Calcitriol and cancer therapy: A missed opportunity

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ABSTRACT

The vitamin D receptor is expressed in most tissues of the body – and the cancers that arise from those tissues. The vitamin D signaling pathway is active in those tissues and cancers. This is at least consistent with the hypothesis that perturbing this signaling may have a favorable effect on the genesis and growth of cancers. Epidemiologic data indicate that vitamin D signaling may be important in the initiation and outcome of a number of types of cancer. Many studies have shown that calcitriol (1,25 dihydroxycholecalciferol) and other vitamin D compounds have antiproliferative, pro-apoptotic, anti-cell migration and antiangiogenic activity in a number of preclinical studies in many different cancer types. Unfortunately, the assessment of the activity of calcitriol or other vitamin D analogues in the treatment of cancer, as single agents or in combination with other anticancer agents has been stymied by the failure to adhere to commonly accepted principles of drug development and clinical trials conduct.

1. Case report

The patient is a 67 yo white man with prostate cancer, diagnosed 7 years previously (T2bN0M0, Gleason 4 + 3 = 7 cancer involving ~30% of the prostate which was removed at robotic prostatectomy. Despite prostatectomy the patient’s prostate specific antigen blood level became detectable 24 months following surgery. Despite irradiation to the prostatic fossa and then androgen deprivation therapy (ADT) with leuprolide + bicalutamide 5 years following prostatectomy PSA was 110 ng/mL and radiographs demonstrated metastases to multiple sites in the boney skeleton (T6 & L1,2 vertebral bodies, both iliac wings, the pubic ramus and the 10th right rib). No visceral or soft tissue metastases were present. Antiandrogen withdrawal resulted in a drop in PSA to 45 ng/mL, but 1 year later the PSA was rising again (78 ng/mL), the patient was asymptomatic and the radiographs demonstrated bone disease, but no visceral or soft tissue metastases. After obtaining informed consent the patient entered an ongoing phase II trial of high dose oral calcitriol + dexamethasone. Oral calcitriol was administered weekly, Monday, Tuesday, and Wednesday (MTW), at a dose of 8 μg, for 1 month, 10 μg every MTW for 1 month, and at 12 μg every MTW thereafter. Dexamethasone at a dose of 4 mg was administered each Sunday, and MTW weekly. Calcium and creatinine were determined weekly and radiographs of the urinary tract were performed every 3 months to monitor for urinary tract stone formation. On day 56 of therapy the PSA had fallen to 30 ng/mL, the radiographs showed no new metastases, stable bone disease and no urinary tract stones. Weekly blood calcium and creatinine concentrations were always within the normal range and no biochemical or subjective toxicity occurred. The patient continued on therapy for a total of 14 months and then with continually stable disease, (PSA 42 ng/mL) the patient elected to cease therapy and was followed receiving only ADT. Is this indication of the therapeutic effect of high dose calcitriol in prostate cancer? (Trump et al., 2006)

2. Introduction

Critical experiments, reported in the 1970s and early 1980s, form the basis for the hypothesis that vitamin D compounds can be used to inhibit the development and progression of several types of cancer:

- treatment with vitamin D compounds was shown to inhibit the development of cancer in a carcinogen-induced model in the hamster
- the vitamin D receptor (VDR) was detected in numerous histotypes of human cancer cells
- treatment of cancer cells with calcitriol (1,25 dihydroxycholecalciferol) in vitro was shown to cause growth arrest (Potter et al., 1985; Moqattash et al., 1985; Freake et al., 1984; Sato et al., 1984; Kuroki et al., 1983; Frampton et al., 1983; Franken et al., 1983; Mccarthy et al., 1983; Honna et al., 1983; Sato et al., 1982; Freake & Macintyre, 1982; Tanaka et al., 1982; Colston et al., 1982; Frampton et al., 1982; Eisman et al., 1981; Sher et al., 1981; Miyaura et al., 1981; Eisman et al., 2006)
Subsequently, hundreds of cholecalciferol analogues have been developed seeking to define more potent compounds and hoping to reduce the propensity to cause hypercalcemia, the only known toxic effect of vitamin D compounds (Van Belle et al., 2014a; González-Pardo et al., 2014; González-Pardo et al., 2013; Duffy et al., 2017; Koefler et al., 1984; Eisman et al., 1986; Murayama et al., 1986; Ostrem et al., 1987; Haq et al., 1993; Binderup et al., 1991). This paper provides an overview of studies of calcitriol and other vitamin D compounds in the treatment of cancer, emphasizing that, while the preclinical data are convincing, clinical development has been slow and disappointing because of errors made in the basic concepts of drug development. While much of the clinical work done in developing calcitriol in cancer has been done in men with prostate cancer, this is likely due to fact that the research teams engaged in the preclinical and clinical work on calcitriol were also engaged in studies in prostate cancer. All existing data indicate that perturbation of the vitamin D signaling axis would be effective in inhibiting a wide variety of cancer types.

3. Biochemistry and molecular biology of vitamin D

Vitamin D is synthesized in the body through a complex series of steps beginning in the skin where ultraviolet light transforms 7-dehydrocholesterol into the vitamin D hormone precursor, cholecalciferol (vitamin D3). Cholecalciferol is subsequently hydroxylated in the liver (yielding 25(OH)D3) and then in the kidney to yield the most active hormone form of these compounds, 1,25 dihydroxycholecalciferol or calcitriol. “Inactivation” of calcitriol occurs primarily by 24-hydroxylation in the kidney yielding 1,24,25(OH)3 cholecalciferol. These hydroxylations are mediated primarily by cytochrome P450 (CYP) enzymes CYP2R1, CYP27B1 and CYP24A1, respectively. While the major sites of metabolism and inactivation are liver and kidneys, tumor cells as well as many other tissues throughout the body and in the microenvironment of tumor cells also metabolize these hormones (Sharifi, 2013; Ishizaki et al., 2013; JH1 et al., 2012).

1,25D3 enters cells by passive diffusion, binds to the vitamin D receptor (VDR) which heterodimerizes with the retinoid X receptor (RXR). This complex binds to promoter regions of vitamin D-responsive genes to modulate the expression of > 2000 genes. The complexities of genetic variants in enzymes responsible for D3 metabolism, protein binding, partner heterodimerization and the multitude of genes modulated by the vitamin D hormone systems suggest that dissection of the role of vitamin D in cancer, as well as other diseases, will require very careful and detailed study.

4. Epidemiologic studies of vitamin D in cancer

An additional reason to examine the role of vitamin D in cancer therapy is the large number of epidemiologic studies linking vitamin D and cancer risk and outcome. Studies in colorectal, breast, lung, non-Hodgkin lymphoma, prostate and bladder cancer – to name only a few cancer types – indicate a higher risk of cancer and poor prognosis of that cancer among individuals with measured or estimated low levels of 25(OH)D3. While there is not uniform concordance among studies seeking to define an association between vitamin D and cancer causation and outcome, this work is ultimately hampered that such studies can only demonstrate “associations” between estimated or measured vitamin D levels, or genotypic variants of proteins important in vitamin D signaling and cancer risk or outcome (Feldman et al., 2014; Grant, 2012; Afsal et al., 2013; Skaaby et al., 2014; Ordóñez-Mena et al., 2013; Ordóñez-Mena et al., 2016; Yin et al., 2013; Ma et al., 2011; Garland & Gorham, 2017; Feng et al., 2017; Liu et al., 2017; Bauer et al., 2013; Gandini et al., 2011). Much greater clarity on the question of the role of vitamin D and cancer risk will be provided by the results of 2 large randomized trials of vitamin D supplementation in large populations of otherwise healthy individuals (Okereke et al., 2018; Neale et al., 2016).

5. Anticancer effects of vitamin D compounds

The biochemical changes associated with anticancer effects of calcitriol treatment of cancer cells have been extensively studied. The exact mechanisms of the antitumor effects of vitamin D compounds are incompletely understood. Calcitriol (1,25 dihydroxycholecalciferol) is the compound most carefully studied, in vitro and in vivo. Calcitriol inhibits tumor growth in association with the following biochemical effects:

- Cell cycle arrest and modulation of CDK inhibitors, such as p21 and p27 (Wang et al., 1996; Simboli-Campbell et al., 1997; Verlinden et al., 1998).
- Induction of apoptosis with PARP cleavage, annexin binding, increased bax/bcl-2 ratio (Bernardi et al., 2001; Welsh, 1994; James et al., 1996; Pintado et al., 1996; Narvaez & Welsh, 1997; Colston & Hansen, 2002).
- Suppression of the “pro-proliferative” signaling molecules such as P-MAPK (ERK1/2), P-AKT, reduction of AKT and MEKK-1 (Johnson et al., 2006).
- Induction of caspase-dependent MEK-cleavage (Albgala et al., 2006; McGuire et al., 2001).

Each of these effects has been described to occur in multiple cancer model systems in vitro and in vivo. In addition to the aforementioned biochemical effects, the following cellular effects which may be accompanied by tumor growth suppression are well described:

- Inhibition of angiogenesis (Miyata et al., 2013; Chung et al., 2009).
- Inhibition of motility and invasion (Hou et al., 2016; Chen et al., 2015a).
- Induction of differentiation (Olssohn et al., 1983; Koefler et al., 1985; Abe et al., 1986).
- Modulation of cytokine/growth factor production by stromal and/or tumor cells (Irani et al., 2015; Chen et al., 2015b; Mohapatra et al., 2015).

In addition, considerable evidence supports the role of vitamin D signaling in immune function and inflammation. Immune dysregulation and inflammation are increasingly recognized as viable targets in cancer therapy and prevention. While the precise role of vitamin D in regulating immune function is still being defined, there are many studies demonstrating the impact of vitamin D signaling on monocyte/macrophage differentiation, T cell function and cytokine production (Gonzalez-Cao et al., 2015; Tran et al., 2017; Byun et al., 2017). Two separate studies in patients with non-Hodgkin lymphoma indicate that low levels of 25(OH)D3 are associated with poor outcome following therapy with cytotoxic chemotherapy + rituximab, an antibody which binds to CD-20 in lymphoma cells (Kelly et al., 2015; Bittenbring et al., 2014). Bittenbring et al. demonstrated that rituximab-mediated killing of lymphoma cells in vitro by an individual’s monocytes was substantially enhanced by restoration of normal vitamin D levels in such individuals (Bittenbring et al., 2014).

Cyclooxygenase-2 (COX-2), the enzyme that catalyzes prostaglandin synthesis, has been extensively investigated as a target in cancer therapy and prevention (Wang et al., 2005; Zha et al., 2001; Gupta et al., 2000; Yoshimura et al., 2000; Krishnan & Feldman, 2010; Sun et al., 2018). COX-2 is overexpressed in putative cancer precursor inflammatory lesions in tissues such as breast and prostate, in a variety of cancer cell lines, as well as in macrophages and other cells in the tumor microenvironment (de Cremoux et al., 2018; Sano et al., 2018). Calcitriol regulates the expression of several genes in the prostaglandin...
pathway; in vitro and in vivo studies demonstrate that calcitriol + nonsteroidal anti-inflammatory agents which inhibit COX-2 potentiate the growth inhibitory effects of calcitriol (Davilal-Gonzalez et al., 2017; Basudhar et al., 2017). $1,25(\text{OH})_2$D analogues have also been described to suppress inflammation as well as COX-2 expression and activity either directly or indirectly (Moreno et al., 2005; Aparna et al., 2008).

Calcitriol may alter androgen metabolism in prostate cancer cells or estrogen metabolism in breast and endometrial cells, providing yet another pathway whereby tumor growth may be disrupted. CYP3A4, CYP3A5, CYP3A43, AKR1C1 another pathway whereby tumor growth may be disrupted. CYP3A4, modulating compounds (metabolism; activity of these enzymes may influence estrogen receptor modulating compounds (e.g. 27-deoxycolesterol), testosterone, dehydroepiandrosterone (DHEA) and androstanediol concentrations intracellularly. Vitamin D compounds may influence the activity of these enzymes in tissues and ultimately modulate the availability of these pro-survival androgenic and estrogenic steroids. There is no conclusive evidence that vitamin D compounds modulate “intracrine” androgen or estrogen metabolism in patients, but preclinical studies are consistent with the hypothesis that this is an additional mechanism whereby $1,25(\text{OH})_2$D compounds may suppress prostate tumor growth (Doherty et al., 2014; Seo et al., 2013; Maguire et al., 2012; Going et al., 2018).

6. Analogues of $1,25(\text{OH})_2$D

Considerable work has been done seeking to delineate analogues of $1,25(\text{OH})_2$D that may have greater antitumor activity and/or less potential to induce hypercalcemia, the only known toxic effect of vitamin D compounds. The analogues EB1089, MC903, 22-oxacalcitriol, BGP-13(a 24-chloro calciotriene-based D$_3$ analogue), R024-2637, 14-nor-14-epi-23-yn-1,25(\text{OH})$_2$D$_3$ (TX 522, inecalcitol) and 19-nor-14,20-bisepi-23-yn-1,25(\text{OH})$_2$D$_3$ (TX 527) are reported to be less likely to cause hypercalcemia than the parent compound calcitriol. Each of these analogues appears to have activity in preclinical cancer models (Skowronska et al., 1995; Campbell et al., 1997; Berkovich et al., 2013; Okamoto et al., 2012; Verlinden et al., 2000; Van Belle et al., 2014b; Ferreira et al., 2013; Verlinden et al., 2013).

For example, phase I and II trials of inecalcitol (TX522) have been completed and demonstrated that (1) 4000 $\mu$g QOD is a safe dose and (2) inecalcitol + docetaxel appears to be superior to docetaxel alone in men with castration resistant prostate cancer (Medioni et al., 2002; Muindi et al., 2009; Fakih et al., 2007; Chan et al., 2008; Muindi et al., 2004). These effects are most pronounced when vitamin D receptor from calcitriol is expressed in many cell types. Synergistic antitumor effects of calcitriol and glucocorticoids have been demonstrated in human prostate cancer xenografts, and the dexamethasone does reduce calcitriol-induced hypercalcemia in patients (McCarry et al., 1983; Trump et al., 2004).

7. Combination therapies with vitamin D compounds

Although calcitriol and analogues have interesting antitumor effects in preclinical models, single agents usually have limited effect in clinical cancer therapy; therefore, calcitriol combination therapies have been widely evaluated.

7.1. Glucocorticoids

Glucocorticoids have direct antitumor effects and block calcitriol-induced hypercalcemia. In addition, glucocorticoids enhance VDR expression in many cell types. Synergistic antitumor effects of calcitriol and glucocorticoids have been demonstrated in human prostate cancer xenografts, and the dexamethasone does reduce calcitriol-induced hypercalcemia in patients (McCarry et al., 1983; Trump et al., 2004).

7.2. Inhibitors of CYP24A1

Non-specific (e.g. ketoconazole and liarazole, isoflavones such as genistein, progesterone) and specific inhibitors of CYP24A1 each accentuate the antitumor activity of calcitriol (Trump et al., 2004; Zhang et al., 2012; Peehl et al., 2002; Swami et al., 2005; Muindi et al., 2010; Chiellini et al., 2012; Lechner et al., 2007; Yee & Simons, 2004; Schuster et al., 2003; Ly et al., 1999; Zhao et al., 1996; Rao et al., 2002; Rodriguez et al., 2016; Lee et al., 2013; Lou & Tuohimaa, 2006; Yee et al., 2006).

7.3. Non-steroidal anti-inflammatory drugs

Prostaglandin synthesis inhibitors may potentiate the activity of vitamin D compounds. A phase II trial of the nonselective inhibitor naproxen and high-dose calcitriol in patients with early recurrent prostate cancer demonstrated apparent activity as evidenced by reduction in PSA doubling time (Srinivas & Feldman, 2009).

7.4. Retinoids

Since calcitriol, VDR, RAR and 9-cisretinoic acid, interact in vitamin D signaling it is plausible to hypothesize that ligands for the retinoid receptors, RARs and RXRs might modify calcitriol activity. Potentiation of calcitriol antitumor effects by retinoid compounds has been well described (Gocce et al., 2012; Mernitz et al., 2007; Mouratidis et al., 2006; Peehl & Feldman, 2004; Ikeda et al., 2003; Elstner et al., 1999).

7.5. Cytotoxic agents

Both in vitro and in vivo, vitamin D compounds can be shown to potentiate the cytotoxicity of many anticancer agents. Calcitriol or its analogues potentiate platinum compounds (cisplatin [Platinol®], carboplatin [Paraplatin®], anthracyclines [doxorubicin [Adriamycin®], mitoxantrone [Novantrone®], topoisomerase Inhibitors (irinotecan [Camptosar®], etoposide [Etoposide®], antimetabolites (cytosine arabinoside [Cytarabine®], gemcitabine [Gemzar®], 5-fluourouracil [Adrucil®]) and taxanes (docetaxel [Docetaxel®] and paclitaxel [Taxol®]). The precise mechanism(s) of this potentiation are not clear (Smith et al., 1999; Johnson et al., 2002; Muindi et al., 2002; Muindi et al., 2005; Muindi et al., 2009; Fakhik et al., 2007; Chan et al., 2008; Muindi et al., 2004). These effects are most pronounced when vitamin D compounds are administered before or simultaneously with the cytotoxic agent.

7.6. Ionizing radiation and photodynamic therapy

Vitamin D compounds potentiate the antitumor effects of ionizing radiation and photodynamic therapy though few clinical studies have been conducted with this approach (Dunlap et al., 2003; Anand et al., 2004).
8. Clinical evaluation of vitamin D compounds in cancer therapy

Many clinical trials have been conducted which have sought to define evidence of clinical benefit of calcitriol and analogues in the treatment of cancer patients (Osborn et al., 1995; Chahda et al., 2010; Trump et al., 2006; Morris et al., 2004; Schwartz et al., 2005; Gross et al., 1998; Beer et al., 2004a; Beer et al., 2003a; Liu et al., 2003; Beer et al., 2001). Suffice it to say, none of these trials has provided strong evidence of benefit or activity. With the benefit of hindsight, several factors have contributed to the failure to demonstrate beneficial effects of calcitriol or analogue therapy in most trials:

- The majority of clinical trials of vitamin D compounds in cancer have been single institution, investigator-initiated trials. Coincidently, because several investigative teams have had interest in prostate cancer, a disproportionate amount of work has been done in men with prostate cancer. While single institution, investigator-initiated trials are not intrinsically flawed, they do tend to be underpowered, involving a relatively small number of patients; in addition, these trials have often tested combinations of calcitriol and other drugs with established antitumor activity (e.g. mitoxantrone, carboplatin, paclitaxel, docetaxel, dexamethasone, cisplatin, ketoconazole) in a given cancer; this confounds assessment of antitumor activity, particularly in small trials.

- The basic principles of anticancer drug development have been incompletely applied in studies of calcitriol and analogues. There has never been clear agreement on the best dose and schedule of calcitriol and analogues. Preclinical studies provide strong evidence for a substantial effect of exposure to calcitriol as a determinant of anticancer activity, especially when combined with cytotoxic agents. Nonetheless many studies have been done with particular attention to estimates of optimal dose or schedule of vitamin D compound.

- An important factor that has hampered the development of vitamin D analogues as cancer therapies has been the lack of interest and commitment to this concept by any pharmaceutical company.

- Absence of patent protection for other than new formulations of calcitriol has been a serious challenge. This is particularly ironic in that the most extensive data on the preclinical effects of vitamin D compounds in cancer models have been developed for calcitriol.

- There has been limited exploration of the activity of vitamin D analogues in cancer (e.g. paricalcitol, EB1089). Commitment to develop these analogues against cancer seems to have been limited by reluctance to commit to a cancer strategy for agents whose primary developmental path has been for chronic renal disease (paricalcitol) or general limitations on resources and interest (EB1089).

Dose and schedule are critical considerations when developing any new therapeutic. A series of studies examining daily oral, subcutaneous QOD and oral QDX3 weekly clearly have shown that the amount of calcitriol that can be administered safely is greatly enhanced by intermittent therapy (see below). However, many clinical studies were done using continuous therapy of every other day dosing or intermittent therapy (see below). However, many clinical studies were done using continuous therapy of every other day dosing – perhaps because vitamin D is a hormone? All studies of daily or QOD therapy have been negative. Preclinical studies of 1,25(OH)2D3 and analogues clearly demonstrate the following principles:

- The anticancer activity of 1,25(OH)2D3 is clearly dose dependent. Based on this concept, clinical studies should be conducting utilizing the maximum safe dose of vitamin D compound.

- Maximum exposure to 1,25(OH)2D3 compounds in animal studies is achieved if these agents are administered on an intermittent schedule.

One would expect these considerations would have been of clinical trials of vitamin D compounds in cancer therapy. As we will see, this has often not been the case.

9. Vitamin D analogues administered before prostatectomy: exploration of biologic effects

There is evidence that vitamin D compounds have biologic effects on human tissues. Several studies evaluated the effect of the administration of vitamin D compounds in the preoperative period before prostatectomy. This study design allows the exploration of clinically relevant dose and biologic response relationships in human tissues. In addition, such data provide evidence that complements preclinical work indicating changes in potentially important biomarkers of biologic effect of vitamin D in human cancer tissues in vivo. Beer and colleagues gave 39 patients either calcitriol weekly (0.5 μg/kg weekly × 4) or placebo and for 4 weeks prior to prostatectomy (Beer et al., 2004b). In the calcitriol group the percentage of cells expressing VDR was lower (75.3%) compared to the patients receiving placebo (98.6%), there was no change in the percentage of cells expressing TGFbeta RII, PTEN, or proliferating cell nuclear antigen (PCNA) (Beer et al., 2004b). Gee and colleagues studied patients randomized to daily doxercalciferol (10 μg QD × 28 days) versus placebo for 28 days prior to radical prostatectomy (Gee et al., 2013). Serum markers examined included vitamin D metabolites, TGFβ1/2, free/total PSA, IGF-1, IGFBP-3, hFGF, and VEGF; tissue markers studied included histology, MIB-1 and TUNEL staining, microvesSEL density and factor VIII, staining, androgen receptor and PSA, VDR expression and nuclear morphometry. TGFβ-2 was the only biomarker significantly altered by doxercalciferol supplementation (~2–4 fold). Wagner and colleagues evaluated the biologic effect of vitamin D3 (cholecalciferol) administration prior to prostatectomy on intraprostatic concentrations of vitamin D metabolites as well as on markers of potential biologic importance (Wagner et al., 2013). 63 patients received either 400 IU, 10,000 IU, or 40,000 IU daily by mouth for up to 4 weeks prior to prostatectomy. In prostate tissue, vitamin D metabolite levels and Ki67 labeling were assessed and serum PTH, and PSA were measured. This study demonstrated the safety of these dosing regimens and showed clearly that tissue and serum levels of vitamin D metabolites, including calcitriol, increased in a dose-dependent manner (P < .03). Vitamin D compound concentrations were highest in the 40,000-IU/d group (P < .03) and tissue vitamin D metabolite levels were correlated with vitamin D serum levels (P < .0001). While Ki67 labeling indices were not different among the dosing groups, there was evidence that intraprostatic calcitriol level was inversely associated with the proliferation marker, Ki67 intensity and percent Ki67 “positive” nuclei in prostate cancer and benign tissue (P < .05). The 2 highest dose supplementation groups were combined in an analysis of the impact of dosing on PTH and PSA serum levels which indicated that high dose D3 administration suppressed PTH and PSA levels (P < .02). This group conducted further exploratory analyses on the tissues available from this clinical trial. VDR was detected in both epithelial and stromal tissues and vitamin D hydroxylases were present only in prostate stromal cells. VDR expression was suppressed in those tissues with the highest 1,25(OH)2D3 content. In specimens with the highest 1,25(OH)2D3 content, epithelial cell IL-6 was highest and stromal cell COX-2 was lowest (Giangreco et al., 2015). These studies in which either calcitriol, doxercalciferol or D3 were administered provided limited evidence of a strong biologic effect, effects were seen – following doses and schedules of calcitriol or doxercalciferol that were not in any way optimized for biologic effect; the data of Wagner and colleagues are consistent with the conclusion that higher concentrations of vitamin D compounds influence the biology of prostate tissues.

Rather than cite each of the several studies that evaluated calcitriol and analogues as single agents and in combination with glucocorticoids, epidermal growth factor receptor antagonists and cytotoxic...
agents, suffice it to say that these studies may be summarized as follows (Osborn et al., 1995; Chadha et al., 2010; Trump et al., 2006; Morris et al., 2004; Schwartz et al., 2005; Gross et al., 1998; Beer et al., 2004a; Beer et al., 2003a; Liu et al., 2003):

- High doses of calcitriol can be given safely to cancer patients
- No unusual or unexpected toxicity has been seen
- No potentiation of cytotoxic agent toxicity has been noted
- The only effect of calcitriol and analogues that has been noted has been mild – moderate transient hypercalcemia.

While enticing suggestions of clinically important anticancer effects have been seen, the limitations of these studies, as noted above (small sample sizes, failure to use biologically optimal or maximally tolerable doses and use in combination with other active agents) no definitive evidence of benefit has been documented.

Before discussing in detail two randomized controlled trials that had the potential to define, clearly, at least in men with castration resistant prostate cancer, the role of calcitriol and cytotoxic chemotherapy, let us consider three characteristics of calcitriol-based anticancer therapy that were confounding to its development:

9.1. Schedule

As noted above, considerable resources were invested in studies of calcitriol and analogues, that were – if maximizing tumor cell exposure to calcitriol was the goal – not likely to be positive. It is clear that the maximum oral dose of calcitriol is 1.5–2.0μg QD (calcitriol exposure = ~14μg/2 weeks) (Osborn et al., 1995). In contrast, intravenous calcitriol at a dose of 74μg weekly is safe and well-tolerated. (calcitriol exposure = 144μg/2 weeks) (Chadha et al., 2010). 125μg/week is safe if combined with dexamethasone (calcitriol exposure = 250μg/2 weeks) (Muindi et al., 2009). Yet many studies of daily or every other day oral calcitriol and analogues have been done. All were negative.

9.2. Dose

A more precise measure of exposure would be drug level achieved in blood or tumor. Muindi and colleagues demonstrated that the systemic exposure to calcitriol achieved in murine models in which calcitriol is effective in suppressing cancer growth can be achieved in humans at high, but safe and tolerable oral and intravenous doses of calcitriol (Muindi et al., 2009; Muindi et al., 2004). As will be discussed below, the largest studies of calcitriol in men with prostate cancer did not use doses high enough to approach these “target exposures”.

9.3. Formulation

As studies of high dose intermittent therapy with calcitriol were conducted it became clear that there is not a suitable formulation of calcitriol for such schedules. Two groups demonstrated that the available oral formulation (Rocaltril®) is inappropriate for high dose administration. Doses of Rocaltril® as high as 40μg QDX3 (daily for 3 successive days) were investigated. This schedule is inconvenient (requiring 80 tablets daily) and pharmaceutically flawed. At doses > 15–20μg QD of Rocaltril® the expected linear relationship between dose administered and plasma levels of calcitriol achieved is lost, apparently due to impaired absorption. Use of the liquid formulation of calcitriol did not overcome this problem (Muindi et al., 2002; Muindi et al., 2005; Liu et al., 2003).

The now defunct pharmaceutical company Novocea developed a new formulation of calcitriol referred to as DN-101. This formulation was carefully studied and did provide a linear relationship between dose and systemic exposure over a wide dose range (Beer et al., 2005; Beer et al., 2007a). With this new formulation in hand, Novocea undertook the evaluation of the potential for calcitriol to enhance the microtubule disrupting agent, docetaxel (Taxotere®) in the treatment of men with castration resistant prostate cancer (CRPC).

DN-101 had a favorable pharmacetic profile: a linear relationship between dose and exposure over a wide dose range. At the 165μg dose, C(max) was 6.21 ± 1.99ng/mL, AUC(0–24) was 41.3 ± 9.77 ng·h/mL and half-life (T1/2) was 16.2 h. Muindi and colleagues noted that AUC and C(max) calcitriol concentrations of 32 ng·h/mL and 9.2 ng/mL are associated with striking antitumor effects in a murine squamous cell carcinoma model (Fig. 1).

Dose escalation in the initial phase 1 trial of single dose administration was halted at 165μg orally × 1 (all human DN-101 development was done employing the oral route of administration) because of the inconvenience of higher doses which would have required >11 capsules to be administered. Might there have been advantage to reformulating the capsules into higher drug content and continuing dose escalation? In the subsequent repeated dosing (weekly, oral administration) phase 1 trial doses of 15, 30, 45, 60 and 75μg were studied. Dose limiting toxicity (DLT) was defined as grade 2 or greater hypercalcemia or grade 3 or greater persistent treatment-related toxicities. The maximum tolerated dose (MTD) was defined as that dose level below which dose limiting toxicity occurred in 2 or more of 6 individuals at a given escalation level. At the 60μg dose 2 of 6 patients experienced asymptomatic grade 2 hypercalcemia (serum calcium of 11.6–12.5mg/dL). 45μg weekly was defined as the MTD of DN-101 and 18 patients were treated with this dose without any dose limiting toxicities. This dose of 45μg weekly was chosen for further development in combination with docetaxel (36mg/m2, weekly) in CRPC defined. Calcitriol had been combined with docetaxel in a phase II trial reported by Beer and colleagues. Prostate-specific antigen (PSA) decline of 50% or greater was seen in 81% of patients in that trial. The calcitriol dose in that study was 0.5μg/kg or ~35μg for a 70kg man – very much in the range of the 45μg weekly dose determined to be the MTD dose of DN-101. The 45μg dose was chosen for further development. The decision as to the definition of dose-limiting toxicity (a single grade 2 elevation in serum calcium without regard to duration of elevation or biologic effects of that elevation) is exceedingly conservative. One could imagine this decision was influenced by the trial of Beer et al. demonstrating a high PSA response rate with a similar calcitriol dose – in a single institution, trial of 37 patients (Beer et al., 2003b).

The extent of toxicity defined as dose limiting (grade 2 hypercalcemia) in the development of DN-101 is substantially less than that usually accepted in studies developing anticancer agents. In 2011 Le Tourneau and colleagues analyzed 155 phase I trials and found that “hypercalcemia” was not specifically noted to have been a DLT in any study, though electrolyte abnormalities were the defining DLT in <5% of trials.

Overall, the great majority of studies employed greater than or equal to

![Calcitriol Area Under Concentration-Time Curve (AUC): Human and Murine Comparisons](image-url)
grade 3 toxicity as a DLT definition, supporting the observation that the MTD and DLT delineation for DN-101 was conservative (Eaton et al., 2016; Le Tourneau et al., 2011). In a trial of weekly intravenous calcitriol + gefitinib and fixed dose dexamethasone, Muindi and colleagues defined DLT as any of the following toxicities attributable to study treatment in the first 4 weeks of treatment: ≥ 3 or 4 toxicity except for grade 3 anemia, ≥ grade 2 or above hypercalcemia (corrected calcium > 11.5 mg/dL) if confirmed on repeat blood draw (> 72 h), any grade 2 or above hypercalcemia that is associated with serious hypercalcemic symptoms, any treatment interruption that lasts for > 2 weeks that is related to treatment toxicity, sustained increase (> 72 h) in creatinine to > 2 × baseline and > 2 mg/dL, and clinical or radiological evidence of new genitourinary stones. With this definition, among 20 patients dose-limiting hypercalcemia was observed in two out of the four patients receiving 163 μg/wk of calcitriol. MTD was defined as 125 μg weekly and at this dose mean (± SE) peak serum calcitriol concentration (Cmax) was 11.17 ± 2.62 ng/mL and the systemic exposure (AU0–72h) was 53.30 ± 10.49 ng h/mL (Muindi et al., 2009). Ramnath and colleagues studied escalating doses of intravenous calcitriol administered every 21 days prior to docetaxel 75 mg/m2 and cisplatin 75 mg/m2 in a phase I/II study. In the phase I portion of this trial intravenous calcitriol doses of 30, 45, 60, and 80 μg/m2 were studied. 14 patients were studied in phase I. At 80 μg/m2/2/4 patients had DLTs of grade 4 neutropenia. Hypercalcemia was not observed. The MTD was determined to be 60 μg/m2. Among 20 evaluable phase II patients, there were 6 partial responses (30%), and 9 patients with stable disease (45%). The role of calcitriol in the myelosuppression seen in this trial is unclear; no hypercalcemia was noted (Ramnath et al., 2013). These data support the hypothesis that the dose of DN-101 chosen for further development was very conservative.

Novocaza conducted two randomized phase trials in men with CRPC: ASCENT I and ASCENT II (Beer et al., 2007b; Scher et al., 2011). The goal of ASCENT I was to determine the PSA response rate (defined as a > 50% decline in PSA for > 1 month) following standard therapy for CRPC (docetaxel 36 mg/m2 weekly intravenously × 4 weeks every 6 weeks) compared to the same dose and schedule of docetaxel + calcitriol (DN-101), 45 μg weekly. Was DN-101 iv or oral? Two hundred fifty patients were randomized. PSA response rates were 63% (DN-101) and 52% (placebo), (P-value = .07). While the primary goal (superior PSA response for DN-101 + docetaxel) of this trial was not achieved, there was a difference in the PSA response rate favoring the DN-101 arm. Survival was not a primary endpoint of this trial, but patients receiving DN-101 did fare better. Median survival for the placebo arm was 16.4 months and for the DN-101 arm 24.5 months. Hazard rate for death in the DN-101 group was 0.67 (p = .04). All toxicities appeared equal between the two arms and no patient experienced hypercalcemia. These results were encouraging, especially in view of the fact that this was a relatively large, randomized trial. Encouragement was derived primarily from a secondary endpoint analysis. Drug approval was not possible based on this trial. A larger trial was initiated (ASCENT II) in order to evaluate the apparent survival advantage of DN-101 + docetaxel.

In the time period between the design of ASCENT trials I and II, the weekly docetaxel regimen employed in ASCENT I clearly was shown to be inferior in terms of patient survival compared to an every three week regimen (75 mg/m2 every three weeks) (Petrylak et al., 2004; Tannock et al., 2004). Petrylak and colleagues reported a trial which compared docetaxel (weekly)/estramustine to mitoxantrone/prednisone in 770 patients with CRPC, and found an improvement in overall survival in the docetaxel arm (median survival 17.5 months for weekly docetaxel/estramustine vs. 15.6 months). Tannock and colleagues reported the TAX327 trial which compared q 3 week docetaxel, weekly docetaxel, and q 3 week mitoxantrone, with prednisone in 1006 patients. The patients who received q 3 week docetaxel had a median survival of 18.9 months (reduction in risk of death = 24%), compared to 17.3 months for weekly docetaxel and 16.4 months for the mitoxantrone arm. In the TAX327 trial the q 3 week docetaxel arm had higher rates of toxicity than the other 2 arms, (neutropenia and neurosensory changes); quality of life data suggest that docetaxel (either weekly or q 3 week) is better tolerated than mitoxantrone. If the designers of the ASCENT trials had adhered strictly to the principles of clinical trial design this change in the standard of care should have been accompanied by a change in the design of ASCENT II. The optimal design would have been docetaxel, 75 mg/m2 q3weeks ± DN-101. However, the ASCENT II trial designers decided to compare the apparently active, experimental treatment arm of ASCENT I (docetaxel, weekly, 36 mg/m2 + DN-101, 45 μg weekly × 4, every 6 weeks) to docetaxel 75 mg/m2 every three weeks + placebo. The enrollment target was 1200 patients. Complete the sentence. The final enrollment target was based on the assumption of a median survival of 18.9 months in the control group (q3weekly docetaxel + placebo) and a hazard ratio for mortality of ≤ 0.78 using a two-sided significance level of 0.05, with a power of 90%. This design tested the hypothesis that calcitriol would make an inferior treatment (weekly docetaxel) superior to a statistically better treatment (every 3 week docetaxel). The reasons for the decision to proceed with this “non-standard” trial design may have included:

(1) Cost – expense would have been required to be sure q3week docetaxel, 75 mg/m2 + 45 μg DN-101 was safe and active.

(2) Time – since the duration of patent protection on a composition of matter patent such as that which protected DN-101, is limited, time invested in resetting the experimental arm would have been a business risk.

(3) “Conviction” that calcitriol was sufficiently active in enhancing docetaxel in CRPC may have played a role.

ASCENT II was initiated on February 9, 2006. On October 31, 2007, when 953 patients had been accrued, the data and safety monitoring board recommended the study be halted. The reason for study interruption was a greater number of deaths in the experimental arm (weekly X4 docetaxel, q6weeks + weekly DN-101) than the control arm (q3weekly docetaxel + placebo). In the experimental (DN-101) arm median overall survival was 17.8 months (95% CI, 16.0 to 19.5) and 20.2 months (95% CI, 18.8 to 23.0) in the docetaxel only arm (log-rank  

P = .002). This trial result has been interpreted to show that calcitriol does not potentiate the antitumor efficacy of docetaxel in CRPC. And, largely, that further studies of vitamin D as a therapeutic agent in cancer were unwarranted. On reflection, ASCENT II could be described more accurately as demonstrating that a calcitriol dose less than that which can be safely administered does not overcome the inferiority of weekly vs. q3 weekly docetaxel. This trial was seriously flawed in two ways:

• The design of ASCENT II violated a basic principle of clinical trial development. The appropriate standard for trial design is standard therapy ± investigational regimen. The scientific conclusion from ASCENT II is that calcitriol at the dose studied was not sufficiently potent to overcome the intrinsic inferiority of the weekly docetaxel regimen.

• There are no data that indicate that the dose of calcitriol administered in ASCENT II was biologically or therapeutically ideal. The dose studied was < 50% of the maximum tolerated dose of calcitriol that can be given on an intermittent schedule. The dose of calcitriol chosen was based on a 37 patient study by Beer and colleagues which demonstrated an 81% response rate in CRPC and the phase 1 study of DN-101 which employed a very conservative definition of MTD.

Ramnath et al. safely administered 80 μg/m2 (~120 μg total dose) of calcitriol intravenously with docetaxel and cisplatin and no patient had hypercalcemia (Ramnath et al., 2013). Myelosuppression was the
limiting toxicity in that trial, likely related solely to the cytotoxic agents. Muindi et al. and Fakih et al. studied intravenous calcitriol weekly with gefitinib (Iressa®), an oral epidermal growth factor tyrosine kinase inhibitor. 74μg weekly alone and 125μg weekly with dexamethasone were the defined “phase II” doses (Muindi et al., 2009; Fakih et al., 2007). The ASCENT I and II trials were done with a dose of calcitriol that was one-quarter to one-half the calcitriol dose that would have been safe. Considerable evidence in preclinical studies indicates that the anticancer effect of calcitriol is dose/exposure related. Aside from the substantial number of studies noted above in prostate cancer and lung cancer, very limited studies have been done with calcitriol or its analogues in other cancers. Each of those studies utilized seocalcitol (EB1089) in breast, colorectal, pancreatic and hepatocellular carcinomas and is limited by concerns related previously: (1) small sample size (2) much less than the MTD of analogue administered (3) use of continuous rather than intermittent dosing regimens (Limaye et al., 2013; Dalhoff et al., 2003; Evans et al., 2002; Gulliford et al., 1998).

10. Summary

There are considerable data indicating the importance of vitamin D signaling in cancer. Vitamin D signaling is a plausible target for the treatment of established cancers – either as vitamin D agents alone or such agents combined with other antineoplastic agents. Unfortunately, there still is limited information regarding the role of vitamin D compounds in the treatment of cancer. Two approaches could be considered to refine the clinical studies of vitamin D in prostate cancer:

(1) Establish dependable biomarkers predictive of vitamin D response. This could facilitate:

- Selection of biologically appropriate or active dose of vitamin D compounds to use therapeutically.
- Selection of patients with a greater likelihood of response. This is the routinely employed approach that has led to the success in developing many so-called “targeted” cancer therapies (Solomon et al., 2014; Drilon et al., 2018; Limaye et al., 2013).

(2) Conduct of well-designed, appropriately powered clinical trials of biologically appropriate doses of vitamin D compounds.

Existing data strongly support the continued development of these approaches.

Transparency document

The Transparency document associated with this article can be found, in online version.
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