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(71) Applicant (for all designated States except US): **VALDERM APS** [DK/DK]; c/o Elisabeth de Darkó, Lundtoftevej 277, DK-2800 Lyngby (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **BYMOSE, Bjarne** [DK/DK]; Kongevejen 75 A, DK-2800 Kgs. Lyngby (DK). **DE DARKÓ, Elisabeth** [DK/DK]; Lundtoftevej 277, DK-2800 Lyngby (DK). **HYLDGAARD, Jørgen** [DK/DK]; Mejsevænget 7, DK 5610 Assens (DK). **BROWN, Marc, Barry** [GB/GB]; 30 Oxhey Road, Watford, Hertfordshire WD19 4QQ (GB). **LIM, Sian, Tiong** [MY/GB]; Hosac, 31 Charlwood Drive, Hoxshott, Surrey KT22 OHB (GB). **BECK, Petra, Helga** [DE/GB]; 15 Longlands Way, Camberley, Surrey GU15 1RH (GB). **GRIFFITH, Hannah, Mary, Talbot** [GB/GB]; Suny-dene, 69 Windsr Road, Chobham, Surrey GU24 8LD (GB).

(74) Agent: **HØIBERG A/S**; St. Kongensgade 59 A, DK-1264 Copenhagen K (DK).

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(54) Title: TREATMENT OF HYPERPROLIFERATIVE CONDITIONS

(57) Abstract: The present invention relates to stable pharmaceutical formulations comprising a solubilized lipophilic anthracycline and the uses thereof in the treatment of clinical conditions, wherein hyperproliferation, such as epithelial hyperproliferation, is a primary factor of the pathogenesis. The pharmaceutical compositions comprises i) a lipophilic anthracycline; ii) one or more oil phase carriers; iii) one or more pharmaceutical acceptable solubilizers; iv) one or more co-surfactants; and the composition further comprises an aqueous phase comprising at least one buffering system having a pH from about pH 2.2 to about pH 6, wherein the water content of the pharmaceutical composition is less than 30%.



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Treatment of hyperproliferative conditions.

Field of invention

5 The present invention relates to stable pharmaceutical formulations comprising a solubilized lipophilic anthracycline and the uses thereof in the treatment of clinical conditions, wherein hyperproliferation, preferably epithelial hyperproliferation, and more preferably hyperproliferating keratinocytes is a primary factor of the pathogenesis.

Background of invention

10 Anthracyclines

Anthracyclines are antibiotics having potent antineoplastic activity, and accordingly they have been used in the treatment of a variety of cancers. Anthracyclines are amongst the most utilised antitumor drugs ever developed. Anthracyclines mediate their anticancer effect in part by targeting topoisomerase II, which leads to DNA
15 damage. Anthracyclines are essential components of several curative drug combinations for the therapy of a number of internal tumors. It is a drug of primary use in the treatment of patients with lymphoma, breast cancer and sarcoma.

The group of anthracyclines for example comprises doxorubicin, valrubicin, epirubicin, daunorubicin and idarubicin. Doxorubicin, daunorubicin, idarubicin and epirubicin are
20 usually administered systemically by intravenous injection or infusion. Systemic administration of these anthracyclines, however, results in a number of undesirable side effects such as cardiotoxicity and bone marrow suppression.

25 Anthracyclines are in general known to be very tissue toxic. For example, paravenous extravasation of doxorubicin results in severe necrosis and immediate measures have to be undertaken to avoid severe local toxicity. It is also known that several anthracyclines are toxic, when applied directly to the skin.

30 Valrubicin is a semisynthetic analogue of doxorubicin and it is developed for the treatment of superficial bladder cancer and approved for such use in the United States. Usually a total of 800 mg is administered by intravesical instillation of two hours +- duration for a total of 6 times once a week. (FDA. Valstar™ (Valrubicin) Sterile Solution. 2009). The approved formulation contains 40 mg/ml Valrubicin in 50%
35 Cremephor®EL (polyoxyl castor oil) and 50% dehydrated alcohol USP. Unopened vials

of VALSTAR™ are stable until the date indicated on the package when stored under refrigerated conditions at 2°-8°C (36°-46°F). Vials should not be heated.

Before administration the approved formulation must be diluted in 0.9% sodium chloride in water. VALSTAR™ diluted in 0.9% Sodium Chloride Injection, USP for administration is stable for 12 hours at temperatures up to 25°C (77°F).

It has been described in an article by Cecilia R.Kjeldsen et al. (C. Rosada et al., *Valrubicin in a topical formulation treats psoriasis in a xenograft transplantation model*, J Invest Dermatol. 2010 Feb;130(2):455-63), that valrubicin in a topical formulation treats psoriasis in a xenograft transplantation model. One of the present inventors is a co-author of the article, and has provided the information, that the topical formulation used in the article is "Formulation A", which is described in example 15 herein.

Hyperproliferative diseases

Several clinical conditions exist where hyperproliferation defined as an abnormal high rate of cell division, is a primary factor of pathogenesis.

Psoriasis is a chronic disease characterised by epidermal hyperproliferation, abnormal cell differentiation with reduced programmed cell death (apoptosis) and parakeratosis, inflammatory infiltration of epidermis and dermis with vascular dilatation resulting in clinical symptoms such as increased thickness of the skin, erythema and scaling. Treatment of psoriasis depends on a number of factors such as the severity of the disease, the type of psoriasis, the body region involved, responsiveness to former treatments, the age and sex of the patient and the patient motivation.

Management of psoriasis comprises a large repertoire of topical or systemic treatments and photo(chemo)therapy. Within recent years biological therapies have provided additional opportunities for systemic treatments.

The mainstay in treatment of psoriasis is topical treatment. The majority of patients (two of three patients) suffer from mild to moderate psoriasis and will benefit from topical treatment as single therapy or in conjunction with other therapies.

First line therapy is topical treatment with vitamin D-analogues or corticosteroids.

Commonly used topical treatments of psoriasis may for example be

- Local treatment with vitamin-D derivative formulations. Vitamin- D analogues have an antiproliferative effect and stimulate cell differentiation although they have a slower onset of action than steroids. Side effects such as burning sensation, itching and erythema are common (1-10%)
- Local treatment with steroids with different strengths. Corticosteroids have an anti-inflammatory effect and a fast onset of action. Common side effects of steroids include thinning of the skin burning sensation and itching. Furthermore, repeat administration of steroids may result in tachyphylaxis or rebound phenomena.
- Coal tar ointment or coal tar baths which have an anti-inflammatory and antiproliferative effect. Common side effects are skin irritation and staining of clothes in addition to allergic and phototoxic responses.
- Topical synthetic retinoids like e.g. Tazarotene, which is available in limited territories only. Tazarotene has anti-inflammatory and antiproliferative effects. Common side effects are erythema, pruritus and scaling of the skin including surrounding normal skin. Retinoids have a known teratogenicity potential.
- Topical calcineurin inhibitors such as Tacrolimus and Pimecrolimus which are macrolide immunosuppressants and inhibit T-cell activation.
- They are mainly used for flexural and facial psoriasis. Common side effects are skin irritation, pruritus and erythema. Treated skin areas must be protected from exposure to UV light.

Patients who are non-responders to the above mentioned treatments may be treated with a systemic treatment. All of these bear a considerable risk for serious side effects, such as hepatotoxicity and nephrotoxicity (methotrexate, cyclosporine), teratogenicity (oral retinoids) and skin cancer (photo/chemotherapy) which limits their long- term use.

Other clinical conditions wherein hyperproliferation is a primary factor of pathogenesis include neoplastic and preneoplastic conditions. For many of these diseases no effective local treatment can be offered apart from surgery. In particular, neoplastic and

preneoplastic conditions of the genitals, reproductive organs, gastrointestinal tract and upper airway tract, lips, oral cavity and nasal cavity are generally treated by surgery.

5 The international patent application WO 03/015759 describes that anthracyclines can be used in the treatment of clinical conditions of body surfaces such as skin and mucosal membranes, wherein abnormal cell differentiation and/or hyperproliferation is a primary factor of the pathogenesis. In particular, the treatment of psoriasis by topical administration of valrubicin comprised in a liquid formulation is disclosed. However, this document is neither concerned with stability of the liquid formulations nor with ability of
10 the anthracyclines to reach target cells.

Accordingly, there is a great need for new improved treatments of clinical conditions, wherein hyperproliferation and/or abnormal cell differentiation is a primary factor of the pathogenesis and that are effective, in such a way that the drug fast and effectively
15 reaches the target cells to be treated. Also there is a need for local treatments, which do not involve use of surgery of these conditions.

Furthermore, there is a documented need for new effective and stable formulations for uncomplicated topical administration to improve patient adherence to treatment
20

Summary of invention

The present invention relates to novel formulations comprising lipophilic anthracyclines that can advantageously be used in local treatment of psoriasis and other clinical conditions, wherein hyperproliferation, such as epithelial hyperproliferation
25 and/abnormal cell differentiation is a primary factor of the pathogenesis. In particular it has been found that the novel formulations according to the present invention provide a very good penetration of the lipophilic anthracyclines through tissue surfaces, such as through the epithelium of epidermis and through mucosal epithelial membranes and thereby a greater part of the anthracycline reaches the cell layers, in which the
30 hyperproliferative target cells are located. Hence, the formulations according to the present invention show excellent release and penetration efficiency as compared to formulations known in the art.

Furthermore, in contrast to formulations of the prior art, the formulations according to
35 the invention are stable even at room temperature.

Hence, it is a first object of the present invention to provide a pharmaceutical composition comprising

- i) a lipophilic anthracycline;
- 5 ii) one or more oil phase carriers;
- iii) one or more pharmaceutical acceptable solubilizers;
- iv) one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from about pH 2.2 to about pH 6, and wherein the water
10 content of the pharmaceutical composition is less than 30%.

It is a second object of the present invention to provide uses of said pharmaceutical compositions comprising a lipophilic anthracycline for the preparation of a medicament for the treatment of a condition associated with hyperproliferation, preferably epithelial
15 hyperproliferation, such as dermal hyperproliferation, preferably epidermal hyperproliferation.

In a third object, the present invention provides methods of treatment of a condition associated with hyperproliferation, preferably epithelial hyperproliferation, more preferably epidermal hyperproliferation in an individual in need thereof comprising
20 administering topically to said individual a pharmaceutical composition comprising

- i) a lipophilic anthracycline;
- ii) one or more oil phase carriers;
- iii) one or more pharmaceutical acceptable solubilizers;
- 25 iv) one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from about pH 2.2 to about pH 6, preferably from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%.

30

It is a further object of the present invention to provide uses of pharmaceutical compositions comprising a lipophilic anthracycline formulated for local administration for the preparation of a medicament for the treatment of a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, nasal cavity, lips,
35 pharynx, larynx or affecting the eye.

It is a further object of the present invention to provide pharmaceutical compositions comprising a lipophilic anthracycline, wherein the composition comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6.0.

5

Additional preferred embodiments of the above mentioned objects of the invention will be further described in detail herein below.

Description of Drawings

10 Figure 1A, shows the dose response effect expressed as % proliferation of DJM-1 cells upon application of increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

15 Figure 1B, shows the dose response effect expressed as % proliferation of HSC-1 cells upon application of increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

20 Figure 1C, shows the dose response effect expressed as % proliferation of HaCaT cells upon application of increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

Figure 2 shows the effect of Valrubicin investigated in three distinct cultures of primary keratinocytes. Proliferation curves are shown at 48 hours.

25 Figure 3A-C shows the effect of valrubicin on progression and treatment of skin cancer in mice *in vivo*. The skin cancer is induced by DMBA/TPA. Figure 3A shows mean number of tumours in the untreated control (=no treatment - triangles), treatment with vehicle only (=Formulation A without Valrubicin - open circles), and treatment with valrubicin in vehicle (=Formulation A - closed circles). Figure 3B shows % mice with
30 tumours in the untreated control (=no treatment - triangles), treatment with vehicle only (=Formulation A without Valrubicin - open circles), and treatment with valrubicin in vehicle (=Formulation A -closed circles). Figure 3C shows tumour volumes in the three groups as measured in mm³.

35 Figure 4A-E shows the semi quantitative clinical psoriasis score in HuSCID mice engrafted with human psoriasis plaque skin and either untreated (diamonds), treated

with vehicle (VLD32 without valrubicin – small squares), treated with valrubicin (VLD32 – triangles), treated with Calcipotriol – large squares) or Bethametasone valerate – medium squares). Fig. 4A-E represents 5 different series using psoriasis plaque skin from 5 different donors.

5

Figure 4F shows the mean value of epidermal skin thickness in μm of the $n=5$ series of engrafted HuSCIDmice after once daily application of treatments for 21 consecutive days. Treatment with the VLD32 formulation is denoted “Valrubicin” in the figure.

10 Figure 5A-I shows the dose-response anti-proliferative effect of valrubicin and doxorubicin expressed as percentage reduction of cell viability of the human SCC cell lines, CaSki (cervix HPV16) (Figs. 5A-C), SW756 (cervix HPV18)(Figs. 5D-F) and CW954 (vulva)(Figs. 5G-I) upon stimulation with compounds for 24-, 48- and 72 hrs. Proliferation curves are shown at 24, 48 and 72 hrs with percentage reduction of cell
15 viability (100% as reference value) at different concentration of compounds applied.

Figure 6 shows a comparison of Valrubicin amount [$\mu\text{g}/\text{cm}^2$] found in the 2 first tape-strips (2TS), 16 tape-strips (Stratum Corneum, 16TS), deeper skin layers (residual skin after stripping) and acceptor medium after 24 hrs exposure of normal skin to the
20 formulations A (JH32) and VDL32. The bars presented are mean value ($n=6$) of amount [$\mu\text{g}/\text{cm}^2$] and standard deviation (SD).

Figure 7 shows a comparison of Valrubicin amount [$\mu\text{g}/\text{cm}^2$] found in the 2 first tape-strips (2TS), 6 tape-strips (Residual Stratum Corneum, 6TS), deeper skin layers
25 (residual skin after stripping) and acceptor medium after 24 hrs exposure of abraded skin to the formulation A (JH32) and VDL32. The bars presented are mean value ($n=6$) of amount [$\mu\text{g}/\text{cm}^2$] and SD.

Figure 8 shows the penetration of Valrubicin into cervical tissue after 24-hour
30 incubation with emulsified gel 4, valrubicin 1%. The diagram shows the concentration profile in the tissue from donor 1. The values shown are the individual values from each Franz cell and arithmetic mean values $\pm\text{SD}$ ($n=3$).

Figure 9 shows the penetration of Valrubicin into cervical tissue after 24-hour
35 incubation with emulsified 4 gel valrubicin 1%. The diagram shows the concentration

profile in the tissue from donor 2. The values shown are the individual values from each Franz cell and the relevant arithmetic mean values $\pm$ SD (n=3).

Detailed description of the invention

- 5 The present invention relates to a pharmaceutical composition comprising
- i) a lipophilic anthracycline;
 - ii) one or more oil phase carriers;
 - iii) one or more pharmaceutical acceptable solubilizers;
 - iv) one or more co-surfactants; and
- 10 wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6.

Further details relating to the different constituents of the pharmaceutical composition will be described herein below.

15

Lipophilic anthracyclines

The anthracyclines of the present invention are in general lipophilic anthracyclines, in particular such lipophilic anthracyclines, which are capable of passing the cell membrane and enter the cytoplasm of cells in a fast manner. Preferably, the lipophilic anthracyclines according to the invention do not enter nucleus of cells to any significant degree.

20

In connection with the present invention the lipophilic anthracyclines may in some instances be characterised by their partition coefficient (P) and log P. The partition coefficient is the ratio of a given compound partitioned between two solvents, traditionally the solvent system is octanol/water. The log P_{octanol} can be measured in a range from -2 to 6, and compounds having a log $P_{\text{octanol/water}}$ of about 0,5 to about 2 may be considered moderately lipophilic, compounds having a log $P_{\text{octanol/water}}$ above 2 may be considered increasingly lipophilic. Hence lipophilic anthracyclines according to the present invention may preferably have a log $P_{\text{octanol/water}}$ value greater than 0.5, preferably greater than 1.0, more preferably greater than 1.5, even more preferably greater than 2.0, yet even more preferably greater than 3.0.

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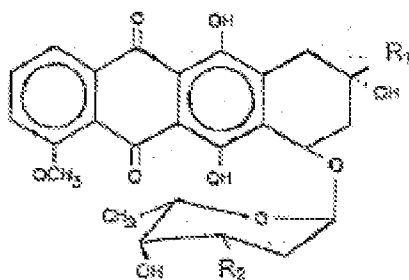
As the pH may have an influence on the determination of the partition coefficient, due to possible ionization of the compound in question, it may be preferred to determine the

35

value at a fixed pH. This may for example be done by the use of octanol and a buffer system, such as e.g. PBS (wherein PBS is phosphate buffered saline, preferably 0.01 M phosphate in 8.5% NaCl, pH 7.2.). In an alternative embodiment of the present invention, the lipophilicity is characterised by the partition coefficient (P) where

5 anthracycline preferably have an octanol/buffer partition coefficient (such as e.g. octanol PBS partition coefficient) greater than 40, more preferably greater than 60, even more preferably greater than 80, and yet even more preferably greater than 100. One useful method for determining the octanol/buffer partition coefficient is described in Panayiotis et al., 1989, Chemistry and Physics of Lipids, 51:105-118.

10 Preferred lipophilic anthracyclines, which can be used in the present invention, may have the general formula (I)



(I)

15 or pharmaceutical acceptable salt, solvates or prodrugs thereof;

wherein R_1 is selected from the group consisting of $-C(O)CH_2-O-(C_1-C_6-acyl)$, $-C(O)CH_2-O-(C_1-C_6-alkyl)$, $-C(O)CH_2-O-(C_1-C_6-alkoxy)$, and $-C(O)CH_2-O-(C_1-C_6-acyl)-(C_1-C_6-alkoxy)$; and

R_2 is selected from the group consisting of $-NH-(C_1-C_6-acyl)$, $-NH-(C_1-C_6-alkyl)$, $-NH-(C_1-C_6-alkoxy)$, $-N(C_1-C_6-acyl)(C_1-C_6-acyl)$, $-N(C_1-C_6-acyl)(C_1-C_6-alkyl)$, $-N(C_1-C_6-acyl)(C_1-C_6-alkoxy)$, $-N(C_1-C_6-alkyl)(C_1-C_6-alkyl)$, $-N(C_1-C_6-alkyl)(C_1-C_6-alkoxy)$, $-N(C_1-C_6-alkoxy)(C_1-C_6-alkoxy)$, -heterocyclyl, $-C(O)CH_2-O-(C_1-C_6-alkyl)$, $-(C_1-C_6-acyl)$, $-NH_2$, $-OH$, and H ; wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of $C_1-C_3-alkyl$, $C_1-C_2-alkoxy$, $-OH$, halogen, $-NH_2$, $-NH-(C_1-C_4-alkyl)$, or $-N(C_1-C_4-alkyl)(C_1-C_4-alkyl)$.

20

25

In a preferred embodiment the lipophilic anthracycline is a compound of the general formula (I).

In relation to the compounds of formula (I), R_1 may preferably be $-\text{C}(\text{O})\text{CH}_2\text{-O-(C}_1\text{-C}_6\text{-acyl)}$; and more preferably R_1 may be $-\text{COCH}_2\text{OCO(CH}_2\text{)}_3\text{CH}_3$.

Alternatively, a preferred group of R_1 may be defined as $-\text{C}(\text{O})\text{CH}_2\text{-O-C}(\text{O})(\text{CH}_2)_n\text{X}$,
5 wherein n is an integer in the range of 1 to 10, preferably in the range of 2 to 5, more preferably in the range of 3 to 4, yet more preferably 3, and X is selected from the group consisting of $-\text{CH}_3$, $-\text{OH}$ and COOH , preferably X is $-\text{CH}_3$.

Furthermore, in relation to the compounds of formula (I), R_2 may preferably be selected
10 from the group consisting of $-\text{NH-(C}_1\text{-C}_6\text{-acyl)}$, $-\text{NH-(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{NH-(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-acyl)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-alkyl)(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{N(C}_1\text{-C}_6\text{-alkyl)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-alkoxy)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{heterocyclyl}$, $-\text{C}(\text{O})\text{CH}_2\text{-O-(C}_1\text{-C}_6\text{-alkyl)}$, and $-(\text{C}_1\text{-C}_6\text{-acyl)}$; wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of
15 $\text{C}_1\text{-C}_3\text{-alkyl}$, $\text{C}_1\text{-C}_2\text{-alkoxy}$, $-\text{OH}$, halogen, $-\text{NH}_2$, $-\text{NH-(C}_1\text{-C}_4\text{-alkyl)}$, or $-\text{N(C}_1\text{-C}_4\text{-alkyl)(C}_1\text{-C}_4\text{-alkyl)}$. More preferably R_2 may be selected from the group consisting of $-\text{NH-(C}_1\text{-C}_6\text{-acyl)}$, $-\text{NH-(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{NH-(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-acyl)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{N(C}_1\text{-C}_6\text{-acyl)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-alkyl)(C}_1\text{-C}_6\text{-alkyl)}$, $-\text{N(C}_1\text{-C}_6\text{-alkyl)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{N(C}_1\text{-C}_6\text{-alkoxy)(C}_1\text{-C}_6\text{-alkoxy)}$, $-\text{heterocyclyl}$, and $-(\text{C}_1\text{-C}_6\text{-acyl)}$;
20 wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of methyl, ethyl, isopropyl, methoxy, ethoxy, isopropoxy, $-\text{OH}$, $-\text{Br}$, $-\text{F}$, $-\text{Cl}$, $-\text{NH}_2$, $-\text{NH-(C}_1\text{-C}_3\text{-alkyl)}$, or $-\text{N(C}_1\text{-C}_3\text{-alkyl)(C}_1\text{-C}_3\text{-alkyl)}$.

In a specific embodiment of the compounds of formula (I) R_2 is selected from the group
25 consisting of $-\text{COCH}_2\text{OCO(CH}_2\text{)}_3\text{CH}_3$, $-\text{COCH}_2\text{OH}$, $-\text{COCH}_3$, $-\text{NH}_2$, $-\text{H}$, $-\text{OH}$, and $-\text{NHCOCF}_3$; wherein any alkyl, acyl, or alkoxy moiety of R_2 optionally is substituted with one or more of $\text{C}_1\text{-C}_3\text{-alkyl}$, $\text{C}_1\text{-C}_2\text{-alkoxy}$, $-\text{OH}$, halogen, $-\text{NH}_2$, $-\text{NH-(C}_1\text{-C}_4\text{-alkyl)}$, or $-\text{N(C}_1\text{-C}_4\text{-alkyl)(C}_1\text{-C}_4\text{-alkyl)}$. In an even more specific embodiment R_2 is selected from the group consisting of $-\text{COCH}_2\text{OCO(CH}_2\text{)}_3\text{CH}_3$, $-\text{COCH}_2\text{OH}$, $-\text{COCH}_3$, and
30 $-\text{NHCOCF}_3$; wherein any alkyl, acyl, or alkoxy moiety of R_2 optionally is substituted with one or more of $\text{C}_1\text{-C}_3\text{-alkyl}$, $\text{C}_1\text{-C}_2\text{-alkoxy}$, $-\text{OH}$, halogen, $-\text{NH}_2$, $-\text{NH-(C}_1\text{-C}_4\text{-alkyl)}$, or $-\text{N(C}_1\text{-C}_4\text{-alkyl)(C}_1\text{-C}_4\text{-alkyl)}$.

In particular, the lipophilic anthracyclines may be selected from the group consisting of
35 OctADR (adriamycin octanoyl-hydrazone), MRA-CN (3'-deamino-3'-(3-cyano-4-

morpholiny)adriamycin), AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof. The lipophilic anthracyclines may also be selected from the group consisting of derivatives of OctADR, MRA-CN, AD32, AD41, AD143, AD194, AD198, AD199, AD201, AD202, AD288, and mixtures thereof. Preferably, the lipophilic anthracycline is valrubicin, which may also be denoted AD32. By the term "derivatives" as used herein is meant a compound, in which one atom or a group of atoms is replaced with another atom or a group of atoms.

10 The structure of OctADR, MRA-CN, AD32, AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288 and the lipophilicity is described by Constantinides et al. (Constantinides, P.P. et al.; Chemistry and Physics of Lipids, 51 (1989) p. 105-118). The above mentioned N-alkyladriamycin (AD41, AD194, AD198, AD199, AD201, AD202, AD288) analogues and N-trifluoro-acetyladiamycin-14-O-hemidipate (AD143) may be synthesised and characterised as described by Israel and colleagues (Israel, M. et al.; J. Med. Chem.; 28; p. 1223-1228; and Israel, M. et al.; US patent 4,610,977).

The size of the lipophilic anthracycline may also be important, because if said molecule is too large, it may be difficult for the molecule to reach the cells, which are afflicted by hyperproliferation. Thus, in embodiments of the invention, wherein the clinical condition to be treated is associated with dermal hyperproliferation, preferably epidermal hyperproliferation, such as epithelial hyperproliferation, then the lipophilic anthracycline is preferably of a size enabling it to pass the skin barrier and penetrate epidermis, to reach the cells in the epidermis in need of treatment. However, the ease of penetration may also be improved by adding a penetration enhancer to the pharmaceutical composition.

Preferred anthracyclines according to the present invention are capable of penetrating into a multilayered epithelium, preferably more than one cell layer, such as approximately 2 cell layers, for example approximately 3 cell layers, such as approximately 4 cell layers, for example approximately 4 to 6 cell layers, such as approximately 6 to 8 cell layers, for example approximately 8 to 10 cell layers, such as approximately 10 to 12 cell layers, for example approximately 12 to 15, such as 15 to 20 cell layers into a multilayered epithelium. Especially it is preferred that 50% by weight of the anthracycline, more preferred 60%, even more preferred 70%, yet even

more preferred 80%, especially preferred 90% by weight of the anthracycline has penetrated at least 2 cell layers, preferably at least 3 cell layers, more preferably at least 4 cell layers, even more preferably in the range of 4 to 20 cell layers, yet more preferably in the range of 6 to 20 cell layers, yet more preferably in the range of 8 to 20, even more preferably in the range of 12 to 20 cell layers.

Additionally, preferred anthracyclines according to the present invention are not locally toxic when applied topically to a body surface of an individual, more preferably, the anthracyclines are not, or are only mildly irritant, when applied to a body surface of an individual in an effective dose.

Whether a compound, such as an anthracycline is not, or only mildly irritant, can be determined as described herein below in the section "Skin irritation".

One example of a preferred lipophilic anthracycline is valrubicin, which is very lipophilic due to the less ionisation compared to other anthracyclines. Accordingly, valrubicin may pass the cell membrane and enter the cytoplasm of cells in a fast manner.

The term "alkyl", as used herein, unless otherwise indicated, includes saturated monovalent hydrocarbon radicals having straight or branched moieties. Examples of alkyl moieties include, but are not limited to, methyl, ethyl, propyl, isopropyl, n-butyl, iso-butyl, sec-butyl, tert-butyl, and neopentyl. Alkyl is preferably C₁-C₆ alkyl, i.e. groups containing from 1 to 6 carbon atoms, and for some embodiments of the present invention, more preferably C₁-C₄ alkyl, such as e.g. C₁-C₃ alkyl.

The term "acyl", as used herein, means an alkyl group as defined above containing at least one oxo moiety (-C=O).

The term "acyl", as used herein, refers to formyl as well as other alkyl substituted carbonyl groups, wherein "alkyl" is as defined above. For example, acyl includes groups such as (C₁-C₆)alkanoyl (e.g., formyl, acetyl, propionyl, butyryl, valeryl, caproyl, t-butylacetyl, etc.).

The term "alkoxy", as used herein, means an -O-alkyl group wherein "alkyl" is as defined above. Examples include, but are not limited to methoxy, ethoxy, propoxy,

isopropoxy, n-butoxy, sec-butoxy, tert-butoxy, pentoxy, 2-pentyloxy, isopentoxy, neopentoxy, hexoxy, 2-hexoxy, 3-hexoxy, and 3-methylpentoxy. Alkoxy is preferably C₁-C₆ alkoxy, i.e. groups containing from 1 to 6 carbon atoms, and for some embodiments of the present invention, more preferably C₁-C₄ alkoxy, such as e.g. C₁-C₂ alkoxy.

The terms "heterocyclyl", as used herein, refer to non-aromatic cyclic groups containing one or more heteroatoms selected from O, S and N. Preferably from one to four heteroatoms, more preferably from one to two heteroatoms. Heterocyclyl groups also include groups that are substituted with one or more oxo moieties. Examples of heterocyclyl include, but are not limited to morpholinyl, piperidinyl, piperazinyl, 1,2,3,6-tetrahydropyridinyl, tetrahydrofuranyl, tetrahydrothienyl, tetrahydropyranyl, tetrahydrothiopyranyl, and thiomorpholinyl. Preferably morpholinyl, as e.g. in MRA-CN.

The term "Halogen", as used herein, includes fluoro, chloro, bromo and iodo.

When it is indicated that any alkyl, acyl, alkoxy, or heterocyclyl moiety are "optionally substituted", the moiety in question may be unsubstituted or optionally substituted with one or more substituents (typically, one to three substituents) independently selected from the group of substituents listed.

The term "pharmaceutical acceptable salt, solvate or prodrug" as used herein refers to those acid and base additions salts, solvates, and prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use without undue toxicity, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention.

Pharmaceutically acceptable acid and base addition salts refers to the relatively non-toxic, inorganic and organic addition salts of compounds of the present invention. These salts can be prepared in situ during the final isolation and purification of the compounds, or by subsequently reacting the purified compound in its free acid or base form with a suitable organic or inorganic compound and isolating the salt thus formed. In so far as the compounds of formula (I) of this invention are basic compounds, they are all capable of forming a wide variety of different salts with various inorganic and

organic acids. Although such salts must be pharmaceutically acceptable for administration to animals, it is often desirable in practice to initially isolate the base compound from the reaction mixture as a pharmaceutically unacceptable salt and then simply convert to the free base compound by treatment with an alkaline reagent and thereafter convert the free base to a pharmaceutically acceptable acid addition salt.

The pharmaceutically acceptable acid addition salts of the basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metal hydroxides, or of organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chloroprocaine, choline, diethanolamine, ethylenediamine, N-methylglucamine, and procaine. The base addition salts of acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in a conventional manner. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present invention.

Salts may be prepared from inorganic acids sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydriodic, phosphorus, and the like. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate

mesylate, glucoheptonate, lactobionate, laurylsulphonate and isethionate salts, and the like. Salts may also be prepared from organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. and the like. Representative salts include acetate, propionate, caprylate, isobutyrate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like. Pharmaceutically acceptable salts may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium and the like, as well as non-toxic ammonium, quaternary ammonium, and amine cations including, but not limited to, ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Also contemplated are the salts of amino acids such as arginate, gluconate, galacturonate, and the like. (See, for example, Berge S.M. et al., "Pharmaceutical Salts," *J. Pharm. Sci.*, 1977;66:1-19 which is incorporated herein by reference.)

The compounds of the present invention may exist in unsolvated forms as well as in solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, are equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention.

The term "prodrug" refers to compounds that are rapidly transformed in vivo to yield the parent compound of the above formulae, for example, by hydrolysis. A thorough discussion is provided in T. Higuchi and V Stella, "Pro-drugs as Novel Delivery Systems," Vol. 14 of the A.C.S. Symposium Series, and in *Bioreversible Carriers in Drug Design*, ed. Edward B. Roche, American Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference. Examples of prodrugs include pharmaceutically acceptable, non-toxic esters of the compounds of the present invention, including C₁-C₆ alkyl esters wherein the alkyl group is a straight or branched chain. Acceptable esters also include C₅-C₇ cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl. C₁-C₄ alkyl esters are preferred. Esters of the compounds of the present invention may be prepared according to

conventional methods "March's Advanced Organic Chemistry, 5th Edition". M. B. Smith & J. March, John Wiley & Sons, 2001.

5 Compounds of formula (I) may contain chiral centers and therefore may exist in different enantiomeric and diastereomeric forms. This invention relates to all optical isomers and all stereoisomers of compounds of the formula (I), both as racemic mixtures and as individual enantiomers and diastereoisomers ((+)- and (-)-optically active forms) of such compounds, and mixtures thereof, and to all pharmaceutical compositions and methods of treatment defined below that contain or employ them,
10 respectively. Individual isomers can be obtained by known methods, such as optical resolution, optically selective reaction, or chromatographic separation in the preparation of the final product or its intermediate.

The pharmaceutical composition according to the present invention may comprise the
15 lipophilic anthracycline in an amount of at least 0.1%, preferably at least 0.5%, more preferably at least 1% of said lipophilic anthracycline (w/w %).

Preferably the pharmaceutical composition according to the present invention may comprise the lipophilic anthracycline in an amount of 0.1 to 10 w/w %, such as e.g.,
20 from 0.1 to 8 w/w %, from 0.1 to 5 w/w %, from 1 to 5 w/w %, from 0.1 to 2.5 w/w %, from 0.1 to 1.5 w/w %, from 0.25 to 1.25 w/w %, from 0.5 to 2.5 w/w %, from 0.5 to 2.0 w/w %, from 0.5 to 1.5 w/w %, or of about 1.0 w/w %. More preferably in an amount of from 0.25 to 1.25 w/w %, and more preferably in an amount of 1.0 w/w %.

25 Pharmaceutical compositions

The pharmaceutical compositions according to the present invention is pharmaceutical compositions comprising

- i) a lipophilic anthracycline;
- ii) one or more oil phase carriers;
- 30 iii) one or more pharmaceutical acceptable solubilizers;
- iv) one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6, and wherein the water content of the pharmaceutical composition is less than 30%.

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The pharmaceutical composition may preferably be in a form selected from the group consisting of a water in oil emulsion, an emulsified gel, a gel, and an oil in water emulsion; more preferably a water in oil emulsion or an emulsified gel. The pharmaceutical composition may also be a composition comprising only a lipid phase, such as an ointment. In one preferred embodiment of the present invention, the composition is a water in oil emulsion. In another preferred embodiment of the present invention the composition is an emulsified gel. In alternative embodiments of the present invention the composition is an ointment, gel or an oil in water emulsion.

10 Pharmaceutical compositions - content of lipophilic anthracycline

The pharmaceutical compositions may comprise more than one different anthracycline, such as 2, for example 3, such as 4, for example 5, such as more than 5 different anthracyclines. Preferably, all of said anthracyclines are lipophilic anthracyclines, preferably any of the lipophilic anthracyclines described herein above in the section "lipophilic anthracycline".

The pharmaceutical compositions may comprise any suitable amount of said lipophilic anthracycline. It is however preferred that all of the lipophilic anthracycline within the compositions is in solution, accordingly the composition should preferably not contain more lipophilic anthracycline than what is soluble in the composition.

In general, the pharmaceutical composition may comprise at least 0.1%, preferably at least 0.5%, more preferably at least 1% of said lipophilic anthracycline. Thus, the composition may comprise in the range of 0.1% to 30%, such as in the range of 0.1% to 20%, for example in the range of 0.1% to 10%, such as in the range of 0.1% to 5%, for example in the range of 0.5% to 30%, such as in the range of 0.5% to 20%, for example in the range of 0.5% to 10%, such as in the range of 0.5% to 5%, for example in the range of 0.5% to 3%, such as in the range of 0.5% to 1.5%, for example in the range of 1% to 30%, such as in the range of 1% to 20%, for example in the range of 1% to 10%, such as in the range of 1% to 5%, for example in the range of 1% to 3%, such as in the range of 1% to 2%, and preferably in the range of 0.25 to 1.25%. The % is a w/w%.

When the pharmaceutical composition is a solution or when the pharmaceutical composition comprises a solution, then said solution may comprise at least 0.1%,

preferably at least 0.5%, more preferably at least 1% of said lipophilic anthracycline. Thus, said solution may comprise in the range of 0.1% to 30%, such as in the range of 0.1% to 20%, for example in the range of 0.1% to 10%, such as in the range of 0.1% to 5%, for example in the range of 0.5% to 30%, such as in the range of 0.5% to 20%, for example in the range of 0.5% to 10%, such as in the range of 0.5% to 5%, for example in the range of 0.5% to 3%, such as in the range of 0.5% to 1.5%, for example in the range of 1% to 30%, such as in the range of 1% to 20%, for example in the range of 1% to 10%, such as in the range of 1% to 5%, for example in the range of 1% to 3%, such as in the range of 1% to 2%.

When the pharmaceutical composition is a gel, cream, lotion or ointment or when the pharmaceutical composition comprises a gel, cream, lotion or ointment, then said solution may comprise at least 0.1%, preferably at least 0.5%, more preferably at least 1% of said lipophilic anthracycline. Preferably, said gel, cream, lotion or ointment may comprise in the range of 0.1% to 10%, such as in the range of 0.1% to 5%, for example in the range of 0.5% to 10%, such as in the range of 0.5% to 5%, for example in the range of 0.5% to 3%, such as in the range of 0.5% to 1.5%, for example in the range of 1% to 10%, such as in the range of 1% to 5%, for example in the range of 1% to 3%, such as in the range of 1% to 2%.

Pharmaceutical compositions – excipients

The present invention discloses that in order for the anthracycline to be effective after local administration, e.g. topical application, it is important that the pharmaceutical composition is formulated in such a way that the anthracycline is kept dissolved when penetrating to the cells to be treated, i.e. to the hyperproliferative cells. Thus, for clinical conditions wherein dermal hyperproliferation, preferably epidermal hyperproliferation, such as e.g. epithelial hyperproliferation, is a primary factor of pathogenesis then it is important that the lipophilic anthracycline is kept dissolved when penetrating the tissue on its passage to the cells in need of treatment, which for example may be the epidermis of the skin or a mucosal epithelial membrane. Consequently, according to the present invention it is most preferred that the lipophilic anthracycline is kept dissolved in a vehicle. It is also preferred that the vehicle does not evaporate, or at least that some of the vehicle does not evaporate when applied to the surface of the skin, but is capable of delivering the anthracycline to the target location.

In order to make sure that the anthracycline is kept dissolved during absorption and penetration, the excipients, such as the oil phase carriers, the solubilizers, and the co-surfactants, used in the formulation of the present invention must be chosen to provide said solubilization. Hence, it is important that the solubility of the lipophilic anthracyclines in at least some of these, preferably in the majority of the individual excipients is of a certain degree. It is also preferred that the lipophilic anthracycline is fully dissolved in parts of the final

Furthermore, the present inventors have found that the pH of the formulation is important to ensure a satisfactory stability of the pharmaceutical composition, including a satisfactory stability of the active compound in question. Interestingly, the present invention discloses that compositions with high pH are not stable at 25°C and 60 RH% (RH = relative humidity). The composition designated "Valstar™" comprising the lipophilic anthracycline valrubicin must be stored at 2-8°C. It has been found by the present inventors that by keeping the pH of the aqueous phase at pH 6 or below, preferably from pH 2.2 to pH 6, then the pharmaceutical composition may be stored at about 25°C and 60 RH%. Accordingly, the pharmaceutical composition according to the present invention comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6.0.

It is even more preferred that the pH of said one or more buffering system is in the range of pH 2.2 to pH 5.0, such as e.g. of pH 2.2 to 4.5, of pH 2.2 to 4.2, more preferably of pH 2.2 to 4.0, even more preferably of pH 2.2 to 3.5, yet even more preferably of pH 2.5 to 3.5, and yet even more preferably of pH 2.5 to 3.0, and most preferably of about pH 2.5, such as pH 2.5.

The term "aqueous phase", as used herein, is intended to mean the aqueous part of the composition, i.e. the aqueous phase may be part of a carrier or excipient system, such as e.g., an oil in water emulsion (o/w), a water in oil emulsion (w/o), or an emulsified gel. The aqueous phase is thus not necessarily a separate aqueous phase as such. As it will be known to the person skilled in the art, it is not immediate possible to obtain a reliable pH measurements on a w/o emulsion, therefore the pH as used herein is specified as the pH of the buffering system of the aqueous phase. Accordingly, if no further aqueous media is added and/or there is no alkaline or acid

compounds present in the formulation, the pH will be that of the added aqueous buffering system.

5 The term "buffering system", as used herein, is intended to mean a mixture of a weak acid and its conjugate base or a weak base and its conjugate acid. The buffering system may furthermore be mixtures of one or more such acid/base pairs. The buffering agent may be any buffering agent, preferably a buffering agent which is not, or only mildly irritant, when applied to skin. Non-limiting examples of buffering agents that may be used alone or in combinations are citric acid (e.g. Citric acid/Sodium
10 citrate), acetic acid (e.g. Acetic acid/Sodium acetate), disodium phosphate (e.g. disodium phosphate/monosodium phosphate), tartaric acid (e.g. tartaric acid/sodium tartrate), malic acid (e.g. malic acid/sodium malate), Glycinate (e.g. Glycine/HCl), or lactic acid (e.g. lactic acid/sodium lactate).

15 Preferably the buffering system may comprise lactic acid, such as e.g. lactic acid – sodium lactate.

In one embodiment of the present invention, the pH of the final pharmaceutical composition is preferably in a range from pH 4.0 to pH 6, as it has been found by the
20 present inventors that lower pH values may provide both stability problems and problems with skin or mucosal membrane irritation. It is to be noted that even if a buffer solution having a pH of about 2.5 is applied in the pharmaceutical formulation according to the present invention, then the skilled person will know that a pH of e.g. pH 4.5 may be obtained in the final pharmaceutical composition by adding the
25 appropriate amount of the buffer solution. For instance, if a composition comprises slightly alkaline constituents and these are to be neutralised at the same time as the composition is to have an additional buffer capacity at a specific pH value, then a buffer solution being more acidic than the desired final pH may be used.

30 The pharmaceutical composition may preferably comprise sufficient of said buffer system in order to obtain a suitable pH in the entire pharmaceutical composition. Accordingly, the pharmaceutical compositions according to the invention may preferably comprise said buffering system as an aqueous solution comprising a buffering compound in the range of 10 to 300 mM, such as e.g., of 10 to 200 mM, of 20
35 to 200 mM, of 30 to 150 mM, of 40 to 150 mM, of 40 to 140 mM, or of 40 to 130 mM.

As mentioned above, the pH in the final pharmaceutical composition may preferably be in a range from pH 4.0 to pH 6, preferably from pH 4.0 to pH 5.5, more preferably from pH 4.0 to pH 5.0, even more preferably from pH 4.2 to pH 4.9. In a specific embodiment of the present invention the pH of the final pharmaceutical composition is in a range of pH 4.2 to about 4.9.

Furthermore, in one preferred embodiment of the invention, where the pharmaceutical compositions is an emulsified gel or a gel and said buffering system is an aqueous solution, then the buffering compound is preferably in a concentration in a range of 10 to 200 mM, such as e.g. of 20 to 150 mM, of 30 to 100 mM, of 30 to 80 mM, or more preferably of 40 to 60 mM, and even more preferably of about 50 mM, yet more preferably 50 mM. In a more preferred embodiment of the present invention where the pharmaceutical compositions is an emulsified gel or a gel and said buffering system is an aqueous solution, the pH of said buffering system is in the range of pH 2.2 to 4.5, such as e.g. of pH 2.2 to 4.2, preferably of pH 2.2 to 4.0, more preferably of pH 2.2 to 3.5, even more preferably of pH 2.5 to 3.5, and yet even more preferably of pH 2.5 to 3.0, and most preferably of about pH 2.5, such as pH 2.5, and the concentration of the one or more buffering compounds is preferably in a range of 20 to 150 mM, such as e.g. of 30 to 100 mM, of 30 to 80 mM, or more preferably of 40 to 60 mM, and even more preferably of about 50 mM, such as 50 mM.

Alternatively, in one preferred embodiment of the invention, where the pharmaceutical compositions is an water in oil emulsion and said buffering system is an aqueous solution, then the buffering compound is preferably in a concentration in a range of 50 to 300 mM, such as e.g., of 50 to 200 mM, e.g. of 50 to 175 mM, e.g. of 60 to 140 mM, or more preferably of 75 to 125 mM, and even more preferably of about 100 mM, such as 100 mM. In a more preferred embodiment of the present invention where the pharmaceutical compositions is an water in oil emulsion and said buffering system is an aqueous solution, the pH of said buffering system is in the range of pH 2.2 to 4.5, such as e.g. of pH 2.2 to 4.2, preferably of pH 2.2 to 4.0, more preferably of pH 2.2 to 3.5, even more preferably of pH 2.5 to 3.5, and yet even more preferably of pH 2.5 to 3.0, and most preferably of about pH 2.5, such as pH 2.5, and the concentration of the one or more buffering compounds is preferably in a range of 50 to 200 mM, such as e.g., of 50 to 175 mM, e.g. of 60 to 140 mM, or more preferably of 75 to 125 mM, and even more preferably of about 100 mM, such as 100 mM.

In a preferred embodiment of the invention the amount of water is minimized. The water content of the pharmaceutical compositions of the invention may preferably be less than 30%, more preferably less than 25%, even more preferably less than 20%, yet even more preferably less than 15%, and yet even more preferably less than 14%. It has for example been found by the present inventors that pharmaceutical compositions according to the invention having a decreased water content are more stable over time. This can for example be seen from the stability study in example 11, where formulation A, having a water content of 35.1%, can be seen to be less stable than formulations VLD32 and emulsified gel 4, having water contents, in the form of the buffer solutions, at about approximately 10% and 13.4%, respectively. The water content of the pharmaceutical formulations of the present invention may preferably be included in the form of an aqueous buffer solution.

The choice of oil phase carriers in the compositions of the present invention is also very important. Thus, preferably the oil phase carrier is chosen so that it may aid to carry the anthracycline through the epidermis and deliver the drug at the cells or within the cells to be treated. As mentioned above the pharmaceutical composition according to the present invention comprises one or more oil phase carriers. Generally, the one or more oil phase carriers is present in an amount of in the range of 10 to 70%, for example in the range of 10 to 60%, preferably in the range 10 to 55 w/w %, such as e.g., of 10 to 50 w/w %, of 12 to 48 w/w %, of 15 to 44 w/w %, or specifically of about 44 w/w % or specifically of about 15 w/w %, such as 44 w/w % or 15 w/w %.

Furthermore, in one preferred embodiment of the invention, where the pharmaceutical compositions is an water in oil emulsion, said oil phase carrier is in an amount of 35 to 55 w/w %, more preferably of 35 to 50 w/w %, even more preferably of 40 to 50 w/w %, and most preferably of about 45 w/w %, such as of about 44 w/w %, for example 45 w/w%, such as 44 w/w %.

Alternatively, in one preferred embodiment of the invention, where the pharmaceutical compositions is an emulsified gel or a gel, said oil phase carrier is in an amount of 10 to 25 w/w %, more preferably of 10 to 20 w/w %, even more preferably of 12 to 18 w/w %, and most preferably of about 15 w/w %, such as 15 w/w%.

In one embodiment of the invention, such as e.g. an oil gel, the pharmaceutical composition may comprise an even larger percentage of said oil phase carrier, such as in the range of 10 to 95%, for example in the range of 10 to 90%, such as in the range of 10 to 80%, preferably in the range of 10 to 70%, such as in the range 10 to 60%.

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Generally the oil phase carrier may be any pharmaceutical acceptable oil phase carrier that will dissolve the active compound and aid carrying the anthracycline through the epidermis. The oil phase carrier may be acylglycerols as for instance a monoacylglycerol, more preferred a diacylglycerol even more preferred a triacylglycerol or mixtures thereof. It is preferred that the fatty acid of the acylglycerol is a saturated or unsaturated fatty acid comprising 8 to 26 carbon atoms, more preferred 12 to 24 carbon atoms, even more preferred 16 to 22 carbon atoms. More preferably the one or more oil phase carriers may be selected from the group consisting of white soft paraffin, hard paraffin, Crodamol (such as e.g. Crodamol GTCC), glycerol monostearate, ceteraryl alcohol, macrogol-20 glycerol monostearate (such as e.g. Tagat S2), and castor oil, or mixtures thereof. Even more preferably the one or more oil phase carriers may be selected from the group consisting of white soft paraffin, hard paraffin, Crodamol (such as e.g. Crodamol GTCC), and mixtures thereof.

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The choice of pharmaceutical acceptable solubilizers in the compositions of the present invention is likewise very important, as the lipophilic anthracyclines need to have a certain solubility in said solubilizer. As mentioned above the pharmaceutical composition according to the present invention comprises one or more pharmaceutical acceptable solubilizers. Generally the pharmaceutical acceptable solubilizers are present in an amount of 10 to 45 w/w %, such as e.g., of 10 to 40 w/w %, of 12 to 38 w/w %, of 15 to 38 w/w %, of 15 to 35 w/w %, or specifically of about 17% or specifically of about 32 %, such as 17% or 32%.

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Furthermore, in one preferred embodiment of the invention, where the pharmaceutical composition is an water in oil emulsion, said solubilizer is in an amount of 10 to 25 w/w %, more preferably of 10 to 20 w/w %, even more preferably of 15 to 20 w/w %, and most preferably of about 17 w/w %, such as 17%.

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Alternatively, in one preferred embodiment of the invention, where the pharmaceutical composition is an emulsified gel or a gel, said solubilizer is in an amount of 20 to 45

w/w %, more preferably of 25 to 35 w/w %, even more preferably of 30 to 35 w/w %, most preferably of about 32 w/w %, such as 32 w/w%.

5 Generally the pharmaceutical acceptable solubilizers may be any pharmaceutical acceptable solubilizers that will dissolve the active compound and aid in carrying the active compound into the epidermis. The solubilizer may be polyvalent alcohols or mixtures of polyvalent alcohols, in particular divalent alcohols or trivalent alcohols or mixtures thereof. More preferably the one or more pharmaceutical acceptable solubilizers may be selected from the group consisting of propylene glycol, glycerol, 10 and cremophor RH40 (polyoxyl 40 hydrogenated castor oil) or mixtures thereof.

At least one of the pharmaceutical acceptable solubilizers may furthermore be characterized as an alcohol comprising at least two free –OH groups. Said solubilizer being an alcohol preferably may be a lower alkyl substituted with at least two –OH 15 groups. More preferably said solubilizer being an alcohol may be selected from the group consisting of glycerol and propylene glycol. The preferred amounts are as specified for the solubilizer immediately herein above.

In order to obtain an appropriate solubility of the lipophilic anthracycline, the 20 pharmaceutical composition according to the present invention furthermore comprises one or more co-surfactants. The co-surfactants may be any co-surfactant giving an acceptable solubility of the active compound. Preferably the one or more co-surfactants may be selected from the group consisting of diethylene glycol monoethyl ether (such as e.g. transcitol P), glycofurol (tetrahydrofurfuryl polyethylenglycol), Propylene Glycol 25 Monolaurate (such as e.g. LauroglycolTM 90), and Propylene Glycol Laurate (such as e.g. LauroglycolTM FCC), or mixtures thereof. More preferably the one or more co-surfactants may comprise diethylene glycol monoethyl ether (such as e.g. transcitol P), and even more preferably transcitol P. Generally the one or more co-surfactants are present in an amount of 5 to 35 w/w %, such as e.g., of 10 to 30 w/w %, of 12 to 27 30 w/w %, of 15 to 25 w/w %, of 5 to 25 w/w %, of 15 to 35 w/w %, or specifically of about 15% or specifically of about 25 %, such as 15% or 25%.

Furthermore, in one preferred embodiment of the invention, where the pharmaceutical compositions is an water in oil emulsion, said co-surfactant is transcitol P in an amount 35 of 5 to 25 w/w %, preferably of about 15 w/w %, such as 15%.

Alternatively, in one preferred embodiment of the invention, where the pharmaceutical composition is an emulsified gel or a gel, said co-surfactant is transcitol P in an amount of 15 to 35 w/w %, preferably of about 25 w/w %, such as 25%.

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At least one of the one or more solubilizers and/or the one or more co-surfactants may furthermore be characterized by

- a) comprises at least one –OH group, and
- b) in addition to the at least one –OH mentioned in a) comprises at least one –O- group; and
- c) has a molecular weight of at the most 300.

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Accordingly, at least one of the one or more solubilizers and/or one of the one or more co-surfactants may be a polyether. Preferably, the polyether may have the general structure $R_1[-O-CH_2-CH_2]_n-R_2$, wherein n is an integer in the range of 2 to 6, preferably 2 to 5, R_1 and R_2 individually are selected from the group consisting of lower alcohols, lower alkyl and 5 to 6 membered rings. More preferably the polyether is selected from the group consisting of diethylene glycol monoethyl ether and glycofurool (Tetrahydrofurfuryl polyethylenglycol). The amount of said polyether in the composition may preferably be in the range of 5 to 50 w/w%, preferably in the range of 10 to 30 w/w%.

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As the solubility of valrubicin in the different excipients is of importance to the present invention the polyether may furthermore be characterized by the solubility of the lipophilic anthracyclines. Accordingly, in a preferred embodiment, the solubility of valrubicin in said polyether is at least 5% w/w, preferably at least 10% w/w, even more preferably at least 20% w/w.

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Likewise of importance is the stability of the pharmaceutical composition, including the stability of the active compound, which may be used in the characterisation of the polyether. Accordingly, the polyether may preferably be a polyether, wherein the percentage peak purity of valrubicin after storage therein for 2 weeks at 25°C and 60 %RH, is at least 80%, preferably at least 85%, more preferably at least 90%.

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The pharmaceutical compositions according to the present invention may further comprise one or more emulsifiers and/or one or more gel forming polymers. The emulsifier may be any emulsifier known to the person skilled in the art that is suitable for pharmaceutical formulations for topical administration. This emulsifier may be an ester originating from an organic acid having 3 to 18 carbon atoms and an alcohol having 3 to 18 carbon atoms and mixtures thereof. The emulsifier may for example be selected from the group consisting of PEG-30 dipolyhydroxystearate, polyglyceryl-2-dipolyhydroxystearate, polyglyceryl-3-diisostearate, lauryl lactate, cetearyl isononanoate, and mixtures thereof. The gel forming polymer may be any gel forming polymer known to the person skilled in the art that is suitable for preparing pharmaceutical gel or emulsified gel formulations.

Generally said one or more emulsifiers and/or one or more gel forming polymers is present in an amount of 0.1 to 10 w/w %, such as e.g., of 0.5 to 8 w/w %, of 1 to 6 w/w %, of 1 to 5 w/w %, of 1 to 4 w/w %, of 1 to 3 w/w %, or specifically of about 2 w/w% or specifically about 2.5%, such as 2 w/w % or 2.5%.

Furthermore, in one embodiment of the invention, where the pharmaceutical compositions is a water in oil emulsion or an oil in water emulsion, it is preferred that the composition comprises one or more emulsifiers in the above specified amounts. More preferably, when the composition is a water in oil emulsion, said one or more emulsifiers may be selected from the group consisting of Dow Corning emulsifier 10, and Abil Em 90. Even more preferably Dow Corning emulsifier 10.

Alternatively, in one embodiment of the invention, where the pharmaceutical compositions is an emulsified gel or a gel, it is preferred that the composition comprises one or more gel forming polymers in the above specified amounts. More preferably, when the composition is an emulsified gel or a gel, said one or more gel forming polymers may be selected from the group consisting of hydroxypropyl cellulose (such as e.g. HPC HF), hydroxyethyl cellulose (such as e.g. HEC HF), Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10. Even more preferably hydroxy propyl cellulose (such as e.g. HPC HF).

The pharmaceutical compositions according to the present invention may further comprise a pharmaceutical acceptable alcohol, preferably ethanol. The alcohol may be

included to aid the solubility, but at the same time it is undesirable to have a large content of said alcohol, such as e.g. ethanol, as this may increase irritation of the skin or other epithelial tissue. Furthermore, if the solubility of the active compound in the composition relies on the presence of the alcohol, then after application the alcohol may evaporate and leave the active compound undissolved, hereby hindering the absorption and the penetration to the relevant tissue. Accordingly, the pharmaceutical acceptable alcohol may be present in an amount of 0.1 to 15 w/w %, such as e.g., of 0.5 to 15 w/w %, of 1 to 13 w/w %, preferably of 2 to 13 w/w %, more preferably of 3 to 13 w/w %, and even more preferably of 4 to 12 w/w %, such as e.g., of 5 to 10 w/w %, or specifically of about 5 w/w % or specifically of about 10 w/w %, such as 5 w/w % or 10 w/w %.

Furthermore, in one preferred embodiment of the invention, where the pharmaceutical compositions is a water in oil emulsion said pharmaceutical acceptable alcohol is present in an amount of 0.1 to 10 w/w %, more preferably of 0.5 to 10 w/w %, even more preferably of 1 to 10 w/w %, yet even more preferably of 2 to 8 w/w %, and specifically of about 5 w/w %, such as 5 w/w %.

Alternatively, in one preferred embodiment of the invention, where the pharmaceutical compositions is an emulsified gel or a gel, said pharmaceutical acceptable alcohol is present in an amount of 1 to 15 w/w %, more preferably of 2 to 15 w/w %, even more preferably of 5 to 15 w/w %, yet even more preferably of 8 to 13 w/w %, and specifically of about 10 w/w %, such as 10 w/w %.

The pharmaceutical compositions according to the present invention may further comprise one or more antioxidants. Generally said one or more antioxidants may be any pharmaceutically acceptable antioxidants known to the skilled person. Preferably said antioxidants are fat soluble antioxidants, due to the lipophilic character of the anthracyclines of the present invention. More preferably said one or more antioxidants may be selected from the group consisting of Ascorbyl palmitate, Alpha tocopherol, and mixtures thereof. Said one or more antioxidants may be present in an amount of 0.01 to 0.15 w/w %, such as e.g. of 0.01 to 0.1 w/w %, of 0.01 to 0.08 w/w %, of 0.02 to 0.08 w/w %, of 0.03 to 0.07 w/w %, or specifically of about 0.05 w/w %, such as 0.05 w/w %.

The pharmaceutical compositions according to the present invention may further comprise one or more stabilizers and/or preservatives. Generally said stabilizers and/or preservatives may be any stabilizers and/or preservatives well known to the person skilled in the art.

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Preferably said one or more stabilizers may at least comprise an EDTA salt, such as EDTA disodium. Said one or more stabilizers may be present in an amount of 0.01 to 0.1 w/w %, such as e.g. of 0.01 to 0.08 w/w %, of 0.01 to 0.06 w/w %, preferably of 0.01 to 0.04 w/w %, or specifically of about 0.02 w/w %, such as 0.02 w/w%.

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Preferably said one or more preservatives is selected from the group consisting of Phenoxy ethanol, sodium benzoate, sorbic acid, potassium sorbate, benzoic acid, and parabens, or mixtures thereof. More preferably said preservative is phenoxy ethanol. Said one or more preservatives may be present in an amount of 0.01 to 5 w/w %, of 0.01 to 2 w/w %, such as e.g., of 0.1 to 1.5 w/w %, preferably of 0.5 to 1.25 w/w %, or specifically of about 1 w/w %, such as 1 w/w%.

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The preferred preservatives may furthermore be characterised according to structure. Accordingly, the preferred preservatives may comprise an aromatic ring substituted with an ether substituted with at least one free -OH group. Furthermore the substituted aromatic may have the general structure: aromatic ring-O-(CH₂)_n-OH, where n is an integer in the range of 1 to 6, preferably 1 to 3. The aromatic ring may furthermore preferably be phenyl, and the preservative may preferably be phenoxyethanol.

20

As the solubility of valrubicin in the different excipients is of importance to the present invention the solubility of valrubicin in said preservative may preferably be at least 5% w/w, preferably at least 10% w/w.

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The stability of the pharmaceutical composition is likewise of importance, including the stability of the active compound, and may be used in the characterisation of the preservative. Accordingly, the preservative may preferably be a preservative, wherein the percentage peak purity of valrubicin after storage therein for 2 weeks at 25°C is at least 80%, preferably at least 85%, more preferably at least 90%.

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It is particularly preferred that the pharmaceutical composition of the present invention further comprises a penetration enhancer, which aids the penetration of the anthracycline through epidermis or mucosal epithelium. By the term "penetration enhancer" as used herein is meant a substance that alters the skin structure thereby allowing other chemical substances to penetrate deeper into the skin or other tissue in question. The penetration enhancer may be present in the aqueous and/or the oily phase and is preferably selected from the group consisting of propylene glycol, panthenol, behenyl alcohol, hyaluronic acid and mixtures thereof. More preferably the penetration enhancer may be selected from the group consisting of propylene glycol, panthenol, behenyl alcohol, and mixtures thereof.

The pharmaceutical compositions according to the present invention may further comprise at least one compound having a molecular weight in the range of 50 and 1000 g/mol, more preferred in the range of 60 and 700 g/mol, even more preferred in the range of 70 and 500 g/mol, where said further compound is selected from the group consisting of aldehydes, ketones, esters, ethers, sugars, and proteins. Any compound known by the skilled person to be suitable for use in compositions for topical application may be used.

As will be appreciated, some component of the compositions of the invention can possess multiple functions. For example, a given component can act as both a solubilizer and a co-solubilizer. In some cases, the function of a given component can be considered singular, even though its properties in other connections may allow multiple functionalities.

The pharmaceutical compositions of the present invention may further comprise one or more additives in addition to the excipients described herein above selected from the group consisting of emollients, humectants, skin preparing agents, preservatives, colouring agents and acidity regulating agents. The acidity regulating agent is preferably any of the buffering systems described herein above. Any suitable agent known by the skilled person may be used in the compositions.

Humectants may be added to the composition. In particular, in embodiments of the invention wherein the pharmaceutical composition is a cream, ointment or lotion, humectants may be added in order to obtain a formulation which may reduce the

dryness of the skin afflicted with said hyperproliferative disorders and to improve patient compliance by having a "good feel". By the term "good feel" as used herein is meant a composition, which when applied to normal skin is non, or only mildly, irritant and preferably may be non greasy. Humectants are characterised by being substances with water attracting properties. Humectants differ in water-binding capacity as well as in ability to penetrate and influence the degree of skin hydration. Examples of humectants, which can be used in the present invention, are propylene glycol, D-pantothenic acid, hyaluronic acid, and alpha-hydroxy acids, such as lactic acid, glycolic acid and tartaric acid. Preferably the humectants may be selected from the group consisting of propylene glycol, D-pantothenic acid, and alpha-hydroxy acids, such as e.g. lactic acid, glycolic acid and tartaric acid.

The pharmaceutical compositions of the present invention may furthermore comprise further additives providing said above-mentioned "good feel", specifically one or more additives giving a smooth feel, ease of spreading and reduced tackiness, such as e.g. Dimethicone 350 (Dow Corning^(R) Q7-9120 Silicone fluid). Said one or more further additives, such as Dimethicone 350, may preferably be present in an amount of 1 to 15 w/w %, such as e.g., of 1 to 12 w/w %, of 1 to 10 w/w %, of 2 to 8 w/w %, of 3 to 7 w/w %; more preferably in an amount of 1 to 10 w/w %; even more preferably of 2 to 8 w/w %; specifically of about 5%.

Furthermore, in one embodiment of the invention, where the pharmaceutical compositions is a water in oil emulsion, it is preferred that the composition comprises Dimethicone 350. In a specific embodiment of the invention, where the pharmaceutical compositions is a water in oil emulsion, the composition comprises Dimethicone 350 in an amount of amount of 1 to 15 w/w %, such as e.g., of 1 to 12 w/w %, of 1 to 10 w/w %, of 2 to 8 w/w %, of 3 to 7 w/w %; more preferably in an amount of 1 to 10 w/w %; even more preferably of 2 to 8 w/w %; specifically of about 5%.

In a further aspect of the present invention the pharmaceutical composition is a water in oil composition comprising an aqueous phase and an oily phase, said oily phase comprising at least one lipophilic anthracycline, an alcohol having a molecular weight between 70 and 500 g/mol, an oil having a molecular weight between 500 and 1100 g/mol, and an emulsifier.

In a specific embodiment of the present invention, the pharmaceutical composition comprises

- i) 0.1-10 w/w % of a lipophilic anthracycline;
- ii) 10-70 w/w %, preferably 10-55 w/w% of one or more oil phase carriers;
- 5 iii) 10-45 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 5-35 w/w % of one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6.0, preferably from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%;

10 said pharmaceutical composition optionally further comprises

- v) 0.1-10 w/w % of one or more emulsifiers and/or gel forming polymers; and

said pharmaceutical composition optionally further comprises

- vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol.

15 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

20 In a further specific embodiment of the present invention, the pharmaceutical composition comprises

- i) 0.1-10 w/w % of a lipophilic anthracycline;
- ii) 10-70 w/w %, preferably 10-55 w/w% of one or more oil phase carriers;
- iii) 10-45 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 5-35 w/w % of one or more co-surfactants;

25 v) 0.1-10 w/w % of one or more emulsifiers and/or gel forming polymers;

- vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6.0, preferably from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%.

30 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In a further specific embodiment of the present invention, the pharmaceutical composition comprises

i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;

ii) 10-50 w/w % of one or more oil phase carriers;

5 iii) 10-40 w/w % of one or more pharmaceutical acceptable solubilizers;

iv) 10-30 w/w % of one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

10 said pharmaceutical composition optionally further comprises

v) 1-5 w/w % of one or more emulsifiers and/or gel forming polymers; and

said pharmaceutical composition optionally further comprises

vi) 1-13 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol. Additionally

said pharmaceutical composition may comprise any further excipients or active

15 compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In a further specific embodiment of the present invention, the pharmaceutical composition comprises

20 composition comprises

i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;

ii) 10-50 w/w % of one or more oil phase carriers;

iii) 10-40 w/w % of one or more pharmaceutical acceptable solubilizers;

iv) 10-30 w/w % of one or more co-surfactants;

25 v) 1-5 w/w % of one or more emulsifiers and/or gel forming polymers;

vi) 1-13 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

30 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In a further specific embodiment of the present invention, the pharmaceutical composition comprises

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 10-50 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof;
- iii) 10-40 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;
- iv) 10-30 w/w % of one or more co-surfactants selected from the group consisting of diethylene glycol monoethyl ether, glycofurol, Propylene Glycol Monolaurate, and Propylene Glycol Laurate, or mixtures thereof; preferably the one or more co-surfactants may comprise diethylene glycol monoethyl ether; and more preferably the one or more co-surfactants may be transcitol P; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

said pharmaceutical composition optionally further comprises

- v) 1-5 w/w % of one or more emulsifiers selected from the group consisting of Dow Corning emulsifier 10, and Abil Em 90, and/or one or more gel forming polymers selected from the group consisting of hydroxypropyl cellulose, hydroxyethyl cellulose, Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10; and

said pharmaceutical composition optionally further comprises

- vi) 1-13 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol.

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. The lipophilic anthracycline may preferably be selected from the group consisting of OctADR, MRA-CN, AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof; more preferably valrubicin. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In a further specific embodiment of the present invention, the pharmaceutical composition comprises

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;

- ii) 10-50 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof;
- iii) 10-40 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;
- 5 iv) 10-30 w/w % of one or more co-surfactants selected from the group consisting of diethylene glycol monoethyl ether, glycofurol, Propylene Glycol Monolaurate, and Propylene Glycol Laurate, or mixtures thereof; preferably the one or more co-surfactants may comprise diethylene glycol monoethyl ether; and more preferably the one or more co-surfactants may be transcitol P;
- 10 v) 1-5 w/w % of one or more emulsifiers selected from the group consisting of Dow Corning emulsifier 10, and Abil Em 90, and/or one or more gel forming polymers selected from the group consisting of hydroxypropyl cellulose, hydroxyethyl cellulose, Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10;
- 15 vi) 1-13 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol; and wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 5.0, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.
- Additionally said pharmaceutical composition may comprise any further excipients or
- 20 active compounds as described herein above or below. The lipophilic anthracycline may preferably be selected from the group consisting of OctADR, MRA-CN, AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof; more preferably valrubicin. This pharmaceutical composition may preferably be used in
- 25 the treatment of conditions as described herein in the section "clinical conditions".

- In a more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising
- i) 0.1-10 w/w % of a lipophilic anthracycline;
- 30 ii) 35-55 w/w % of one or more oil phase carriers;
- iii) 10-25 w/w % of one or more pharmaceutical acceptable solubilizers;
- vi) 5-25 w/w % of one or more co-surfactants; and
- wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of
- 35 the pharmaceutical composition is less than 30%, preferably less than 20%;

said pharmaceutical composition optionally further comprises

v) 0.1-10 w/w % of one or more emulsifiers; and

said pharmaceutical composition optionally further comprises

vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol.

5 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

10 In a more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising

i) 0.1-10 w/w % of a lipophilic anthracycline;

ii) 35-55 w/w % of one or more oil phase carriers;

iii) 10-25 w/w % of one or more pharmaceutical acceptable solubilizers;

15 vi) 5-25 w/w % of one or more co-surfactants;

v) 0.1-10 w/w % of one or more emulsifiers;

vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

20 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

25 In an even more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising

i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;

ii) 40-50 w/w % of one or more oil phase carriers;

30 iii) 10-20 w/w % of one or more pharmaceutical acceptable solubilizers;

vi) 5-25 w/w % of one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

35 said pharmaceutical composition optionally further comprises

- v) 1-5 w/w % of one or more emulsifiers; and
- said pharmaceutical composition optionally further comprises
- vi) 1-10 w/w % of a pharmaceutical acceptable alcohol.

5 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

10 In an even more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 40-50 w/w % of one or more oil phase carriers;
- iii) 10-20 w/w % of one or more pharmaceutical acceptable solubilizers;
- vi) 5-25 w/w % of one or more co-surfactants;

15 v) 1-5 w/w % of one or more emulsifiers;

- vi) 1-10 w/w % of a pharmaceutical acceptable alcohol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

20 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

25 In an even more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 40-50 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof;

30 iii) 10-20 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;

- vi) 5-25 w/w % of one or more co-surfactants comprising diethylene glycol monoethyl ether; and more preferably the one or more co-surfactants may be transcitol P; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

said pharmaceutical composition optionally further comprises

- 5 v) 1-5 w/w % of one or more emulsifiers selected from the group consisting of Dow Corning emulsifier 10 and Abil Em 90, preferably Dow Corning emulsifier 10; and

said pharmaceutical composition optionally further comprises

- vi) 1-10 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol.

- 10 Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. The lipophilic anthracycline may preferably be selected from the group consisting of OctADR, MRA-CN, AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof;
- 15 more preferably valrubicin. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In an even more specific embodiment of the present invention, the pharmaceutical composition is a water in oil emulsion comprising

- 20 i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 40-50 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof;
- iii) 10-20 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;
- 25 vi) 5-25 w/w % of one or more co-surfactants comprising diethylene glycol monoethyl ether; and more preferably the one or more co-surfactants may be transcitol P;
- v) 1-5 w/w % of one or more emulsifiers selected from the group consisting of Dow Corning emulsifier 10 and Abil Em 90, preferably Dow Corning emulsifier 10;
- 30 vi) 1-10 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

Additionally said pharmaceutical composition may comprise any further excipients or

35 active compounds as described herein above or below. The lipophilic anthracycline

may preferably be selected from the group consisting of OctADR, MRA-CN, AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof; more preferably valrubicin. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In an alternative more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-10 w/w % of a lipophilic anthracycline;
- ii) 10-25 w/w % of one or more oil phase carriers;
- iii) 20-45 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 15-35 w/w % of one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%; said pharmaceutical composition optionally further comprises

- v) 0.1-10 w/w % of one or more gel forming polymers;
- vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol.

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In an alternative more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-10 w/w % of a lipophilic anthracycline;
- ii) 10-25 w/w % of one or more oil phase carriers;
- iii) 20-45 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 15-35 w/w % of one or more co-surfactants;
- v) 0.1-10 w/w % of one or more gel forming polymers;
- vi) 0.1-15 w/w % of a pharmaceutical acceptable alcohol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

5

In an alternative even more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 10-20 w/w % of one or more oil phase carriers;
- 10 iii) 25-35 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 15-35 w/w % of one or more co-surfactants; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

15

said pharmaceutical composition optionally further comprises

- v) 1-5 w/w % of one or more gel forming polymers;
- said pharmaceutical composition optionally further comprises
- vi) 5-15 w/w % of a pharmaceutical acceptable alcohol.

20

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

25

In an alternative even more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 10-20 w/w % of one or more oil phase carriers;
- iii) 25-35 w/w % of one or more pharmaceutical acceptable solubilizers;
- iv) 15-35 w/w % of one or more co-surfactants;
- 30 v) 1-5 w/w % of one or more gel forming polymers;
- vi) 5-15 w/w % of a pharmaceutical acceptable alcohol; and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

5

In an alternative even more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
 - ii) 10-20 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof, more preferably the oil phase carrier may be Crodamol;
 - iii) 25-35 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;
 - iv) 15-35 w/w % of one or more co-surfactants comprising diethylene glycol monoethyl ether; more preferably the one or more co-surfactants may be transcutoyl P;
- and

wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%;

said pharmaceutical composition optionally further comprises

- v) 1-5 w/w % of one or more gel forming polymers selected from the group consisting of hydroxypropyl cellulose, hydroxyethyl cellulose, Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10, preferably HPC HF;

said pharmaceutical composition optionally further comprises

- vi) 5-15 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol.

Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. The lipophilic anthracycline may preferably be selected from the group consisting of OctADR, MRA-CN, AD32

(valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof; more preferably valrubicin. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

In an alternative even more specific embodiment of the present invention, the pharmaceutical composition is an emulsified gel comprising

- i) 0.1-2.5 w/w %, preferably 0.25-1.25 w/w % of a lipophilic anthracycline;
- ii) 10-20 w/w % of one or more oil phase carriers selected from the group consisting of white soft paraffin, hard paraffin, Crodamol, and mixtures thereof, more preferably the oil phase carrier may be Crodamol;
- 5 iii) 25-35 w/w % of one or more pharmaceutical acceptable solubilisers selected from the group consisting of propylene glycol, glycerol, and cremophor RH40, or mixtures thereof;
- iv) 15-35 w/w % of one or more co-surfactants comprising diethylene glycol monoethyl ether; more preferably the one or more co-surfactants may be transcitol P;
- 10 v) 1-5 w/w % of one or more gel forming polymers selected from the group consisting of hydroxypropyl cellulose, hydroxyethyl cellulose, Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10, preferably HPC HF;
- vi) 5-15 w/w % of a pharmaceutical acceptable alcohol, preferably ethanol; and wherein the composition further comprises an aqueous phase comprising at least one
- 15 buffering system having a pH from pH 2.2 to pH 4.2, and wherein the water content of the pharmaceutical composition is less than 30%, preferably less than 20%.
- Additionally said pharmaceutical composition may comprise any further excipients or active compounds as described herein above or below. The lipophilic anthracycline may preferably be selected from the group consisting of OctADR, MRA-CN, AD32
- 20 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or mixtures thereof, or pharmaceutical acceptable salts, solvates or prodrugs thereof; more preferably valrubicin. This pharmaceutical composition may preferably be used in the treatment of conditions as described herein in the section "clinical conditions".

25 Pharmaceutical compositions - stability

It is an important feature of the pharmaceutical compositions according to the invention that said compositions are stable, even upon storage at room temperature.

Thus, in a pharmaceutical composition according to the invention then preferably the

30 percentage peak purity of the lipophilic anthracycline (such as valrubicin) comprised therein is at least 70%, more preferably at least 80%, yet more preferably at least 90%, yet more preferably at least 95% after storage for 3 months at 25°C and 60 %RH. It is also preferred that the percentage recovery of said lipophilic anthracycline (such as valrubicin) is at least 80%, preferably at least 90%, more preferably at least 95%, even

35 more preferably at least 98%, and yet even more preferably 99% after storage for 3

months at 25°C and 60 %RH. In a specific embodiment of the present invention the percentage recovery of said lipophilic anthracycline (such as valrubicin) is at least 90% after storage of the pharmaceutical composition according to the invention at 25°C and 60 %RH for up to and including 12 months, preferably at least 95% after storage for 12 months at 25°C and 60 %RH.

The stability of the pharmaceutical formulations according to the present invention may additionally be increase by storage at 2-8°C, i.e. maximum 8°C. The stability at both 25°C and 2-8°C can be seen from example 11 herein. In a specific embodiment of the present invention, the percentage recovery of said lipophilic anthracycline (such as valrubicin) is at least 90% after storage of the pharmaceutical composition according to the invention at 2-8°C for up to and including 12 months, preferably at least 95%, more preferably at least 98%, even more preferably at least 99%, yet even more preferably 99.5%, yet even more preferably 99.9% after storage for 12 months at 2-8°C.

More preferably in a pharmaceutical composition according to the invention the percentage peak purity of the lipophilic anthracycline (such as valrubicin) comprised therein is at least 70%, more preferably at least 80%, yet more preferably at least 90%, even more preferably at least 95% after storage for 4 months at 25°C. It is also preferred that the percentage recovery of said lipophilic anthracycline (such as valrubicin) is at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and yet even more preferably 99% after storage for 4 months at 25°C.

It is furthermore preferred that the compositions are stable even after long term storage. Thus preferably in a pharmaceutical composition according to the invention then the percentage peak purity of the lipophilic anthracycline (such as valrubicin) comprised therein is at least 70%, more preferably at least 80%, yet more preferably at least 90%, after storage for 6 months at 25°C. It is also preferred that the percentage recovery of said lipophilic anthracycline (such as valrubicin) is at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 98%, and yet even more preferably 99% after storage for 6 months at 25°C.

It is preferred that the pharmaceutical compositions according to the invention comprise one or more excipients in which the lipophilic anthracyclines of the invention are stable.

5 Thus, it is preferred that the pharmaceutical compositions of the invention comprises at least one polyether (also termed co-surfactant, see herein above), which is a polyether, wherein the percentage peak purity of a lipophilic anthracycline (such as valrubicin) comprised therein is at least 80%, preferably at least 85%, more preferably at least 90% after storage therein for 2 weeks at 25°C.

10

Likewise it is preferred that the pharmaceutical compositions of the invention comprises at least one preservative, wherein the percentage peak purity of a lipophilic anthracycline (such as valrubicin) comprised therein is at least 80%, preferably at least 85%, more preferably at least 90% after storage therein for 2 weeks at 25°C.

15

Pharmaceutical compositions - solubility

As mentioned herein above it is preferred that the pharmaceutical compositions according to the invention comprise solubilized lipophilic anthracycline. This may improve the ability of the lipophilic anthracycline to reach the target of disease.

20

Thus, the solubility of a lipophilic anthracycline according to the invention (preferably of valrubicin) in said composition is preferably at least 0.5% w/w, yet more preferably at least 0.6% w/w, yet more preferably at least 0.7% w/w, yet more preferably at least 0.8% w/w, yet more preferably at least 0.9% w/w, yet more preferably at least 1% w/w in the pharmaceutical composition according to the invention.

25

It is thus preferred that the pharmaceutical compositions according to the invention comprise one or more excipients in which the lipophilic anthracyclines of the invention are soluble.

30

Thus, it is preferred that the pharmaceutical compositions of the invention comprises at least one polyether (also termed co-surfactant, see herein above), wherein the solubility of a lipophilic anthracycline (preferably valrubicin) in said polyether is at least 5% w/w, preferably at least 10% w/w, even more preferably at least 20% w/w.

35

Thus, it is preferred that the pharmaceutical compositions of the invention comprises at least one preservative, wherein the solubility of a lipophilic anthracycline (preferably valrubicin) in said preservative is at least 5% w/w, preferably at least 10% w/w.

5 Pharmaceutical compositions – colour masking agent

Due to the genuine red color of anthracyclines, the composition itself will be coloured if no action in order to mask the colour is taken. For example, if valrubicin is present in the composition, the composition will be bright red. Some patients may find it unpleasant to use a red formulation, in particular when applied for a skin disorder
10 characterized by erythema. Furthermore, in particular a red skin colour may be undesirable and even impair quality of life. The present invention also provides pharmaceutical formulations comprising one or more colour masking agents, which in particular may be added to pharmaceutical formulations of the invention comprising a red lipophilic anthracycline. In particular, pharmaceutical formulations for administration
15 to the skin (such as gels, creams, lotions or ointments) may comprise at least one colour masking agent. The colour masking agent may in one embodiment be one or more dyes capable of masking red colour.

The colour masking agents are themselves dyes and preferably the pharmaceutical
20 compositions may comprise:

- i) at least a green dye; or
- ii) at least one blue dye and at least one yellow dye.

In this embodiment the pharmaceutical composition will appear brownish and/or
25 reddish.

The dyes may be any dye, preferably a dye which is not or only mildly irritant when applied to skin. Methods for determining whether a dye is irritant to skin are described herein below in the section "Skin irritation".

30 Preferably, the green dye is selected from the group consisting of FD&C green No. 3, D&C green No. 5, D&C green No.6 and D&C green No.8. Preferably, the blue dye is selected from the group consisting of FD&C blue, (such as FD&C blue No. 1), D&C blue No. 4, FD&C blue No 2 and D&C blue No. 9. Preferably, the yellow dye is selected from the group consisting of FD&C yellow No 5, FD&C yellow No. 6, D&C yellow No. 7,
35 D&C yellow No. 8, D&C yellow No. 10, Sicovit Quinoline yellow and D&C yellow No. 11

In a preferred embodiment of the pharmaceutical compositions the at least one colour masking agent is selected from the group consisting of the colour masking agents FD&C blue and Sicovit Quinoline yellow.

5

Pharmaceutical compositions – specific formulations

Examples of specific formulations according to the present invention are given in the examples herein below.

10 The pharmaceutical compositions may be formulated in a number of different ways depending on the condition to be treated, the individual to be treated and the location of disease. Accordingly, the pharmaceutical composition is preferably formulated according to the need of the specific embodiment of the present invention.

15 For example the pharmaceutical composition may be in a form selected from the group consisting of vaginal cups, creams, ointments, gels, lotions, foams, pastes, sticks, patches, membranes, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries, suppositories, vaginal sponges, sprays, eye drops, and inhalators.

20

Additionally, the pharmaceutical composition may be in a form selected from the group consisting of vaginal cups, creams, ointments, gels, lotions, foams, pastes, sticks, patches, membranes, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries, suppositories, vaginal sponges, sprays, eye drops,
25 nasal drops and inhalators.

25

The pharmaceutical composition according to the present invention may preferably be a composition comprising a gel, an emulsified gel, an ointment, a cream, a foam, a solution or a lotion. The pharmaceutical composition may furthermore preferably be in
30 a form selected from the group consisting of a gel, an ointment, a cream, a foam, a solution or a lotion. The pharmaceutical composition may more preferably be in a form selected from the group consisting of a gel, an ointment, a cream, a solution and a lotion.

30

35 Alternatively the pharmaceutical compositions may be in the form of vaginal cups, patches, vaginal tampons, vaginal pessaries and/or vaginal sponges and may

optionally comprise a gel, an ointment, a cream, a foam, a solution and/or a lotion comprising the lipophilic anthracycline, as described herein above.

5 Furthermore the pharmaceutical composition may for example be in a form selected from the group consisting of vaginal cups, patches, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries, suppositories and vaginal sponges comprising said gel, ointment, cream, foam, solution or lotion. The solution, gel, cream and/or ointment may be any of the gels, ointments, crèmes, foams, solutions and/or lotions described herein above.

10

In a specific embodiment according to the invention, the pharmaceutical composition is in the form of a solution or cream or gel comprising said lipophilic anthracycline. In this specific embodiment, the pharmaceutical composition may further be in a form selected from the group consisting of patches, bandages, vaginal tampons, vaginal capsules, vaginal sponges, sprays, eye drops and inhalators comprising said solution.

15

In particular, patches, bandages, vaginal cups, patches, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries, suppositories and vaginal sponges may comprise a gel, foam, lotion, cream or ointment comprising a lipophilic anthracycline and a buffer with a pH of at the most 6 (i.e. any of the gels, foams, lotions, creams or ointments described herein in this section above).

20

Patches may take many different forms. Typically a patch comprises a polymer and an adhesive. The lipophilic anthracycline may be deposited on the patch in the form of a solution, gel, cream and/or ointment. The patch may be an occlusive patch. The patch may be in the form of a thin membrane. This may in particular be the case for administration of lipophilic anthracycline to a mucosal membrane, for example for nasal administration, for administration to the mouth or for buccal administration, or for administration to the genital region.

25

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Sticks as used herein may be any stick. Typically the stick will comprise a stiff formulation comprising a high amount of oil phase carrier allowing easy application of the formulation to a limited body surface area. The stick may for example be a lipstick, or a stick useful for application to the skin or a mucosal membrane.

35

Pharmaceutical compositions containing an anthracycline according to the present invention may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 1995, edited by E. W. Martin, Mack Publishing Company, 19th edition, Easton, Pa, unless otherwise described herein

5

Solutions, creams, ointments or gels according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with the aid of suitable machinery, with a greasy or non-greasy base, such as known to the person skilled in the art. Surprisingly, it has been found that the formulations according to the present invention show a much higher stability during storage than other known formulations.

10

Clinical conditions

15

The clinical condition to be treated according to the present invention is any clinical condition associated with hyperproliferation, i.e. cell hyperproliferation, preferably epithelial hyperproliferation and/or abnormal cell differentiation. Preferably, the clinical condition is associated with hyperproliferation of a local body area, preferably a local body area accessible without invasive means. More preferably, the clinical condition is associated with hyperproliferation of epithelial cells in skin and/or mucosal membranes.

20

In one preferred embodiment of the invention, the clinical condition is a clinical condition wherein hyperproliferation, such as e.g. epithelial hyperproliferation, epidermal hyperproliferation, hyperproliferation of keratinocytes, or dermal proliferation of small vessels; preferably epithelial hyperproliferation, epidermal hyperproliferation and hyperproliferation of keratinocytes; more preferably epithelial hyperproliferation or hyperproliferation of keratinocytes; even more preferably epithelial hyperproliferation; yet even more preferably epidermal hyperproliferation, is a primary factor of the pathogenesis.

25

30

Thus, the condition may be associated with epidermal hyperproliferation and/or epidermal pre-neoplastic and/or neoplastic processes. Preferably, the clinical condition is a clinical condition wherein epidermal hyperproliferation is a primary factor of the pathogenesis. Hyperproliferation of epidermis may for example involve hyperproliferation of stratum basale.

35

For example the condition according to the present invention may be selected from the group consisting of psoriasis, actinic keratosis, seborrheic keratosis, lichen planus (lichen ruber planus), basocellular carcinoma of the skin, squamous cellular carcinoma of the skin, planocellular carcinoma of the skin, verruca vulgaris, condyloma acuminata of skin or mucosal membranes, lichen sclerosis, cutaneous T-cell lymphomas, cutaneous metastasis, Karposi sarcoma and cicatricial hypertrophy.

Additionally the condition according to the present invention may be selected from the group consisting of psoriasis, actinic keratosis, seborrheic keratosis, lichen planus (lichen ruber planus), basocellular carcinoma of the skin, squamous cellular carcinoma of the skin or mucosal membranes, planocellular carcinoma of the skin, verruca vulgaris, condyloma acuminata of skin or mucosal membranes, lichen sclerosis, cutaneous T-cell lymphomas, cutaneous metastasis, Karposi sarcoma and cicatricial hypertrophy.

In one embodiment the clinical condition is cutaneous metastasis. The primary tumour from which the metastasis is derived may be any tumour; for example the primary tumour may be breast cancer.

In one preferred embodiment, the clinical condition to be treated with lipophilic anthracyclines formulated as described by the pharmaceutical compositions of the present invention is a condition associated with hyperproliferation, preferably epidermal hyperproliferation, preferably a condition associated with psoriasis, and more preferably psoriasis vulgaris. When the condition to be treated is related to an epidermal hyperproliferation (e.g. psoriasis, skin cancer, basal cell carcinoma, malignant melanoma, cutaneous metastasis, hyperproliferating keratinocytes), and accordingly is located on the skin, then it may be preferred to use a pharmaceutical composition according to the present invention in the form of a water in oil emulsion.

The term psoriasis according to the present invention includes all types of psoriasis known to the person skilled in the art. For example psoriasis may be selected from the group consisting of psoriasis vulgaris, guttate psoriasis, flexural psoriasis, erythrodermic psoriasis, generalised pustular psoriasis and localised pustular psoriasis, preferably psoriasis vulgaris, guttate psoriasis, flexural psoriasis, and localised pustular psoriasis. Additionally, psoriasis may be selected from the group consisting of psoriasis

vulgaris, guttate psoriasis, flexural psoriasis, erythrodermic psoriasis, generalised pustular psoriasis and localised pustular psoriasis, preferably psoriasis vulgaris, guttate psoriasis, flexural psoriasis, nail psoriasis and localised pustular psoriasis. The psoriasis to be treated according to the present invention may be mild, moderate, more
5 severe or very severe psoriasis, such as psoriasis wherein less than 2 percent, for example 2% to 5%, such as 5% to 10%, for example 10% to 15%, such as more than 15% of the skin is affected.

10 In another preferred embodiment of the invention the clinical condition is a clinical condition wherein hyperproliferation of mucosa, preferably hyperproliferation of epithelial cells of mucosa is a primary factor of the pathogenesis. When the condition to be treated is related to mucosa it may be preferred to use a pharmaceutical composition according to the present invention in the form of an emulsified gel or a gel, non-limiting example are the formulations described in examples 9, 10, and 16 herein.

15 Thus, in a very preferred embodiment the clinical conditions to be treated with lipophilic anthracyclines formulated as described in the pharmaceutical compositions of the present invention are neoplastic or preneoplastic conditions affecting the epithelium of the genitals, reproductive organs, lips, oral cavity, nasal cavity, pharynx, larynx,
20 trachea, or bronchi, or affecting the eye. Additionally, the clinical conditions to be treated with lipophilic anthracyclines may be squamous cell carcinomas or cancers of the genitals, reproductive organs, the lower gastrointestinal tract and/or the eyes. More preferably the clinical conditions to be treated with lipophilic anthracyclines may be cancers of the genitals, reproductive organs, the lower gastrointestinal tract and/or the
25 eyes.

Within the meaning of the present invention the genitals may preferably be selected from the group consisting of penis, cervix, vagina and vulva.

30 Thus, the clinical condition may be neoplastic or preneoplastic conditions of the penis, preferably carcinoma of the penis (penile carcinoma), for example squamous cell carcinoma of the penis, such as squamous cell carcinoma originating in the glans or foreskin. The clinical condition may also be condylomas of the penis. Currently, the only effective treatment of penile cancer is surgery, optionally in combination with other
35 treatments. The present invention provides a non-invasive method for treating

neoplastic or preneoplastic conditions of the penis by administering a lipophilic anthracycline to the penis, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from neoplastic or preneoplastic condition of the penis may be selected from the group consisting of patches, gels, creams or ointments. The formulations may be administered with or without occlusion, e.g. an occlusive bandage, plaster etc.

The clinical condition may also be neoplastic or preneoplastic conditions of the vulva, for example neoplastic or preneoplastic conditions such as vulvar intraepithelial neoplasia (VIN) or dysplasia of the labia majora or the labia minora. Thus, the clinical condition may be vulvar cancer, for example squamous cell carcinoma originating from vulvar tissue, such as from the epithelium of vulvar tissue. The vulvar cancer may also be basal cell carcinoma of the vulva. The clinical condition may also be condylomas of the vulva. Currently, vulvar cancer is treated by surgery. The present invention provides a non-invasive method for treating neoplastic or preneoplastic conditions of the vulva by administering a lipophilic anthracycline to the vulva, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from neoplastic or preneoplastic condition of the vulva may be selected from the group consisting of patches, sticks, gels, creams and ointments.

The clinical condition may also be neoplastic or preneoplastic conditions of the vagina, preferably vaginal cancer, for example carcinoma in situ or squamous cell carcinoma of the vagina. The clinical condition may also be condylomas of the vagina, in particular of the areas surrounding the vaginal entrance. The present invention provides a non-invasive method for treating neoplastic or preneoplastic conditions of the vagina by administering a lipophilic anthracycline to the vagina, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred

formulations for administration to patients suffering from neoplastic or preneoplastic conditions of the vagina may be selected from the group consisting of vaginal cups, creams, gels, patches, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries and vaginal sponges.

5

Within the meaning of the present invention the reproductive organs may preferably be selected from the groups consisting of uterus and the cervix, preferably the cervix. The present invention provides a non-invasive method for treating cervical cancer by administering a lipophilic anthracycline to the cervix, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from cervical intraepithelial neoplasia I to III (CIN I to CIN III), carcinoma in situ or squamous cervical carcinoma, may be selected from the group consisting of vaginal cups, creams, gels, emulsified gels, ointments, suppositories, patches, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries and vaginal sponges.

The lower gastrointestinal tract preferably comprises or more preferably consists of the part of the lower gastrointestinal tract which is readily accessible by non-invasive means. Thus, the clinical condition may be a neoplastic or preneoplastic condition of the anus or the perianal area. The clinical condition may thus for example be polyps of the anus or perianal area, condylomas of the anus or perianal area, anal cancer or perianal cancer. Said anal cancer or perianal cancer may for example be a squamous cell carcinoma or a basal cell carcinoma. The present invention provides a non-invasive method for treating neoplastic or preneoplastic condition of the anus or the perianal area by administering a lipophilic anthracycline to the anus or perianal area, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from neoplastic or preneoplastic condition of the anus or the perianal area may be selected from the group consisting of suppositories, patches, gels, emulsified gels, sticks, creams and ointments.

35

The clinical condition according to the present invention may also be a neoplastic or preneoplastic condition of the eyes, such as papillomas of the eye or cancers of the eye. Within the meaning of the present invention the eye includes the globe, the orbit and the adnexal structures. Thus, the clinical condition may for example be orbital, ,
5 basal cell carcinoma of the eyelid, conjunctival squamous cell carcinoma of the eye, ,medulloepithelioma,. The present invention provides a non-invasive method for treating neoplastic or preneoplastic condition of the eye by administering a lipophilic anthracycline to the eye, preferably locally to the affected site. More preferably said lipophilic anthracycline is administered in a pharmaceutical formulation according to the
10 invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from neoplastic or preneoplastic condition of the eye may be selected from the group consisting of eye drops and eye ointments.

15 In one embodiment of the invention, the clinical condition is a neoplastic or preneoplastic condition of the upper airway tract. The upper airway tract according to the invention comprises and more preferably consists of the lips, nasal cavity, the oral cavity, larynx, pharynx, bronchi and trachea. Thus, the clinical condition may for example be a cancer of the upper airway tract or papillomas of the upper airway tract.
20 Accordingly, the clinical condition may for example be a cancer of the lips, cancer of the nasal cavity, cancer of the oral cavity, cancer of the tongue and/or cancer on the vocal cords. Papillomas of the upper airway tract may for example be Laryngeal papillomatosis. The clinical condition may also be erythroplakia, leukoplakia, carcinoma in situ in the mouth. Preferably, for treatment of neoplastic or preneoplastic conditions
25 of the upper airway tract the lipophilic anthracycline is administered in a pharmaceutical formulation according to the invention as described herein in the section "Pharmaceutical compositions" including the subsections of that section. Very preferred formulations for administration to patients suffering from neoplastic or preneoplastic condition of the upper airway tract may be selected from the group consisting of
30 ointments, sprays, and patches; additionally, these may be selected from the group consisting of ointments, sprays, sticks, and patches.

The clinical condition to be treated with the lipophilic anthracyclines according to the present invention (preferably formulated as described herein in the section
35 "Pharmaceutical compositions" including the subsections of that section) may be a

clinical condition associated with or preferably caused by infection with human papilloma virus (HPV). Several different clinical conditions may be associated with or caused by HPV infection. Thus, the clinical condition may for example be papillomas, such as condylomas.

5

The present invention specifically relates to the use of the pharmaceutical compositions of the invention for treatment of a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, lips, oral cavity, nasal cavity, pharynx, larynx, trachea, or bronchi, or affecting the eye. In one embodiment the neoplastic or preneoplastic condition may furthermore be a condition associated with an infection with Human Papilloma virus (HPV) and/or it may be a neoplastic or preneoplastic condition selected from the group consisting of vulva squamous cell carcinoma, cervical cancer, vaginal cancer, penis cancer, perianal cancer, anogenital cancer and squamous cell carcinoma of the nasal cavity, lips, oral cavity, the pharynx, the larynx, the trachea, the bronchi or the eye. In another embodiment the neoplastic or preneoplastic condition to be treated with the pharmaceutical compositions of the invention is a condition associated with an infection with Human Papilloma virus (HPV) and/or it is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, perianal squamous cell carcinoma, squamous cell carcinoma of the nasal cavity, squamous cell carcinoma of the oral cavity, squamous cell carcinoma of the lips, squamous cell carcinoma of the pharynx, squamous cell carcinoma of the larynx, squamous cell carcinoma of the trachea, squamous cell carcinoma of the bronchi, and squamous cell carcinoma of the eye.

In a further embodiment of the present invention, the neoplastic or preneoplastic condition to be treated with the pharmaceutical compositions of the invention is a condition associated with an infection with Human Papilloma virus (HPV) and/or it is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, and perianal squamous cell carcinoma. Specifically the neoplastic or preneoplastic condition may be condylomas on the genitals or reproductive organs.

A specific aspect of the present invention relates to the use of a pharmaceutical composition comprising a lipophilic anthracycline (as defined herein) formulated for local administration for treatment of a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, perianal region, lips, nasal cavity, pharynx, larynx, trachea, or bronchi, or affecting the eye. In this aspect the neoplastic or preneoplastic condition may furthermore be a condition associated with an infection with Human Papilloma virus (HPV) and/or it may be a neoplastic or preneoplastic condition selected from the group consisting of vulvacarcinoma, cervical cancer, vaginal cancer, penis cancer, perianal cancer, anogenital cancer and squamous cell carcinoma of the nasal cavity, the pharynx, the larynx, the trachea, the bronchi or the eye. In another embodiment of this aspect of the invention, the neoplastic or preneoplastic condition to be treated is a condition associated with an infection with Human Papilloma virus (HPV) and/or it is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, perianal squamous cell carcinoma, squamous cell carcinoma of the nasal cavity, squamous cell carcinoma of the lips, squamous cell carcinoma of the pharynx, squamous cell carcinoma of the larynx, squamous cell carcinoma of the trachea, squamous cell carcinoma of the bronchi, and squamous cell carcinoma of the eye.

In a further embodiment of this aspect of the present invention, the neoplastic or preneoplastic condition is a condition associated with an infection with Human Papilloma virus (HPV) and/or it is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, and perianal squamous cell carcinoma. Specifically the neoplastic or preneoplastic condition may be condylomas on the genitals or reproductive organs.

The present invention furthermore relates to a method of treating a condition associated with hyperproliferation, such as e.g. epithelial hyperproliferation, epidermal hyperproliferation, hyperproliferation of keratinocytes, or dermal proliferation of small vessels; preferably epithelial hyperproliferation, epidermal hyperproliferation and

hyperproliferation of keratinocytes; more preferably epithelial hyperproliferation or hyperproliferation of keratinocytes; even more preferably epithelial hyperproliferation; yet even more preferably epidermal hyperproliferation in an individual in need thereof comprising administering topically to said individual a pharmaceutical composition

5 comprising

- i) a lipophilic anthracycline;
- ii) one or more oil phase carriers;
- iii) one or more pharmaceutical acceptable solubilizers;
- iv) one or more co-surfactants; and

10 wherein the composition further comprises an aqueous phase comprising at least one buffering system having a pH from pH 2.2 to pH 6, and wherein the water content of the pharmaceutical composition is less than 30%. Further details relating to this method may be found herein in the sections relating to the pharmaceutical composition, the clinical conditions, administration forms, combination therapy, and
15 skin irritation.

The present invention furthermore relates to a method of treating a condition associated with hyperproliferation, such as e.g. epithelial hyperproliferation, epidermal hyperproliferation, hyperproliferation of keratinocytes, or dermal proliferation of small
20 vessels; preferably epithelial hyperproliferation, epidermal hyperproliferation and hyperproliferation of keratinocytes; more preferably epithelial hyperproliferation or hyperproliferation of keratinocytes; even more preferably epithelial hyperproliferation; yet even more preferably epidermal hyperproliferation in an individual in need thereof comprising administering topically to said individual a pharmaceutical composition
25 comprising a lipophilic anthracycline formulated for local administration. Preferably the condition to be treated may be a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, nasal cavity, pharynx, larynx, trachea, or bronchi, or affecting the eye. In one embodiment the neoplastic or preneoplastic condition may furthermore be a condition associated with an infection with Human
30 Papilloma virus (HPV) and/or it may be a neoplastic or preneoplastic condition selected from the group consisting of vulva carcinoma, cervical cancer, vaginal cancer, penis cancer, perianal cancer, anogenital cancer and squamous cell carcinoma of the nasal cavity, the pharynx, the larynx, the trachea, the bronchi or the eye. In another embodiment of this aspect of the invention, the neoplastic or preneoplastic condition is
35 a condition associated with an infection with Human Papilloma virus (HPV) and/or it is

a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, perianal squamous cell carcinoma, squamous cell carcinoma of the nasal cavity, squamous cell carcinoma of the lips, squamous cell carcinoma of the pharynx, squamous cell carcinoma of the larynx, squamous cell carcinoma of the trachea, squamous cell carcinoma of the bronchi, and squamous cell carcinoma of the eye.

10 In a further embodiment of this method of the present invention, the neoplastic or preneoplastic condition is a condition associated with an infection with Human Papilloma virus (HPV) and/or it is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease),
15 squamous cell carcinoma of vagina, penile squamous cell carcinoma, and perianal squamous cell carcinoma. Specifically the neoplastic or preneoplastic condition may be condylomas on the genitals or reproductive organs.

Administration forms

The individual to be treated according to the present invention is preferably an
20 individual suffering from a condition associated with hyperproliferation, for example epithelial hyperproliferation. The individual may be any animal, however, preferably the individual is a mammal, more preferably a human being.

The treatment may be ameliorating treatment and/or the treatment may be curative
25 treatment and/or the treatment may be prophylactic treatment. In some embodiments of the present invention, the treatment may abolish or relieve some or all of the symptoms of the condition during treatment and/or for a specific period of time after cessation of treatment, but then one or more symptoms may reappear. For example, the symptoms may reappear about 1 day, such as about 2 days, for example about 3
30 days, such as about 3 to 5 days, for example about 5 to 7 days, such as about 7 to 10 days, for example about 10 to 15 days, such as about 15 to 20 days, for example about 20 to 30 days, such as about 30 to 60 days, for example about 60 to 120 days, such as more than 120 days after cessation of treatment. In cases, where the symptoms reappear the treatment is preferably resumed.

5 The pharmaceutical formulations according to the present invention are preferably formulated for local administration, such as e.g. to the skin, to an epithelial surface, to the mucosa of e.g. the nasal cavity etc., accordingly, it is preferred to administer the formulations/compositions topically.

10 Topical administration according to the present invention should be understood as local administration directly to the site of disease. Preferably, topical administration results in that the majority of the active compound, i.e. anthracycline, is not systemically absorbed and hence substantially only capable of exerting its effect locally at the site of application. Preferably, systemic absorption is less than 10%, such as less than 8%, for example less than 6%, such as less than 5% for example less than 4%, such as less than 3%, for example less than 2%, such as less than 1%. Even more preferably systemic uptake is less than 10 ng/ml, more preferably less than 5 ng/ml, even more
15 preferably less than 1 ng/ml as measured in blood of a patient receiving treatment.

Preferably, administration of the pharmaceutical compositions according to the present invention does not result in any severe malaise or any severe irritation, more preferably administration does not result in any significant nuisance to the individual to be treated,
20 most preferably, administration does result in only mild and/or no malaise, irritation and/or nuisance.

Administration frequency will depend on the particular clinical condition to be treated and the particular formulation of the pharmaceutical composition. In general however, the pharmaceutical composition as described herein is formulation for administration
25 once or twice daily, preferably once daily.

Combination therapy

30 The pharmaceutical compositions according to the present invention may comprise one or more different active components in addition to the anthracycline.

In one preferred embodiment the pharmaceutical composition furthermore comprises a second active component. The second active component may be any active component known to the person skilled in the art. In a particularly preferred
35 embodiment, the second active component is selected from the group of active components, which are known to be active against the condition to be treated.

Accordingly, when the condition is psoriasis, the second active component may be a component which is known in the art to be effective against psoriasis. For example the second active component may be selected from the group consisting of steroids,
5 vitamin-D analogues, vitamin- A analogues, vitamin D, vitamin A, calcineurin inhibitors, salicylic acid, methotrexate, and cyclosporine, anthraline and coal tar.

The methods of treatment disclosed by the present invention may also be combined with one or more second treatments. In one embodiment of the present invention, the
10 second treatment may be treatment with a different anthracycline, so that the method comprises treatment with 2, for example 3, such as 4, for example 5, such as more than 5 different anthracyclines.

However, the second treatment according to the present invention may also be
15 treatment which is not administration of an anthracycline. Preferably, the second treatment(s) may be selected from the group of treatments, which are known to be active against the condition to be treated.

Accordingly, when the condition is psoriasis the second treatment may be a treatment,
20 which is known in the art to be effective against psoriasis, when the condition is e.g. a cancer disease the second treatment may be an anticancer treatment. For example the second treatment may be selected from the group consisting of treatment with sunlight, ultraviolet light B (UVB) or PUVA. In particular, the anthracycline according to the present invention may sensitise the individual towards treatment with sunlight,
25 ultraviolet light B (UVB) or PUVA, in a manner such as lower amount of light and/or irradiation is required to obtain the desired effect. Furthermore, the second treatment may be administration of at least one compound selected from the group consisting of steroids, coal tar, vitamin-D analogues, vitamin D, vitamin A analogous, vitamin A, anthralin, calcineurin inhibitors, salicylic acid, methotrexate, and cyclosporin. The
30 second treatment may furthermore be a treatment with biologics, such as e.g., adalimumab, etanercept or infliximab (TNF- α inhibitors), Ustekinumab (IL12/23 inhibitor) and others.

The anthracycline and the second treatment may be administered simultaneously or
35 sequentially in any order. In one embodiment, the treatments are administered in a

rotational manner, such as one treatment is administered for a specific predetermined amount of time, after which the second treatment is administered for a specific predetermined amount of time, after which the first treatment is administered again and so forth. Rotational treatment may also comprise more than 2, such as 3, for example 4, such as 5, for example more than 5 different treatments.

Each of the different treatments may be given once or more than once as described for the anthracyclines herein above.

10 Skin irritation

As described herein above, it is preferred that the pharmaceutical compositions according to the invention are not or only mildly irritant when applied to skin of a subject. Furthermore, it is preferred that the lipophilic anthracyclines and other compounds (such as dyes or buffering agents) comprised in the pharmaceutical compositions according to the invention are not or only mildly irritant when applied to skin or mucosa of a subject.

Several methods for determining irritation are available, for example a visual assessment or a more recent human 4-h patch test. When the irritation is determined by visual assessment by an observer, a score of skin irritation is provided on a scale from 0 to 9. The visual assessment is preferably carried out by one or more trained observer(s). A score of 0 on such a scale indicates that the pharmaceutical formulation or the anthracycline is not irritant, whereas a score of in the range of 1 to 3 on such a scale indicates that the pharmaceutical formulation or the anthracycline is mildly irritant. Preferably, the assessment is made in the range of 23 to 76 hours after application of the pharmaceutical formulation or the anthracycline onto the skin of said subject, more preferably in the range of 23 to 48 hours after application of the pharmaceutical formulation or the anthracycline or other compound onto the skin of said subject.

The subject may be any mammal, for example mini pigs, preferably the subject is a human being.

Preferably, the scale used is as follows:

0	no reaction
1	marginal reaction

- | | | |
|----|---|--|
| | 2 | slight perceptible erythema |
| | 3 | a greater than slight reaction which is not sufficient to be classed as distinct |
| | 4 | distinct erythema |
| 5 | 5 | a greater than distinct reaction which is insufficient to be classed as well developed |
| | 6 | well developed, possibly spreading erythema |
| | 7 | a greater reaction which is not sufficient to be classed as strong |
| | 8 | strong, deep erythema, which may extend beyond the treatment site |
| 10 | 9 | a more intense reaction than above |

In a preferred embodiment of the invention, the composition when applied to skin has a score for skin irritation of at the most 3 on a scale from 0 to 9, wherein 0 is no reaction and 9 is the strongest reaction.

15

In one embodiment of the present invention skin irritation is determined as described in Basketter et al., Contact Dermatitis, 1997, 37:218-220.

20

Alternatively, skin irritation may be determined by a standard human 4-h patch test as described in D. A. Basketter et al, Contact Dermatitis, 2004, 51:1-4: "Determination of skin irritation potential in the human 4-h patch test". The assessment of the skin reaction is graded as follows:

- | | | |
|----|-----|--|
| | 0 | No reaction |
| 25 | + | Weakly positive reaction (usually characterized by mild erythema or dryness across most of the treatment site) |
| | ++ | Moderately positive reaction (usually distinct erythema possibly spreading beyond the treatment site) |
| | +++ | Strongly positive reaction (strong, often spreading erythema with oedema) |

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35

Accordingly, the pharmaceutical compositions according to the present invention is preferably not, or only mildly irritant when applied to skin, as corresponding to a grading of 0 or + on the human 4-h patch scale. In one embodiment of the present invention the pharmaceutical compositions, when applied to skin has a grading in the human 4-h patch test of at the most +, on a scale from 0 to +++, wherein 0 is no reaction and +++ is the strongest reaction.

Examples

Example 1

Anthracyclines, incl. Valrubicin, decreases cell proliferation *in vitro*

- 5 DJM-1, HSC-1, two human skin Squamous Cell Carcinoma cell lines, HaCaT cell lines, (human spontaneously transformed normal keratinocytes) were cultured for 24h and 48h in the presence of Valrubicin, AD41 and Doxorubicin in increasing concentrations as indicated ($\mu\text{g/ml}$).
- 10 Cells were propagated in DMEM (Gibco) with addition of 10% Fetal Bovine Serum (Gibco) and a cocktail of antibiotics: Penicillin, Streptomycin, and Gentamycin (Bie&Berntsen, Denmark) and kept in standard growing condition at 37°C and 90% humidity.
- 15 2000 and 4000 cells were seeded per well in a 96 well/plate in quadruplicate. At day 1 from seeding the cells were exposed to Valrubicin or AD41 in acetone solution, and doxorubicin as positive control at the doses of 0.01 $\mu\text{g/ml}$, 0.01 $\mu\text{g/ml}$, 0.05 $\mu\text{g/ml}$, 0.1 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$ 1 $\mu\text{g/ml}$, 5 $\mu\text{g/ml}$ and 10 $\mu\text{g/ml}$.
- 20 The final concentration of acetone in the cell culture medium was of 0.01%, thus the control medium for these two drugs contained the same acetone concentration. It has been previously shown that this acetone concentration did not affect cell proliferation. Doxorubicin was used as a water solution, thus its control culture was grown in medium alone.
- 25 At 24 hour, 48 hour and 72 hour viability was assessed as a measure of luminescence produced with the CellTiter-Glo® viability assay kit (Promega, USA). Effect on proliferation was then evaluated as per cent of reduction in viability. The percent of proliferation is estimated by the viability of cells in the treated cultures versus the
- 30 respective control (given value 100%). Valrubicin decreased cell proliferation in all cell lines at 48h at the three higher doses. Similar effect is seen with AD41, however higher concentrations are required. Doxorubicin is, as expected, the most effective drug showing an effect on proliferation already at 24h.

Figure 1A, shows the dose response effect expressed as % proliferation of DJM-1 cells against increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

- 5 Figure 1B, shows the dose response effect expressed as % proliferation of HSC-1 cells against increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

- 10 Figure 1C, shows the dose response effect expressed as % proliferation of HaCaT cells against increasing amount of compounds. From left to right: Valrubicin 24 h; Valrubicin 48 h; AD41 24 h; AD41 48 h; Doxorubicin 24 hr; Doxorubicin 48 hr.

Example 2

- 15 Three distinct keratinocyte cultures were established *in vitro* and prepared with 4000 cells per well in a 96 well/plate. Following an overnight incubation, valrubicin was added in increasing concentration and proliferation assessed after 24, 48, and 72 hours essentially as described in Example 1. Proliferation curves for the three keratinocyte cultures are shown at 48 hours (Figure 2) and demonstrate the anti-proliferative effect of valrubicin on primary keratinocytes.

20

Example 3

Effect of valrubicin on progression and treatment of skin cancer in mice *in vivo*

- 25 Skin cancer is induced in BALB/6J mice by a two step treatment with DMBA and TPA. DMBA creates mutations in the skin cells and TPA stimulates growths of these cells. Some of the formed papillomas will become malignant (SCC). This DMBA/TPA skin cancer model is used worldwide. The BALB/6J is used instead of C57BL/6J due to a reported increased formation of papillomas (More, 1999).

- 30 Mice were treated with DMBA and following TPA for 7 weeks where the development of initial tumors were observed. Mice were then divided into three treatment groups and subjected to treatment: Group 1 received one daily topical treatment with Valrubicin cream (Formulation A (JH32) as described in Example 15) (n = 13), group 2 received vehicle cream (Formulation A (JH32) without Valrubicin) (n= 13), and group 3 received no treatment (n = 13).

35

Figure 3 shows the results. Tumors were counted once weekly and expressed as the mean number of tumors per treatment group (figure 3(a)). In each group, the percentage of mice with tumors was calculated (figure 3(b)). Tumor size was measured with a vernier caliper, and the volume calculated as $V = 4/3 * \pi * d_1 * d_2 * d_3$ where d_{1-3} are the three measured cross-sectional diameters (figure 3(c)).

The animals treated with Valrubicin Formulation A had significantly less tumours and also the number of mice having tumours was significantly reduced after treatment with Valrubicin Formulation A.

Example 4

Valrubicin water-in-oil emulsion, VLD32

Excipient	Phase	VLD32 (% w/w)
White soft paraffin	Oil	21.00
Hard paraffin	Oil	3.00
Crodamol GTCC	Oil	20.00
Dow Corning Emulsifier 10	Oil	2.00
Dimethicone 350	N/A	5.00
Lactic acid buffer pH 2.5 (100mM) coloured solution	Aqueous	9.93
Transcutol® P	Aqueous	15.00
Propylene glycol	Aqueous	14.00
Ethanol	Aqueous	5.00
Phenoxyethanol	Aqueous	1.00
Glycerol	Aqueous	3.00
Alpha tocopherol	Oil	0.03
Ascorbyl palmitate	Oil	0.02
EDTA disodium	Aqueous	0.02
Valrubicin	Aqueous	1.00
Total	N/A	100.00

N/A = not applicable

This is a very preferred formulation according to the invention.

Lactic acid – sodium lactate buffers pH 2.5, 100 mM were prepared by preparing two primary solutions. A 0.2 M solution of lactic acid was prepared by weighing 1.8 g of lactic acid into a 100 ml volumetric flask, and making up to volume with deionised water. A 0.2 M solution of sodium lactate was prepared by weighing 2.24 g of sodium lactate in a 100 ml volumetric flask and making up to volume with deionised water. The Lactic acid – sodium lactate buffer, pH 2.5, was prepared by adding 60 ml of the 0.2 M solution of lactic acid and 40 ml of the 0.2 M sodium lactate solution to a 200 ml volumetric flask and making up to volume with deionised water. The pH of the solution was determined and either reduced using 0.2 M lactic acid solution or increased using 0.1 M sodium lactate.

The coloured solutions were prepared by weighing 0.00875 g and 0.10625 g of FD & C blue and D&C yellow into a suitably sized container, to the container was added approximately 49.885 g of the pH 2.5 lactic acid – sodium lactate buffer.

The water soluble excipients were weighed into a suitably sized container (aqueous phase) and the oil soluble ingredients were weighed into a separate suitably sized container (oil phase). Valrubicin was dissolved in phenoxyethanol prior to addition of the remaining aqueous phase (where applicable). The oil phase excipients were heated at approximately 65°C in a temperature controlled water bath until all excipients had melted. The aqueous phase was heated until it had reached approximately 65°C. The aqueous phase was added very slowly to the oil phase while the oil phase was stirred vigorously. The emulsion was then homogenised using a Silverson homogeniser for 1 – 2 min. The formulation was stirred by hand using a spatula until the temperature of the formulation had reached approximately ambient temperature. The pH of the final composition was measured to approximately pH 4.7 – 4.8.

Example 5**Valrubicin water-in-oil emulsion, VLD42**

Excipient	Phase	VLD42 (% w/w)
White soft paraffin	Oil	21.00
Hard paraffin	Oil	3.00
Crodamol GTCC	Oil	20.00
Dow Corning Emulsifier 10	Oil	2.00
Dimethicone 350	N/A	5.00
Glycine-HCl buffer pH 2.5 (100mM) coloured solution	Aqueous	9.93
Transcutol® P	Aqueous	15.00
Propylene glycol	Aqueous	14.00
Ethanol	Aqueous	5.00
Phenoxyethanol	Aqueous	1.00
Glycerol	Aqueous	3.00
Alpha tocopherol	Oil	0.03
Ascorbyl palmitate	Oil	0.02
EDTA disodium	Aqueous	0.02
Valrubicin	Aqueous	1.00
Total	N/A	100.00

N/A = not applicable

The formulation was prepared as described in example 4, except for the buffer which was prepared as follows:

5

Glycine – HCl buffer pH 2.5, 100 mM were prepared by preparing two primary solutions. A 0.2 M solution of HCL was prepared by weighing 4 g of 1 M HCl into a 20 ml volumetric flask, and making up to volume with deionised water. A 0.2 M solution of glycine was prepared by weighing 0.75 g glycine in a 50 ml volumetric flask and making up to volume with deionised water. The glycine – HCl buffer, pH 2.5, was prepared by adding 15 ml of the 0.2 M solution of HCl and 50 ml of the 0.2 M glycine solution to a 100 ml volumetric flask and making up to volume with deionised water. The pH of the solution was determined and was either reduced with 0.1 M HCl or increased using 0.1 M glycine if required.

10

15

The coloured solutions were prepared by weighing 0.00875 g and 0.10625 g of FD & C blue and D&C yellow into a suitably sized container, to the container was added approximately 49.885 g of the pH 2.5 Glycine-HCl buffer.

The pH of the final composition was measured to approximately pH 4.1 – 4.2.

5

Example 6

Valrubicin water-in-oil emulsion, VLD34

Excipient	Phase	VLD34 (% w/w)
White soft paraffin	Oil	32.50
Crodamol GTCC	Oil	11.50
Dow Corning Emulsifier 10	Oil	2.20
Dimethicone 350	N/A	5.00
Lactic acid buffer pH 2.5 (100mM)	Aqueous	1.23
Coloured Lactic acid buffer solution	Aqueous	10.00
Transcutol® P	Aqueous	16.50
Propylene glycol	Aqueous	15.50
Phenoxyethanol	Aqueous	1.00
Glycerol	Aqueous	3.50
Alpha tocopherol	Oil	0.03
Ascorbyl palmitate	Oil	0.02
EDTA disodium	Aqueous	0.02
Valrubicin	Aqueous	1.00
Total	N/A	100.00

N/A = not applicable

The formulation was prepared as described in example 4.

10 The pH of the final composition was measured to approximately pH 4.4 - 4.6.

15

Example 7**Valrubicin water-in-oil emulsion, VLD35**

Excipient	Phase	VLD35 (% w/w)
White soft paraffin	Oil	32.50
Crodamol GTCC	Oil	11.50
Dow Corning Emulsifier 10	Oil	2.20
Dimethicone 350	N/A	5.00
Glycine-HCl buffer pH 2.5 (100mM)	Aqueous	1.23
Coloured Glycine-HCl buffer solution	Aqueous	10.00
Transcutol® P	Aqueous	16.50
Propylene glycol	Aqueous	15.50
Phenoxyethanol	Aqueous	1.00
Glycerol	Aqueous	3.50
Alpha tocopherol	Oil	0.03
Ascorbyl palmitate	Oil	0.02
EDTA disodium	Aqueous	0.02
Valrubicin	Aqueous	1.00
Total	N/A	100.00

N/A = not applicable

The formulation was prepared as described in example 5.

- 5 The pH of the final composition was measured to approximately pH 4.4.

Example 8**Treatment of psoriasis**

- 5 series of HuSCID mice were included in this study
- 10 • Keratome biopsies were taken from distinct psoriatic donors and transported to the animal facility (0 °C). Each series represent one donor. The following day, the keratomes are cut in smaller pieces before transplantation on the back of anesthetized SCID mice (6-8 weeks old).
- After a healing period of approx.. 10 days, the animals were divided into 5
15 different groups and treated topically once daily for 3 weeks (21 days: Monday to Monday or 19 days: Monday to Friday). The groups were as follows:
Group 1 of 3 mice: Valrubicin cream (VLD32 as described in Example 4)

Group 2 of 2 mice: Vehicle cream (Same as VLD32 as described in Example 4, but lacking Valrubicin)

Group 3 of 2 mice: Untreated

Group 4 of 3 mice: Calcipotriol

5 Group 5 of 2 mice: Betamethasone valerate

- At study end biopsies of 4mm were taken from the center of the graft and kept in 4% PFA until paraffin embedding.
- The rest of the graft was snap-frozen in liquid nitrogen for potential further investigation.
- 10 • Subjective assessment of psoriasis resolution was determined using a 4-point semi-quantitative clinical psoriasis score (redness, scaling and thickness of the skin) with a range from 0 to 3, wherein 3 is severe, and 0 is no psoriasis.
- Objective assessment of reduction of skin thickness of psoriasis plaques was determined by measurement of histological rete lengths of biopsies of treated skin.
- 15

This example verifies the effect of Valrubicin cream (VLD32) in ameliorating psoriasis. Also the cream vehicle without active ingredient was being tested. As monotherapy, valrubicin cream was compared with conventional topical psoriasis treatments, namely Calcipotriol and Betamethasone valerate which were included as positive controls in all 5 series. All treatment groups were compared to an untreated group confirming psoriasis presence at the end of the 3 weeks.

20

Figure 4A-F are showing the results of all 5 series. In all series treatment with VLD32 (denoted "Valrubicin" in the figure) was at least as effective as treatment with either Calcipotriol or Betnovat. However, in series 2, 3 and 4 treatment with VLD32 was significantly better than treatment with either Calcipotriol or Betamethasone valerate after 21 days. Treatment with VLD32 was significantly more effective than treatment with vehicle cream without Valrubicin in all 5 series.

25

Also Valrubicin in formulation A (content as described in Example 15) was tested in the psoriasis xenograft transplantation model, where human psoriasis plaque skin was transplanted onto SCID mice maintaining the characteristics of psoriasis. Mice engrafted with psoriatic plaque skin were divided into four treatment groups: vehicle cream (formulation A without valrubicin), untreated, valrubicin cream (formulation A), and clobetasol propionate (the strongest corticosteroid approved for topical treatment

30

35

for psoriasis). All treatments were applied once daily to the human grafted skin in the psoriasis xenograft transplantation model for twelve days using the semi-quantitative clinical psoriasis 4-point score with a range from 0 to 3 as described above.

- 5 The semi-quantitative clinical psoriasis score was significantly reduced after 12 days treatment in the valrubicin group (Formulation A) (1.7 ± 0.1 ; mean $\pm$ SEM, $p < 0.01$) compared to the vehicle group (2.2 ± 0.1 ; mean $\pm$ SEM), indicating remission of psoriasis. Similarly, the semi-quantitative clinical psoriasis score in the clobetasol propionate treated group (positive control) was reduced to 1.5 ± 0.1 (mean $\pm$ SEM).

10

Figure 4F shows the mean value of epidermal skin thickness in μm of the $n=5$ series of engrafted HuSCIDmice after once daily application of treatments for 21 consecutive days. Treatment with the VLD32 formulation is denoted "Valrubicin" in the figure.

15

Example 9

Valrubicin emulsified gel, Emulgel 1A

Excipient	Emulgel 1A (% w/w)
Lactic acid buffer pH 4.5 (50mM)	11.43
Lactic acid buffer coloured 5-fold	2.00
Transcutol® P	25.00
Propylene glycol	20.00
Ethanol	10.00
Glycerol	10.00
Phenoxyethanol	1.00
HPC HF	2.50
Alpha tocopherol	0.03
Ascorbyl palmitate	0.02
EDTA disodium	0.02
Crodamol GTCC	15.00
Cremophor RH40 = Croduret 50 special	2.00
Valrubicin	1.00
Total	100.00

The formulation was prepared as described in example 16, except for the buffer which was prepared as follows:

The pH 4.5 lactate buffer was prepared by preparing two primary solutions. A 0.1 M solution of lactic acid was prepared by weighing 0.9 g of lactic acid into a 100 ml volumetric flask, and making up to volume with deionised water. A 0.1 M solution of sodium lactate was prepared by weighing 1.12 g of sodium lactate in a 100 ml volumetric flask and making up to volume with deionised water. The lactate buffer, pH 4.5, was prepared by adding 6 ml of the 0.1 M solution of lactic acid and 44 ml of the 0.1 M sodium lactate solution to a 100 ml volumetric flask and making up to volume with deionised water. The pH of the solution was determined and adjusted if required.

10

A coloured buffer solution was prepared by weighing 0.04375 g and 0.53125 g of FD & C blue and D&C yellow into a suitably sized container, to the container was added approximately 49.425 g of the pH 4.5 lactic acid buffer. The pH of the final composition was measured to approximately pH 6.4.

15

Example 10

Valrubicin emulsified gel, Emulgel 2A

Excipient	Emulgel 2A (% w/w)
Lactic acid buffer pH 4.5 (50mM)	16.93
Lactic acid buffer coloured 5-fold	2.00
Transcutol® P	20.00
Propylene glycol	20.00
Ethanol	10.00
Glycerol	10.00
Phenoxyethanol	1.00
HPC HF	2.50
Alpha tocopherol	0.03
Ascorbyl palmitate	0.02
EDTA disodium	0.02
Crodamol GTCC	15.00
Cremophor RH40 = Croduret 50 special	2.00
Valrubicin	0.50
Total	100.00

The formulation was prepared as described in example 8, and in example 9 (with regard to the buffer).

The pH of the final composition was measured to approximately pH 6.4.2

5 **Example 11**

Short and long term stability experiments of Valrubicin in formulations

- The percentage recovery of valrubicin, and the percentage peak purity of valrubicin from different formulations were determined to t=0, t=1 month, t=3 months, t=9 months and t= 12 months at 2-8°C, 25°C and 60 % RH, and in some cases at 40°C. The
- 10 content of Valrubicin was determined by extracting the compound from the formulation and determining the concentration. The extraction procedure used was dependant on the formulation type (W/O or Emulgel) and the target was to have approximately 100 mg/ml Valrubicin in the samples analysed by HPLC.
- 15 Extraction of Valrubicin from W/O formulations (VLD32). In triplicate, approximately 100 mg of each formulation was accurately weighed into a 10 ml volumetric flask. Isopropyl alcohol (HPLC grade, 5 ml) was added to the volumetric flask and the flask vortex mixed for 2 min to disperse the formulation. The volumetric flask was made up with water (MilliQ,) and stirred at ambient temperature for approximately 2 h. Approximately
- 20 1.5 ml of the solution was transferred to an Eppendorf tube and centrifuged at 13000 rpm for 5 min in a Heraeus Biofuge pico centrifuge (rotor PP 1/99 # 3325). A sample (approximately 1 ml) of the supernatant was transferred to a glass HPLC vial and analysed by HPLC.
- 25 In triplicate, approximately 100 mg of emulsified gel 4 containing 1% Valrubicin was accurately weighed into a 10 ml volumetric flask. The volumetric flask was made up to volume with acetonitrile and the flask vortex mixed for 1 min to disperse the gel. The volumetric flask was stirred for approximately 4 h at ambient temperature.
- 30 Approximately 1.5 ml of the solution was transferred to an Eppendorf tube and centrifuged at 13000 rpm for 5 min in a Heraeus Biofuge pico centrifuge (rotor PP 1/99 # 3325) according to SOP 3051. A sample (approximately 1 ml) of the supernatant was transferred to a glass HPLC vial and analysed by HPLC.

The percentage recovery of Valrubicin from the formulations was acceptable for all

formulations at $t=0$. At $t=0$ the peak purity of Valrubicin was observed to be approximately 99% in all samples. The percentage recovery of Valrubicin from the formulations at the 1 month and 3 months time points were considered acceptable (Table 1A). The percentage recovery of Valrubicin from the formulations from the longer term (6, 9 and 12 months) time points was likewise considered acceptable (Table 1B). For the 12 months time point are both data for glass and High Density Polyethylene (HDPE) containers.

Table 1A

Formulation	Batch number	Percentage <i>w/w</i> valrubicin (% recovery)				
		t=0, mean $\pm$ SD, n=9	t=1 month, mean $\pm$ SEM, n=3		t=3 months, mean $\pm$ SEM, n=3	
			2 – 8°C	25°C / 60% RH	2 – 8°C	25°C / 60% RH
VLD32 1.0% valrubicin formulation	7077/001	1.08 $\pm$ 0.01 (107.65 $\pm$ 1.36)	1.07 $\pm$ 0.01 (106.69 $\pm$ 1.17)	1.07 $\pm$ 0.01 (106.74 $\pm$ 1.38)	1.07 $\pm$ 0.00 (106.56 $\pm$ 0.06)	1.05 $\pm$ 0.00 (104.81 $\pm$ 0.07)
Emulsified gel 4 1.0% valrubicin formulation	7078/001	1.02 $\pm$ 0.01 (101.70 $\pm$ 0.98)	1.03 $\pm$ 0.01 (102.92 $\pm$ 0.37)	1.03 $\pm$ 0.00 (102.51 $\pm$ 0.26)	1.02 $\pm$ 0.01 (102.34 $\pm$ 0.66)	1.01 $\pm$ 0.01 (101.02 $\pm$ 0.61)

Table 1B

Formulation		Percentage w/w valrubicin (% recovery)	
		VLD32 1.0% valrubicin formulation	Emulsified gel 4 1.0% valrubicin formulation
Batch number		7077/001	7078/001
t=0, mean $\pm$ SD, n=9		1.08 $\pm$ 0.01 (107.65 $\pm$ 1.36)	1.02 $\pm$ 0.01 (101.70 $\pm$ 0.98)
t=1 month,	2 – 8°C	1.07 $\pm$ 0.01 (106.69 $\pm$ 1.17)	1.03 $\pm$ 0.01 (102.92 $\pm$ 0.37)
Mean $\pm$ SEM, n=3	25°C/60 RH	1.07 $\pm$ 0.01 (106.74 $\pm$ 1.38)	1.03 $\pm$ 0.00 (102.51 $\pm$ 0.26)
t=3 month,	2 – 8°C	1.07 $\pm$ 0.00 (106.56 $\pm$ 0.06)	1.02 $\pm$ 0.01 (102.34 $\pm$ 0.66)
Mean $\pm$ SEM, n=3	25°C/60 RH	1.05 $\pm$ 0.00 (104.81 $\pm$ 0.07)	1.01 $\pm$ 0.01 (101.02 $\pm$ 0.61)
t=6 month,	2 – 8°C	1.04 $\pm$ 0.01 (104.34 $\pm$ 0.99)	1.02 $\pm$ 0.00 (102.14 $\pm$ 0.06)
Mean $\pm$ SEM, n=3	25°C/60 RH	1.02 $\pm$ 0.01 (102.03 $\pm$ 0.83)	1.00 $\pm$ 0.00 (100.05 $\pm$ 0.18)
t=9 month,	2 – 8°C	1.05 $\pm$ 0.01 (104.61 $\pm$ 1.04)	1.06 $\pm$ 0.02 (105.63 $\pm$ 2.10)
Mean $\pm$ SEM, n=3	25°C/60 RH	1.01 $\pm$ 0.02 (100.51 $\pm$ 2.42)	0.97 $\pm$ 0.01 (97.21 $\pm$ 0.70)
t=12 month HDPE	2 – 8°C	1.05 $\pm$ 0.02 (105.41 $\pm$ 2.19)	1.01 $\pm$ 0.01 (101.38 $\pm$ 0.75)
Mean $\pm$ SEM, n=3	25°C/60 RH	1.00 $\pm$ 0.01 (99.53 $\pm$ 0.52)	0.95 $\pm$ 0.00 (95.09 $\pm$ 0.11)
t=12 month glass	2 – 8°C	1.07 $\pm$ 0.05 (106.91 $\pm$ 4.50)	1.01 $\pm$ 0.01 (100.72 $\pm$ 1.14)
Mean $\pm$ SEM, n=3	25°C/60 RH	0.97 $\pm$ 0.02 (97.03 $\pm$ 1.52)	0.96 $\pm$ 0.01 (96.00 $\pm$ 1.07)

It was observed that in general the percentage recovery for formulation A, (table 2) at 3 months at 25°C, was considerably lower at 3 months than the percentage recovery for VLD32 and Emulsified gel 4 at same time point and temperature. Furthermore the percentage recovery for formulation A, at both 1 month and 3 months, at 40°C was considerably lower (89-99%) at 40°C in comparison to 25°C (98 – 101%). Accordingly, the formulations VLD32 and Emulsified gel 4 showed a considerably better stability profile.

Table 2

Formulation A, Valrubicin, 1.0%	25C/60%RH	40C/75%RH
0 months	102,3	102,3
1 month	101,2	99,4
3 months	98,8	89,1

Example 12**Solubility of Valrubicin in solvents**

In order to determine which solvents (also termed oil phase carriers, solubilizers and co-surfactants herein elsewhere) were useful for preparing pharmaceutical

- 5 compositions according to the present invention comprising lipophilic anthracyclines, and especially for valrubicin, solubility studies were performed. The solvents in which valrubicin showed high solubility (> 10%) were ethanol, benzyl alcohol, PEG 400, glycofuranol, phenoxyethanol and Transcutol. The solubility of valrubicin was observed to be low (< 0.2 %) in cyclomethicone, glycerol, IPP, IPM and water.

10

Table 3

Excipient	Solubility of Valrubicin (% w/w)
Arlasolve DMI	>8.52
Benzyl alcohol	>38.70
Butylene glycol	0.26 – 0.39
Castor oil	0.21 – 0.31
Crodamol GTCC (Medium chain triglyceride)	0.19 – 0.29
Cyclomethicone	<0.05
Dimethicone 350 CST	<0.05
Ethanol	>39.68
Glycerol	0.09 – 0.16
Glycofurol	>18.88
Isopropyl myristate (IPM)	0.08 – 0.10
Isopropyl palmitate (IPP)	0.13 – 0.17
Isostearic acid	<0.26
Polyethylene glycol (PEG 400)	>28.16
Phenoxyethanol	>11.8
Lauroglycol® 90 (Propylene Glycol Monolaurate)	1.06 – 2.24
Propylene glycol	0.31 – 0.42
Transcutol® P (Diethylene glycol monoethyl ether)	>27.36
Water	<0.21
20:80 PEG: Buffer pH 4.0	<0.68

20:80 PEG: Buffer pH 5.0	<0.60
20:80 PEG: Buffer pH 6.0	<0.53
20:80 PEG: Buffer pH 7.0	<0.52
20:80 PEG: Buffer pH 8.0	<0.46

Example 13**Stability of Valrubicin in solvents**

5 In order to determine which solvents (also termed oil phase carriers, solubilizers and co-surfactants herein elsewhere) were useful for preparing pharmaceutical compositions according to the present invention comprising lipophilic anthracyclines, and especially for valrubicin, stability studies where performed.

10 The percentage peak purity of valrubicin in different solvents were determined to t=0, t=2 weeks, and t=4 weeks, at both 25°C and 40°C. Valrubicin was observed to remain stable (100% peak purity) in Lauroglycol® 90, benzyl alcohol, Crodamol GTCC and phenoxyethanol at 25 and 40°C for two weeks. No additional peaks were observed at 25°C in 50% ethanol in pH 4 buffer. At the four week time point, Valrubicin was
15 observed to be stable (peak purity of 100%) after storage in Crodamol GTCC, benzyl alcohol and phenoxyethanol.

The peak purity of Valrubicin was observed to decrease with higher pH, whereby significant loss of Valrubicin peak purity was observed at pH 5 and above. Valrubicin also showed a decrease in peak purity after storage in PEG 400, Propylene glycol,
20 Transcutol® P, ethanol and Arlasolve DMI.

Table 4

Formulation	Percentage Peak Purity (mean $\pm$ SD, n=3)				
	t=0	t=2 weeks		T=4 weeks	
		25°C	40°C	25°C	40°C
50:50 Ethanol: pH 4 buffer	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	89.72 $\pm$ 0.24	94.83 $\pm$ 2.83	77.96 $\pm$ 2.51
50:50 Ethanol: pH 5 buffer	100.00 $\pm$ 0.00	90.14 $\pm$ 0.05	77.72 $\pm$ 1.50	90.27 $\pm$ 0.27	53.49 $\pm$ 0.15
50:50 Ethanol: pH 6 buffer	100.00 $\pm$ 0.00	82.29 $\pm$ 0.80	33.79 $\pm$ 0.22	62.93 $\pm$ 0.51	9.27 $\pm$ 0.42
50:50 Ethanol: pH 7 buffer	100.00 $\pm$ 0.00	28.71 $\pm$ 0.51	0.54 $\pm$ 0.02	8.15 $\pm$ 0.26	Peak not present
Benzyl alcohol	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Castor oil	91.26 $\pm$ 3.90	91.26 $\pm$ 3.90	80.47 $\pm$ 0.25	N/A	N/A
Crodamol GTCC	100.00 $\pm$ 0.00	97.73 $\pm$ 1.18	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00
Ethanol	100.00 $\pm$ 0.00	98.43 $\pm$ 0.04	95.00 $\pm$ 0.05	97.64 $\pm$ 0.03	92.72 $\pm$ 0.07
Lauroglycol	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	99.44 $\pm$ 0.49	99.97 $\pm$ 0.06
PEG 400	100.00 $\pm$ 0.00	92.35 $\pm$ 0.22	73.09 $\pm$ 0.21	80.86 $\pm$ 0.78	34.89 $\pm$ 0.64
Propylene glycol	100.00 $\pm$ 0.00	98.66 $\pm$ 0.12	95.09 $\pm$ 0.30	88.29 $\pm$ 0.30	72.47 $\pm$ 0.57
Transcutol® P	100.00 $\pm$ 0.00	98.09 $\pm$ 0.19	93.32 $\pm$ 0.07	99.71 $\pm$ 0.50	86.91 $\pm$ 0.62
Arlasolve DMI	100.00 $\pm$ 0.00	63.82 $\pm$ 1.62	39.34 $\pm$ 0.35	98.00 $\pm$ 0.15	47.93 $\pm$ 0.41
Phenoxy-ethanol	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00	100.00 $\pm$ 0.00

Example 14**Dissolution tests**

- 5 The dissolution of valrubicin in different solvents was measured. In a first test, X mg of valrubicin was attempted dissolved in Y mg solvent. The results of these initial tests are listed in table 5 below. Then, based on the knowledge gained in the first test, the

maximum amount of valrubicin was estimated by use of a visual colorimetric evaluation of the intensity of the red colour, which appears whenever valrubicin is dissolved in a solvent. In order to be sure that the dissolution estimated in fact was the maximum amount of valrubicin, which can be dissolved, all samples were stored under various conditions and/or heated to 90°C and then slowly cooled to ambient temperature. All visual estimates were carried out at 20°C after a long period of storage.

Table 5.

Results of dissolution test, in which valrubicin was dissolved in different solvents.

Solvent	Valrubicin attempted dissolved (%)	Visual estimation of maximum dissolution of valrubicin (%)
70% ethanol	20	20
Lauryl lactate	8.7	8.7
Oleic acid	n.m.	8?
Sorbitan laurate	n.m.	7
Diisopropyl adipate	6	6
Propylene glycol	5.38 / 7.67	6-7 % (after heating)
Panthenol 100%	5	5
Decyl glycoside, Plantaren 2000	5	5
Lactic acid	5.14	4.5
Diisopropyl sebacate	20	3
Castor oil	1.98 / 2.51	2.20
Safflower oil	2.85 / 3.23	2% ?
Cetearyl isononanoate Cetiol SN	3.15	0.2
Glycerol	5.0	0.1
Behenyl alcohol	n.m.	n.m.
Water	0	n.m.

10 n.m.: not measured

Based on the above results, the formulations of Example 15 were prepared.

Example 15**Water-in-oil formulations**

Excipient	JH 14 (%)	JH 15 (%)
<u>Oil phase</u>		
Dehymuls LE	3.0	3.0
Dehymuls PGHP	3.0	3.0
Lameform TGI	1.0	1.0
Lanette 22	9.0	9.1
Cetiol SN	9.9	10.0
Propylene glycol	5.5	8.1
Vitamin E acetate	0.1	0.1
D-Panthenol 50	5.0	2.6
Castor oil	14.9	15.1
Valrubicin	1.0	0
Lactic acid 80%, food gr.	2.5	2.5
<u>Water phase</u>		
Water	42.6	43.0
MgSO ₄	1.0	1.0
Hyaluronic acid	0.5	0.5
Sodium benzoate	0.6	0.6
Potassium sorbate	0.2	0.2
Total	100	100

JH12: valrubicin dissolved in castor oil, Panthenol, Lameform, propylene glycol, lactic
5 acid, Dehymuls LE: 2 g was dissolved in 54%, 4.2%.

The formulations were prepared by first preparing the oil phase and the water phase
separately. The oil phase was prepared by dissolving valrubicin and then mixing said
mixture with the rest of the constituents of the oil phase. The water phase was
10 prepared by dissolving all substituent of the water phase in the water. Finally, the
water-in-oil formulation was prepared by mixing the water phase into the oil phase.

	Formulation A (JH32)	
	<u>Water phase:</u>	
	Water	35.1
	FD&C Yellow No. 5 powder	0.1
5	FDC Blue no. 1 powder (DA01)	0.01
	Sodium benzoate, NF, EP	0.3
	Sorbic acid, powder, NF	0.2
	Magnesium sulphate heptahydrate,	1.1
	Sodium hyaluronate	0.5
10	<u>Oil phase</u>	
	Arlacel P135	3.2
	Dehymuls PGPH	3.2
	Propylglyceryl-3-diisostearate	1.4
15	Ceraphyl 31 lauryl lactate	4.3
	Castor oil	16.0
	Propylene glycol	8.5
	D Panthenol	2.7
	Vitamin E acetate	0.01
20	Lactic acid	2.3
	Valrubicin	1.0
	Lanette 22	9.6
	Cetearyl isononanoate	10.6
25	Total	100

The pH of formulation A has been measured to approximately pH 3.7.

Example 16

- 30 EMULSIFIED GEL 4A with valrubicin and emulsified gel 4P, which is the formulation without active ingredient.

Excipient	Emulsified gel 4 F	Emulsified gel 4 A
	(Theoretical composition, %, w/w)	
Lactic acid buffer pH 2.5	12.43	11.43
Lactic acid buffer pH 2.5 5-fold dye solution	2.00	2.00
Transcutol® P	25.00	25.00
Propylene glycol	20.00	20.00
Ethanol	10.00	10.00
Glycerol	10.00	10.00
Phenoxyethanol	1.00	1.00
HPC HF	2.50	2.50
Alpha tocopherol	0.03	0.03
Ascorbyl palmitate	0.02	0.02
EDTA disodium	0.02	0.02
Crodamol GTCC	15.00	15.00
Cremophor RH40 = Croduret 50 special	2.00	2.00
Valrubicin	0.00	1.00
Total	100.00	100.00

The buffer and the coloured solution were prepared as described in example 4.

Valrubicin was weighed into a suitably sized container and dissolved in phenoxyethanol and ethanol prior to the addition of Transcutol® P, propylene glycol, alpha tocopherol, ascorbyl palmitate and EDTA disodium. The solution was stirred until Valrubicin and the anti-oxidants had dissolved, prior to addition of the gelling agent (HPC HF). The gels were allowed to hydrate for a minimum of 3 h at ambient temperature. The Crodamol GTCC and Cremophor RH40 were weighed into a suitably sized container and heated at approximately 65°C until the Cremophor RH40 had melted. The Cremophor RH40 and Crodamol GTCC were added to the gel and stirred thoroughly using a Lab egg stirrer, followed by vigorous hand stirring. The pH of the final composition was measured to approximately pH 4.5 – 4.8.

EMULSIFIED GEL 5A with valrubicin and emulsified gel 5P, which is the formulation without active ingredient.

5

Excipient	Emulsified gel 5 P	Emulsified gel 5 A
	(Theoretical composition, %, w/w)	
Lactic acid buffer pH 2.5	17.43	16.93
Lactic acid buffer pH 2.5 5-fold dye solution	2.00	2.00
Transcutol® P	20.00	20.00
Propylene glycol	20.00	20.00
Ethanol	10.00	10.00
Glycerol	10.00	10.00
Phenoxyethanol	1.00	1.00
HPC HF	2.50	2.50
Alpha tocopherol	0.03	0.03
Ascorbyl palmitate	0.02	0.02
EDTA disodium	0.02	0.02
Crodamol GTCC	15.00	15.00
Cremophor RH40 = Croduret 50 special	2.00	2.00
Valrubicin	0.00	0.50
Total	100.00	100.00

The pH of the final composition was measured to approximately pH 4.3 – 4.6.

Example 17

10

Mass Balance study

Methods:

15

Individually calibrated Franz cells with an average surface area and volume of approximately 0.6 cm² and 2 ml, respectively, was employed to determine the permeation of valrubicin. The receptor compartment was filled with receiver fluid. The formulation (approximately 10 mg) was weighed onto the rubber plunger end of a 1 ml syringe, using the rubber plunger the formulations was spread out on the surface of the

epidermal membrane, the plunger was re-weighed to ensure that approximately 6 mg (10 mg/cm²) mg was applied to the surface of the epidermal membrane. Any formulation remaining on the plunger was determined by immersing the plunger into a glass vial containing 5 ml of solvent (80% acetonitrile in 20% water) and assay via HPLC.

Five formulations were employed during the final skin permeation and mass balance experiments, these were as follows:

- VLD32 – 1.0% w/w valrubicin (see Example 4 for content of VLD32)
- VLD43 – 0.5% w/w valrubicin (see Example 5 for content of VLD42)
- Emulsified gel 4A – 1.0% w/w valrubicin (see Example 16 for content of Emulsified gel 4A)
- Emulsified gel 5A – 0.5% w/w valrubicin (see Example 6 for content of Emulsified gel 5A)
- Comparator (Formulation A) - 1.0% w/w valrubicin (see Example 15 for content of Formulation A (JH32))

Assessments:

Following the application of each formulation, receiver fluid (200 µl) was removed from the receiver compartment of each Franz cell via the sampling arm after 2, 4, 6, 8, 24, 30 and 48 h and analysed via HPLC.

In summary, valrubicin was recovered and analysed via HPLC from the following components:

- Valrubicin remaining on the surface of the applicator (AP) collected after application of the formulation,
- Valrubicin remaining on surface of epidermal membrane (S),
- Valrubicin retained in the *stratum corneum* (only applicable in the intact epidermal membrane, SC)
- Valrubicin present in the epidermal membrane (EM).
- Valrubicin present in the interface (IF), this consisted of the Franz cell swabs and filter paper.
- Valrubicin permeated into receiver fluid (P), collected during the permeation experiment.

Results:

Upon application of valrubicin containing formulations to intact human epidermal membrane (Table 6), approximately 6.9 and 1.6 µg of valrubicin were recovered from the *stratum corneum*

5 after application of the VLD32 and VLD43, respectively. The amount of valrubicin recovered from the *stratum corneum* after application of VLD32 was significantly higher ($p > 0.005$ using Bonferroni correction) than all other formulations applied to the intact human epidermal membrane. The amount of valrubicin recovered from the *stratum corneum* was approximately 2.3 and 1.2 µg from the cells applied with Emulsified gel 4
10 and Emulsified gel 5.

No detectable valrubicin was observed in the *stratum corneum* after application of the Comparator formulation.

When VLD32 was applied to perturbed epidermal membrane approximately 8.1 µg of
15 valrubicin was recovered from the epidermal membrane, this was significantly greater ($p > 0.005$ using Bonferroni correction) than the amount recovered after application with all formulations with the exception of VLD43. Approximately 5.0 µg of valrubicin was recovered from the epidermal membrane after application of VLD43, this value was significantly greater ($p > 0.005$ using Bonferroni correction) than the amount recovered
20 after application of Emulsified gel 4 and Emulsified gel 5 of approximately 1.9 and 2.1 µg of valrubicin, respectively. After application of the comparator formulation to the perturbed epidermal membrane approximately 4 µg of Valrubicin was recovered from the epidermal membrane.

25 Table 6 shows the amount of Valrubicin recovered from the epidermal membrane (EM) 48 h after application of Valrubicin containing formulations to the intact and perturbed human epidermal membrane. In addition the amount of Valrubicin recovered from the *stratum corneum* 48 h after application of valrubicin containing formulations to the intact human epidermal membrane is indicated.

30

Table 6

Formulation	Epidermal membrane	Amount of Valrubicin recovered (μg , mean $\pm$ SE, n=3-4)	
		<i>Stratum corneum</i>	Epidermal membrane
VLD32	Intact	6.94 ± 0.09	$0.27 \pm 0.14^*$
	Perturbed	N/A	8.12 ± 0.92
VLD43	Intact	$1.64 \pm 0.68^*$	$0.32 \pm 0.19^*$
	Perturbed	N/A	4.98 ± 0.18
Emulsified gel 4	Intact	$2.25 \pm 0.56^*$	0.00 ± 0.00
	Perturbed	N/A	$1.89 \pm 0.38^*$
Emulsified gel 5	Intact	$1.22 \pm 0.63^*$	0.00 ± 0.00
	Perturbed	N/A	$2.11 \pm 0.27^*$
Comparator	Intact	0.00 ± 0.00	0.00 ± 0.00
	Perturbed	N/A	4.07 ± 0.40

At or below the LOQ of the analytical method (approximately $0.7 \mu\text{g/mL}$ in 80:20 acetonitrile:water)

Example 18

5 Effect of Valrubicin on proliferation of the human squamous carcinoma (SCC) cell lines: Ca Ski, SW756, and SW954

Aim of the study:

To investigate the dose-response effect on CaSki, SW756 and SW954 cell proliferation upon stimulation with increasing concentrations of valrubicin and doxorubicin for 24, 48
10 and 72 hrs respectively.

Cell Cultures:

CaSki (cervix SCC, HPV16), SW756 (cervix SCC HPV18), and SW954 (vulva SCC) cells from human cell lines were cultured in the presence of vehicle, valrubicin, or
15 doxorubicin. Valrubicin and doxorubicin were added in increasing concentrations: 0.02 $\mu\text{g/mL}$, 0.04 $\mu\text{g/mL}$, 0.08 $\mu\text{g/mL}$, 0.16 $\mu\text{g/mL}$, 0.32 $\mu\text{g/mL}$ and 0.64 $\mu\text{g/mL}$.

The CellTiter-Glo® viability assay kit (Promega, USA) was employed to assess the effect of valrubicin and doxorubicin on cell proliferation.

Cells were seeded in a concentration of 2000 cells (6500 cells/cm², CaSki, population doubling = 1/day), 8000 cells (26000 cells/cm², SW756, population doubling = 0.5/day), and 10000 cells (32500 cells/cm², SW954, population doubling = 0.25/day) per well in a 96 well/plate in quadruplicate and treated the following day. Effect on proliferation was evaluated as percent of reduction in viability.

Results:

Figures 5A-C show that valrubicin and doxorubicin have a significant dose-dependant anti-proliferative effect on CaSki cells (cervix SCC, HPV16) after 48- and 72 hrs of stimulation. After 72 hrs of stimulation with valrubicin, cell viability was reduced to <50% upon stimulation with the highest concentration. Doxorubicin demonstrates a greater anti-proliferative effect than valrubicin at the 48- and 72 hrs assessments.

Figures 5D-F show that valrubicin and doxorubicin have a significant dose-dependant anti-proliferative effect on SW756 cells (cervix SCC, HPV18) after 48- and 72 hrs of stimulation. After 72 hrs of stimulation with valrubicin, cell viability was reduced to <50% upon stimulation with the highest concentration. Doxorubicin demonstrates a greater anti-proliferative effect than valrubicin at the 48- and 72 hrs assessments.

Figures 5G-I show that valrubicin and doxorubicin have a significant dose-dependant anti-proliferative effect on SW954cells (vulva SCC) after 48- and 72 hrs of stimulation. After 72 hrs of stimulation with valrubicin, cell viability was reduced to 25% upon stimulation with the highest concentration. Doxorubicin demonstrates a greater anti-proliferative effect than valrubicin at the 48- and 72 hr. assessments.

Conclusion:

Valrubicin and doxorubin demonstrate a significant dose-dependant anti-proliferative effect on human CaSki cells (cervix SCC, HPV16), SW756 cells (cervix SCC, HPV18) and SW954cells (vulva SCC) after 48-and 72 hr stimulation.

Valrubicin demonstrates a consistent dose-dependant reduction of cell viability in all the human SCC cell lines investigated after 48- and 72 hrs of stimulation with the greatest effect, expressed as reduction of cell viability, to 25% at the highest concentration in the SW954 cells (vulva SCC) which indicates that valrubicin may be attractive for topical use in said conditions, currently treated with surgical intervention as first line therapy.

In all cell series doxorubicin demonstrated a higher reduction in cell viability confirming results in previous studies. However, as doxorubicin is extremely toxic to tissue and skin upon extravasations, it can not be considered for topical treatment of said conditions.

5

Example 19

Penetration study – human skin

Methods:

The experiment was performed using normal and abraded abdominal human skin. To simulate damaged skin, the skin sample was stripped ten times with Tape film (19 mm wide) to partially remove the Stratum Corneum before insertion into the Franz diffusion cells. Individually calibrated Franz cells (FZ) with a diffusion area and acceptor volume of approximately 3 cm² and 20 ml, respectively, was employed to determine the penetration of valrubicin. The receptor compartment was filled with acceptor fluid. Each formulation (30 mg +/- 5%) was applied to the diffusion skin area and homogenously spread on the skin surface using a spatula. The application was followed by massaging simulating the real application conditions. The Franz diffusion cells were covered by Parafilm® during the 24 hour period.

Two formulations were employed during the skin penetration experiments, these were as follows:

- VLD32 – 1.0% w/w valrubicin (see Example 4 for content of VLD32)
- Formulation A – 1.0% w/w valrubicin (see Example 15 for content of Formulation A)

Assessments:

At the end of the penetration experiments the remaining valrubicin content in the residual formulation in the donor skin was determined by HPCL for each FZ. The formulation remaining on the skin surface was collected with cotton swabs with aid of extraction medium (ACN/water 60:40 v/v %) and transferred into glass tubes with an extraction medium.

After removing the residual test product, the concentration of the test substance in the skin samples was quantified. Two segmenting techniques: tape stripping and cryosectioning, were applied to separate different skin layers parallel to the upper surface of the sample and thus provide information on the extent to which the test compound had penetrated into the different skin areas. The upper epidermal layers of

the skin were stripped off using Tape film. In total 18 tape strips was performed per each skin biopsy of the intact skin and 8 tape strips for the damaged skin.

The first two strips were separately considered as non penetrated drug due to potential contamination by residual valrubicin on the surface of the skin according to the recommendations of the Guidelines "FDA Guidance on assessment of Bioavailability/Bioequivalence of topical dermatological products". All samples were extracted with 2 mL extraction media by shaking for 1 hour at 200 rpm at RT and quantified by HPLC.

Penetration studies were performed in six-fold per formulation with damaged and normal skin.

Following the application of each formulation, receiver fluid was removed from the receiver compartment of each Franz cell via the sampling arm after 24h and analysed via HPLC.

In summary, valrubicin was recovered and analysed via HPLC from the following components:

- Valrubicin remaining on surface of epidermal membrane (skin surface),
- Valrubicin in the epidermis after 2 tape strips (2TS)
- Valrubicin in the epidermal membrane after 16 tape strips (normal skin)(16TS) or 6 tape strips (damaged skin*)(6TS)
- Valrubicin in the deeper skin layers (epidermis without SC)
- Valrubicin in the acceptor fluid (acceptor), collected during the penetration experiment.

* The skin was 10 times stripped prior to the start of the experiment in order to simulate damaged skin

Results:

Upon application of valrubicin containing formulations to intact human skin, the drug amount measured after 24 hours in the samples after 16 times stripping was almost 3-fold higher for the formulation VDL32 ($0.35 \pm 0.11 \text{ mg/cm}^2$) in comparison to the Formulation A ($0.13 \pm 0.06 \text{ mg/cm}^2$). After the incubation time no drug could be detected in the residual skin after stripping (deeper skin layers) and acceptor medium. Figure 6 shows the results for intact skin, and is a comparison of Valrubicin amount [$\mu\text{g/cm}^2$] found in the 2 first tape-strips (2TS), 16 tape-strips (Stratum Corneum, 16TS),

deeper skin layers (residual skin after stripping) and acceptor medium from the formulations A (JH32) and VDL32. The bars presented are mean value (n=6) of amount [$\mu\text{g}/\text{cm}^2$] and standard deviation (SD).

- 5 Upon application of valrubicin containing formulations to damaged human skin, the drug amount measured after 24 hours for the samples after 6 times stripping was approximately 3-fold higher for the formulation VDL32 ($0.32 \pm 0.05 \text{ mg}/\text{cm}^2$) in comparison to the Formulation A ($0.11 \pm 0.02 \text{ mg}/\text{cm}^2$). The measured drug amount in the deeper skin layers was also clearly higher for VDL32 ($0.08 \pm 0.06 \text{ mg}/\text{cm}^2$) in
10 comparison to Formula ($0.01 \pm 0.01 \text{ mg}/\text{cm}^2$). No Valrubicin could be detected in the acceptor medium during the experiment with damaged skin.

- Figure 7 shows the results for damaged skin, and is a comparison of Valrubicin amount [$\mu\text{g}/\text{cm}^2$] found in the 2 first tape-strips (2TS), 6 tape-strips (Residual Stratum Corneum, 6TS), deeper skin layers (residual skin after stripping) and acceptor medium from the
15 formulations Formulation A (JH32) and VDL32. The bars presented are mean value (n=6) of amount [$\mu\text{g}/\text{cm}^2$] and SD.

Example 20

20 Penetration study – human cervical tissue

Methods:

- The experiment was performed using pre-menopausal human cervical tissue from 2 donors. Individually calibrated Franz cells with a diffusion area and acceptor volume of approximately $0,14 \text{ cm}^2$ and $5,5 \text{ ml}$, respectively, was employed to determine the
25 penetration of valrubicin. The receptor compartment was filled with acceptor fluid. The formulation, approximately 30 mg , was pipetted onto the surface of the tissue and left there for 24 hours.

One formulation was employed during the skin penetration experiments:

- 30 Emulsified gel 4 – $1.0\% \text{ w/w}$ valrubicin (see Example 16 for content of emulsified gel 4)

- After the incubation time the residual formulation was removed by rinsing twice with extraction medium ($2 \times 100 \mu\text{L}$) and the concentration of the test substance was quantified. Acceptor medium samples were also collected. For the tissue samples,
35 cryosection was used as segmenting technique to separate different tissue layers

parallel to the upper surface of the sample and thus provide information on the extent to which the test compound had penetrated into the tissue. The tissue was frozen at – 20 °C before cryosegmentation. After segmenting all samples were extracted with ACN/water 60:40 (v/v %) and quantified by LC-UV.

5

In summary, valrubicin was recovered and analysed from the following components:

- Tissue surface (300 mm)
- Segment 1 (350 mm)
- Segment 2 (400 mm)
- 10 • Segment 3 (600 mm)
- Deeper tissue or rest (3000 mm)
- Acceptor fluid (acceptor 24 hrs)

Results:

15 The results show that the drug substantially remains in the tissue surface, hereby indicating that the formulation is suitable for local application and treatment of localized conditions, e.g. cervical hyperproliferative conditions such as pre-neoplasia, carcinoma in situ.

20 In donor 1, the drug amount penetrated was 2.03 ± 0.13 mg which corresponds to 0.66 ± 0.55 % of the total drug amount applied. After the incubation time of 24 hours the drug substance quantified in the deeper segments (segment 1, segment 2, segment 3 and deeper tissue or rest) was 0.19 ± 0.06 % of the total amount applied.

25 Figure 8 shows the penetration of Valrubicin into cervical tissue after 24-hour incubation with emulsified gel 4, valrubicin 1%. The diagram shows the concentration profile in the tissue. The values shown are the individual values from each Franz cell and arithmetic mean values $\pm$ SD (n=3).

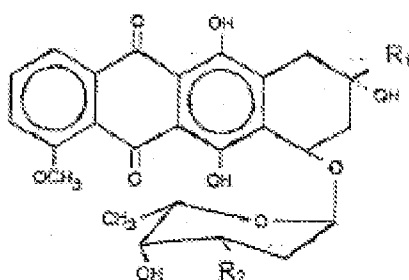
30 The results from the second experiment employing donor tissue 2 have confirmed the findings from the first experiment employing donor tissue 1. Valrubicin was mostly concentrated in the tissue surface (1.45 ± 0.95 μ g which corresponds to 0.48 ± 0.32 % of the total drug amount applied) after the incubation time of 24 hours, whereas the drug substance quantified in the deeper segments (segment 1, segment 2, segment 3
35 and deeper tissue or rest) was 0.17 ± 0.12 % of the total amount applied. The values

from the mass balance were within the limits recommended by the SSPC 2006 Guideline for the in vitro dermal absorption ($100 \pm 15\%$).

- 5 Figure 9 shows the penetration of Valrubicin into cervical tissue after 24-hour incubation with emulsified 4 gel valrubicin 1%. The diagram shows the concentration profile in the tissue. The values shown are the individual values from each Franz cell and the relevant arithmetic mean values $\pm$ SD (n=3).

Claims

1. A pharmaceutical composition comprising
i) a lipophilic anthracycline;
5 ii) one or more oil phase carriers;
iii) one or more pharmaceutical acceptable solubilizers;
iv) one or more co-surfactants; and
wherein the composition further comprises an aqueous phase comprising at least one
buffering system having a pH from pH 2.2 to pH 6, and wherein the water content of
10 the pharmaceutical composition is less than 30%.
2. The pharmaceutical composition according to claim 1, wherein the lipophilic anthracycline is selected from the group consisting of OctADR, MRA-CN, AD32 (valrubicin), AD41, AD143, AD194, AD198, AD199, AD201, AD202, and AD288, or
15 mixtures thereof, or pharmaceutical acceptable salt, solvates or prodrugs thereof.
3. The pharmaceutical composition according to claim 1, wherein the lipophilic anthracycline is valrubicin.
- 20 4. The pharmaceutical composition according to claim 1, wherein the lipophilic anthracycline has an log P octanol/water value greater than 0.5, preferably greater than 1.0, more preferably greater than 1.5, even more preferably greater than 2.0, yet even more preferably greater than 3.0.
- 25 5. The pharmaceutical composition according to any of claims 1 and 4, wherein the lipophilic anthracycline has the general formula (I):



(I)

pharmaceutical acceptable salt, solvates or prodrugs thereof;

wherein R_1 is selected from the group consisting of $-C(O)CH_2O-(C_1-C_6\text{-acyl})$, $-C(O)CH_2O-(C_1-C_6\text{-alkyl})$, $-C(O)CH_2O-(C_1-C_6\text{-alkoxy})$, and $-C(O)CH_2O-(C_1-C_6\text{-acyl})-(C_1-C_6\text{-alkoxy})$; and

R_2 is selected from the group consisting of $-NH-(C_1-C_6\text{-acyl})$, $-NH-(C_1-C_6\text{-alkyl})$, $-NH-(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-acyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkoxy})(C_1-C_6\text{-alkoxy})$, heterocyclyl, $-C(O)CH_2O-(C_1-C_6\text{-alkyl})$, $-(C_1-C_6\text{-acyl})$, $-NH_2$, $-OH$, and H ; wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of $C_1-C_3\text{-alkyl}$, $C_1-C_2\text{-alkoxy}$, $-OH$, halogen, $-NH_2$, $-NH-(C_1-C_4\text{-alkyl})$, or $-N(C_1-C_4\text{-alkyl})(C_1-C_4\text{-alkyl})$.

6. The pharmaceutical composition according to claim 5, wherein R_1 is $-C(O)CH_2O-(C_1-C_6\text{-acyl})$; more preferably R_1 is $-COCH_2OCO(CH_2)_3CH_3$.

7. The pharmaceutical composition according to claim 5, wherein R_2 is selected from the group consisting of $-NH-(C_1-C_6\text{-acyl})$, $-NH-(C_1-C_6\text{-alkyl})$, $-NH-(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-acyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkoxy})(C_1-C_6\text{-alkoxy})$, heterocyclyl, $-C(O)CH_2O-(C_1-C_6\text{-alkyl})$, and $-(C_1-C_6\text{-acyl})$; wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of $C_1-C_3\text{-alkyl}$, $C_1-C_2\text{-alkoxy}$, $-OH$, halogen, $-NH_2$, $-NH-(C_1-C_4\text{-alkyl})$, or $-N(C_1-C_4\text{-alkyl})(C_1-C_4\text{-alkyl})$.

8. The pharmaceutical composition according to claim 5, wherein R_2 is selected from the group consisting of $-NH-(C_1-C_6\text{-acyl})$, $-NH-(C_1-C_6\text{-alkyl})$, $-NH-(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-acyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-acyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkyl})$, $-N(C_1-C_6\text{-alkyl})(C_1-C_6\text{-alkoxy})$, $-N(C_1-C_6\text{-alkoxy})(C_1-C_6\text{-alkoxy})$, heterocyclyl, and $-(C_1-C_6\text{-acyl})$; wherein any alkyl, acyl, alkoxy, or heterocyclyl moiety of R_2 optionally is substituted with one or more of methyl, ethyl, isopropyl, methoxy, ethoxy, isopropoxy, $-OH$, $-Br$, $-F$, $-Cl$, $-NH_2$, $-NH-(C_1-C_3\text{-alkyl})$, or $-N(C_1-C_3\text{-alkyl})(C_1-C_3\text{-alkyl})$.

9. The pharmaceutical composition according to claim 5, wherein R_2 is selected from the group consisting of $-COCH_2OCO(CH_2)_3CH_3$, $-COCH_2OH$, $-COCH_3$, $-NH_2$, $-H$, $-OH$, and $-NHCOCF_3$; wherein any alkyl, acyl, or alkoxy moiety of R_2 optionally is substituted

with one or more of C₁-C₃-alkyl, C₁-C₂-alkoxy, -OH, halogen, -NH₂, -NH-(C₁-C₄-alkyl), or -N(C₁-C₄-alkyl)(C₁-C₄-alkyl).

- 5 10. The pharmaceutical composition according to claim 5, wherein R₂ is selected from the group consisting of -COCH₂OCO(CH₂)₃CH₃, -COCH₂OH, -COCH₃, and -NHCOCF₃; wherein any alkyl, acyl, or alkoxy moiety of R₂ optionally is substituted with one or more of C₁-C₃-alkyl, C₁-C₂-alkoxy, -OH, halogen, -NH₂, -NH-(C₁-C₄-alkyl), or -N(C₁-C₄-alkyl)(C₁-C₄-alkyl).
- 10 11. The pharmaceutical composition according to any of the preceding claims, wherein the composition comprises at least 0.1%, preferably at least 0.5%, more preferably at least 1% of said lipophilic anthracycline.
- 15 12. The pharmaceutical composition according to any of the preceding claims, wherein the composition comprises said lipophilic anthracycline in an amount of 0.1 to 10 w/w %, such as e.g., from 0.1 to 8 w/w %, from 0.1 to 5 w/w %, from 0.25 to 1.25 w/w %, from 1 to 5 w/w %, from 0.1 to 2.5 w/w %, from 0.1 to 1.5 w/w %, from 0.5 to 2.5 w/w %, from 0.5 to 2.0 w/w %, from 0.5 to 1.5 w/w %, or of about 1.0 w/w %.
- 20 13. The pharmaceutical composition according to any of the preceding claims, wherein the composition is in a form selected from the group consisting of a water in oil emulsion, an emulsified gel, a gel, and an oil in water emulsion.
- 25 14. The pharmaceutical compositions according to any of the preceding claims, wherein the pH of said one or more buffering system is in the range of pH 2.2 to pH 5.0, such as e.g. of pH 2.2 to 4.5, of pH 2.2 to 4.2, of pH 2.2 to 4.0, of pH 2.2 to 3.5, of pH 2.5 to 3.5, of pH 2.5 to 3.0, or about pH 2.5.
- 30 15. The pharmaceutical compositions according to any of the preceding claims, wherein said buffering system is an aqueous solution comprising a buffering compound in the range of 10 to 300 mM, such as e.g., of 10 to 200 mM, of 20 to 200 mM, of 30 to 150 mM, of 40 to 150 mM, of 40 to 140 mM, or of 40 to 130 mM.
- 35 16. The pharmaceutical compositions according to any of the preceding claims, wherein the composition is an emulsified gel or a gel and said buffering system is an

aqueous solution comprising a buffering compound in a concentration of at about 50 mM.

5 17. The pharmaceutical compositions according to any of claims 1-15, wherein the composition is a water in oil emulsion and said buffering system is an aqueous solution comprising a buffering compound in a concentration of at about 100 mM.

10 18. The pharmaceutical compositions according to any of the preceding claims, wherein the buffering system is lactic acid – sodium lactate.

19. The pharmaceutical compositions according to any of the preceding claims, wherein said one or more oil phase carriers is present in an amount of 10 to 55 w/w %, such as e.g., of 10 to 50 w/w %, of 12 to 48 w/w %, of 15 to 44 w/w %, or specifically of about 44 w/w % or specifically of about 15 w/w %.

15 20. The pharmaceutical compositions according to any of claims 1-15, 17-19 wherein the composition is a water in oil emulsion and the oil phase carrier is in an amount of 35 to 55 w/w %, such as e.g., of 35 to 50 w/w %, of 40 to 50 w/w %, preferably of about 45 w/w %, such as of about 44 w/w %.

20 21. The pharmaceutical compositions according to any of claims 1-16, 18-19, wherein the composition is an emulsified gel or a gel and the oil phase carrier is in an amount of 10 to 25 w/w %, such as e.g., of 10 to 20 w/w %, of 12 to 18 w/w %, preferably of about 15 w/w %.

25 22. The pharmaceutical compositions according to any of the preceding claims, wherein said one or more oil phase carriers is selected from the group consisting of white soft paraffin, hard paraffin, Crodamol (such as e.g. Crodamol GTCC), glycerol monostearate, ceteraryl alcohol, macrogol-20 glycerol monostearate (such as e.g.
30 Tagat S2), and castor oil, or mixtures thereof.

23. The pharmaceutical compositions according to any of the preceding claims, wherein said one or more pharmaceutical acceptable solubilizers is present in an amount of 10 to 45 w/w %, such as e.g., of 10 to 40 w/w %, of 12 to 38 w/w %, of 15 to
35 38 w/w %, of 15 to 35 w/w %, or specifically of about 17% or specifically of about 32 %.

24. The pharmaceutical compositions according to any of claims 1-15,17-20,22-23,
wherein the composition is an water in oil emulsion and the solubilizer is in an amount
of 10 to 25 w/w %, such as e.g., of 10 to 20 w/w %, of 15 to 20 w/w %, preferably of
5 about 17 w/w %.

25. The pharmaceutical compositions according to any of claims 1-16,18-19,21-23,
wherein the composition is an emulsified gel or a gel and the solubilizer is in an amount
of 20 to 45 w/w %, such as e.g., of 25 to 35 w/w %, of 30 to 35 w/w %, preferably of
10 about 32 w/w %.

26. The pharmaceutical compositions according to any of the preceding claims,
wherein said one or more pharmaceutical acceptable solubilizers is selected from the
group consisting of propylene glycol, glycerol, and cremophor RH40 (polyoxyl 40
15 hydrogenated castor oil) or mixtures thereof.

27. The pharmaceutical compositions according to any of the preceding claims,
wherein said one or more co-surfactants is present in an amount of 5 to 35 w/w %,
such as e.g., of 10 to 30 w/w %, of 12 to 27 w/w %, of 15 to 25 w/w %, of 5 to 25 w/w
20 %, of 15 to 35 w/w %, or specifically of about 15% or specifically of about 25 %.

28. The pharmaceutical compositions according to any of the preceding claims,
wherein said one or more co-surfactants is selected from the group consisting of
diethylene glycol monoethyl ether (such as e.g. transcitol P), glycofurool
25 (tetrahydrofurfuryl polyethylenglycol), Propylene Glycol Monolaurate (such as e.g.
Lauroglycol™ 90), and Propylene Glycol Laurate (such as e.g. Lauroglycol™ FCC), or
mixtures thereof.

29. The pharmaceutical compositions according to any of claims 1-15,17-20,22-24,26-
30 28, wherein the composition is a water in oil emulsion and the co-surfactant is
transcitol P in an amount of 5 to 25 w/w %, preferably of about 15 w/w %.

30. The pharmaceutical compositions according to any of claims 1-16,18-19,21-23,25-
28, wherein the composition is an emulsified gel or a gel and the co-surfactant is
35 transcitol P in an amount of 15 to 35 w/w %, preferably of about 25 w/w %.

31. The pharmaceutical compositions according to any of the preceding claims, further comprising one or more emulsifiers and/or one or more gel forming polymers.
- 5 32. The pharmaceutical compositions according to claim 31, wherein said one or more emulsifiers and/or one or more gel forming polymers is present in an amount of 0.1 to 10 w/w %, such as e.g., of 0.5 to 8 w/w %, of 1 to 6 w/w %, of 1 to 5 w/w %, of 1 to 4 w/w %, of 1 to 3 w/w %, or specifically of about 2 w/w% or specifically about 2.5%.
- 10 33. The pharmaceutical compositions according to any of claims 31-32, wherein the composition is a water in oil emulsion and said one or more emulsifiers is selected from the group consisting of Dow Corning emulsifier 10, and Abil Em 90.
- 15 34. The pharmaceutical compositions according to any of claims 31-32, wherein the composition is an emulsified gel or a gel and said one or more gel forming polymers is selected from the group consisting of hydroxypropyl cellulose (such as e.g. HPC HF), hydroxyethyl cellulose (such as e.g. HEC HF), Sodium Polyacrylate, Cetyl Hydroxy Ethyl Cellulose, and Polyquaternium 10; preferably hydroxy propyl cellulose.
- 20 35. The pharmaceutical compositions according to any of the preceding claims, further comprising a pharmaceutical acceptable alcohol, preferably ethanol.
- 25 36. The pharmaceutical compositions according to claim 35, wherein said pharmaceutical acceptable alcohol is present in an amount of 0.1 to 15 w/w %, such as e.g., of 0.5 to 15 w/w %, of 1 to 13 w/w %, of 2 to 13 w/w %, of 3 to 13 w/w %, of 4 to 12 w/w %, of 5 to 10 w/w %, or specifically of about 5 w/w % or specifically of about 10 w/w %.
- 30 37. The pharmaceutical compositions according to any of claims 35-36, wherein the composition is a water in oil emulsion and said pharmaceutical acceptable alcohol is present in an amount of 0.1 to 10 w/w %, such as e.g., of 0.5 to 10 w/w %, of 1 to 10 w/w %, of 2 to 8 w/w %, or specifically of 5 w/w %.
- 35 38. The pharmaceutical compositions according to any of claims 35-36, wherein the composition is an emulsified gel or a gel and said pharmaceutical acceptable alcohol is

present in an amount of 1 to 15 w/w %, such as e.g., of 2 to 15 w/w %, of 5 to 15 w/w %, of 8 to 13 w/w %, or specifically of about 10 w/w %.

5 39. The pharmaceutical compositions according to any of the preceding claims, further comprising one or more antioxidants.

40. The pharmaceutical compositions according to claim 39, wherein said one or more antioxidants is present in an amount of 0.01 to 0.15 w/w %, such as e.g. of 0.01 to 0.1 w/w %, of 0.01 to 0.08 w/w %, of 0.02 to 0.08 w/w %, of 0.03 to 0.07 w/w %, or
10 specifically of about 0.05 w/w %.

41. The pharmaceutical compositions according to any of claims 39-40, wherein said one or more antioxidants is selected from the group consisting of Ascorbyl palmitate, Alpha tocopherol, and mixtures thereof.
15

42. The pharmaceutical compositions according to any of the preceding claims, further comprising one or more stabilizers and/or preservatives.

43. The pharmaceutical compositions according to claim 42, wherein said one or more stabilizers is present in an amount of 0.01 to 0.1 w/w %, such as e.g. of 0.01 to 0.08 w/w %, of 0.01 to 0.06 w/w %, of 0.01 to 0.04 w/w %, or specifically of about 0.02 w/w %.
20

44. The pharmaceutical compositions according to claim 42, wherein said one or more stabilizers at least comprises EDTA disodium.
25

45. The pharmaceutical compositions according to any of claims 42-44, wherein said one or more preservatives is present in an amount of 0.01 to 2 w/w %, such as e.g., of 0.1 to 1.5 w/w %, of 0.5 to 1.25 w/w %, or specifically of about 1 w/w %.
30

46. The pharmaceutical compositions according to any of claims 42-45, wherein said one or more preservatives is selected from the group consisting of Phenoxy ethanol, sodium benzoate, sorbic acid, potassium sorbate, benzoic acid, and parabens, or mixtures thereof.
35

47. The pharmaceutical composition according to any of claims 1-15,17-20,22-24,26-29,31-33,35-37,39-46, wherein the composition is a water in oil emulsion.
- 5 48. The pharmaceutical composition according to any of claims 1-16,18-19,21-23,25-28,30-32,34-36,38-46, wherein the composition is an emulsified gel.
49. The pharmaceutical composition according to any of claims 1-15,18-19,22-23,26-28,31-32,35-36,39-46, wherein the composition is a gel.
- 10 50. The pharmaceutical composition according to any of claims 1-15,18-19,22-23,26-28,31-32,35-36,39-46, wherein the composition is an oil in water emulsion.
- 15 51. The pharmaceutical composition according to any of the preceding claims, wherein the composition comprises a gel, an ointment, a cream, a foam, a solution, a lotion or a stick.
52. The pharmaceutical composition according to any of the preceding claims, wherein the composition is in a form selected from the group consisting of a gel, an ointment, a cream, a foam, a solution or a lotion.
- 20 53. The pharmaceutical composition according to any one of the preceding claims, wherein the composition is in a form selected from the group consisting of vaginal cups, patches, lotions, vaginal rings, vaginal tampons, vaginal strips, vaginal capsules, vaginal tablets, vaginal pessaries, suppositories and vaginal sponges comprising said gel, ointment, cream, foam, solution, or lotion.
- 25 54. The pharmaceutical composition according to any of the preceding claims, wherein the composition comprises a solution comprising said lipophilic anthracycline.
- 30 55. The pharmaceutical composition according to claim 54, wherein the pharmaceutical composition is in a form selected from the group consisting of vaginal tampons, vaginal capsules, vaginal sponges, sprays, eye drops and inhalators comprising said solution.
- 35 56. The pharmaceutical composition according to any of the preceding claims, wherein the one or more solubilizers and/or the one or more co-surfactants

- a) comprises at least one –OH group, and
- b) in addition to the at least one –OH mentioned in a) comprises at least one –O- group; and
- c) has a molecular weight of at the most 300.

5

57. The pharmaceutical composition according to claim 56, wherein at least one of the one or more solubilizers and/or one of the one or more co-surfactants is a polyether.

10

58. The pharmaceutical composition according to claim 57, wherein the polyether has the general structure $R_1[-O-CH_2-CH_2]_n-R_2$, wherein n is an integer in the range of 2 to 6, preferably 2 to 5, R_1 and R_2 individually are selected from the group consisting of lower alcohols, lower alkyl and 5 to 6 membered rings.

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59. The pharmaceutical composition according to claim 57, wherein the polyether is selected from the group consisting of diethylene glycol monoethyl ether and glycofurool (Tetrahydrofurfuryl polyethylenglycol).

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60. The pharmaceutical composition according to any of claims 56-59, wherein the solubility of valrubicin in said polyether is at least 5% w/w, preferably at least 10% w/w, even more preferably at least 20% w/w.

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61. The pharmaceutical composition according to any of claims 56-60, wherein the polyether is a polyether, wherein the percentage peak purity of valrubicin after storage therein for 2 weeks at 25°C is at least 80%, preferably at least 85%, more preferably at least 90%.

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62. The pharmaceutical composition according to any one of claims 56-61, wherein the composition comprises in the range of 5 to 50%, preferably in the range of 10 to 30% of said polyether.

63. The pharmaceutical composition according to any of the preceding claims, wherein at least one of the one or more solubilizers is an alcohol comprising at least two free –OH groups.

64. The pharmaceutical composition according to any of the preceding claims, wherein the composition further comprises at least one colour masking agent.

5 65. The pharmaceutical composition according to claim 64, wherein one or more colour masking agents are selected from the group consisting of FD&C blue and Sicovit Quinoline yellow.

10 66. The pharmaceutical composition according to any of the preceding claims, wherein said composition when applied to skin has a grading in the human 4-h patch test of at the most +, on a scale from 0 to +++, wherein 0 is no reaction and +++ is the strongest reaction.

15 67. The pharmaceutical composition according to any of the preceding claims, wherein the solubility of a lipophilic anthracycline, preferably of valrubicin, in said composition is at least 0.5% w/w.

68. The pharmaceutical composition according to any of the preceding claims, wherein the solubility of said lipophilic anthracycline in the composition is at least 1% w/w.

20 69. The pharmaceutical composition according to any of the preceding claims, wherein the percentage peak purity of the lipophilic anthracycline after storage of the composition for 3 months at 25°C is at least 90%, preferably at least 95%.

25 70. The pharmaceutical composition according to any of the preceding claims, wherein the percentage recovery of said lipophilic anthracycline after storage of the composition for 3 months at 25°C is at least 99%, preferably at least 99.5%.

30 71. The pharmaceutical composition according to any of the preceding claims, wherein the composition is for treatment of a condition associated with hyperproliferation, preferably epithelial hyperproliferation, preferably epidermal hyperproliferation.

72. The pharmaceutical composition according to claim 71, wherein the condition is associated with psoriasis.

73. The pharmaceutical composition according to any of claims 1-70, wherein the composition is for treatment of a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, nasal cavity, lips, oral cavity, pharynx, larynx, trachea, or bronchi, or affecting the eye.

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74. A pharmaceutical composition comprising a lipophilic anthracycline formulated for local administration for treatment of a neoplastic or preneoplastic condition affecting the epithelium of genitals, reproductive organs, nasal cavity, lips, pharynx, larynx, trachea, or bronchi, or affecting the eye.

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75. The pharmaceutical composition according to any of claims 73-74, wherein the neoplastic or preneoplastic condition is a condition associated with an infection with Human Papilloma virus (HPV).

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76. The pharmaceutical composition according to any of claims 73-75, wherein the neoplastic or preneoplastic condition is a carcinoma in situ or a squamous cell carcinoma selected from the group consisting of cervical intraepithelial neoplasia (CIN), vulvar intraepithelial neoplasia (VIN), squamous cell carcinoma in situ (Bowens disease), squamous cell carcinoma of vagina, penile squamous cell carcinoma, perianal squamous cell carcinoma, squamous cell carcinoma of the nasal cavity, squamous cell carcinoma of the lips, squamous cell carcinoma of the pharynx, squamous cell carcinoma of the larynx, squamous cell carcinoma of the trachea, squamous cell carcinoma of the bronchi, and squamous cell carcinoma of the eye.

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77. The pharmaceutical composition according to any one of claims 73-76, wherein the neoplastic or preneoplastic condition is condylomas on the genitals or reproductive organs.

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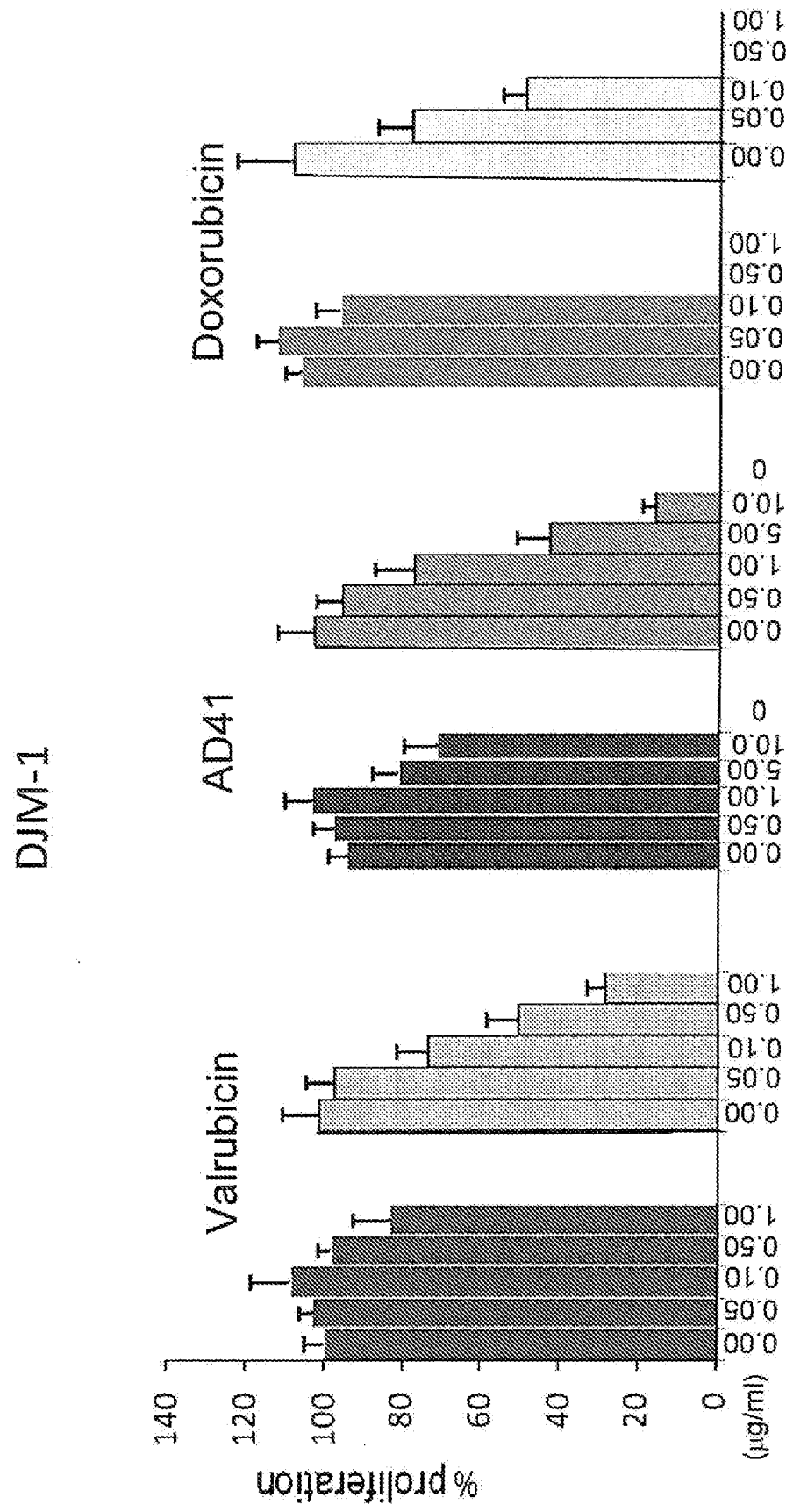


Fig. 1A

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HSC-1

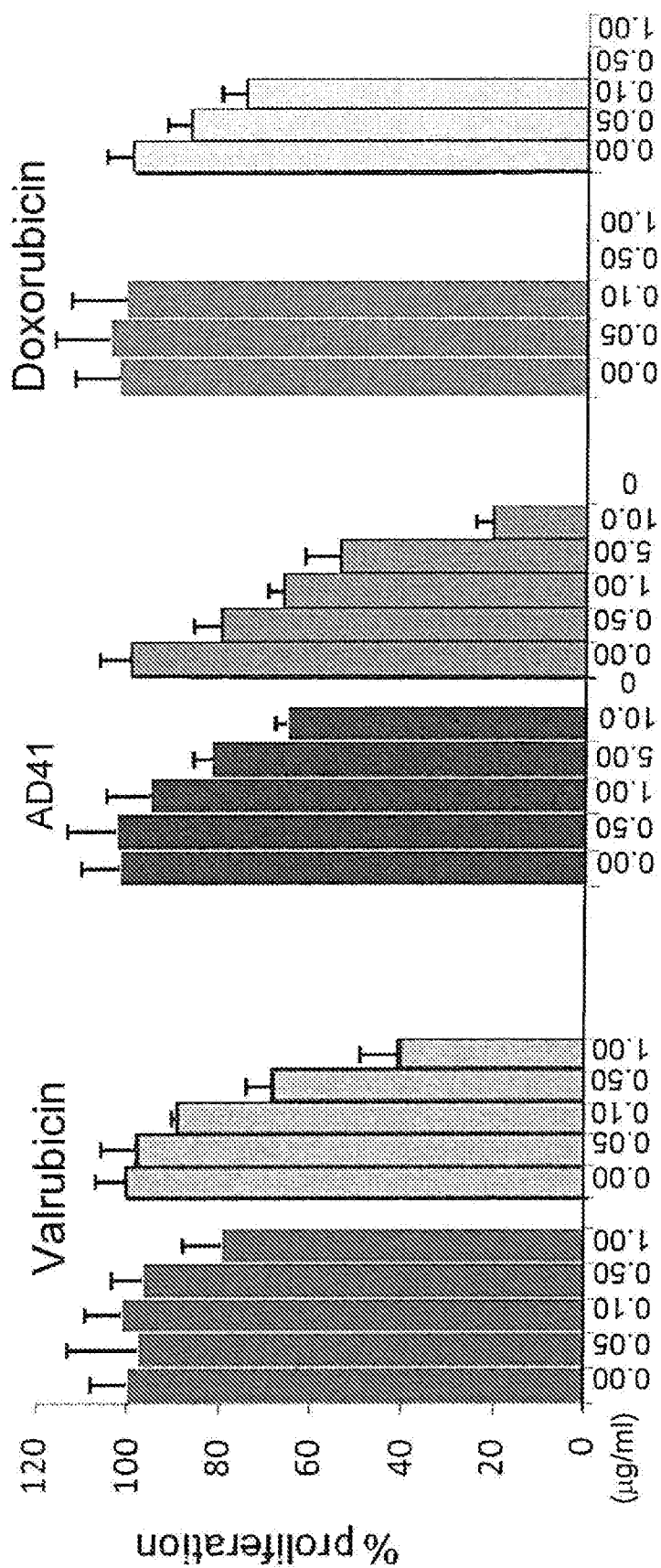


Fig. 1B

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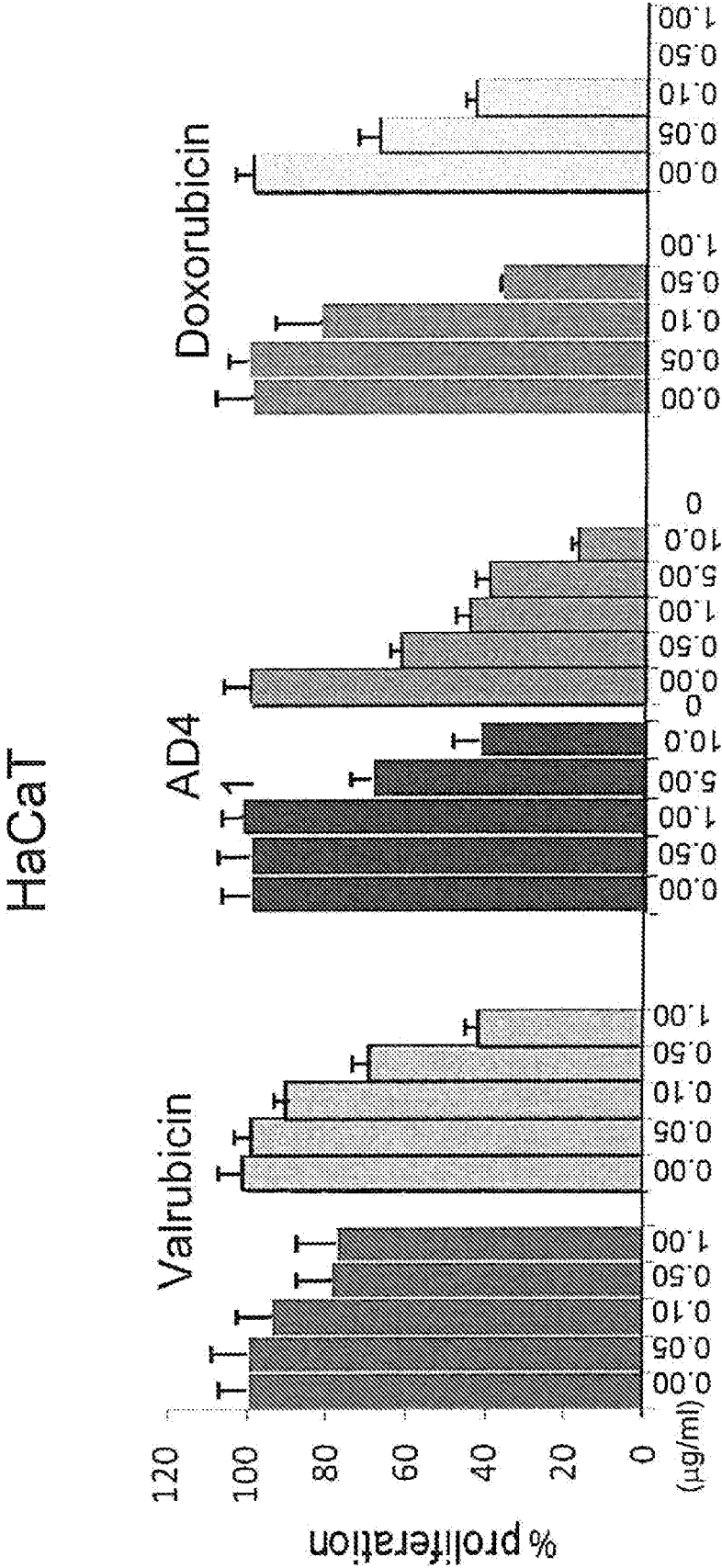


Fig. 1C

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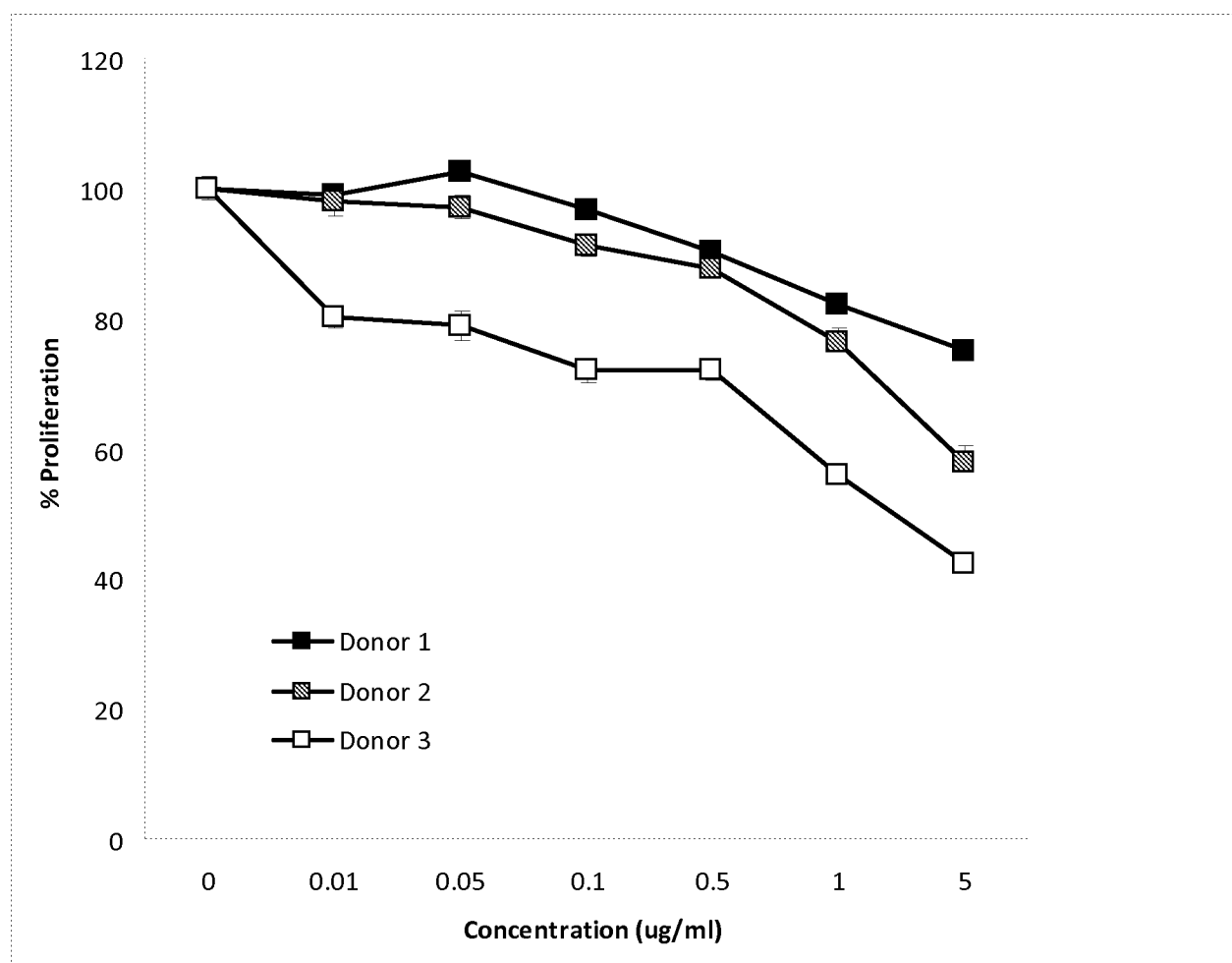


Fig. 2

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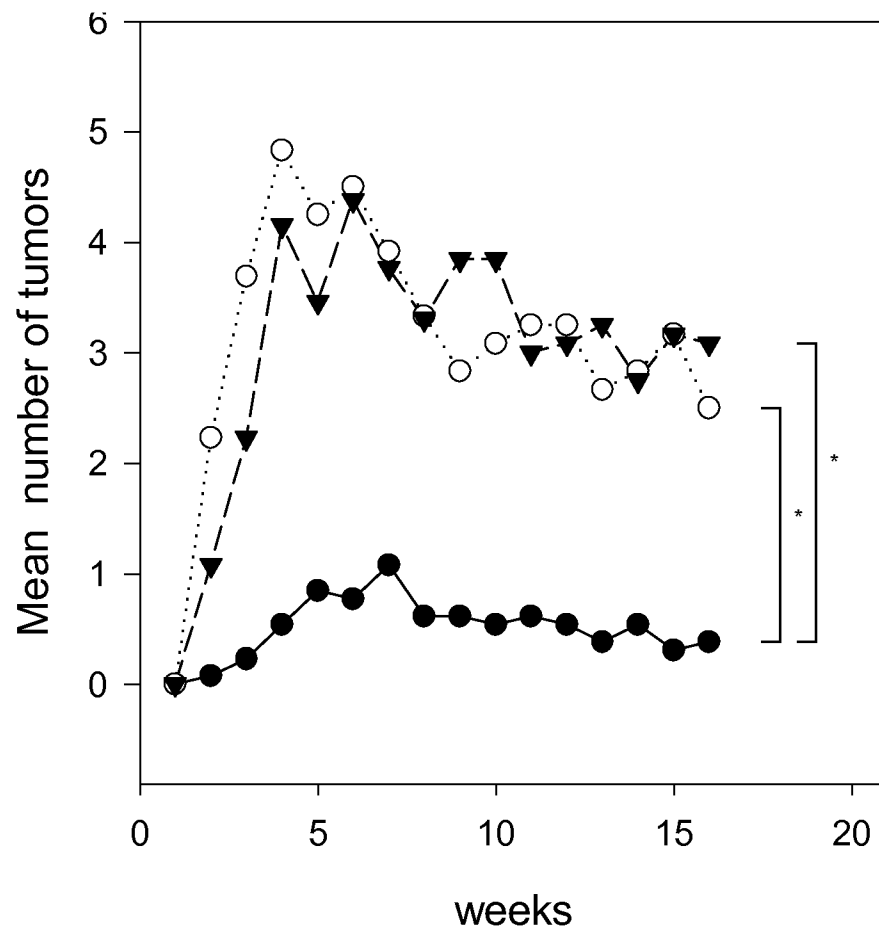


Fig. 3A

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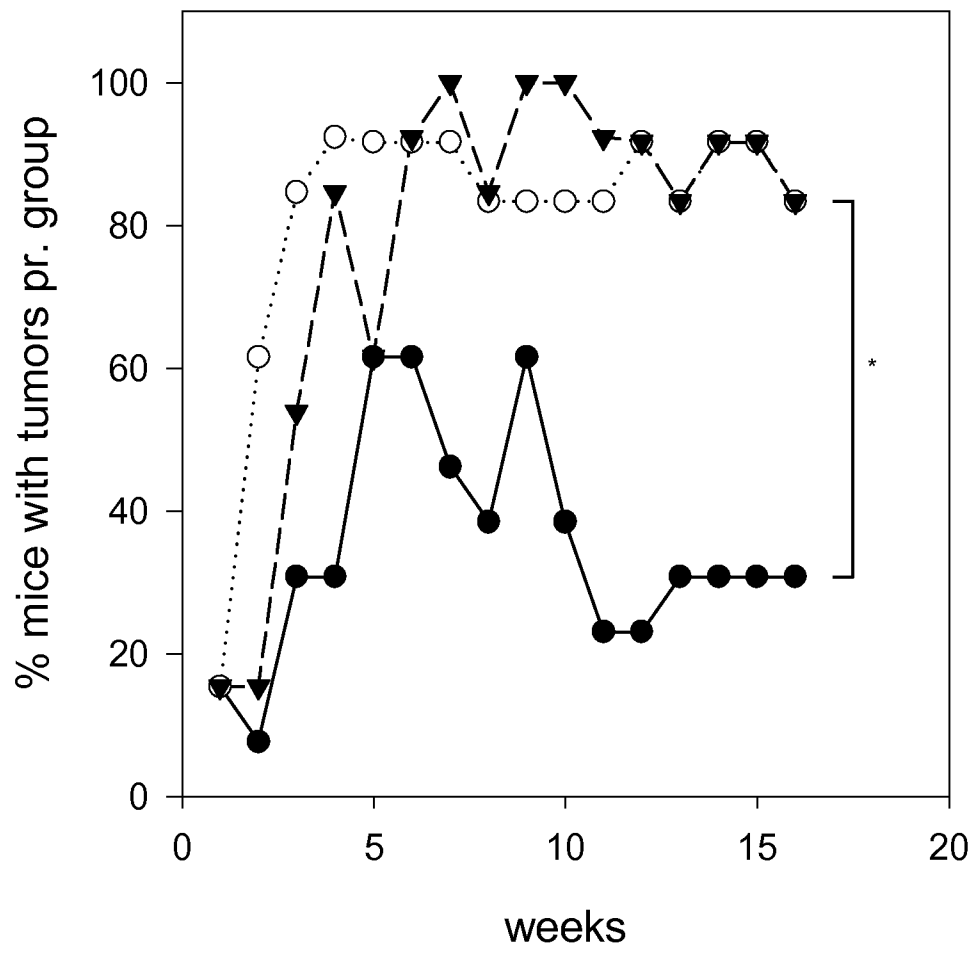


Fig. 3B

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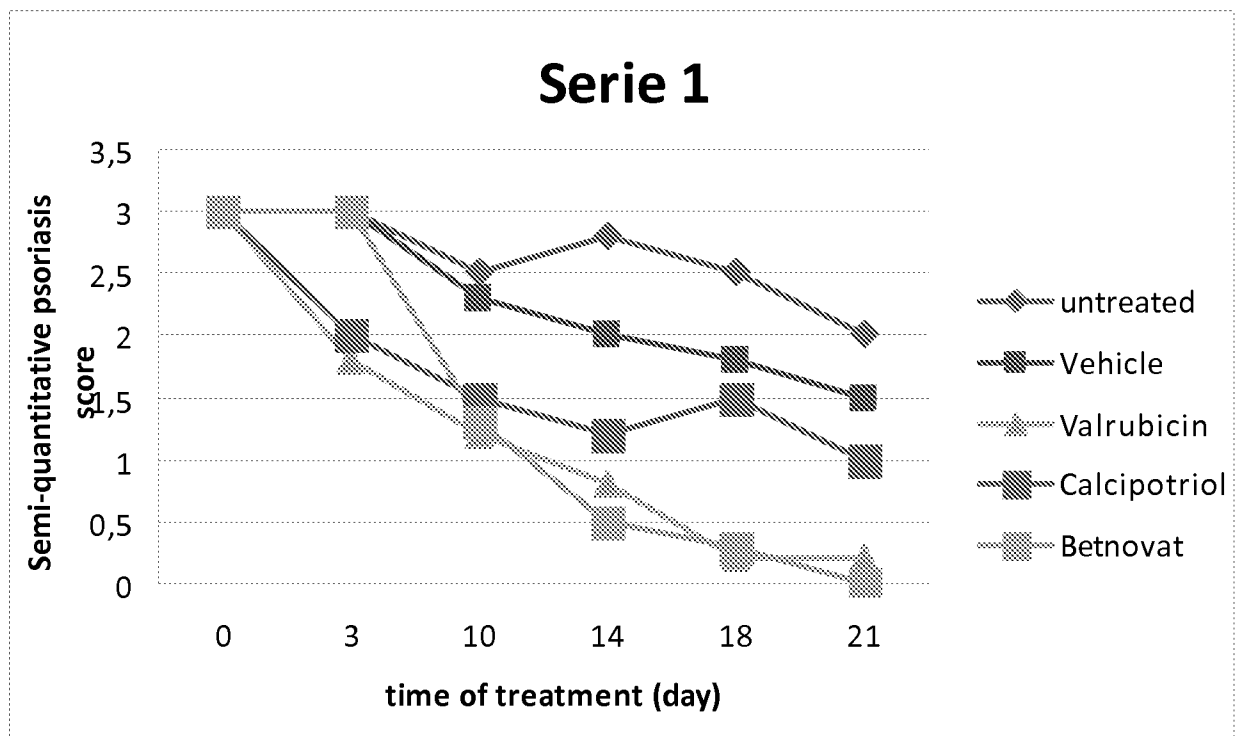


Fig. 4A

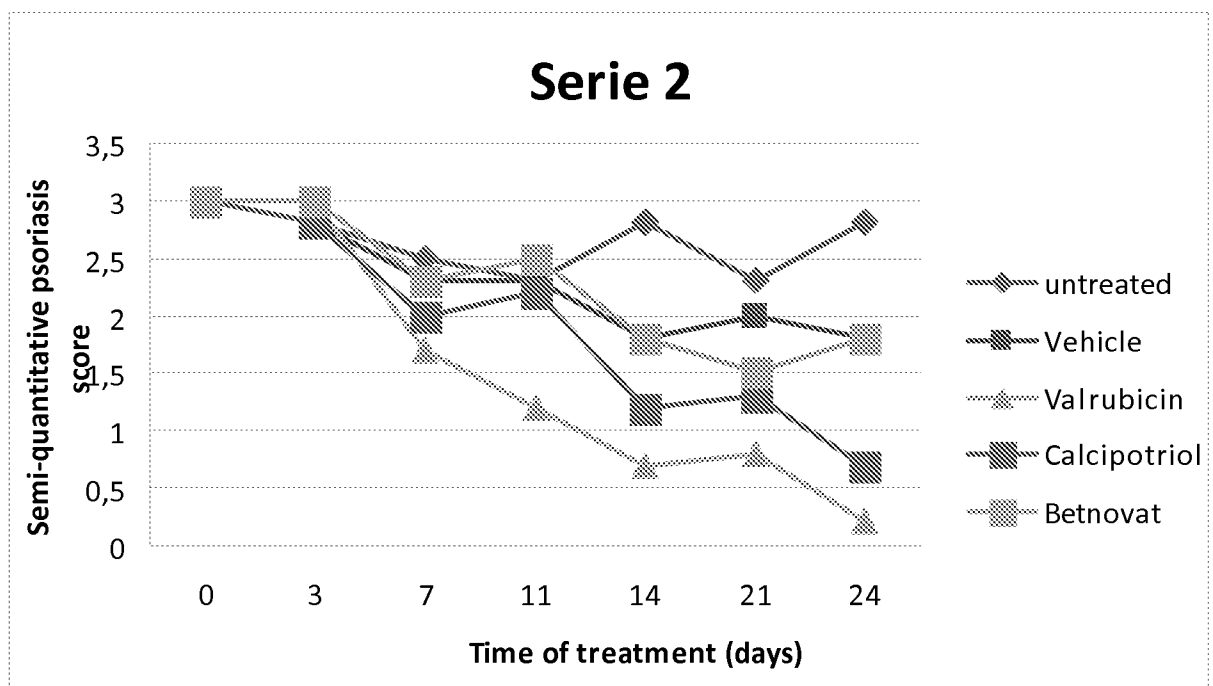


Fig. 4B

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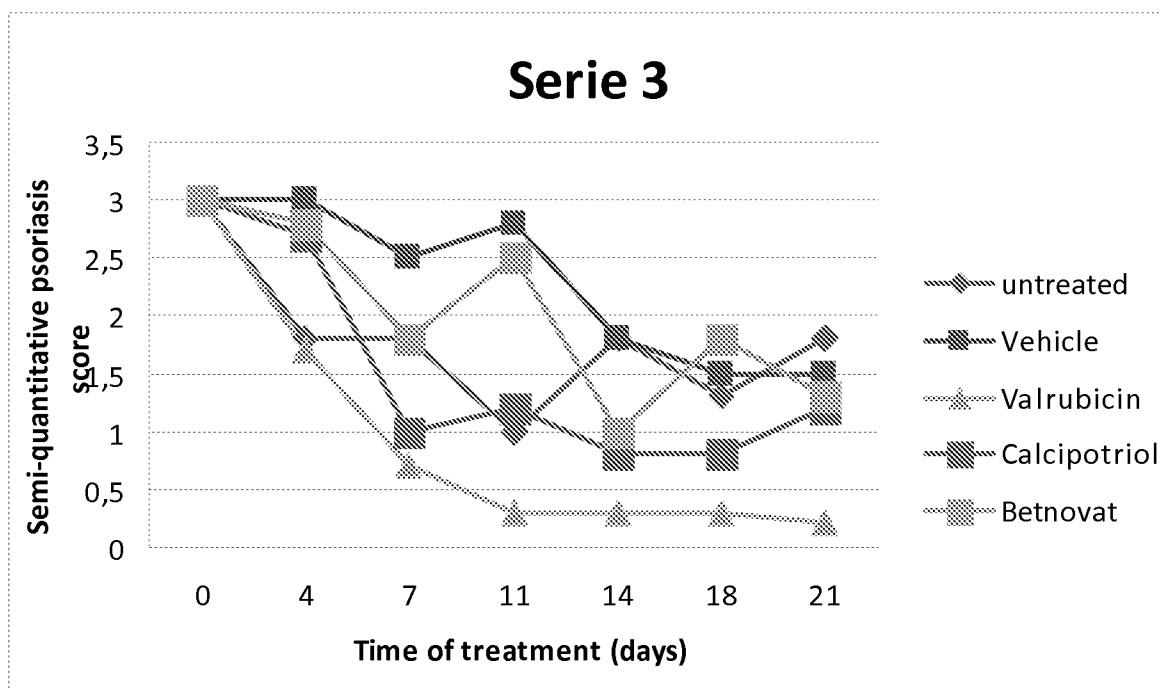


Fig. 4C

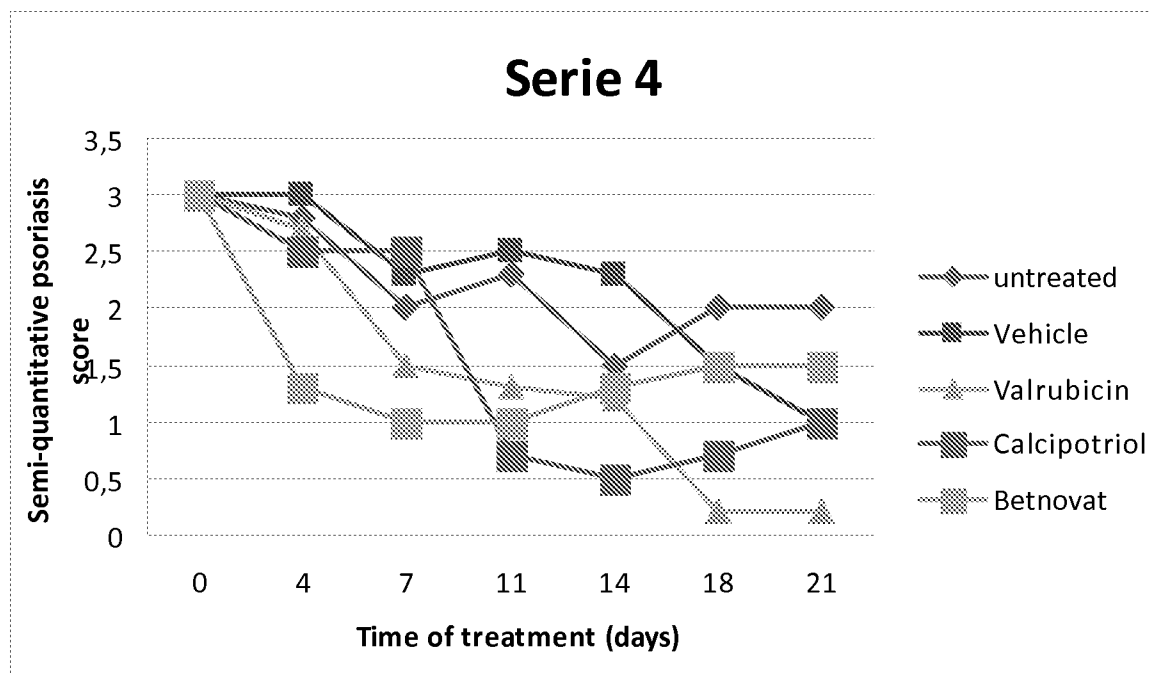


Fig. 4D

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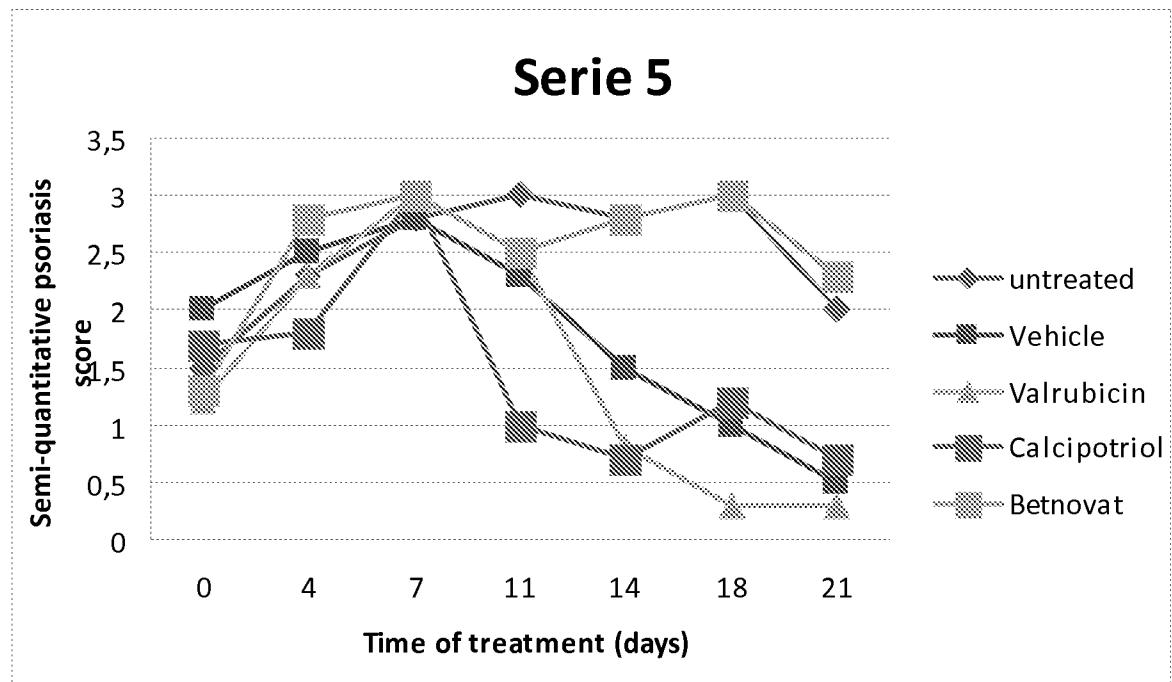


Fig. 4E

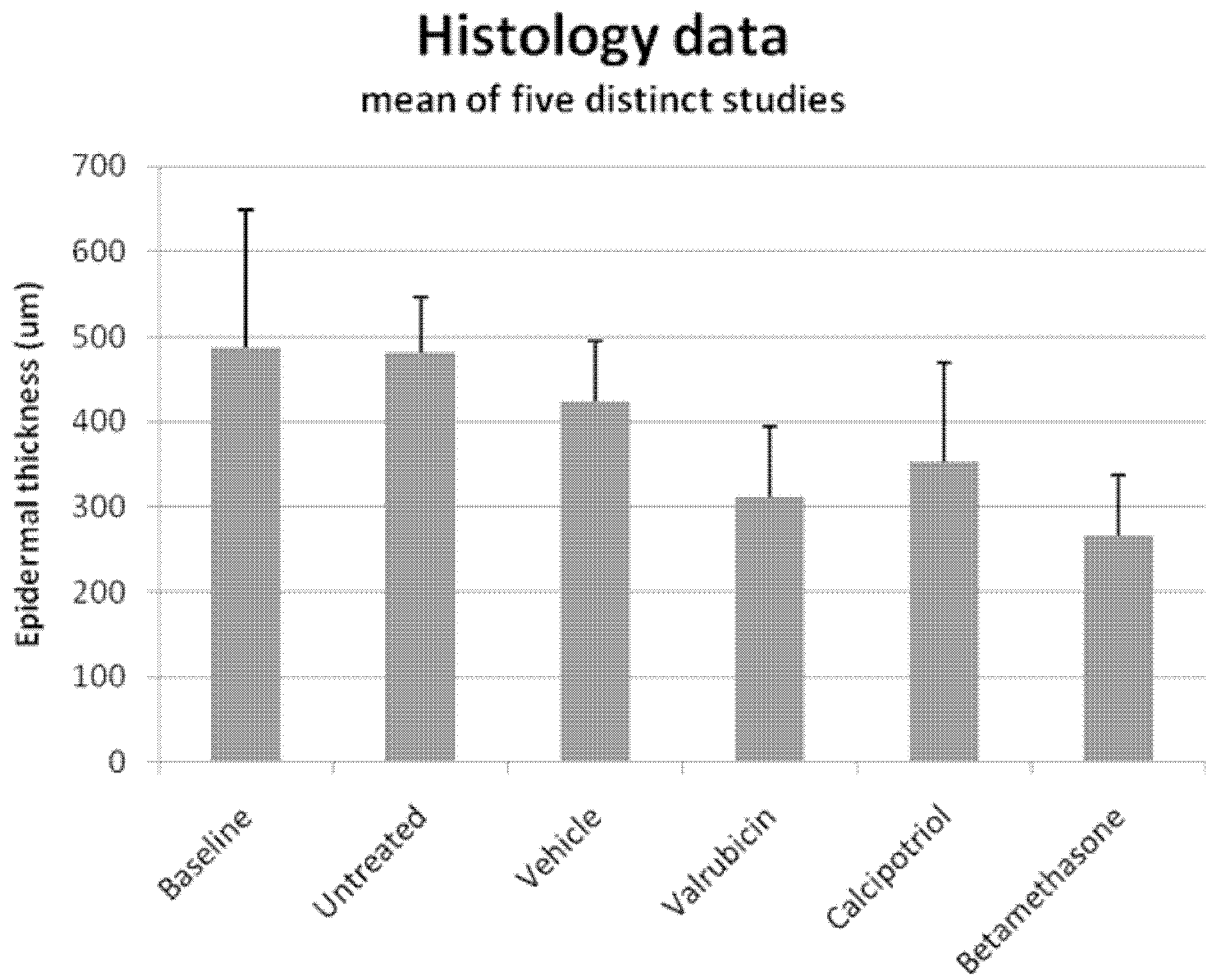


Fig. 4F

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Ca Ski cells (cervix SCC, HPV 16) 24 hr

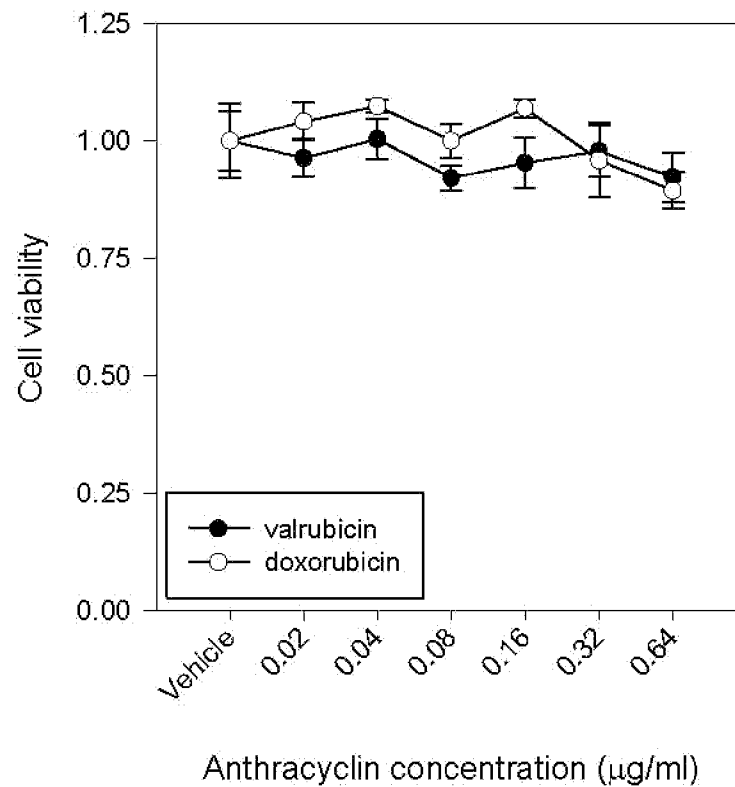


Fig. 5A

Ca Ski cells (cervix SCC, HPV 16) 48 hr

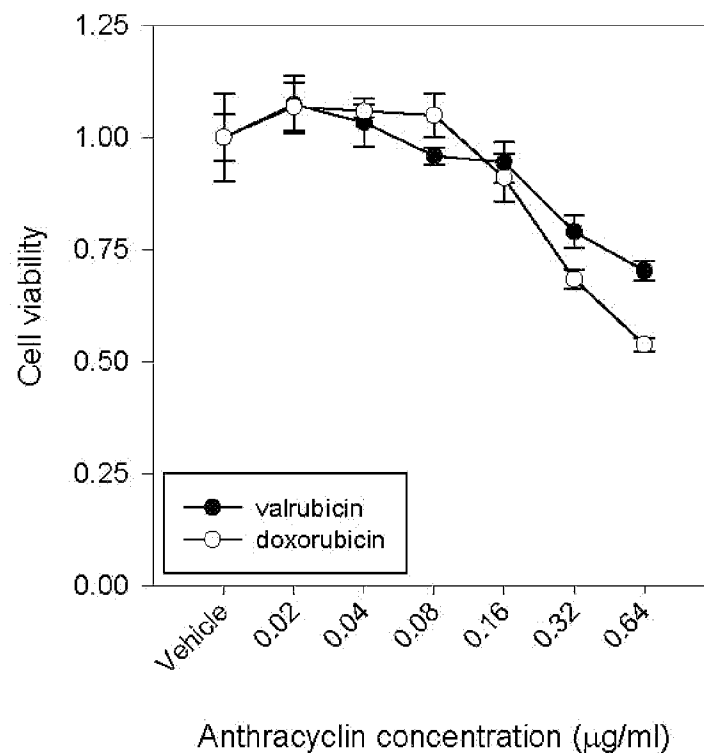


Fig. 5B

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Ca Ski cells (cervix SCC, HPV 16) 72 hr

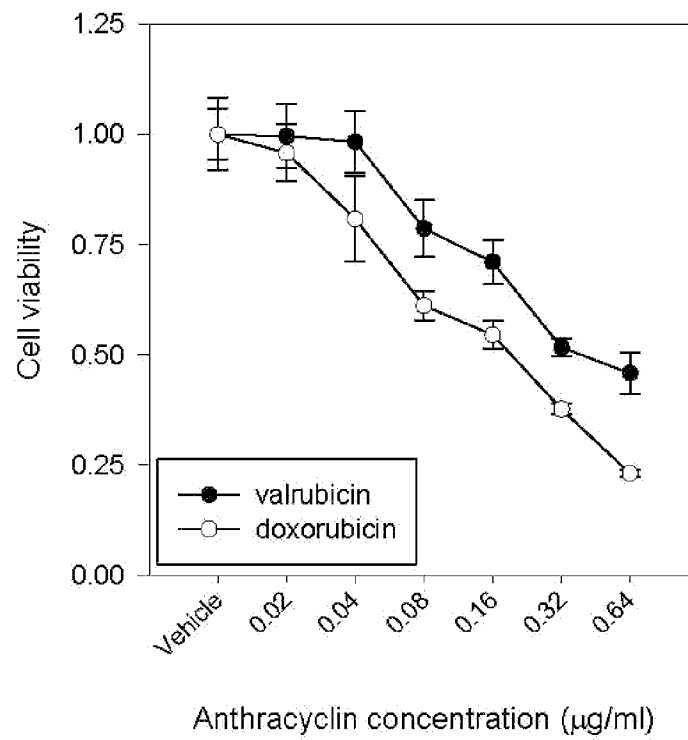


Fig. 5C

SW756 (cervix SCC, HPV 18) cells 24 hr

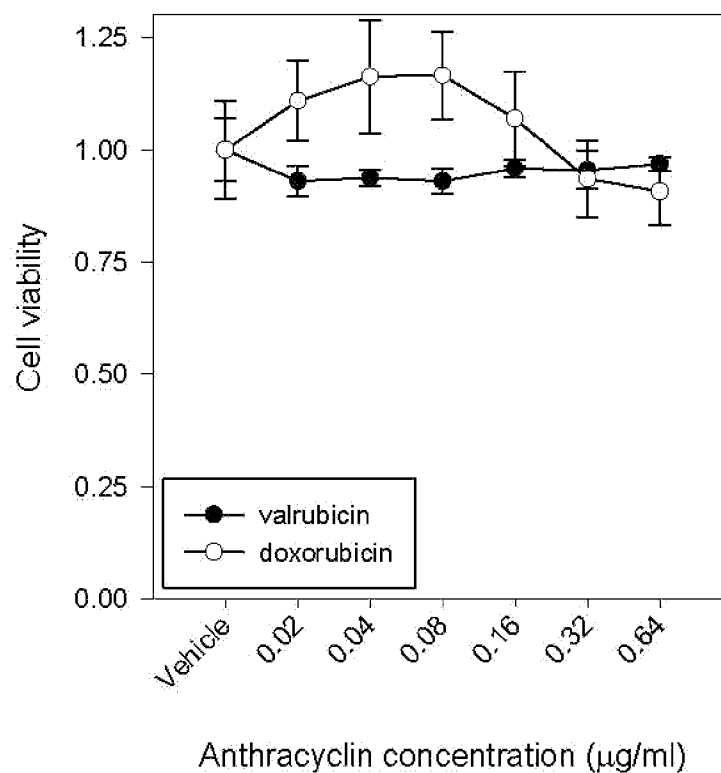


Fig. 5D

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SW756 (cervix SCC, HPV 18) cells 48 hr

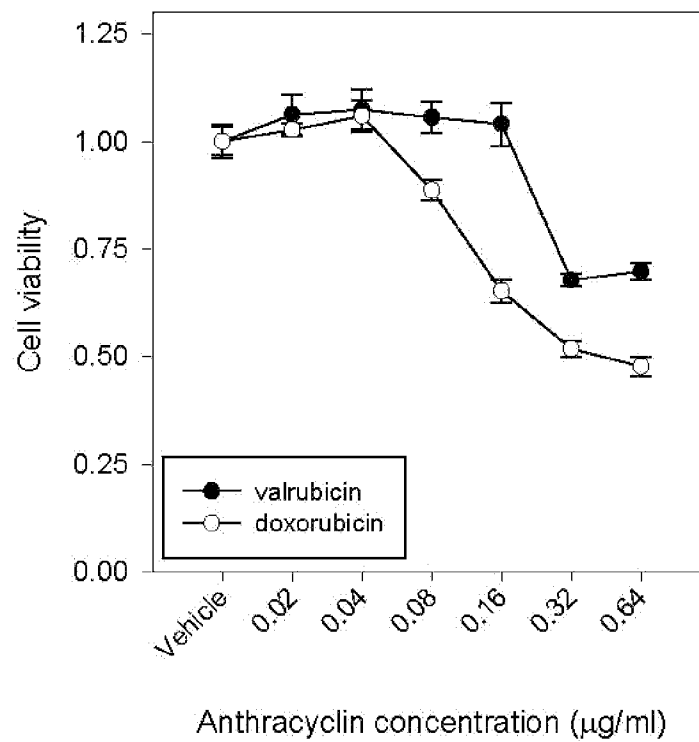


Fig. 5E

SW756 (cervix SCC, HPV 18) cells 72 hr

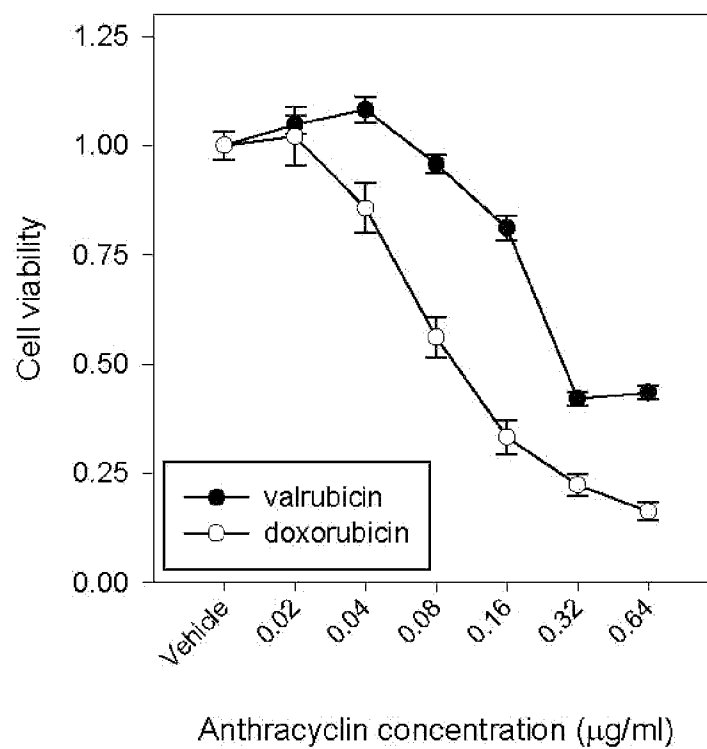


Fig. 5F

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SW954 (vulva SCC) cells 24 hr

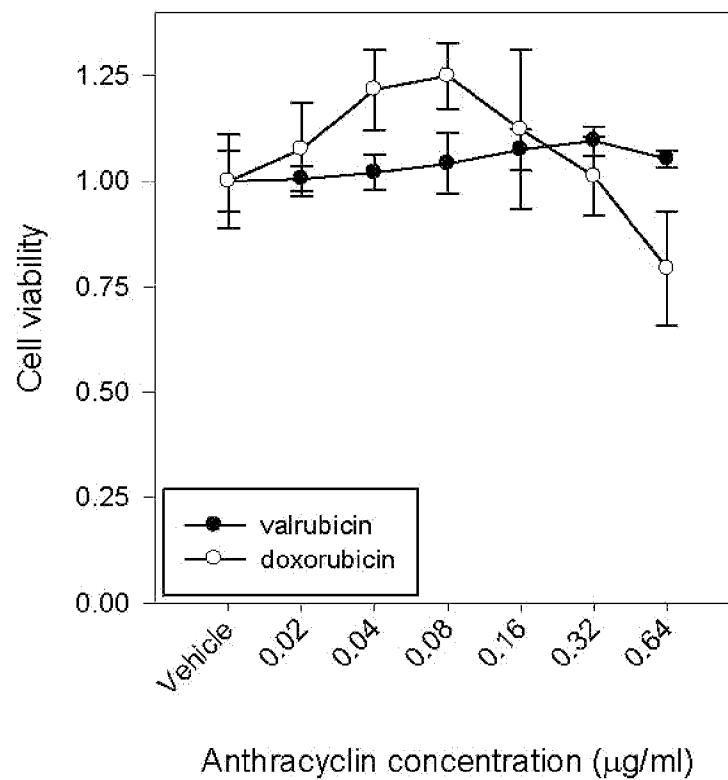


Fig. 5G

SW954 (vulva SCC) cells 48 hr

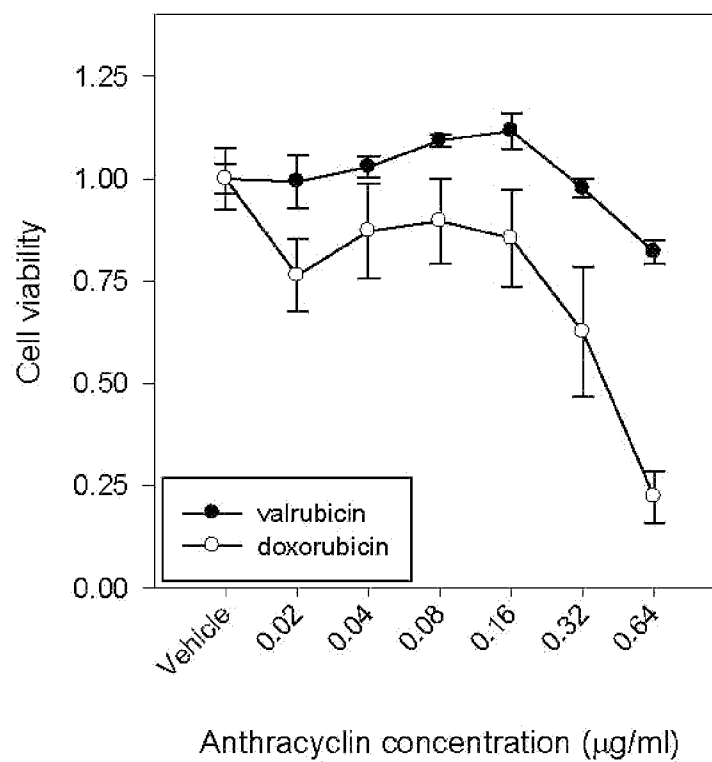


Fig. 5H

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SW954 (vulva SCC) cells 72 hr

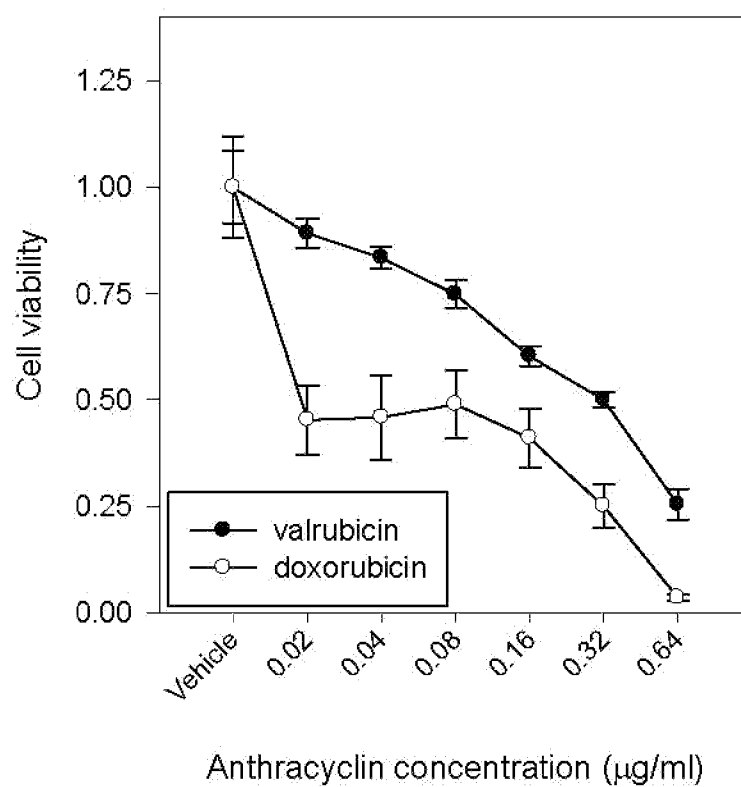


Fig. 5I

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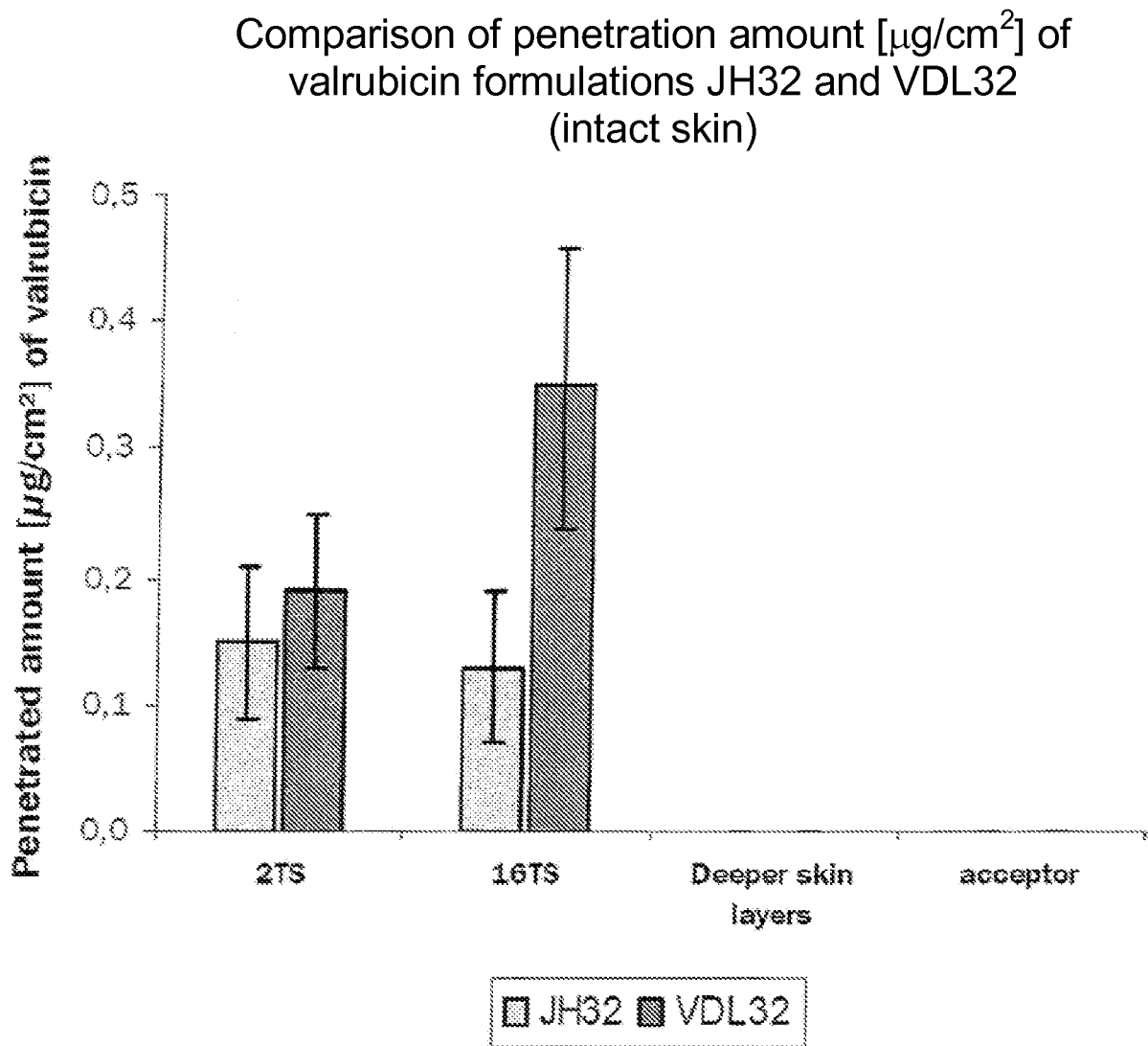


Fig. 6

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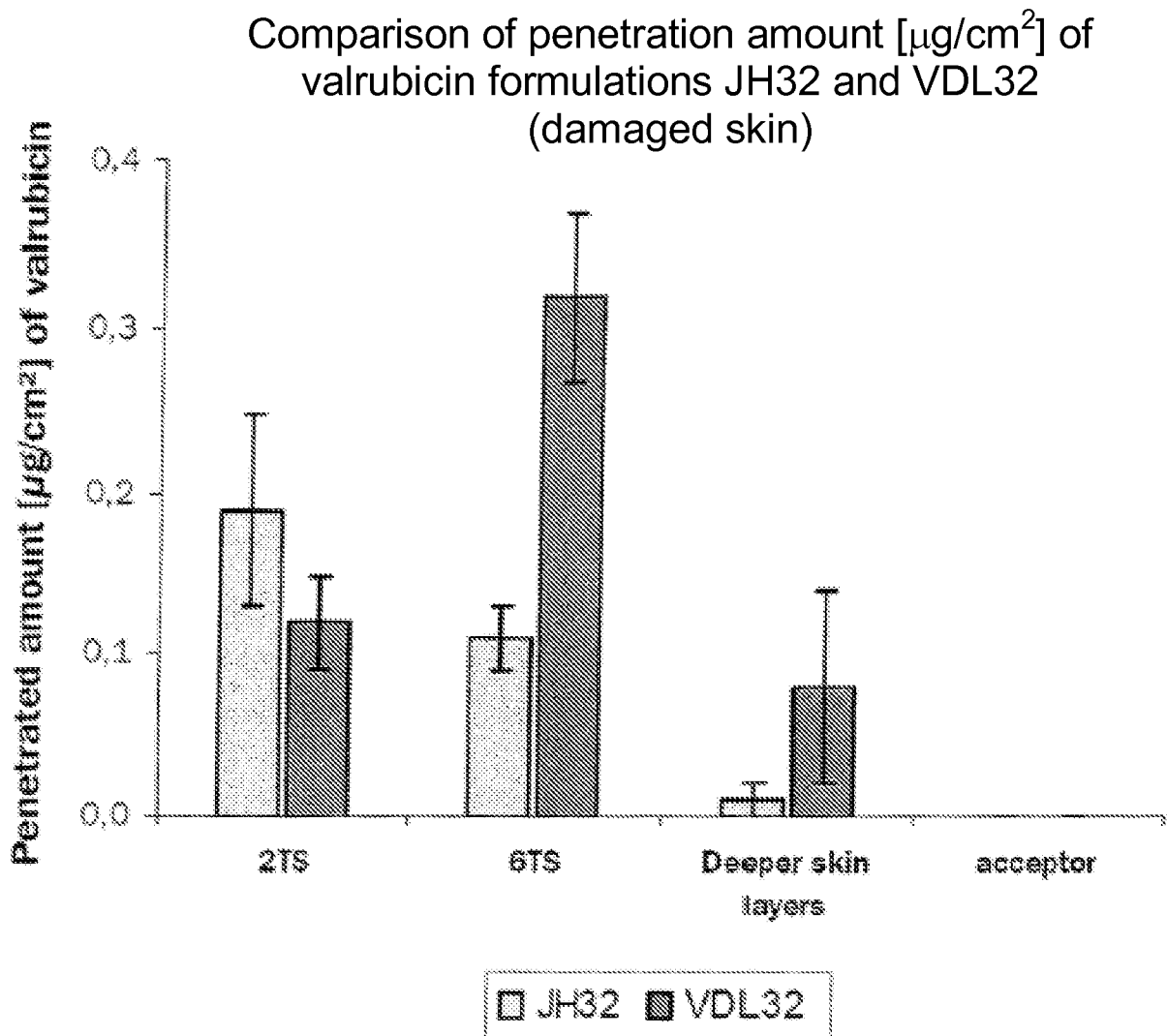


Fig. 7

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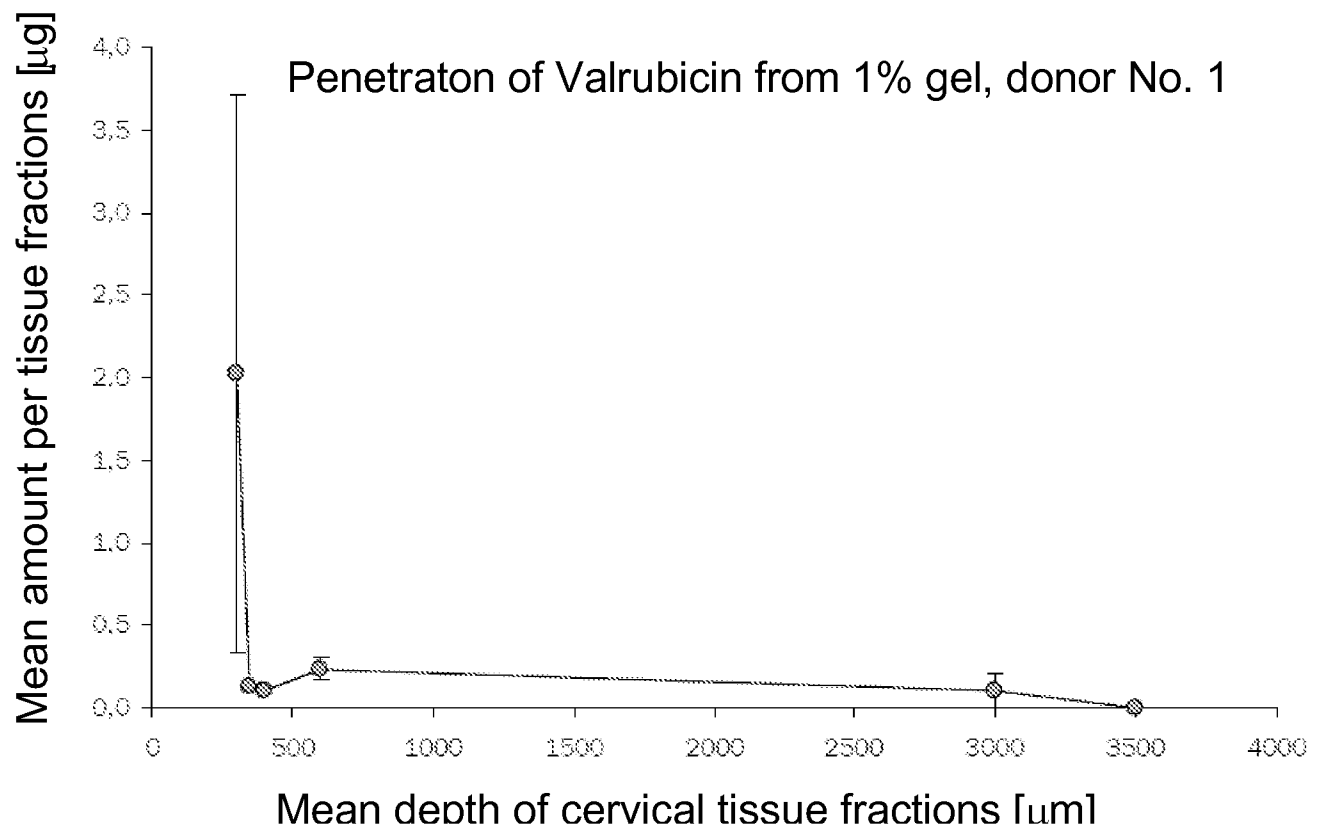


Fig. 8

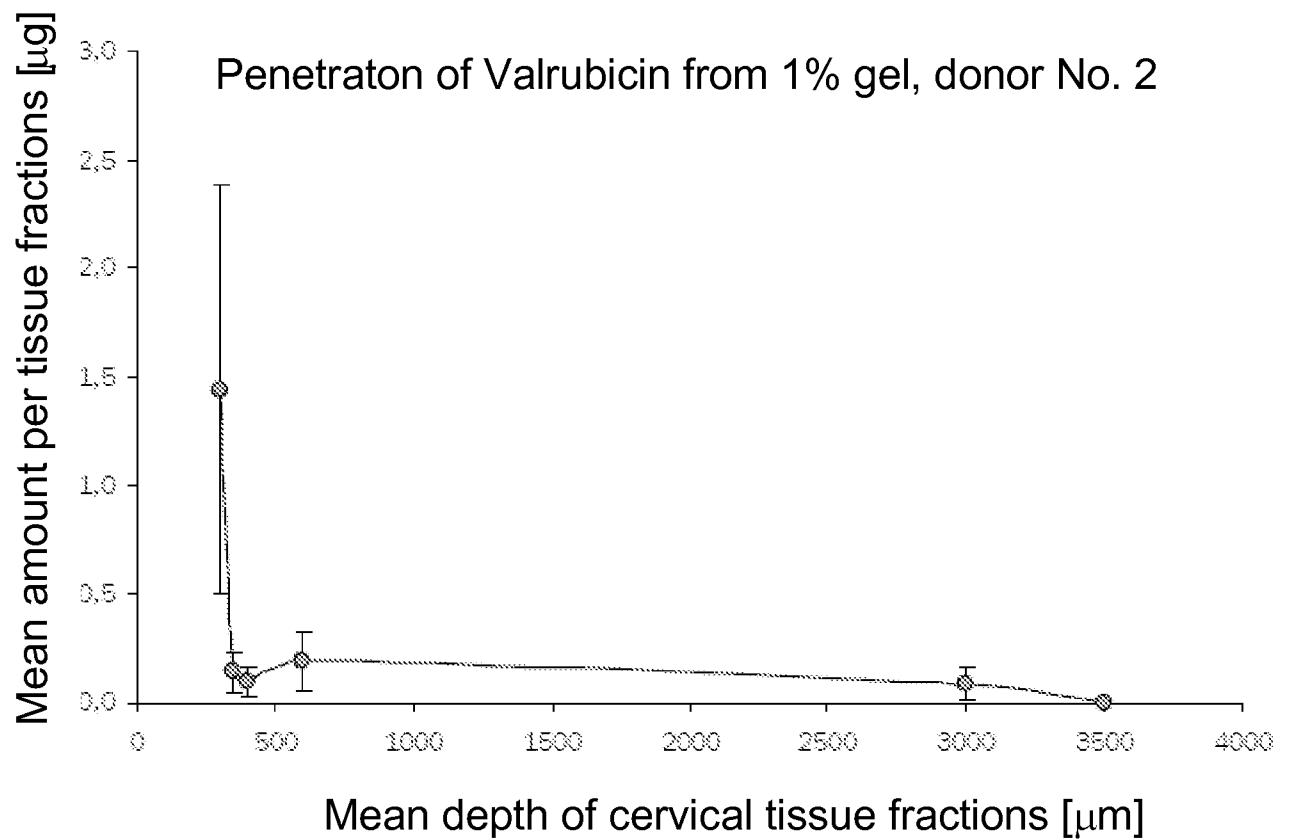


Fig. 9