glutathione-S-transferase and glutathione peroxidase are necessary [84].

Triterpenes, particularly lupeol, but also betulin and ursolic acid are known for their antioxidative potential [85–90]. They do not act as a classical antioxidant; however, triterpenes activate the enzymatic system by increasing the activity of SOD as well as CAT and glutathione S-transferase and glutathione peroxidase [90–92].

In detail, lupeol especially displays convincing effects particularly on chronic inflammatory diseases such as chronic arthritis, but also as a chemoprotective agent. When arthritic rats were treated orally with lupeol (50 mg/kg body weight daily for 8 days), a significant decrease of the inflammatory symptoms was observed, while the activity of the antioxidative enzymes SOD and CAT were elevated [93]. Another positive effect of lupeol is documented in the case of hyperoxaluria in rats [94]. The excess of oxalate causes a high oxidative stress on the renal tissue. Similar to lupeol, betulin (35 mg/kg body weight daily for 21 days) normalises the glutathione status, increases the SOD and CAT activity [90] and decreases the peroxidation of erythrocyte membrane lipids as well as normalises the activity of membrane bound ATPases [92]. A third indication that has been investigated is hypercholesterolemia. Lupeol (50 mg/kg body weight daily) normalises the lipid profile and activates the body's own antioxidative system followed by a decrease of oxidative stress in rats. This results in a protection of renal tissue. In this case the antioxidative effect of lupeol is called cardioprotective or renalprotective [87,95,96]. In the majority of *in vivo* studies lupeol and also betulin were administered orally daily without any adverse effects at a dosage of 35–50 mg/kg body weight, which is a high dose. Considering an average human body weight of 60 kg, the application of 3 g of the drug per day would be necessary.

The diseases mentioned are not directly related to the development of cancer, but they illustrate the antioxidative activity of lupeol or betulin. This seems to be not only a central part of their biological activity but also the basic mechanism of their chemopreventive effects which may avoid cancer development.

Substances that cause cell damage, particularly DNA damage or induce chronic inflammations are potential carcinogens. Agents that shield the organism from these attacks are called chemopre-

ventive. Recently lupeol was termed as a chemopreventive agent, reviewed by Chaturvedi and colleagues [7]. This includes hepatoprotective effects protecting liver cells from cadmium, 7,12-dimethylbenz(a)anthracene (DMBA) or hepatotoxic aflatoxins [97–100] or cardioprotective activity shielding cardiac tissue from cyclophosphamide induced cardiotoxicity [88] by oral administration of lupeol. Furthermore, a chemoprotective effect of topically applied lupeol was observed when skin was treated with benzoylperoxides [89, 101] or DMBA [102]. All these potential carcinogenic agents cause oxidative stress, deplete glutathione and decrease the activity of antioxidant enzymes. Lupeol regenerates the glutathione pool along with an elevation in the activities of the antioxidising enzymes and anti-oxidants.

Further cytoprotective effects are also known for betulin and triterpene acids. *In vitro* pretreatment with 2 and 22  $\mu\text{M}$  ursolic acid protects human lymphocytes against UVB-induced lipid peroxidation and DNA-damage concentration-dependently [86]. Various *in vitro* studies revealed hepatoprotective effects of betulin, betulinic, ursolic and oleanolic acid against cadmium or ethanol-induced toxicity in HepG2 cells (2–11  $\mu\text{M}$ ) [103, 104]. It should be mentioned that these effects occur using subtoxic triterpene concentrations depending on the cell type. Recently published data showed chemopreventive activity of oleanolic and ursolic acid also *in vivo*. Rats treated orally with 1,2-dimethylhydrazine developed colon associated carcinogenic dysplasia caused by agent-induced oxidative stress. Simultaneous oral administration of 25 mg/kg body weight oleanolic or ursolic acid decreases the appearance of cell damage [105].

Further experiments focussed on hepatoprotective effects have identified a possible key target for the antioxidative effect of triterpenes. Oleanolic acid treatment dramatically increased expression of the transcription factor nuclear factor E2-related factor 2 (Nrf2) [106]. Regulatory regions of the genes for cytoprotective enzymes such as glutathione S-transferase or SOD contain the antioxidant response element (ARE), which is activated upon binding of Nrf2. Nrf2 has been shown to be essential in the upregulation of these genes in response to oxidative stress [107]. The mechanism of increasing Nrf2 expression by oleanolic acid has not been clarified so far. One hypothesis might be the generation of ROS that triggers the antioxidative cascade including Nrf2 expression. For oleanolic acid treatment at concentrations of 25  $\mu\text{M}$ , accumulation of ROS in an astrocytoma cell line resulting in apoptosis has been reported [20], but it was interpreted only in the context of apoptosis induction. Also betulinic acid (20–100  $\mu\text{M}$ ) showed ROS generation at concentrations resulting in apoptosis [18, 24]. Therefore the question arises, whether generation of ROS could be induced by triterpenes or especially by triterpene acids used in a subtoxic concentration, and if this could lead to an activation of the antioxidative system.

Taken together, lupeol and betulin, as well as triterpene acids, such as oleanolic acid and ursolic acid, display an antioxidative activity. The underlying mechanism is the modulation of the body's own enzymatic antioxidative system including enzymes such as SOD and CAT, as well as glutathione S-transferase, perhaps triggered by the activation of Nrf2 and leading to a generally elevated antioxidant status of the organism. The antioxidative effect of triterpenes seems to be a central part of their biological activity and may also be useful as a preventive strategy in the case of cancer.

## Redifferentiation



Differentiation and proliferation of a cell are mutually exclusive. Differentiation takes place in the G0 state of a cell. In order to proliferate, reentering the cell cycle is necessary. Thus, achieving differentiation of a still proliferating cancer cell is one possible approach of cancer treatment. In this respect, compounds that influence and enhance differentiation processes in cells are promising candidates for cancer therapy. In case of triterpenes, very interesting data reveal the promotion of differentiation of healthy cells such as keratinocytes and also of the redifferentiation of tumour cells.

With regard to differentiation of healthy cells, it was shown that betulinic acid induces differentiation in normal keratinocytes *in vitro* at concentrations of 9–18  $\mu\text{M}$ , including an upregulation of filaggrin and involucrin [11]. Likewise, oleanolic acid, but not ursolic acid, increases the expression of these two differentiation markers *in vivo* in mouse skin disrupted by tape stripping. Oleanolic acid not only upregulated structural proteins localised in the spinous/granular layers in the epidermis, but also the cornified envelope formation as the final product of terminal differentiation was increased and the barrier function of the epidermis was regenerated, as measured by a decreased transepidermal water loss [108]. Similar results were obtained with a highly purified triterpene dry extract from birch bark consisting of betulin (80%), betulinic acid, oleanolic acid, and lupeol (1–4%). Normal human keratinocytes and human skin explants were treated with 10  $\mu\text{g}/\text{mL}$  of the extract. Besides the upregulation of early and late differentiation markers such as loricrin, filaggrin, involucrin, transglutaminase, and keratin 10, as well as increased Notch2 expression, also a typical degradation of DNA strictly limited to the distal stratum granulosum cells was observed [109].

One possible signalling cascade activated by triterpenes is the peroxisome proliferator activated receptor- $\alpha$  (PPAR- $\alpha$ ) pathway, which is involved in the regulation of lipid metabolism in the epidermis [110]. Oleanolic acid and ursolic acid topically applied on disrupted mouse skin improved the recovery of permeability barrier functions and stimulated epidermal differentiation via PPAR- $\alpha$  expression [108, 111]. Another hypothesis is the selective upregulation of the transient receptor potential channel 6 (TRPC6) by triterpenes in primary keratinocytes [109]. TRPC6 is a calcium channel mediating differentiation by regulating the  $\text{Ca}^{2+}$  influx into the keratinocytes [112].

Besides the upregulation of differentiation markers, or the activation of corresponding signalling pathways, morphological changes are also an important part of the differentiation of many cell types and, therefore, a useful additional marker to recognise differentiation. Thus, the morphological change of primary keratinocytes into a typical hexagonal corneocyte shape under triterpene acid treatment encourages the suggestion of their differentiation-inducing efficacy [11, 108]. Also mammary epithelial cells of rats as well as tumour cells, such as tetracarcinoma stem cells [113], showed morphological changes when treated with oleanolic acid or ursolic acid [114]. Not only triterpene acids induce such morphological changes, but also treatment with lupeol results in modifications of the cell shape, e.g., of the mouse melanoma cell line B16 2F2, and an inhibition of the ability of the cells to migrate, thus reducing their malignant potential [115]. The morphological changes mediated by alterations of the cytoskeleton are accompanied by the degradation and reconstruction of microfilaments consisting of actin, whereas the actin content of the cells remains constant [20, 115, 116]. Up to now it is not clear whether

these changes are directly induced by triterpenoids or are a result of the activated differentiation process. But it should be mentioned that morphological changes could also be observed in the apoptosis process induced by triterpenes [20]. Therefore the interpretation of this phenomenon must be carefully evaluated.

Moreover, differentiation is also indicated by regaining or developing special cell type specific properties. For example, myeloid leukaemia cells (HL-60) amplify 1- $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> induced monocytic marker expression such as CD11b or CD14 when treated additionally with betulinic acid [117]. Oleanolic acid decreases the proliferation rate of M1 mouse carcinoma cells and human leukaemia cells (HL-60) while phagocytotic activity is increased. And lupeol as well as betulin and betulinic acid induce melanogenesis in B16 2F2 mouse melanoma cells [118].

The ability to induce differentiation or redifferentiation, instead of acting as a cytotoxic substance, is always a question of the available triterpene concentration and depends also on cell type and cell status [109]. Until now some mechanistic investigations have been carried out only in keratinocytes, revealing two possible targets: PPAR- $\alpha$  and TRPC6. For other cell types, the observations were only of the differentiation process or the outcome, for example, morphological changes, developing special abilities such as phagocytotic activity or expressing differentiation markers such as CD11b or CD14 in the case of myeloid leukaemia cells. The differentiation data are still fragmentary, but here the potential of triterpenes seems to be worth investigating in detail.

### Anticancer Activity *in vivo*

The relevance of *in vitro* results can only be judged against subsequent *in vivo* studies. Because of this, the strong interest on pentacyclic triterpenes as anticancer agents did not start until betulinic acid was found to be effective *in vivo* against melanoma by Pisha et al. in 1995 [119].

Bioavailability is the precondition of *in vivo* effects. Only a few pharmacokinetic studies have been published. BA was found in various tissues 24 h after i. p. administration (500 mg/kg; mouse) and reached its highest concentration in perirenal fat. Peak serum concentration of 4.0  $\mu$ g/mL was observed at 0.23 h after application [120]. BE reaches a saturation concentration of 138 ng/mL within 4 h after i. p. administration to rats [121]. These relatively low serum levels can be explained by the low solubility in water (BA, OA: 0.02  $\mu$ g/mL and BE < 0.1  $\mu$ g/mL) [62,122]. It is known that OA is able to bind to plasmin and albumin [17,21], so binding phenomena could support the bioavailability. Thus the prediction of *in vivo* effects and the concentrations necessary for them may be unreliable if based on *in vitro* results.

For instance, cytotoxicity was shown for lupeol combating testosterone-induced prostate enlargement in mice, by inducing apoptosis in the hypodiploid regions, and in tumours with human prostate origin in a xenograft model [28,123]. Recent findings showed that lupeol (40 mg/kg body weight thrice a week) also inhibits the growth of highly aggressive human metastatic melanoma cells (451Lu) in an athymic nude mouse xenograft model. Immunohistochemical analysis of tumour tissue revealed that animals receiving lupeol exhibit decreased Ki67 and PCNA-positive cells, suggesting an antiproliferative effect of lupeol. This correlated with a decreased number of cyclin D1, cyclin D2 and Cdk2 positive cells and an elevated level of p21 protein compared to the control mice. The latter result indicates that lupeol causes

growth arrest in melanoma tumours by interfering with the cyclin/cdk2/p21 complex activity [124].

Modern cancer treatment also includes modulation of the immune system. As noted above, *in vitro* a broad spectrum of immune modulations by triterpenes is observed. Indeed, this could be confirmed in some *in vivo* studies, e.g., in a study using melanoma (B16.F10) bearing mice (C57BL/6). I. p. injected ursolic or oleanolic acid (50  $\mu$ mol/kg body weight, for 5 days) was found to produce enhanced natural killer cell activity and increased the cytokine IL-2 that promotes the lytic activity of NK cells. In addition, antibody-dependent cell mediated cytotoxicity (ADCC) as well as antibody-dependent complement-mediated cytotoxicity (ACC) were enhanced. According to the expected anticancer effect, the elevated levels of GM-CSF and IL-6 in tumour-bearing control animals were also reduced by the treatment with ursolic acid [125]. Another study showed lupeol efficacy in a TPA induced mouse skin tumourigenesis model (CD1) by its anti-inflammatory activity. Prior topical application of 1–2 mg/animal lupeol resulted in the inhibition of the TPA induced activation of PI3K and NF- $\kappa$ B and in an inhibition of COX-2 and iNOS protein expression. The mice showed significant reduced tumour incidence, lower tumour burden and a delay in the latency period for tumour appearance [76].

While the antiangiogenic effect of ursolic acid *in vitro* was still being discussed, Lee et al. (2001) proposed this kind of anticancer effect based on *in vivo* studies. Reduced oxygen consumption after treatment as well as a significant decreased tumour interstitial fluid and blood pressure were obtained after i. p. application of 100 mg/kg body weight ursolic acid. This was accompanied by an inhibited tumour growth of a murine fibrosarcoma (FSall) [126]. The inhibitory action of ursolic acid on urokinase as observed *in vitro* was assumed to be important with respect not only to the antiangiogenic effect but also to the suppression of tumour-invasion and metastasis. An *in vivo* study on B16 mouse melanoma treated C57BL/6 mice showed the complete inhibition of lung colonisation after 50 mg/kg ursolic acid administered i. p. daily, immediately after tumour injection during 16 consecutive days. The authors proposed that besides the *in vitro* observed urokinase inhibition activity of ursolic acid, an inhibition of cathepsin B, which represents another possible drug target for the suppression of tumour invasion and metastasis, may also play a crucial role [41]. Comparable results with respect to tumour metastasis were found for betulinic acid (10 mg/kg body weight per day) when used in a similar experimental setup [127]. As already mentioned above, betulinic acid targets VEGF expression in a prostate cancer mouse model and induces the selective proteasome-mediated degradation of transcription factor specificity protein Sp1, Sp3 and Sp4 [47].

Another approach for the usage of triterpenes in cancer-therapy is their combination with established chemotherapeutics. *In vivo* studies using an orthotopic metastatic nude mouse model of oral tongue squamous cell carcinoma showed that lupeol (2 mg/kg body weight) dramatically decreased the tumour volume and suppressed local metastasis without side effects. It was 3-fold more effective than cisplatin, a commonly used chemotherapeutic agent with severe side effects. Surprisingly, lupeol in combination with low-dose cisplatin was 13-fold more potent than lupeol alone and up to 40-fold more than cisplatin alone, certainly without side effects in the animal model used [128]. In another study betulinic acid augmented the inhibitory effect of vincristine, the major chemotherapeutic agent used for the treatment of melanoma [127]. Combination with triterpenes allowed reduction of the

concentration of the chemotherapeutic agent, without losing the effectiveness of treatment, while side effects were decreased. As mentioned previously, up to now there has been only very limited experience in the treatment of human cancer patients with the listed triterpenoids. It should be mentioned, that in animal studies dosages of between 10 and 100 mg/kg body weight are applied which is between 0.6 and 6 g per patient per application (60 kg body weight assumed). This is an unusually high amount for a drug, and the feasibility and the relevance of this dose is questionable. Nevertheless, betulinic acid is currently undergoing a phase II clinical trial for dysplastic melanocytic naevus (web site: ClinicalTrials.gov). Beside this, recent data show the successful topical treatment of precancerous lesions, namely actinic keratoses, with a triterpene dry extract (TE) of the outer bark of birch as mentioned above [37]. Immunohistochemical investigations of biopsies before and after a three month treatment with TE showed a downstaging of the actinic keratosis and a re-organised epidermal structure.

It has been possible to confirm *in vivo* all the general effects of the triterpenes discussed that were predicted based on *in vitro* data (inducing apoptosis, anti-inflammatory, antiangiogenic and antioxidative effects), except for the redifferentiation effect. In this case, the observation of the downstaging of actinic keratosis lesions after a three month treatment with triterpenes in a clinical trial has been the only observation available until now, but there is no experimental work. Thus it is too soon to claim a re-differentiation effect for the triterpenes in cancer treatment; however it is a promising field of triterpene research.

Administration of drugs also raises the question of their toxicity *in vivo*. Pentacyclic triterpenes of the lupane, oleanane and ursane group are considered as relatively nontoxic drugs. A recently published subchronic toxicity study showed that intraperitoneal and subcutaneous administration of a triterpene mixture (80% betulin, betulinic acid, lupeol, oleanolic acid, erythrodiol 1–4%) produces no toxic effects [121]. This is in line with previously published data for single triterpenes. For example, *i.p.* administered oleanolic acid has a LD50 of 1500 mg/mL in mice [129] and a single *s.c.* dose of 1000 mg/mL caused no toxic effects in rats [130]. Also *i.p.* administration of 500 mg/kg body weight betulinic acid in suspension caused no toxicity [131]. As summarised previously, administration of betulin or lupeol at concentrations of 35–50 mg/kg body weight also produces no toxic effects.

## Conclusion

▼  
Cancer is a disease with multiple etiological factors and multiple oncogenes are involved in its pathogenesis. Therefore, beside a combination of different treatment strategies, multifunctional agents with multiple targets also offer a more rational approach than single ones to both its prevention and therapy.

The literature survey reveals that lupeol, betulin, betulinic acid, oleanolic and ursolic acid are multitarget agents (► Fig. 2). They fit to the concept of modern cancer therapy, by treating cancer from different sides, including the tumour environment and the immune system.

But parallel testing of these compounds revealed differences in their efficacy in several assays. Therefore, the combination of different triterpenes may be a way to improve their potential as multitarget drugs. Plants like white birch, rosemary, or mistletoe offer different natural compositions of triterpenes, and their triterpene extracts may be used as starting material for further pharmaceutical development.

Another possible application is combining triterpenes with already commonly used chemotherapeutic agents. This may allow lowering the chemotherapeutic dose without loss of efficacy but hopefully brings with it less adverse effects and may even give synergistic effects.

The pharmacological potential of triterpenes for cancer treatment seems to be high, although up to now no clinical trial has been published using triterpenes in cancer therapy. This may be explained by their almost complete insolubility in water [5, 122]. But this galenic problem can be solved by derivatisation, complexation [132, 133], or liposomal formulation [134]. Another problem might be that triterpenes often provide only moderate effects *in vitro*, perhaps due to their poor solubility [122] and the use of solvents such as dimethylsulfoxide (DMSO) that are not inert. In our experience, using 1% DMSO in cell culture medium, a maximum concentration between 20 and 40 µg/ml triterpenes is possible without crystallisation but it has to be carefully checked in each case. Nonetheless they exhibit convincing effects when applied *in vivo*, as seen in the case of the birch bark TE extract. Further, the high dose that is used in animal tests might be an obstacle to transferring it to a therapeutic dose used in human treatment. Up to now, only two clinical trials have been carried out with these substances, one treating actinic keratosis and another dysplastic melanocytic naevus. In each case topical ointments were used. Internal application, such as *s.c.* or *i.v.*, has not yet been tested in human cancer treatment.

Looking into future, there remains some tasks to do in order to tap the full potential of these triterpenes. A lot of effects are not fully understood like the antioxidative efficacy or the differentiation effects. Another point is that the strength of each single compound is not defined detailed enough, to find their optimal domain or to compose them. The differences between the experimental settings, the used concentrations, time courses or the various cell lines, with different metabolic background and varying cell culture parameters, makes it difficult to compare and combine results to get an idea of the underlying mechanisms. Therefore, parallel-testing of triterpenes in a standardised setting would be preferable. Due to their insolubility in water the bio-availability is not given in an optimal way, nevertheless, *in vivo* effects are observed. This may be improved by optimising the galenic form or the application route.

Their chemopreventive activity makes triterpenes interesting in another field. As secondary plant metabolites they are present in food. For example, due to the common use of olives and herbs such as rosemary of the Lamiaceae family, the Mediterranean diet is high in triterpenes. It is not proven yet, whether they are responsible for the beneficial effects of this nutrition on health.

In conclusion pentacyclic triterpenes are a rich natural pool of promising anticancer drugs as well as chemopreventive agents. Further, they are available in high amounts also in industrial waste products like birch bark or the pulpy residue from apples. This and their promising pharmacological effects warrants further pharmaceutical development and clinical investigations to conclude the puzzle of their biological activities.

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