Agents Promoting Cellular Differentiation

In general, cells that are less differentiated have a greater tendency to proliferation. This is most clearly seen with embryonal, and especially stem cells. As cells mature, they become more differentiated into specific cell types, with a consequent significant decrease in proliferative ability. Cancer cells tend to regress to a less differentiated phenotype. Those that are the least differentiated tend to be show greater aggressive malignant properties. This is evident in the pathological description of cancer cells, often described on a range of well differentiated to poorly differentiated or anaplastic. These processes of differentiation are largely under genetic control.

Various natural substances such as retinoids and different forms of Vitamin D, as well as numerous medications, including agents typically used for other illnesses, such as the glitazones for diabetes and fibrates for lipid abnormalities effect different nuclear receptors, which have the effect of stimulating genes which promote cellular differentiation. Retinoids stimulate retinoid receptors (RAR and RXR), certain classes of Vitamin D stimulate vitamin D receptors (VDR), and glitazones and fibrates stimulates peroxisome proliferation activation receptors (PPAR). There is often an interaction, and synergism between these different substances and their receptors. This is particularly important because there is dose limiting toxicity for these agents, such as hypercalcemia for Vitamin D and liver toxicity for Vitamin A. These toxicities have greatly limited their clinical usefulness when employed as single agents. Their potential synergism between themselves (1,2,3 4), synergism with some conventional chemotherapy agents (5) as well as other natural substances (6), raises the hope that they could be given in non toxic dosages and be of benefit in an integrative treatment program.

Synergism

A general principal in integrative cancer treatment is that most agents have more than one clinical action and effect, and these effects are often linked to each other. Vitamin D, as a cogent example, has the ability to (a) induce differentiation, (b) induce apoptosis, (c) induce the activity of the tumor suppressors p21 or p27, (d) inhibit angiogenesis, and (e) inhibit cell migration (7). Retinoids show similar effects, as well as having a reciprocal inhibitory relationship with NFkB, a cancer promoting transcription factor (8). These actions are linked, as for example, p21, as a tumor suppressor gene, induces apoptosis. Another important principle in integrative cancer treatment is that single agents, though likely to show only slight activity when used alone, display synergistic and more comprehensive effects when used together with other agents. This principle is also understood and used in conventional cancer treatment, as most chemotherapy regimens employ multiple agents with different modes of action. This synergism also allows lower doses of single agents and limits toxicity. This is especially highlighted in discussing differentiation inducing vitamins, as they have potential toxicity when used in higher doses.

Retinoids

Retinoids such as the forms found in food and supplements (retinyl ester such as retinyl palmitate), 9 and 13 cis retinoic acid, as well as pharmacological forms such as all trans retinoic acid (ATRA), exert their effect by effecting the transcription of a variety of genes critical to cellular proliferation and differentiation. Retinoids are transported to the cellular nucleus, where they effect the retinoic acid receptors, RAR and RXR. These receptors also have the effect of increasing the DNA binding affinity to other receptors such as the Vitamin D receptor, and PPAR (peroxisome proliferator activated receptor). (9). These actions have the effect of promoting cellular differentiation and decreasing proliferation. They have also been shown to promote apoptosis and cell cycle arrest through degradation and decreased formation of stimulatory proteins. These actions involve the proteosome/ubiquitin pathway (10), a pathway of great interest because of, among other things, its central role in the production of NFkB, a transcription factor which promotes cellular proliferation, and is central in chemotherapy resistance.

Retinoids have been studied in various malignancies, and have significant efficacy in acute promyelocytic leukemia, and leukoplakia, a premalignant condition. They have been shown to effect a very wide range of cells, such as breast (11). A number of experimental and clinical studies have been performed in the past two decades with retinoids showing that they inhibit or reverse the carcinogenic process in some organs, including hematological malignancies as well as premalignant and malignant lesions in the oral cavity, head and neck, breast, skin and liver (12). Despite these encouraging findings, the clinical use of retinoids, as a single agent, has been relatively disappointing. Goodman completed a phase II trial of oral retinol (200,000 units/m2) in 65 patients with advanced cancer, with only minimal success. Six of sixty five patients showed a limited response (13). Pastorino treated patients with curatively resected stage 1 lung cancer with Vitamin A, 300,000 iu for twelve months, observing for the occurrence of second malignancies in this group of patients with a significant smoking history. There was some decrease noted after 46 months (14). Lippmann published a similar study, however using a synthetic retinoid, isoretinoin, and found no difference in the development of second malignancies (15). As different agents were used, it's difficult to draw a firm conclusion about the ability of vitamin A alone to prevent recurrent or second malignancies in this group of patients.

As opposed to the use of a single agent, most integrative oncology regimens would use vitamin A in combination with other agents, taking advantage of the synergistic effect. In this context, numerous studies have shown synergistic benefit. Koga showed breast cancer cells treated with vitamin A had a synergistic benefit when cotreated with vitamin D and/or antiestrogens (2). Konopleva showed an enhanced differentiation and proapoptotic effect on leukemic cells when vitamin A analogues were combined with PPAR agonists (16). A combination of interferon alpha and isoretinoin was shown to be of benefit to patients with refractory hematological malignancies (17).

Issues related to vitamin A toxicity are of importance. Acute vitamin A toxicity is rare, and requires extremely high doses, usually over 500,000 iu. There is significant toxicity in pregnant women and children, and high doses should be avoided in these populations. Chronic vitamin A toxicity is documented to occur with prolonged dosing of as low as 25,000 iu/day, but most authors quote > 50,000 iu/day for periods of a year or more. Early signs of toxicity include anorexia, headache, blurred vision, loss of hair, bleeding lips, cracking and peeling skin, muscular stiffness and pain (18). Liver enzymes and retinol levels should be followed periodically in patients on moderate to high doses. It is also known that very prolonged uses of 5-10,000 iu/day predisposes to the development of osteoporosis. These issues need to be considered in the light of the specific oncological clinical situation. For instance, in patients with progressive malignancies, uses of 50,000 iu/day for up to a year can be considered, along with a comprehensive treatment program. It is also possible, that the use of lower doses, when combined with other differentiating agents like vitamin D, can give similar beneficial effects as higher doses of vitamin A used alone.

Vitamin D

Vitamin D has generally been considered in relation to bone and mineral metabolism. Vitamin D has numerous other actions. Deficiencies have been associated with an increased prevalence of hypertension and multiple sclerosis. In the realm of oncology, lower serum levels of vitamin D have been associated with an increased incidence of various malignancies, including breast (19), colorectal (20), prostate and pancreas (21). Vitamin D has numerous physiologic actions which can be important in the treatment of malignancies, including "an increase in G_0/G_1 arrest, induction of apoptosis and differentiation, and modulation of expression of growth factor receptors" (22), as well as important effects on cell differentiation. It has also been shown to stabilize DNA in a murine lymphoma model, leading to a decrease in mutations as well as increased survival (23).

An in vitro study with numerous differentiating agents, including two forms of vitamin D, showed an inhibition of proliferation on head and neck tumor cells (4). Gross, et al. (24) studied patients with early stage prostate cancer, who received calcitriol (1-25 OH vitamin D) after initial surgery or radiation therapy. They found a significantly decreased rate of PSA rise. Dosages ranged from 0.5 to 2.5 mcg/day, depending on the calcium levels obtained during treatment. Beer et al. (5), have performed a series of studies using high oral doses of calcitriol given weekly to patients with prostate and other cancers, in an attempt to avoid the potential hypercalcemia that can occur with daily doses. They began with low doses, and escalated doses as tolerated, finding that the optimal dose was .5mcg/kg/week. This allowed the peak 1-25OH vitamin D levels to exceed 1ng/ml, which, from in vitro studies, has been felt to be necessary to show a clinical effect. They also report on a phase 2 study in patients with androgen unresponsive prostate cancer, who were treated with a combination of Taxol and weekly high dose calcitriol. This showed improved responses regarding PSA and tumor shrinkage compared to historical controls with Taxol alone (5). Another study, however, found unimpressive results when calcitriol was combined with zoledronate and dexamethasone in progressive prostate cancer patients (25). However, in this study, calcitriol was given at much lower daily oral doses than the weekly schedule studied by Beer. Trouillas (26) studied 11 patients with aggressive brain tumors (10 with glioblastoma multiforme and 1 with anaplastic astrocytoma) who were treated in a phase II trial involving surgery or biopsy, radiotherapy (64 Gy), chemotherapy with VM26-CCNU or fotemustine, and alfacalcidol (a synthetic Vit D analogue which is a precursor to calcitriol) at the daily dose of 0.04 mcg/kg. Three of the 11 patients were long-term survivors (still alive after 7,5 and 4 years).

Due to the concern of hypercalcemia, attempts to circumvent this adverse reaction have also included the development and use of vitamin D analogues (26,27,28), with mixed effects so far. Other dosing schedules, such as the weekly schedule discussed above, are being studied. A comprehensive article discussing optimal dosing, optimal blood levels, and controversies regarding toxicity should be consulted (29). In this review, Vieth presents data suggesting that usual recommendations for dosages and serum levels are much lower than optimal. He suggests that a normal blood level of vitamin D is greater than 100 mmol/liter , as opposed to the typical stated ranges of 20-80. He also states that toxic states have not been documented until the blood level is >200. (Note that vitamin D levels are also often reported as ng/ml. 200 mmol/l is about equivalent of 85ng/ml).He also states that daily intake of vitamin D3 of 4000 iu/day is safe. This is significantly higher than the usual recommended doses of 400-800 iu/day.

In addition to dosage changes, synergism has been noted with numerous other agents, including retinoids, genistein (6), vitamin E (30), curcumin and NSAIDS (31).

As discussed above under synergism, the use of these differentiating agents in combination has the potential to improve efficacy, while at the same time limiting the potential for adverse effects.

Peroxisome Proliferator Activated Receptor Agonists (PPAR agonists)

PPARs are nuclear receptors closely connected with the RAR, RXR and vitamin D receptors discussed above. Stimulation of the vitamin A receptors can have a reciprocal stimulatory effect on the vitamin D receptor and PPARs. The PPAR of most interest is PPAR gamma.

Various substances act as ligands for PPAR gamma, combining with it and leading to signal transduction and gene stimulation. They include, of great interest, the glitazone family of medications, commonly used in diabetic patients to increase insulin sensitivity. Other agonists include long chain fatty acids and eicosanoids. Many agents, including NSAIDS and COX 2 inhibitors have the effect of stimulating PPARs.

PPAR agonists have been shown to have numerous relevant clinical effects besides differentiation, including inhibition of NFkB, proapoptotic, antiproliferative, and antiangiogenetic actions (32).

PPAR agonists have been studied in a wide range of tumor types, including carcinomas (33), lymphomas (34,35), and sarcomas (36). Patients with medium to high grade liposarcomas, where the state of differentiation is of great importance, were treated with troglitazone, and an improved state of tumor differentiation was noted.

Practical Aspects of Differentiation Therapy

As with most integrative treatment regimens, multiagent protocols are usually employed. There are numerous rational reasons to employ multiagent protocols in the attempt to promote differentiation in malignant cells. Nature itself suggests that this should be done, as the three nuclear receptor groups discussed above, are in close anatomic proximation, and stimulation of one group has reciprocal effects on the other groups, through the process of dimerization.

Issues of toxicity, especially with the fat soluble vitamins A and D are important considerations. The dosages of vitamin A are likely to be very dependent on the time of the course of treatment. With extended treatment courses, clinical and laboratory monitoring is of importance. Vitamin D is usually given in doses far below the optimal range. Nevertheless, high doses, and the calcitriol form particularly, can induce hypercalcemia. Various treatment regimens, using different forms of natural vitamin D (such as D3 available OTC, and calcitriol;1-25 OH Vit D, which is available only by prescription), and vitamin D synthetic analogues, as well as different treatment schedules, including once weekly doses, are actively being studied. The forms used, and dosages prescribed could depend on the clinical stage and treatment goal. For instance, patients in a relatively stable, wait and see stage could be treated with 25-OH regimens as described below. The two clinical studies using Vit D for patients in a more progressive stage, in association with active conventional treatment (prostate cancer and brain gliomas), used 1-25 OH forms (calcitriol for prostate cancer and a calcitriol precursor, alfacalcidol, for brain gliomas). Levels of calcium and Vitamin D should be monitored at frequent intervals. It should be noted that most laboratories report normal ranges of Vitamin D which are probably significantly lower than the optimal levels. One should aim for a range of 50-100 ng/ml, or 100-180 mmol/liter. The use of PPAR agonists involves at present mainly prescription medications primarily used for diabetics, the glitazones.

In addition to the synergistic effects noted between these three groups of agents, numerous other substances, including antioxidants and anti-inflammatory agents, have been shown to increase the benefits. Therefore the use of differentiation agents should be combined with the general integrative treatment protocol outlined elsewhere.

Dosage recommendations, with the above mentioned considerations and precautions are as follows:

A) **Vitamin A** – retinol levels and liver enzymes should be followed
Short term use – 100,000-300,000 iu/day, not to exceed 1-2 months
Medium term use – 40,000-50,000 iu/day, for up to 1 year
Long term use – 20,000 iu/d, for up to 5 years

B) **Vitamin D –** 25-OH levels, and serum Calcium levels should be followed and doses adjusted accordingly
Vit D2: 50,000 i.u. po weekly for 6-8 weeks and reevaluate
Vit D3: 1,000 – 6,000 iu/day
Calcitriol (1-25OH vit D): .5mcg/kg weekly (used in clinical trial for treatment of prostate cancer. A precursor, alfacalcidol, was used as an active treatment for brain gliomas.

C) **PPAR agonists**
Consider the use of glitazones, with appropriate blood sugar monitoring.

Bibliography

1) Breitman TR, He RY. Combinations of retinoic acid with either sodium butyrate, dimethyl sulfoxide, or hexamethylene bisacetamide synergistically induce differentiation of the human myeloid leukemia cell line HL60. Cancer Res. 50(19):6268-73, 1990.

2) Koga M, Sutherland RL. Retinoic acid acts synergistically with 1,25-dihydroxyvitamin D3 or antioestrogen to inhibit T-47D human breast cancer cell proliferation. J Steroid Biochem Mol Biol. 39(4A):455-60, 1991.

3) James SY, Williams MA, Kelsey SM, Newland AC, Colston KW. The role of vitamin D derivatives and retinoids in the differentiation of human leukaemia cells. Biochemical Pharmacology. 54:625-34, 1997.

4) Satake K, Takagi E, Ishii A, Kato Y, Imagawa Y, Kimura Y, Tsukuda M. Anti-tumor effect of vitamin A and D on head and neck squamous cell carcinoma. Auris Nasus Larynx. 30(4):403-12, 2003.

5) Beer TM, Myrthue A. Calcitriol in cancer treatment: from the lab to the clinic. Mol Cancer Ther. 3(3):373-81, 2004.

6) Rao A, Coan A, Welsh JE, Barclay WW, Koumenis C, Cramer SD. Vitamin D receptor and p21/WAF1 are targets of genistein and 1,25-dihydroxyvitamin D3 in human prostate cancer cells. Cancer Res. 64(6):2143-7, 2004.

7) Boik J. Natural compounds in cancer therapy. Princeton, MN: Oregon Medical Press, 2001, p. 323.

8) Andela VB, Rosier RN. The proteosome inhibitor MG132 attenuates retinoic acid receptor trans-activation and enhances trans-repression of nuclear factor kappaB. Potential relevance to chemo-preventive interventions with retinoids. Mol Cancer. 3(1):8, 2004.

9) Heber D, Blackburn GL, Go VLW, Holland JF. Nutritional oncology. San Diego: Academic Press, 1999, p. 501.

10) Dragnev KH, Petty WJ. Dmitrovsky E. Retinoid targets in cancer therapy and chemoprevention. Cancer Biology & Therapy. 2(4 Suppl 1):S150-6, 2003.

11) Czeczuga-Semeniuk E, Anchim T, Dzieciol J, Dabrowska M, Wolczynski S. Can transforming growth factor-beta1 and retinoids modify the activity of estradiol and antiestrogens in MCF-7 breast cancer cells? Acta Biochim Pol. 51(3):733-45, 2004.

12) Okuno M, Kojima S, Matsushima-Nishiwaki R, Tsurumi H, Muto Y, Friedman SL, Moriwaki H. Retinoids in cancer chemoprevention. Curr Cancer Drug Targets. 4(3):285-98, 2004.

13) Goodman GE. Phase II trial of retinol in patients with advanced cancer. Cancer Treat Rep. 70(8):1023-4, 1986.

14) Pastorino U, Infante M, Maioli M, Chiesa G, Buyse M, Firket P, Rosmentz N, Clerici M, Soresi E, Valente M, et al. Adjuvant treatment of stage I lung cancer with high-dose vitamin A. J Clin Oncol. 11(7):1216-22, 1993.

15) Lippman SM, Lee JJ, Karp DD, Vokes EE, Benner SE, Goodman GE, Khuri FR, Marks R, Winn RJ, Fry WW, Graziano G, Gandara D, Okawara G, Woodhouse CL, Williams B, Perez C, Kim HW, Lotan R, Roth J, Ki Hong W. Randomized phase III intergroup trial of isotretinoin to prevent second primary tumors in stage I non-small-cell lung cancer. J Natl Cancer Inst. 93(8): 605-618, 2001.

16) Konopleva M, Elstner E, McQueen TJ, Tsao T, Sudarikov A, Hu W, Schober WD, Wang RY, Chism D, Kornblau SM, Younes A, Collins SJ, Koeffler HP, Andreeff M. Peroxisome proliferator-activated receptor gamma and retinoid X receptor ligands are potent inducers of differentiation and apoptosis in leukemias. Mol Cancer Ther. 3(10):1249-1262, 2004.

17) Tsimberidou AM, Giles F, Romaguera J, Duvic M, Kurzrock R. Activity of interferon-alpha and isotretinoin in patients with advanced, refractory lymphoid malignancies. Cancer. 100(3):574-80, 2004.

18) Pizzorno JE, Murray MT. Textbook of natural medicine, 2nd ed. New York: Churchill Livingstone, 1999, chapter 122.

19) Berube S, Diorio C, Verhoek-Oftedahl W, Brisson J. Vitamin D, calcium, and mammographic breast densities. Cancer Epidemiol Biomarkers Prev. 13(9):1466-72, 2004.

20) Courtney ED, Melville DM, Leicester RJ. Review article: chemoprevention of colorectal cancer. Alimentary Pharmacology & Therapeutics. 19(1): 1-24, 2004.

21) Vanchieri C. Studies shedding light on vitamin D and cancer. Journal of the National Cancer Institute. 96(10):735-736, 2004.

22) Sarkar A, Saha BK, Basak R, Mukhopadhyay I, Karmakar R, Chatterjee M. Anticlastogenic potential of 1alpha,25-dihydroxyvitamin D3 in murine lymphoma. Cancer Lett. 150(1):1-13, 2000.

23) Trump DL, Hershberger PA, Bernardi RJ, Ahmed S, Muindi J, Fakih M, Yu WD, Johnson CS. Anti-tumor activity of calcitriol: pre-clinical and clinical studies. Journal of Steroid Biochemistry and Molecular Biology. 89-90(1-5):519-526, 2004.

24) Gross C, Stamey T, Hancock S, Feldman D. Treatment of early recurrent prostate cancer with 1,25-dihydroxyvitamin D3 (calcitriol) J Urol. 159(6):2035-9, 1998; discussion 2039-40. Erratum in: J Urol. 160(3 Pt 1):840, 1998.

25) Morris MJ, Smaletz O, Solit D, Kelly WK, Slovin S, Flombaum C, Curley T, Delacruz A, Schwartz L, Fleisher M, Zhu A, Diani M, Fallon M, Scher HI. High-dose calcitriol, zoledronate, and dexamethasone for the treatment of progressive prostate carcinoma. Cancer. 100(9):1868-75, 2004.

26) Trouillas P, Honnorat J, Bret P, Jouvet A, Gerard JP. Redifferentiation therapy in brain tumors: long-lasting complete regression of glioblastomas and an anaplastic astrocytoma under long term 1-alpha-hydroxycholecalciferol. J Neurooncol. 51(1):57-66, 2001.

27) Albert DM, Kumar A, Strugnell SA, Darjatmoko SR, Lokken JM, Lindstrom MJ, Patel S. Effectiveness of vitamin D analogues in treating large tumors and during prolonged use in murine retinoblastoma models. Arch Ophthalmol. 122(9):1357-62, 2004.

28) Hansen CM, Hamberg KJ, Binderup E, Binderup L. Seocalcitol (EB 1089): a vitamin D analogue of anti-cancer potential. Background, design, synthesis, pre-clinical and clinical evaluation. Curr Pharm Des. 6(7):803-28, 2000.

29) Vieth, R. Effect of Pseudomonas fluorescens on beef discoloration and oxymyoglobin oxidation in vitro. Am J Clin Nutr. 69 842-56, 1999.

30) Sokoloski JA, Hodnick WF, Mayne ST, Cinquina C, Kim CS, Sartorelli AC. Induction of the differentiation of HL-60 promyelocytic leukemia cells by vitamin E and other antioxidants in combination with low levels of vitamin D3: possible relationship to NF-kappaB. Leukemia. 11(9):1546-1553, 1997.

31) Danilenko M, Studzinski GP. Enhancement by other compounds of the anti-cancer activity of vitamin D(3) and its analogs. Experimental Cell Research. 298(2):339-358, 2004.

32) Grommes C, Landreth GE, Heneka MT. Antineoplastic effects of peroxisome proliferator-activated receptor gamma agonists. Lancet Oncol. 5(7):419-29, 2004.

33) Sakamoto A, Yokoyama Y, Umemoto M, Futagami M, Sakamoto T, Bing X, Mizunuma H. British Journal of Cancer. 91(4):633-8, 2004.

34) Padilla J, Kaur K, Harris SG, Phipps RP. PPAR-gamma-mediated regulation of normal and malignant B lineage cells. Ann N Y Acad Sci. 2000 Apr;905:97-109.

35) Padilla J, Kaur K, Cao HJ, Smith TJ, Phipps RP. Peroxisome proliferator activator receptor-gamma agonists and 15-deoxy-Delta(12,14)(12,14)-PGJ(2) induce apoptosis in normal and malignant B-lineage cells. Journal of Immunology. 165(12):6941-8, 2000.

36) Demetri GD, Fletcher CD, Mueller E, Sarraf P, Naujoks R, Campbell N, Spiegelman BM, Singer S. Induction of solid tumor differentiation by the peroxisome proliferator-activated receptor-gamma ligand troglitazone in patients with liposarcoma . Proc Natl Acad Sci USA. 96(7):3951-6, 1999.