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Pharmaceutical Composition

Background of the Invention

The present invention is concerned with pharmaceutical compositions suitable for the treatment of cancer, and in particular, with pharmaceutical compositions containing vitamin D or a precursor, analogue or metabolite thereof and the use of these compositions in the treatment of a tumor in a subject.

Vitamin D is an isoprenoid compound made up of activated 5-carbon units. The most abundant form of vitamin D is vitamin D₃, or cholecalciferol. Vitamin D₃ arises from biosynthesis of 7-dehydrocholesterol, an intermediate in cholesterol biosynthesis. Vitamin D₃ is metabolised in the liver to 25-hydroxycholecalciferol [25(OH)D₃] which is a major form of Vitamin D circulating in the blood compartment. 25(OH)D₃ is converted by the kidney to produce two principal dihydroxylated metabolites, namely, 1,25-dihydroxycholecalciferol [1,25(OH)₂D₃] and 24,25-dihydroxycholecalciferol [24R,25(OH)₂D₃].

1,25(OH)₂D₃ is the most biologically active naturally occurring form of vitamin D₃ and is transported in the bloodstream to its major site of action in the mucosal cells of the intestine, where calcium absorption is stimulated. Thus vitamin D₃ may be regarded as a prohormone because it is converted to a metabolite that acts analogously to a steroid hormone. It regulates calcium and phosphorous metabolism particularly in the synthesis of the inorganic matrix of bones.

Systemic administration of high doses of vitamin D₃ or its metabolites is limited by the production of hypercalcaemia. This has led to the development of analogues of activated vitamin D (D₃) which have a greater effect on cell growth than on calcium metabolism. These compounds have been found to be effective in the inhibition of growth of breast, rectal, colorectal and prostate cancer cells.

Disclosure of the Invention

We have found that tumors in certain organs, for example, primary and secondary tumors in the liver, can be treated by regional delivery of high concentrations of $1,25(\text{OH})_2\text{D}_3$ to the affected organ without giving rise to hypercalcaemia. Applicant's co-pending International Application No. 5 PCT/AU98/00440, the disclosure of which is incorporated herein by reference, discloses a method for the treatment of liver cancer by means of regional delivery of vitamin D or its metabolite or analogues to the liver.

The effect on tumors is very dose dependent and there is therefore 10 advantage in delivering higher concentrations of vitamin D compounds such as $1,25(\text{OH})_2\text{D}_3$. However, the limited solubility of vitamin D and its precursors, analogues and metabolites in a conventional carrier such as water places an upper limit on the concentration of the compound that can be delivered to the organ. Delivery of vitamin D_3 intra-arterially in a 15 conventional carrier limits the vitamin D_3 concentration in the blood going to the liver to at most 10^{-7} mole per litre.

We have found that very high concentrations of vitamin D compound such as $1,25(\text{OH})_2\text{D}_3$ can be achieved by dissolving $1,25(\text{OH})_2\text{D}_3$ in a lipid, for example, an iodised or non-iodised oil. A further advantage of using an oil as 20 the carrier for the vitamin D compound is that some oils are concentrated in certain cancers allowing the achievement of very high tumor concentrations of vitamin D_3 . Moreover, we believe that the use of a lipid as the carrier for the vitamin D compound results in a sustained antiproliferative activity of the vitamin D compound and the compound is retained for considerably 25 longer periods within the tumor.

Accordingly, in a first aspect, the present invention provides a pharmaceutical composition suitable for use in the treatment of cancer cells in an organ by regional delivery of the composition to the organ, the composition including a vitamin D compound and a pharmaceutically

acceptable lipid, wherein the concentration of the vitamin D compound in the composition is greater than about 1×10^{-7} mole per litre.

By the term "vitamin D compound" we include the biologically active and inactive forms of vitamin D. The vitamin D compound may be a precursor, metabolite or analogue of vitamin D. The vitamin D compound may be any analogue having anti-tumor properties. The vitamin D compound may be cholecalciferol, $25(\text{OH})\text{D}_3$ or $1,25(\text{OH})_2\text{D}_3$. Examples of analogues of vitamin D include, but are not restricted to, EB1089, OCT (22-oxa- $1,25(\text{OH})_2\text{D}_3$), 1α $25(\text{OH})_2$, 22,24 diene, 24, 26, 27 trihomo D_3 , MC903 (calcipotriol) and KH1060, $1,25(\text{OH})_2$ -16-ene 23-yne vitamin D_3 and its hexadeutero form. The vitamin D compound may be vitamin D_5 or an analogue thereof.

Preferably the concentration of vitamin D compound in the composition of the invention is at least about 1×10^{-6} mole per litre. The concentration of vitamin D compound may be at least about 1×10^{-5} mole per litre. The concentration may be at least about 1×10^{-4} mole per litre. The concentration may be at least 1×10^{-3} mole per litre. A preferred concentration of the vitamin D compound is about 1×10^{-6} to 50×10^{-5} mole per litre.

Preferably the lipid used is one for which the tumor is avid so that high concentrations of the vitamin D compound are delivered to the tumor. To determine whether the tumor being treated is lipid avid or not, a small dose of lipid may be given into the hepatic artery by a percutaneous or surgically placed catheter. A tumor that is not lipid avid is unlikely to benefit from the treatment to the same extent as a tumor that is avid to the the lipid used.

The pharmaceutically acceptable lipid may be an oil. A non-iodised oil is preferred. The non-iodised oil may be any pharmaceutically acceptable oil in which the vitamin D compound is soluble. The non-iodised oil may be a vegetable oil. The oil may be, for example, poppy seed oil, soybean oil, sesame oil, safflower oil, peanut oil, cremophore, Liposyn or Intralipid. The

oil may be derived from shark liver oil including squalane and squalene. The lipid may be medium chain triglycerides (MCT).

5 An iodised oil such as iodised poppy seed oil (lipiodol) can be used, however, the iodine has the effect of making the vitamin D compound more light sensitive.

The lipid may be in the form of an emulsion of these oils prepared with pharmaceutically acceptable emulsifying agents including, but not limited to, natural and synthetic phospholipids, Spans, Tweens or Pluronics.

10 We have found that very high concentrations of activated vitamin D₃ in lipiodol can be achieved. For example, 2 mg of 1,25(OH)₂D₃ can be readily dissolved in one ml of iodised poppy seed oil.

15 Apart from providing a composition that is more effective in the treatment of tumors, the higher concentrations of vitamin D compounds achievable in the composition of the invention provide a reservoir of the vitamin D compound, which is released over time, thus allowing for "one shot" administration of the composition.

20 Furthermore, because the composition of the invention is capable of providing higher concentrations of vitamin D, even non-active forms of vitamin D may be used to treat tumors. Although at low concentrations these non-active forms may not be effective treating tumors, at very high concentrations they may become effective.

25 The composition of the invention may further include one or more other components. The present composition may contain a component that is capable of increasing vitamin D receptor expression. The composition may include an estrogen, estrogen-like compound or estrogen antagonist. The component may be tamoxifen.

The composition may be used to treat primary or secondary tumors in any organ to which the composition can be administered by regional delivery.

Accordingly, the present invention further extends to a method for the treatment of a tumor in an organ in a subject, the method including regional administration to the organ of a composition in accordance with the present invention.

5 The regional delivery may be by means of intra-arterial delivery, for example, by intra-arterial infusion. Alternatively, the composition of the invention may be delivered to the portal vein. The tumor may be a primary or secondary tumor. The organ treated may be the liver, breast, prostate, bone tumor or kidney. The composition of the invention may also be used to treat
10 colorectal cancer or sarcomas. The method and composition of the present invention are particularly suitable for the treatment of hepatoma.

 The vitamin D compound may be dissolved in lipid as described above, for example, iodised or non-iodised poppy seed oil to the desired concentration of vitamin D compound.

15 The method of the invention may be repeated at monthly, or other, intervals. It will be readily apparent that the composition and method of the present invention provide the capability of delivering very high local concentration of vitamin D substance delivered by the composition of the present invention will achieve tumor control and whilst avoiding
20 hypercalcaemia.

 In order to more fully describe the invention we provide the following non-limiting examples.

Brief Description of the Drawings

25 Figure 1 is a photograph taken under a microscope showing uptake of lipiodol by HepG2 cells exposed to a medium with 1% lipiodol or without lipiodol (control). Lipiodol is stained red using the Oil red O technique.

 Figure 2 is a graph showing [³H]thymidine incorporation in HepG2 cells after 5 days treatment with different doses of vitamin D₃ in normal

medium (—●—) or in 1% lipiodol (—○—). Values represent mean $\pm$ s.e.m. (***) = $p < 0.001$ using unpaired t-test).

Figure 3 provides bar graphs depicting cell counts per well (% of control) after HepG2 cells were exposed to vitamin D₃ for 5 days with different doses of vitamin D₃ dissolved in normal medium (a) or in 1% lipiodol (b). Values represent mean $\pm$ s.e.m. (***) = $p < 0.001$ using unpaired t-test).

Figure 4 provides graphs showing [³H]thymidine incorporation in HepG2 cells after one day exposure to different doses of vitamin D₃ in normal medium (—■—) or in 1% lipiodol (—□—), followed by 4 days exposure to medium without the drug. Values represent mean $\pm$ s.e.m. (***) = $p < 0.001$ using unpaired t-test.

Figure 5 provides bar graphs showing cell counts per well (% control) after HepG2 cells were exposed for 1 day to different doses of vitamin D₃ dissolved in normal medium (a) or in 1% lipiodol (b), followed by 4 days exposure to medium without the drug. values represent mean $\pm$ s.e.m. (***) = $p < 0.001$ using unpaired t-test).

Figure 6 provides bar graphs showing the 1 hour post injection level of radioactive vitamin D₃ in the liver, tumor and plasma of Sprague-Dawley rats following administration of vitamin D₃/Tween, vitamin D₃/MCT and vitamin D₃/lipiodol respectively as described in paragraph 2 below (*In vivo* studies in tumor bearing rats).

Figure 7 is a bar graph giving a summary of the 1 hour results shown in Figure 6.

Figure 8 provides bar graphs showing the 1 day post injection level of radioactive vitamin D₃ in the liver, tumor and plasma of Sprague-Dawley rats following of vitamin D₃/Tween, vitamin D₃/MCT and vitamin D₃/lipiodol respectively as described in paragraph 2.

Figure 9 is a bar graph giving a summary of the 1 day results shown in Figure 8.

Figure 10 provides bar graphs showing the 3 day post injection level of radioactive vitamin D₃ in the liver, tumor and plasma of Sprague-Dawley rats following administration of vitamin D₃/Tween, vitamin D₃/MCT and vitamin D₃/lipiodol respectively as described in paragraph 2.

5 Figure 11 is a bar graph giving a summary of the 3 day results shown in Figure 10.

Figure 12 provides graphs showing the effect of intra-hepatic arterial administration of vitamin D₃ (50µg) on AFP and calcium levels in a patient with HCC.

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Modes for carrying out the Invention

1 Cell culture studies

1.1 Materials and methods:

1.1.1 Uptake studies

For the uptake studies, monolayers of HepG2 cells were grown on sterile microscope cover glass, placed in 6 well tissue culture plates. Cells were exposed (24 h) to culture media (MEM - 5% FBS) containing 1% lipiodol and stained by an oil- red O impregnation technique. This briefly involved fixation of the cells by 10% formalin followed by staining, differentiating and counter staining. Cells were thoroughly washed with phosphate buffer solution (pH 7.4), and water through out the fixation procedure. After fixation, cells were viewed and photographed under the microscope.

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1.1.2 Cell proliferation assays

Following exposure of cells to vitamin D₃ dissolved in normal or lipiodol (1% v/v) containing media, [³H]thymidine incorporation assay and the trypan blue exclusion cell count methods were used to determine cell proliferation. To do this, cells were plated in 24 well tissue culture plates at 10⁴ cells per well and incubated for 24 h in an incubator at 37°C with humidified 5% CO₂ atmosphere. The, medium was replaced with one containing vitamin D₃(10⁻¹¹ to 10⁻⁷ M) dissolved in either the normal medium

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or first dissolved in lipiodol and then mixed thoroughly with the medium to make a final lipiodol concentration of 1% (v/v). For acute dose studies, cells were exposed to one of the two drug containing media for 24 h, which were then replaced with drug free medium for the next four days. Whilst, in
5 chronic treatment, cells were exposed for 5 days to different concentrations of the drug dissolved in one of the media described above. The media were changed on alternate days. At the end of treatment period, cell cultures were assayed for thymidine incorporation by the addition of 0.1 μ Ci of
10 [3 H]thymidine to each well for the last 4 h of culture. The amount of radioactivity incorporated into trichloroacetic acid precipitable material was determined using a β -scintillation counter. Results are plotted as percentage inhibition relative to control. The control cells for the vitamin D₃/lipiodol group were treated with a lipiodol-containing medium (1%).

For the cell count assay, cells were plated in six well plates at a
15 concentration of 5×10^4 cells per well. Cell treatment procedure was as described for the thymidine assay. At the end of the treatment period (5 days), cells were trypsinised and counted with a hemocytometer. All counts were obtained in quadruplicate and each experiment was repeated at least twice. Unpaired Student's t-test was used for comparisons of treated versus
20 control values and $p < 0.05$ was considered to represent a significant difference.

1.2 Results

HepG2 cells exposed to lipiodol consistently demonstrated multiple
25 intracellular red vesicles of lipiodol on oil-red O impregnation, while, there was no sign of lipiodol in control cells (Fig 1).

When treated chronically, HepG2 cells showed significant reduction in thymidine incorporation at all concentrations of vitamin D₃ and in both delivery systems used. However, in cells exposed to the vitamin D₃/lipiodol
30 delivery system, the degree of inhibition of thymidine incorporation was significantly greater (Fig 2).

Similarly, upon chronic treatment of cells with either of the test media, cell proliferation was inhibited resulting in a dose dependant decrease in cell number (Fig 3). In acute studies, where cells were only exposed to the
35 medium containing the drug for 1 day and to the medium without the drug for a further 4 days, compared to control, cells treated with vitamin D₃ made

up in the medium alone, did not show a reduction in thymidine incorporation while those exposed to vitamin D₃ in lipiodol, showed significant reductions in thymidine incorporation (Fig 4). In the cell count method, a significant reduction in cell number was observed in wells exposed to the 10⁻⁷ M concentration of vitamin D₃, while the vitamin D₃ lipiodol treated plates, wells exposed to 10⁻⁹, 10⁻⁸ and 10⁻⁷ M all showed significant reductions in the number of cells available within the wells (Fig 5).

Note:

1. The experiments described above were all also repeated using 1% MCT instead of 1% lipiodol in an exactly similar manner. The results obtained for MCT too, are also very similar to those of lipiodol showing significantly greater inhibition of proliferation in cells exposed to vitamin D₃/MCT compared to those exposed to vitamin D₃/medium.

2. In order to find an appropriate cell line, which would grow in the rat tumor and also be responsive to vitamin D₃, rat cell lines, Novikoff and HTC were screened for responsiveness to vitamin D₃ (10⁻¹¹ - 10⁻⁶ M). Results obtained show that, Novikoff is a non responsive cell line to vitamin D₃, while with HTC, an approximately 50% inhibition of proliferation was achieved with the 10⁻⁶ M concentration of the drug.

2. In vivo studies in tumor bearing rats

2.1 Materials and methods:

Male 300-350 gr. Sprague-Dawley rats were given a general anaesthetic (halothane) and a laparotomy performed. Then 5 x 10⁶ Novikoff cells suspended in 100 µl of medium was instilled beneath the liver capsule using a 26G3/8-tuberculin needle. The abdominal incision was then closed with gut suture.

All procedures were carried out under general anaesthesia and before reversing anaesthesia, animals were given a subcutaneous injection of the lipiodol analgesic buprenorphine (0.025 mg/kg).

Animals were then left for 10 days with food and water available *ad libitum* and monitored daily to assess health and well being. After this

period, animals were randomly allocated to one of the treatment groups (n=6 per group) and lapratomy was performed again. A catheter was inserted into the hepatic artery of the animal, through which radiolabelled vitamin D₃ or vehicle injections were carried out. The different experimental groups treated were as follows:

- Groups 1-3: receiving 100 µl of lipiodol
- Groups 4-6: receiving vitamin D₃ (hot + cold) in 100 µl of lipiodol
- Groups 7-9: receiving 100 µl of MCT
- Groups 10-12: receiving vitamin D₃ (hot + cold) in 100 µl of MCT
- Groups 13-15: receiving 100 µl of Tween
- Groups 16-18: receiving vitamin D₃ (hot + cold) in 100 µl of Tween

After administration of the drug, the catheter was removed and the incision sutured. Animals were euthanased at 1 hour, 1, and 3 days post drug (or vehicle) administration. Just before euthanasing, 3 ml of blood was drawn through cardiac puncture from each animal.

Post mortem, the liver and the tumor of each animal was excised, weighed and frozen in the freezer until analysis. After tissue homogenization, liver, tumor or plasma was extracted with dichloromethane. The amount of hot vitamin D₃ present in each sample was determined using a β-counter.

2.2 Results

Results obtained are presented in Figures 6-11. From these results it can be seen that at 1 hour the level of radioactivity is high in all three samples of liver, tumor and plasma. While, in the 1 and 3 day samples the difference between tumor and those of liver and plasma is very different with much higher levels being available in the tumor for both lipiodol and MCT. Summary of the results for each time point (Figures 7,9 and 11) clearly shows that in each case the level of radioactivity (vitamin D or metabolites) was greater in lipiodol treated animals. This in fact is a confirmation of the results obtained in the in vitro studies, showing that vitamin D₃ dissolved in lipiodol or MCT is taken up, retained and eventually released within the

tumor. While, vitamin D₃ dissolved in a normal vehicle like Tween is rapidly cleared from the tumor and the body.

3. Human studies: Phase 1 clinical trial

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3.1 Patient treatment

Patients suffering from primary HCC were selected according to the inclusion/exclusion criteria. After conduction of all medical and paramedical tests, patients received a single dose of 50 µg vitamin D₃ dissolved in 5 ml of lipiodol through the hepatic artery administered by an expert radiologist. Drug formulation was carried out under complete aseptic conditions in the oncology pharmacy unit of the hospital. Every 24 hours post drug administration, blood samples were withdrawn for the determination of serum AFP, calcium and vitamin D₃ levels daily for 3 days after which the patient was discharged from the hospital and visited once a week for 4 weeks (in total after drug administration).

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3.2 Results

Results obtained for AFP and calcium levels in the one patient who has completed the treatment so far are presented in figure 12.

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It can be seen that the AFP levels dropped significantly, reached the lowest level around day 7 and remained low till day 15 after which it was back to pretreatment levels at around day 28. With regards to the calcium level, there was a sharp rise in this between days 2-4 but it was back to normal by day 7.

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Discussion

Comparing the results for the different delivery systems clearly shows an enhanced and lasting inhibitory activity for vitamin D₃ in the lipiodol delivery system. This could be due to a number of factors including uptake retention and sustained release of vitamin D₃ from lipiodol. Previous reports (1,2) employing different techniques, have shown the uptake of lipiodol by HCCs. Our results in this section, using HepG2 cell line and oil-red O

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impregnation technique clearly demonstrates the abundant uptake of lipiodol by these cells which is presumably brought about by endocytosis.

It has been shown that, the oil is retained within the cells as for the life of the cell (1). The prolonged intracellular retention of lipiodol appears to be a specific capability of hepatoma cells, as normal liver cells appear to take up and excrete it rapidly in a few days as compared to the very long retention of it by HCCs (2). Consequently, it may be hypothesized that the increased inhibitory activity observed during chronic treatment with vitamin D₃/lipiodol, might partly be due to the accumulation of lipiodol and hence vitamin D₃ within the cell. However, as can be seen from the results, this alone cannot account for the difference obtained between the two delivery systems. Another contributing factor might be the higher stability of vitamin D₃ in lipiodol as this may prevent extensive degradation and rapid exposure of the drug to the metabolizing enzymes (3). This can particularly be true in the present setting where vitamin D₃ is a drug which due to its chemical structure is vulnerable to extensive metabolism and to add to this, HepG2 is a cell line with extensive metabolizing capacities (4).

The data in the acute treatment experiments where a 24-h exposure of cells to a single dose of vitamin D₃/lipiodol results in significant inhibition of proliferation, may perhaps indicate the sustained release of vitamin D₃ from the intracellular lipiodol depots. This might well be the reason behind the differences observed in the greater and lasting inhibitory activity of vitamin D₃ in lipiodol or MCT compared to that of vitamin D₃ in the medium alone. Results obtained in the Novikoff tumor bearing rats point put to the same point and confirm the validity of the cell culture studies in that vitamin D₃ in lipiodol and MCT, is taken up, retained and gradually released from the tumor HCC cells. However, as Novikoff is a vitamin D₃ resistant cell line, efficacy experiments could not be conducted in this model.

Although too early to comment, but results obtained in the one patient treated so far with the 50 µg dose of vitamin D₃/5ml lipiodol is in line with the experimental data obtained in cell culture and rat studies. Plasma sample assay for vitamin D₃ levels (currently ongoing), should clearly show the behaviour of vitamin D₃/lipiodol in HCC.

In conclusion, the results presented above suggest that, vitamin D₃ dissolved in lipiodol produce an increased and sustained antiproliferative activity in the human HCC cell line HepG2 and is retained for considerably

longer periods within the tumor as shown by the liver tumor experiments in the rat.

References:

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It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A pharmaceutical composition suitable for use in the treatment of a tumor in an organ by regional delivery of the composition to the organ, the composition including a vitamin D compound in a pharmaceutically acceptable lipid, wherein the concentration of the vitamin D compound in the composition is greater than about 1×10^{-7} mole per litre.
2. A composition according to claim 1 wherein the vitamin D compound is vitamin D or a precursor, metabolite or analogue thereof.
3. A composition according to claim 1 or claim 2 wherein the concentration of the vitamin D compound in the composition is at least about 1×10^{-6} mole per litre.
4. A composition according to claim 3 wherein the concentration of the vitamin D compound is at least about 1×10^{-5} mole per litre.
5. A composition according to claim 4 wherein the concentration of the vitamin D compound is at least 1×10^{-4} mole per litre.
6. A composition according to claim 5 wherein the concentration of the vitamin D compound is at least 1×10^{-3} mole per litre.
7. A composition according to any one of the preceding claims wherein the vitamin D compound is selected from the group vitamin D_3 , $25(OH)D_3$, $1,25(OH)_2D_3$ and $24R, 25(OH)_2D_3$ or a precursor, metabolite or analogue thereof.
8. A composition according to any one of claims 1 to 6 wherein the vitamin D compound is selected from the group EB1089, OCT (22-oxa-1,25(OH) $_2D_3$), $1\alpha 25(OH)_2$, 22,24 diene, 24, 26, 27 trihomo D_3 , MC903

(calcipotriol) and KH1060, 1,25(OH)₂-16-ene 23-yne vitamin D₃ and its hexadeutero form.

5 9. A composition according to any one of claims 1 to 6 wherein the vitamin D compound is vitamin D₅ or an analogue or metabolite thereof.

10. A composition according to any one of the preceding claims wherein the lipid is a lipid for which the tumor is avid.

10 11. A composition in accordance with any one of the preceding claims wherein the lipid is a pharmaceutically acceptable oil.

12. A composition according to claim 11 wherein the oil is a non-iodised oil
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13. A composition according to claim 12 wherein the oil is poppy seed oil.

14. A composition according to claim 11 wherein the oil is an iodised oil.

20 15. A composition according to claim 14 wherein the oil is iodised poppy seed oil (lipiodol).

16. A composition according to any one of the preceding claims wherein the composition further includes a component for increasing vitamin D
25 receptor expression.

17. A composition according to claim 16 wherein the further component is an estrogen, estrogen-like compound or estrogen antagonist.

18. A composition according to claim 16 wherein the further component is tamoxifen.

5 19. A method for the treatment of a tumor in an organ in a subject, the method including regional administration to the organ of a composition in accordance with any one of the preceding claims.

10 20. A method according to any one of claim 19 wherein the organ treated is selected from the group liver, breast, prostate, bone tumor and kidney.

21. A method according to claim 20 wherein the organ is the liver.

15 22. A method according to any one of claims 19 to 21 wherein the regional delivery is by means of intra-arterial delivery.

23. A method according to claim 20 of claim 21 wherein the composition is delivered to the portal vein.

20 24. A method according to any one of claims 19 to 23 wherein the tumor is a primary or secondary tumor.

25 25. A method according to any one of claims 19 to 24 wherein the tumor is a hepatoma.

26. A method according to any one of claims 19 to 24 wherein tumor is a colorectal cancer or sarcoma.

30 27. A method according to claim 25 wherein the regional administration is by intra-hepatic arterial infusion.

28. A method according to any one of claims 18 to 26 wherein the composition is delivered as a one shot administration.