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The Vitamin D Endocrine System: Manipulation of Structure-Function Relationships to Provide Opportunities for Development of New Cancer Chemopreventive and Immunosuppressive Agents

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Abstract Biological responses mediated by vitamin D occur as a consequence of the integrated actions of the vitamin D endocrine system. The vitamin D endocrine system is characterized by the sequential two-step metabolism of vitamin D to $1\alpha,25(OH)_2D_3$ by the liver and kidney, and by the ability to generate biological responses in over 30 target tissues through nuclear receptor (nVDR) regulation of gene transcription and nongenomic pathways. It is now clear that the vitamin D endocrine system embraces many more target tissues than simply the intestine, bone and kidney. Notable additions to this list of tissues containing the nVDR include pancreatic B cells, pituitary gland, breast tissue, placenta, lymphocytes, keratinocytes, colon, and prostate, as well as many cancer cell lines. In addition to the classical actions of $1\alpha,25(OH)_2D_3$ on mediating calcium homeostasis, this seco steroid has been identified as a potent stimulator of cell differentiation as well as an inhibitor of proliferation.

Over the past decade at least 400 analogs of $1\alpha,25(OH)_2D_3$ have been chemically synthesized and their biological properties systematically explored in a variety of assays which quantified both their calcemic effects and cell differentiating potential. The objective has been to identify new analogs devoid of the classical calcemic consequences of high doses of $1\alpha,25(OH)_2D_3$, namely hypercalcemia, soft tissue calcification and nephrocalcinosis. As a consequence, several analogs of $1\alpha,25(OH)_2D_3$ have recently been identified and are discussed in this paper for consideration as possible chemotherapeutic agents for acute promyelocytic leukemia, breast, colon, and prostate cancer, or as immunosuppressive agents with possible beneficial structure-activity profiles for use in cardiac allografts, autoimmune graft rejection, lupus erythematosus and psoriasis. © 1995 Wiley-Liss, Inc.

Key words: Breast cancer, immunosuppressant, leukemia, psoriasis, $1\alpha,25(OH)_2D_3$, vitamin D

It is now firmly established that $1\alpha,25(OH)_2D_3$ is the principal hormonally active agent of the vitamin D endocrine system [1,2]. This seco steroid is now known to produce biological effects both via interaction with nuclear receptors (nVDR) which regulate gene transcription [3] and via signal transduction pathways, probably involving a membrane receptor [4], to generate rapid biological responses believed to be independent of direct interaction with the genome [5,6]. Approximately 30 tissues are known to possess the nVDR for $1\alpha,25(OH)_2D_3$, and over 50 genes are believed to be regulated via $1\alpha,25(OH)_2D_3$ receptor complexes [3,7]. In addition, an emerging array of acute responses are known to be generated via the agonist $1\alpha,25(OH)_2D_3$; these include transcaltachia, the rapid stimulation of intestinal Ca^{2+} absorption [8], rapid changes in phospholipid metabolism in the intestine [9], and activa-

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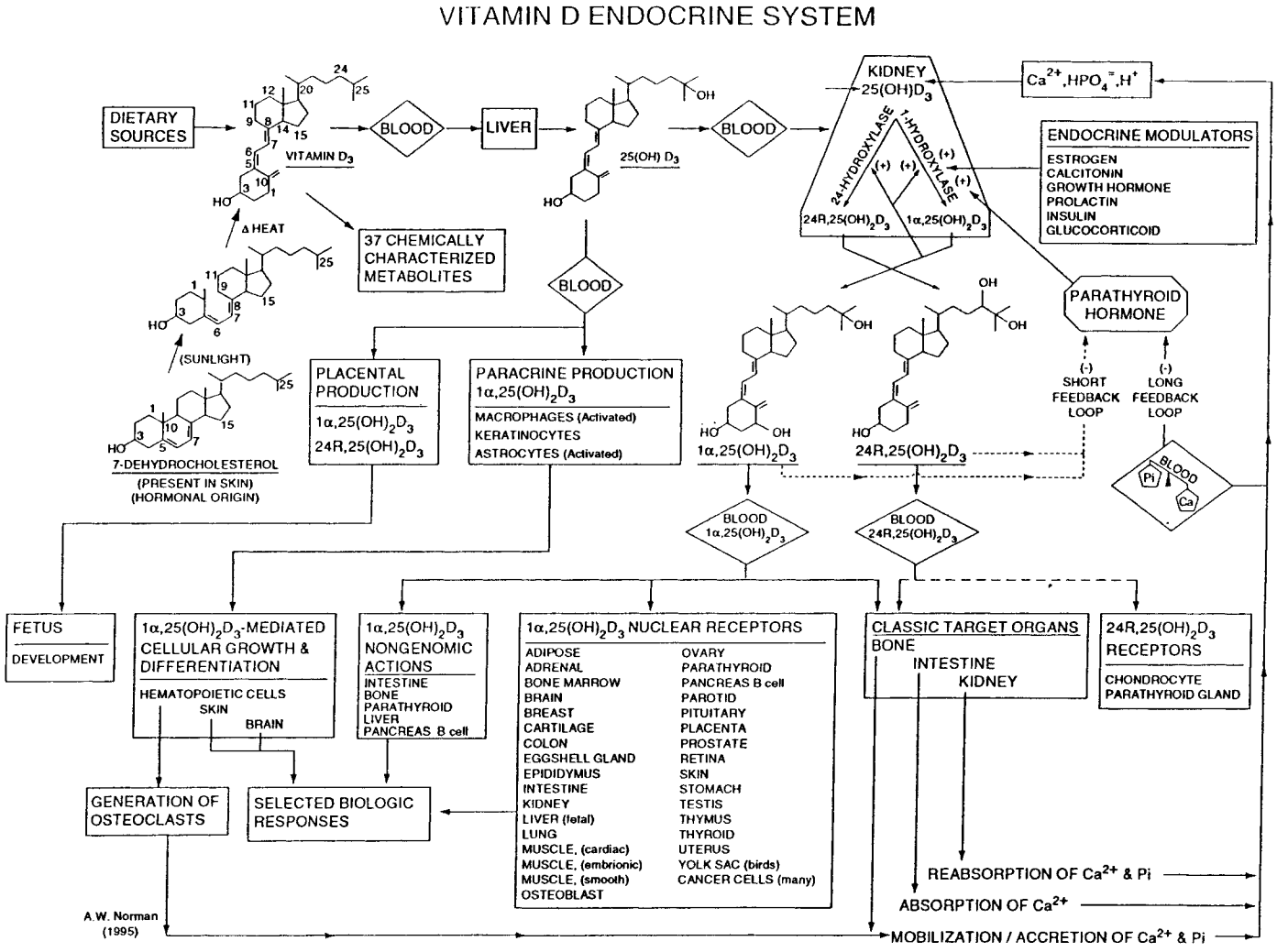


Fig. 1. Overview of the vitamin D endocrine system. The kidney functioning as an endocrine gland serves as the principal source of the circulating $1\alpha,25(OH)_2D_3$; however, both the placenta and three paracrine systems can also biosynthesize this steroid hormone. Target organs and target cells by definition contain nuclear receptors for $1\alpha,25(OH)_2D_3$ [nVDR] (see box). In addition, $1\alpha,25(OH)_2D_3$ also generates biological effects via nongenomic pathways; the site of these actions are summarized in a box. UV denotes ultraviolet light. The precise biological role(s) of $24R,25(OH)_2D_3$ are not yet defined.

tion of the Ca^{2+} message system via opening of Ca^{2+} channels in both ROS 17/2.8 cells [10] and intestine [11].

The extraordinarily broad sphere of influence of the vitamin D endocrine system (see Fig. 1) calls for determination of whether all biological effects are mediated via one invariant ligand, namely $1\alpha,25(OH)_2D_3$. $1\alpha,25(OH)_2D_3$ is unusually conformationally mobile [12]; it possesses unusual side chain flexibility, the capability for rotation about the 6,7 carbon-carbon single bond of the seco B ring [13], and chair-chair interconversion of the cyclohexane-like A ring [14]. Thus extensive efforts have been directed at the possibility of synthesizing analogs of $1\alpha,25(OH)_2D_3$ which, in the same animal, could initiate selective or differential biological responses in components of the vitamin D endocrine system [15].

One property of $1\alpha,25(OH)_2D_3$ that was not predicted based on its discovery as the calcium homeostatic steroid hormone [16] is its extraordinary potency in mediating cell differentiation and inhibiting proliferation of a wide variety of cell types [3], such as hematopoietic cells, cancer cells, and the epidermis [2]. However, the potent calcemic activity of $1\alpha,25(OH)_2D_3$, which can lead to hypercalcemia, soft tissue calcification and nephrocalcinosis, makes it impossible to directly utilize $1\alpha,25(OH)_2D_3$ in a clinical setting to treat various cancers or skin disorders [17]. Thus, identifying analogs of $1\alpha,25(OH)_2D_3$ capable of initiating selective biological responses would identify a potent differentiating agent in the cell type of interest which is devoid of the deleterious calcemic side effects.

Our laboratory, in collaboration with Professors H. L. Henry, W. H. Okamura, and H. P. Koeffler, has formally evaluated structure-function relationships in the vitamin D endocrine system using a library of over 400 chemically synthesized analogs of $1\alpha,25(OH)_2D_3$. As documented in Figure 2, a systematic modification of all portions of the $1\alpha,25(OH)_2D_3$ molecule has been effected; this includes alteration of the side chain and the C/D, B, and A rings.

This library of analogs has been systematically evaluated by the four-step screening strategy described in Figure 3. The original objective was an economical yet scientifically justifiable profile of a given analogue's biological properties to identify those that were potent differentiating agents of human leukemic cells, but devoid of unwanted calcemic effects. The same screening matrix has also been useful to screen analogs for other cancer cell lines. Details of the screening strategy are given in the legend to Figure 3. We have reported the results of our structure-function assessment in several publications [18–20].

As a consequence of the systematic application of this screening matrix, we have identified a candidate drug to treat acute promyelocytic leu-

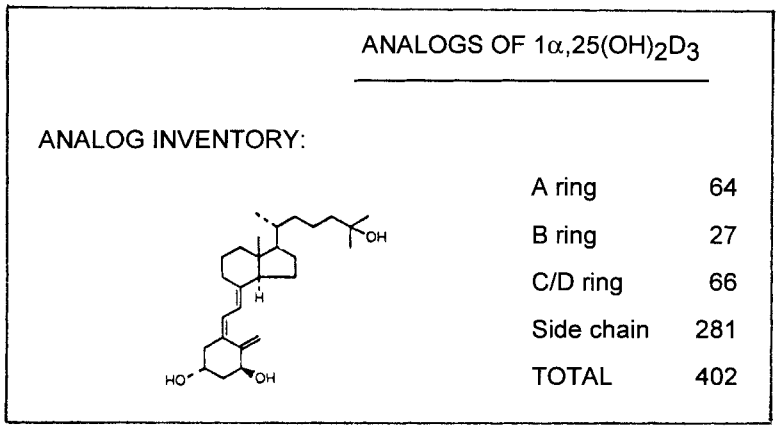


Fig. 2. Inventory of analogs of $1\alpha,25(OH)_2D_3$. These analogs have been generously made available by W. H. Okamura, M. Uskokovic, L. Binderup, W. Dauben, S. Ishizuka, A. Mourino, Y. Nishii, G. Posner and S. Wilson. The structure-activity relationships of this analog library have been reviewed.

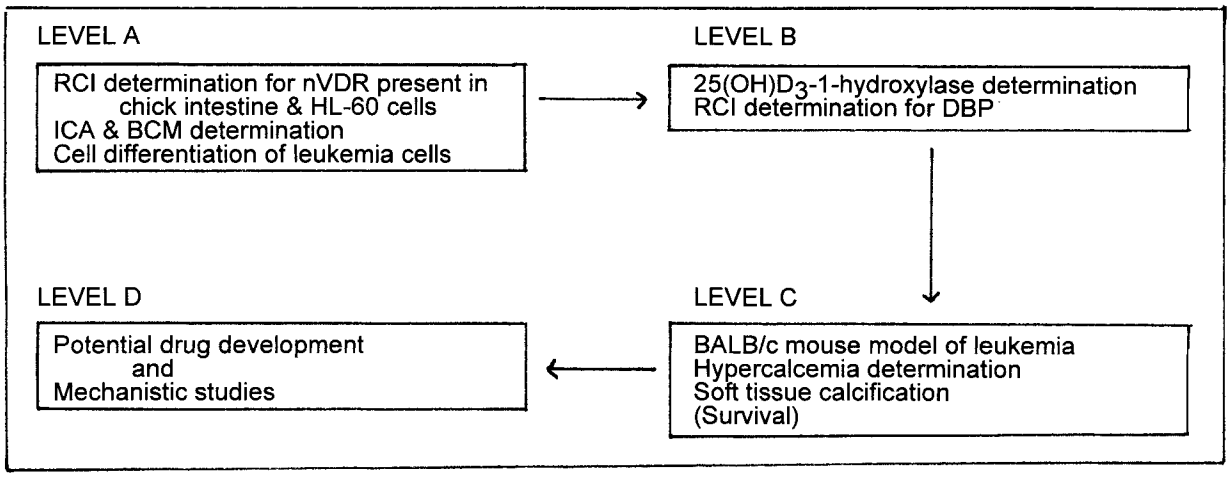
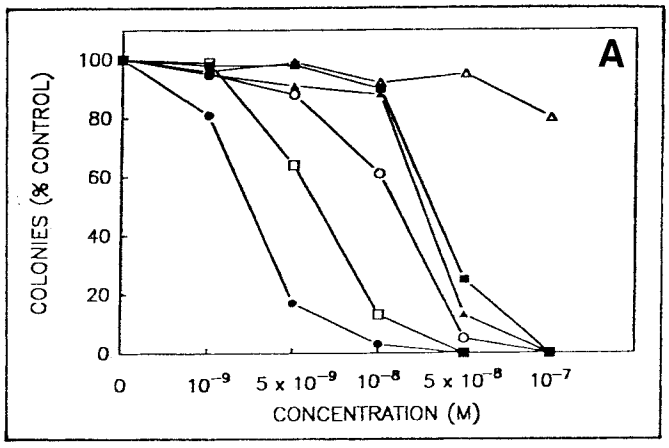


Fig. 3. Vitamin D analog screening strategy. The starting point in the evaluation is an assessment of an analog's ability to bind to the chick intestinal nuclear $1\alpha,25(OH)_2D_3$ receptor (nVDR) and to the HL-60 nVDR. The result is expressed as RCI = Relative Competitive Index (RCI) which is a measure of the relative affinity of an analog for binding to the nuclear VDR; in this assay, the RCI for $1\alpha,25(OH)_2D_3$ is by definition 100%. Other cancer cell lines may be substituted for HL-60 cells. In general, for an analog to advance from Level A to Level B, it should be at least as active as $1\alpha,25(OH)_2D_3$ in promoting cell differentiation in HL-60 cells but have a significantly reduced stimulation of both intestinal Ca^{2+} absorption (ICA) and bone Ca^{2+} mobilizing (BCM) activity. Promotion of an analog from Level B to Level C is for analogs which do not have an obvious adverse effect on the activity of renal $25(OH)D_3$-1-hydroxylase [28]. In addition, the RCI determination for binding to the plasma transport protein, the vitamin D binding protein (DBP), is effected at Level B. Evaluation of an analog at Level C is reserved for the limited number of analogs which may be serious candidates for consideration for a clinical trial. The "survival" assay relates to screening of analogs of $1\alpha,25(OH)_2D_3$ in a BALB/c mouse model of leukemia [22]. However, other animal disease models may be substituted. Level D is designed to provide assessment of the mechanistic *modus operandi* of those very few analogs which might be nominated for a clinical trial. For analog **V** [$1\alpha,25(OH)_2$-16-ene-23-yne-D_3] see reference [15] and for analog **BT** [$1\alpha,24(OH)_2$-24-cyclopropyl-D_3] see reference [24]. The $25(OH)D_3$-1-hydroxylase is an *in vitro* determination of the ability of kidney homogenate from an animal dosed with a test analog to convert $25(OH)D_3$ into $1\alpha,25(OH)_2D_3$ [28]. Hypercalcemia is assessed via determination of the serum Ca^{2+} level [22]. Soft tissue calcification is an assay that quantifies the soft tissue deposition of $^{45}Ca^{2+}$ after chronic administration of either $1\alpha,25(OH)_2D_3$ or the test analog [29].

kemia. We have reported that analog V, [$1\alpha,25$-$(OH)_2$-16-ene-23-yne-D_3], is 7-fold more potent than the parent $1\alpha,25(OH)_2D_3$ with respect to inhibition of the clonal proliferation of HL-60 cells, as well as induction of differentiation of HL-60 promyelocytes, with only 2–3% of the calcemic activity of $1\alpha,25(OH)_2D_3$ (see Fig. 4A) [21]. In an animal model of leukemia (Fig. 4B) we demonstrated that mice treated with $1\alpha,25(OH)_2$-16-ene-23-yne-D_3 had a significantly longer survival time than mice treated with the highest noncalcemic dose of $1\alpha,25(OH)_2D_3$ [22].

As stated in Figure 3, the objective of Level 4 screening is to provide some mechanistic insight into the operative biological mechanism(s) which allow a given analog to separate adverse from beneficial biological effects. Figure 4 emphasizes three major explanations put forth by a variety of investigators [23,24]. Certainly in the case of analog V, we have demonstrated both an increased "free" concentration in the plasma as well as marked selective target organ localization [15], which apparently generate a therapeutic advantage for analog V over $1\alpha,25(OH)_2D_3$.

A number of other laboratories and pharmaceutical companies have also carefully screened $1\alpha,25(OH)_2D_3$ analogs with the objective of developing useful drugs. The results of these ef-



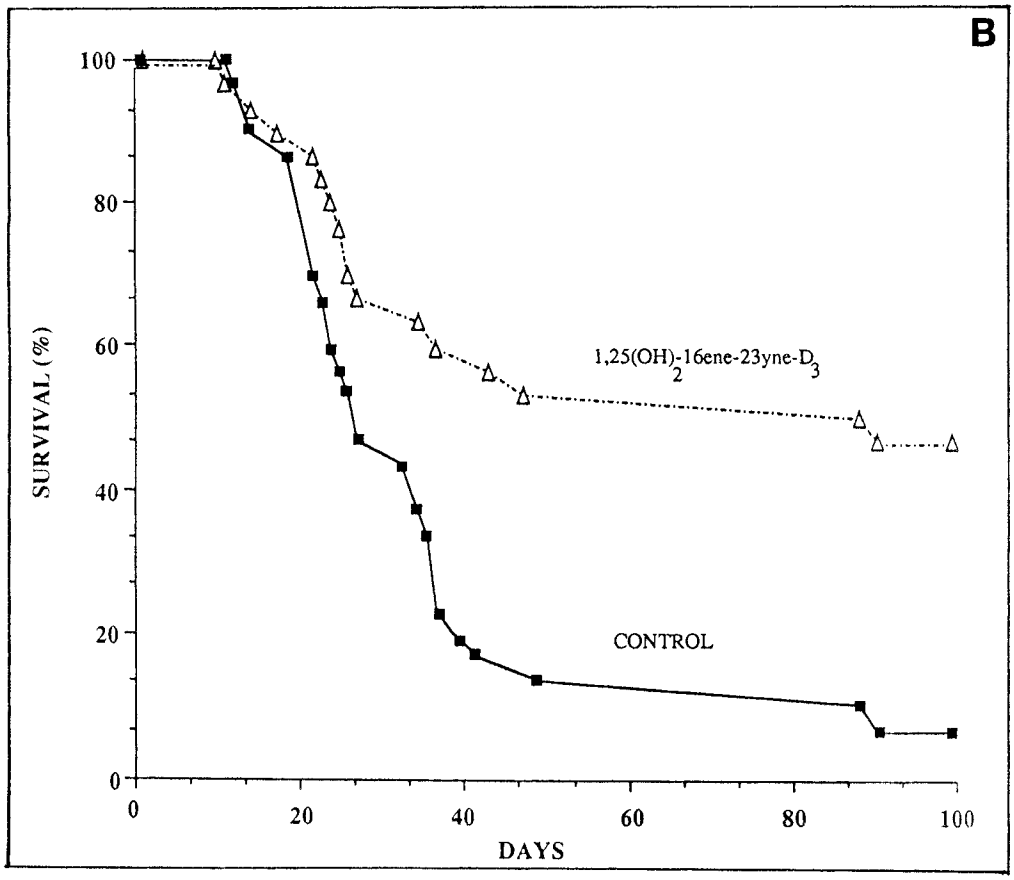


Fig. 4. Biological profiling of analog **V** [1α,25(OH)$_2$-16-ene-23-yne-D$_3$] for acute myelocytic leukemia [21]. (Panel A) Dose response of vitamin D analogs for clonal proliferation of HL-60 cells. Results are expressed as a percentage of control plates not exposed to 1α,25(OH)$_2$D$_3$ compounds. O = 1α,25(OH)$_2$D$_3$; ● = analog **V** [1α,25(OH)$_2$-16-ene-23-yne-D$_3$]; Δ = analog **AT** [25(OH)$_2$-16-ene-23-yne-D$_3$]; □ = analog **AV** [1,25(OH)$_2$-16-ene-23-yne-D$_3$-26,26,26,27,27,27-d$_6$]; Δ = analog **AU** [1-F-25(OH)-16-ene-23-yne-D$_3$]; ■ = analog **AW** [1-F-25(OH)-16-ene-23-yne-D$_3$-26,26,26,27,27,27-d$_6$]. (Panel B) Survival of BALB/c mice inoculated with syngeneic WEHI 3BD+ leukemic cells and treated with analog **V** [1α,25(OH)$_2$-16-ene-23-yne-D$_3$] [22]. Mice received either 0.8 μg of analog **V** (Δ) or diluent (■) control in 0.1 ml *ip qod.* Thirty mice were in each group.

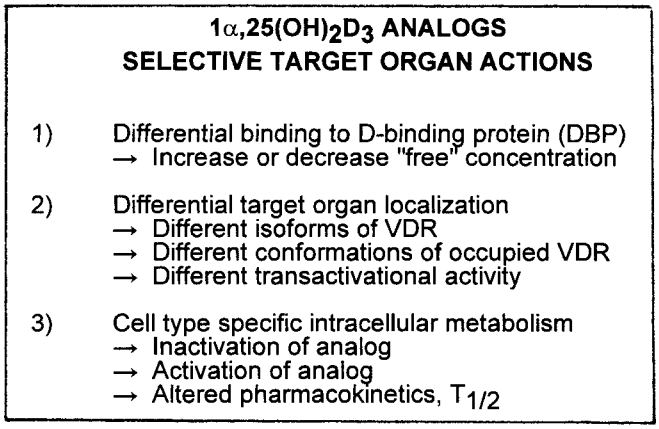


Fig. 5. Proposed biological mechanisms by which selective biological responses to analogs of $1\alpha,25(OH)_2D_3$ are generated. See text for a description and references.

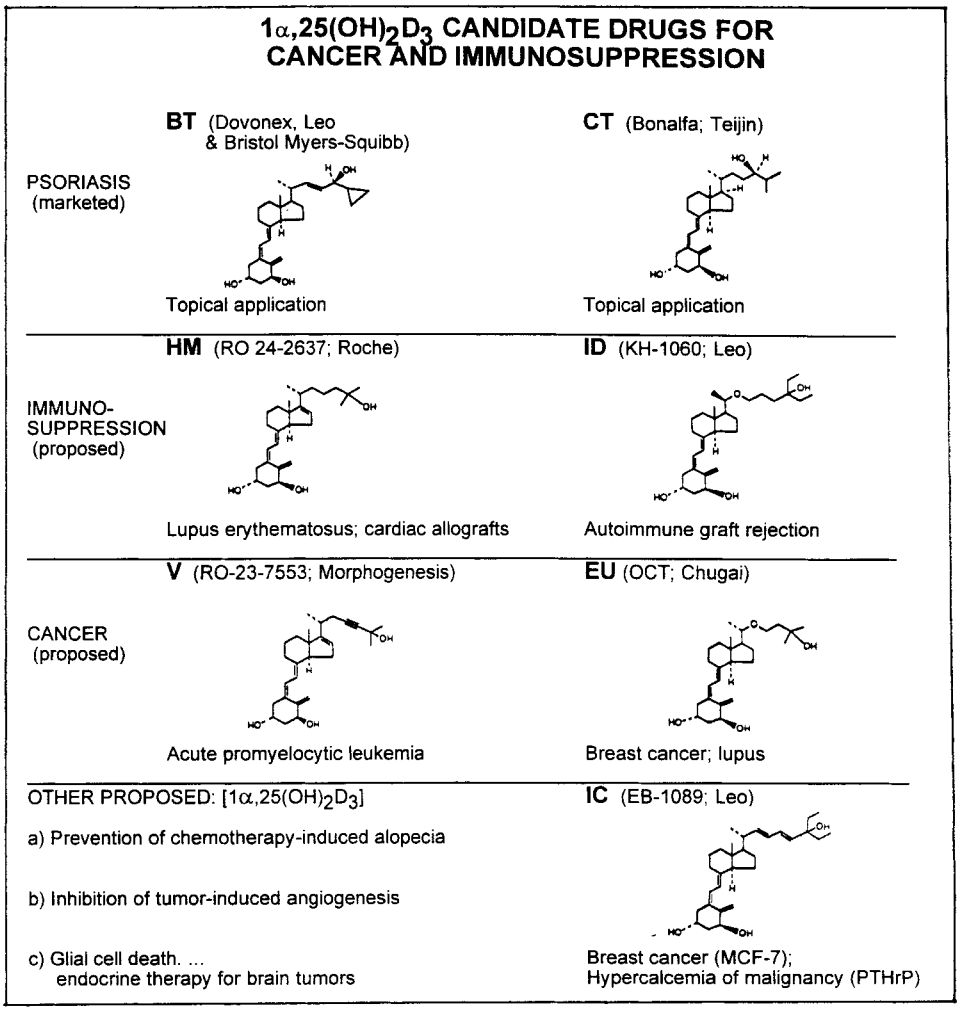


Fig. 6. $1\alpha,25(OH)_2D_3$ analog candidate drugs for consideration for use in psoriasis, as immunosuppressants, or in cancer. The relevant literature citations for each analog are as follows: analog **BT** [30,31]; analog **CT** [32,33]; analog **HM** [34]; analog **ID** [35]; analog **V** [21,36]; analog **EU** [37]; and analog **IC** for breast cancer [38] or hypercalcemia of malignancy [39].

forts, which have applications to psoriasis, cancer and immunology, are summarized in Figure 6. For each of the $1\alpha,25(OH)_2D_3$ analogs presented, a growing scientific literature documents and describes the potential for clinical application (see legend to Figure 6). Already two new drugs are marketed for psoriasis (Dovonex® = analog BT = $1\alpha,24(OH)_2$-24-cyclopropyl-D_3; Bonalfa® = CT = $1\alpha,24(OH)_2D_3$). Other suggested uses of $1\alpha,25(OH)_2D_3$ analogs in the cancer field include prevention of chemotherapy-induced alopecia [25], inhibition of tumor-induced angiogenesis [26], and as an endocrine therapy for glial cell-related brain tumors [27].

It will be interesting to observe over the next decade to what extent the manipulation of $1\alpha,25(OH)_2D_3$ structure-function relationships is successful with regard to development of new drugs for psoriasis, cancer, immunology and other targets such as osteoporosis and suppression of parathyroid hormone in the secondary hyperparathyroidism frequently associated with chronic renal failure.

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