

Vitamin A:

Wang, J. and A. Yen (2004). "A novel retinoic acid-responsive element regulates retinoic acid-induced BLR1 expression." *Mol Cell Biol* **24**(6): 2423-43.

The mechanism of action of retinoic acid (RA) is of broad relevance to cell and developmental biology, nutrition, and cancer chemotherapy. **RA is known to induce expression of the Burkitt's lymphoma receptor 1 (BLR1) gene which propels RA-induced cell cycle arrest and differentiation of HL-60 human myeloblastic leukemia cells, motivating the present analysis of transcriptional regulation of blr1 expression by RA.** The RA-treated HL-60 cells used here expressed all RA receptor (RAR) and retinoid X receptor (RXR) subtypes (as detected by Northern analysis) except RXR γ . Treatment with RAR- and RXR-selective ligands showed that RAR α synergized with RXR α to transcriptionally activate blr1 expression. A 5'-flanking region capable of supporting RA-induced blr1 activation in HL-60 cells was found to contain a 205-bp sequence in the distal portion that was necessary for transcriptional activation by RA. Within this sequence DNase I footprinting revealed that RA induced binding of a nuclear protein complex to an element containing two GT boxes. Electromobility shift assays (EMSAs) and supershift assays showed that this element bound recombinant RAR α and RXR α . Without RA there was neither complex binding nor transcriptional activation. Both GT boxes were needed for binding the complex, and mutation of either GT box caused the loss of transcriptional activation by RA. The ability of this cis-acting RAR-RXR binding element to activate transcription in response to RA also depended on downstream sequences where an octamer transcription factor 1 (Oct1) site and a nuclear factor of activated T cells (NFATc) site between this element and the transcriptional start, as well as a cyclic AMP response element binding factor (CREB) site between the transcriptional start and first exon of the blr1 gene, were necessary. Each of these sites bound its corresponding transcription factor. A transcription factor-transcription factor binding array analysis of nuclear lysate from RA-treated cells indicated several prominent RAR α binding partners; among these, Oct1, NFATc3, and CREB2 were identified by competition EMSA and supershift and chromatin immunoprecipitation assays as components of the complex. RA upregulated expression of these three factors. In sum the results of the present study indicate that RA-induced expression of blr1 expression depends on a novel RA response element. This cis-acting element approximately 1 kb upstream of the transcriptional start consists of two GT boxes that bind RAR and RXR in a nuclear protein complex that also contains Oct1, NFATc3, and CREB2 bound to their cognate downstream consensus binding sites.

High, K. P., C. Legault, et al. (2002). **"Low plasma concentrations of retinol and alpha-tocopherol in hematopoietic stem cell transplant recipients: the effect of mucositis and the risk of infection."** *Am J Clin Nutr* **76**(6): 1358-66.

BACKGROUND: Although vitamin deficiencies are rare in the United States, acute reductions in concentrations of plasma retinol (vitamin A) or alpha-tocopherol (vitamin E) have been associated with impaired immune responses in some clinical settings. OBJECTIVE: **The objectives were to determine the plasma concentrations of retinol and alpha-tocopherol in patients undergoing dose-intensive therapy and hematopoietic stem cell transplant and to examine the association of plasma concentrations with clinical outcomes reflecting immunity.** DESIGN:

This was an observational trial of 120 consecutive recipients of hematopoietic stem cell transplant and a multivariate analysis of plasma vitamin concentrations, mucositis, infections in the first 30 d, and herpes zoster infections in the first year after hematopoietic stem cell transplant. RESULTS: Plasma retinol and alpha-tocopherol concentrations declined from baseline to day 7, typically recovering without specific replacement toward baseline by day 14. **The severity of mucositis was a strong predictor of low plasma retinol on day 7** (P = 0.001). Eighty-two patients (68%) had at least one plasma retinol concentration ≤ 1.05 micro mol/L, a concentration previously determined to be of immunologic significance, during the peritransplant period (day -8 to day 14). Men more frequently acquired herpes zoster than women, and men who developed hyporetinolemia (≤ 1.05 micro mol/L) had a significantly higher risk of herpes zoster (OR: 6.6; 95% CI: 1.5, 29.6). Plasma alpha-tocopherol was not associated with any clinical event measured in this study.

CONCLUSION: **Hyporetinolemia is common, particularly in subjects with severe mucositis, and is associated with an increased risk of herpes zoster infection in recipients of hematopoietic stem cell transplant.** Additional investigations are required to determine whether these findings indicate a causal relation.

Battle, T. E., M. S. Roberson, et al. (2001). "Retinoic acid-induced blr1 expression requires RARalpha, RXR, and MAPK activation and uses ERK2 but not JNK/SAPK to accelerate cell differentiation." *Eur J Cell Biol* **80**(1): 59-67.

Upstream signaling requirements of retinoic acid (RA)-induced blr1 expression and downstream signaling consequences of blr1 over-expression in a human myeloid leukemia cell line demonstrate that mitogen-activated protein kinase (MAPK) signaling complexes are involved in both avenues. **RA-induced myeloid differentiation and G1/G0 growth arrest of HL-60 cells is known to require the activation of the RARalpha and RXR retinoid receptors, as well as activation of the MAPK, ERK2.** Transcriptional activation of the Burkitt's lymphoma receptor 1 (blr1) gene occurs early during RA-induced differentiation of

HL-60 cells and requires these same three activating processes. The use of retinoid ligands that activate either the RARalpha or the RXR retinoid receptors revealed that blr1 mRNA induction was detectable only when both RARalpha and RXR were activated. Neither the RARalpha nor RXR selective ligands alone induced expression of blr1, but the combination of the two ligands induced the expression of blr1 to the same extent as RA. The MAPKK (MEK) inhibitor, PD98059, was used to determine whether extracellular signal-regulated kinase (ERK2) activation was necessary for induction of blr1 mRNA. PD98059 inhibited induced blr1 mRNA expression, due to RA or activated RARalpha plus RXR ligands, indicating that ERK2 activation is necessary for blr1 mRNA expression. Previous studies showed that ectopic expression of blr1 also caused increased MAPK activation, in particular ERK2, and subsequently accelerated RA-induced differentiation and G1/G0 growth arrest. Inhibition of ERK2 activation inhibited differentiation of blr1 transfectants, suggesting that the accelerated differentiation reflected blr1-enhanced ERK2 activation. The present data also demonstrate that ectopic expression of blr1 increased JNK/SAPK activity, but JNK/ SAPK activation was not needed for accelerated RA-induced differentiation and growth arrest. **The results show that the signals known to be required for HL-60 differentiation, activated RARalpha, RXR, and ERK2, are necessary for blr1 mRNA expression. Downstream consequences of blr1 overexpression include enhanced MAPK signaling.**

Koskela, K., T. T. Pelliniemi, et al. (2004). "Treatment of multiple myeloma with all-trans retinoic acid alone and in combination with chemotherapy: a phase I/II trial." *Leuk Lymphoma* 45(4): 749-54.

All-trans retinoic acid (ATRA) is a derivative of vitamin A. ATRA inhibits the growth of human myeloma cell lines and freshly isolated myeloma cells in vitro mainly by down-regulating interleukin-6 receptor. Clinically, however, ATRA alone has not been efficacious and adverse events, notably hypercalcemia, have been common. In the present study **10 patients with stable multiple myeloma after conventional chemotherapy received ATRA alone for 2 months, followed by a combination of ATRA and the chemotherapy regimen during which no further reduction of the paraprotein had occurred. The purpose of the combination therapy was to sensitize the myeloma cells with ATRA to chemotherapy by blocking the growth-promoting effect of IL-6.** Although ATRA was well tolerated, ATRA alone lacked clinical efficacy. **The combination therapy resulted minimal responses in 4 patients and relatively long progression-free survival in 4 patients was achieved.** In 3 of these responding patients serum concentrations of interleukin-6 and/or soluble interleukin-6 receptor were elevated prior to the study. The bone marrow cells of responding patients

were sensitive to ATRA in vitro. These results show that ATRA alone is not effective to treat multiple myeloma. **There may be some beneficial effect of ATRA in combination chemotherapy in selected patients who have activated IL-6 signaling.**

Drisko, J. A., J. Chapman, et al. (2003). "The use of antioxidant therapies during chemotherapy." *Gynecol Oncol* **88**(3): 434-9.

OBJECTIVE: At the present time, many cancer patients combine some form of complementary and alternative medicine therapies with their conventional therapies. The most common choice of these therapies is the use of antioxidants. **RESULTS: A review of four common antioxidants is undertaken, which includes vitamin E (mixed tocopherols and tocotrienols), beta-carotene (natural mixed carotenoids), vitamin C (ascorbic acid), and vitamin A (retinoic acid).** Antioxidants act as electron acceptors as well as therapeutic biologic response modifiers. **Despite the fact that chemotherapy-induced formation of free radicals is well-demonstrated, chemotherapy-induced cytotoxicity in general does not seem to depend on formation of reactive oxygen species.** **CONCLUSIONS: Currently, evidence is growing that antioxidants may provide some benefit when combined with certain types of chemotherapy.** Because of the potential for positive benefits, a randomized controlled trial evaluating the safety and efficacy of adding antioxidants to chemotherapy in newly diagnosed ovarian cancer is underway at the University of Kansas Medical Center.

Prasad, K. N., A. Kumar, et al. (1999). "High doses of multiple antioxidant vitamins: essential ingredients in improving the efficacy of standard cancer therapy." *J Am Coll Nutr* **18**(1): 13-25.

Numerous articles and several reviews have been published on the role of antioxidants, and diet and lifestyle modifications in cancer prevention. **However, the potential role of these factors in the management of human cancer have been largely ignored. Extensive in vitro studies and limited in vivo studies have revealed that individual antioxidants such as vitamin A (retinoids), vitamin E (primarily alpha-tocopheryl succinate), vitamin C (primarily sodium ascorbate) and carotenoids (primarily polar carotenoids) induce cell differentiation and growth inhibition to various degrees in rodent and human cancer cells by complex mechanisms.** The proposed mechanisms for these effects include inhibition of protein kinase C activity, prostaglandin E1-stimulated adenylate cyclase activity, expression of c-myc, H-ras, and a transcription factor (E2F), and induction of transforming growth factor-beta and p21 genes. **Furthermore, antioxidant vitamins individually or in combination enhance the growth-inhibitory effects of x-irradiation,**

chemotherapeutic agents, hyperthermia, and biological response modifiers on tumor cells, primarily in vitro. These vitamins, individually, also reduce the toxicity of several standard tumor therapeutic agents on normal cells. Low fat and high fiber diets can further enhance the efficacy of standard cancer therapeutic agents; the proposed mechanisms for these effects include the production of increased levels of butyric acid and binding of potential mutagens in the gastrointestinal tract by high fiber and reduced levels of growth promoting agents such as prostaglandins, certain fatty acids and estrogen by low fat. **We propose, therefore, a working hypothesis that multiple antioxidant vitamin supplements together with diet and lifestyle modifications may improve the efficacy of standard and experimental cancer therapies.**

Singh, D. K. and S. M. Lippman (1998). "Cancer chemoprevention. Part 1: Retinoids and carotenoids and other classic antioxidants." Oncology (Williston Park) **12**(11): 1643-53, 1657-8; discussion 1659-60.

Cancer chemoprevention is the use of specific natural or synthetic substances with the objective of reversing, suppressing, or preventing carcinogenic progression to invasive cancer. Currently, numerous chemopreventive agents are in various stages of development and testing. Part 1 of this two-part series provides an overview of issues unique to chemoprevention trials, including the use of surrogate biomarkers as end points. **This is followed by a discussion of the retinoids**, such as all-trans-retinoic acid (ATRA [Vesanoid]), 9-cis-retinoic acid (9cRA), and isotretinoin (Accutane), **and the carotenoids** (e.g., beta-carotene and lycopene) **and other "classic" antioxidants (e.g., vitamins E and C and selenium).** Research on these agents will be delineated by disease site when applicable. Part 2, which will appear in next month's issue, will focus on hormonally mediated chemopreventive agents, such as tamoxifen (Nolvadex), finasteride (Proscar), oral contraceptives, and dehydroepiandrosterone (DHEA). Part 2 also will cover nonantioxidant natural agents, such as calcium, the polyphenols, the isothiocyanates, and genistein; nonsteroidal anti-inflammatory drugs (NSAIDS), such as celecoxib, sulindac sulfone, and aspirin; difluromethylornithine (DFMO [Eflornithine]); oltipraz; and N-acetylcysteine.

Toma, S., P. L. Losardo, et al. (1995). "Effectiveness of beta-carotene in cancer chemoprevention." Eur J Cancer Prev **4**(3): 213-24.

This article reviews the current knowledge on the cancer-preventive potential of beta-carotene, a precursor of vitamin A, and plentiful in fruits and vegetables, which has been studied widely as a promising

chemopreventive agent in reducing the risk of cancer in humans. Several retrospective and prospective epidemiological investigations have demonstrated that a diet rich in micronutrients such as vitamins, carotenoids and selenium, could prevent the arising, in 'high-risk' patients, of precancerous and neoplastic lesions of specific sites, particularly of the upper aerodigestive tract. **Numerous in vitro expressions have been performed in order to verify the true role played by this agent on cell proliferation and differentiation; until now, findings have been very encouraging, uniformly showing the beta-carotene can affect carcinogenesis, particularly in early stages, through an antigenotoxic action. Antioxidant functions, immunomodulatory effects and control of intercellular messages via gap junctions are possible action mechanisms of the ability of beta-carotene to block the carcinogenetic process.** In vivo animal studies partially confirm the results obtained in vitro showing that beta-carotene is able to reduce the induce cancer development; moreover, the association of the carotenoid with other microelements, such as **vitamins E, C and glutathione often appears to be more effective than each agent used alone.** From a clinical point of view, beta-carotene appears an 'ideal' agent to be used in chemoprevention trials in humans, although optimal doses and intake methods need to be better defined; its almost zero toxicity permits the long-term administration of the drug, a vital condition for its anti-cancer activity, with good patient compliance. Human intervention studies performed so far, both randomized and uncontrolled clinical trials, have showed positive findings in specific cancer sites such as oral cavity, head and neck and colon; less consistent or negative are results on skin, lung and oesophagus cancer. The ongoing studies will provide more answer on these issues. A definitive evaluation of the ability of beta-carotene to prevent cancer in human requires further controlled trials; studies on a larger spectrum of cancer sites and different stages of disease must be encouraged. In addition, further investigation on biomarkers related to cancer risk and cancer incidence are necessary, particularly focused on the measurements for genotoxic damage, eg micronuclei, that may provide a valid and 'easy' marker for early stage carcinogenesis.

Prasad, K. N., J. Edwards-Prasad, et al. (1993). "Vitamins regulate gene expression and induce differentiation and growth inhibition in cancer cells. Their relevance in cancer prevention." Arch Otolaryngol Head Neck Surg **119**(10): 1133-40.

Although several hypotheses for human carcinogenesis have been proposed, the specific genetic changes that cause normal cells to become cancer cells have not been identified. In spite of uncertainties regarding the mechanisms of carcinogenesis, several vitamins such as beta-carotene and vitamins A, C, and E, which can reduce the risk of cancer,

have been identified, using animal and in vitro models of carcinogenesis. These studies have led to a hypothesis that the supplemental intake of these vitamins may reduce the risk of cancer. This hypothesis in humans can be tested only by intervention trials that are in progress. Prospective and retrospective case-controlled experimental designs are not suitable for testing the above hypothesis. **The fact that some vitamins induce cell differentiation and/or growth inhibition in tumor cells in culture suggests that the use of these vitamins in cancer prevention has a cellular basis. In addition to having a direct effect on tumor cells, vitamins such as alpha-tocopheryl succinate and beta-carotene enhance the effect of other agents that induce differentiation in tumor cells. Some vitamins like beta-carotene, retinoic acid, alpha-tocopheryl succinate, and vitamin D also regulate the expressions of certain oncogenes and cellular genes.** These are exciting new functions of vitamins that nobody could have predicted only a few years ago.

Seifter, E., G. Rettura, et al. (1983). "Regression of C3HBA mouse tumor due to X-ray therapy combined with supplemental beta-carotene or vitamin A." J Natl Cancer Inst **71**(2): 409-17.

Male CBA/J mice, ingesting a vitamin A- and beta-carotene-sufficient laboratory chow, were inoculated in a hind limb with 2×10^5 C3HBA adenocarcinoma cells. When the mean tumor size was 6.2 mm, the mice were divided randomly into groups; some groups received supplemental vitamin A or beta-carotene, some received 3,000 rad local radiation to the tumor, and others received both radiation and one of the supplements. All mice that received only radiation or one of the dietary supplements died within 3 months. **When local irradiation and supplemental vitamin A or beta-carotene were coupled, "complete" tumor regression occurred in every case (12/12), and tumor regrowth in and death of the mice occurred in only 1 of 12 in each of these groups during the succeeding 12 months.** One year after irradiation and dietary supplementation, half the surviving mice were switched back to the control chow. During the next year, none of the mice remaining on the vitamin A or beta-carotene supplements developed tumors; however, of 6 mice switched from vitamin A, 5 had tumors that reappeared. In contrast, tumors recurred in only 2 of 6 mice after they were switched from beta-carotene. **A second experiment yielded similar results. These results show that both vitamin A and beta-carotene supplementation added remarkably to the antitumor effect of local irradiation.** beta-Carotene supplementation produced a greater residual antitumor action than vitamin A supplementation after the supplements were discontinued, which may have been due to greater tissue storage of beta-carotene.

Thatcher, N., G. Blackledge, et al. (1980). "Advanced recurrent squamous cell carcinoma of the head and neck. Results of a chemotherapeutic regimen with adriamycin, bleomycin, 5-fluorouracil, methotrexate, and vitamin A." Cancer 46(6): 1324-8.

Twenty-five patients with advanced squamous cell carcinoma of the head and neck were entered into this study. All patients had previously been irradiated and the majority had also undergone surgery for recurrent tumor. **A low-dose regimen consisting of adriamycin, bleomycin, 5-fluorouracil, methotrexate, and vitamin A was prescribed,** the median number of courses was four and a total of 95 were administered. Ten patients (40%) achieved objective responses (7 partial, 3 complete). The median duration of response was 14 weeks (range, nine to 60 weeks) with a median survival time of 38.5 weeks (range, eight to 72 weeks). The nonresponding patient group's survival time was significantly reduced ($P = 0.002$; median, 12 weeks; range, three to 40 weeks). The treatment was given on an outpatient basis and no serious hematologic toxic reactions were encountered. Mucositis was uncommon. **This regimen produced an acceptable response rate without serious side-effects. The use of Vitamin A may have helped to prevent further impairment of the patient's nutritional status by ameliorating drug-induced mucositis.**

Naziroglu, M., A. Karaoglu, et al. (2004). "Selenium and high dose vitamin E administration protects cisplatin-induced oxidative damage to renal, liver and lens tissues in rats." Toxicology 195(2-3): 221-30.

Cisplatin is one of the most active cytotoxic agents in the treatment of cancer but its clinical use is associated with nephrotoxicity. **Several studies suggest that supplementation with antioxidant can influence cisplatin induced nephrotoxicity.** In the present study, we investigated the effect of selenium with high dose vitamin E administration on lipid peroxidation (MDA) and scavenging enzyme activity in kidneys, liver and lens of cisplatin-induced toxicity in rats. Forty female Wistar rats were used. They were randomly divided into five groups. The first and second groups were used as control and cisplatin (6 mg/kg BW) intraperitoneally administered groups. Groups III, IV and V received intraperitoneally five doses of selenium (1.5 mg/kg BW) and a high dose of vitamin E (1000 mg/kg BW) combination before, simultaneously and after with cisplatin, respectively. Glutathione peroxidase (GSH-Px), vitamin E and beta-carotene levels in the kidney, lens and liver, vitamin A and reduced glutathione (GSH) levels in the kidney were significantly ($P < 0.05$ to < 0.001) lower in the cisplatin group than in the control whereas there was a significant increase in kidney, liver and lens MDA levels in rats treated with cisplatin. **The decreased antioxidant enzymes and vitamins and increased MDA levels in the kidney, lens and liver of animals administered with cisplatin were significantly ($P < 0.05$ to < 0.001)**

improved with selenium and a high dose vitamin E injection. In conclusion, this data demonstrates that there is an increase in lipid peroxidation in the kidney, liver and lens of animals administered with cisplatin whereas there is a decrease in antioxidant vitamins and enzymes. However, intraperitoneally injected selenium combined with a high dose of vitamin E seem to produce a significant improvement on antioxidants concentrations in rats treated before, simultaneously and after with cisplatin. **The selenium with high dose vitamin E injection may play a role in preventing cisplatin-induced nephropathy and cataract formation in cancer patient.**

Whelan, R. L., K. D. Horvath, et al. (1999). "Vitamin and calcium supplement use is associated with decreased adenoma recurrence in patients with a previous history of neoplasia." *Dis Colon Rectum* **42**(2): 212-7.

INTRODUCTION: Although some have suggested that certain vitamins or calcium supplements may reduce adenoma recurrence, our own prior retrospective study found no such effects. The purpose of this case-control study was to further investigate whether regular vitamin or calcium supplement intake influenced the incidence of recurrent adenomatous polyps in patients with previous neoplasia who were undergoing follow-up colonoscopy. **METHODS:** This study enrolled 1,162 patients who underwent colonoscopy by one of three surgeons at Columbia-Presbyterian Medical Center in New York City between March 1993 and February 1997. Of these patients 448 (250 males) had a previous diagnosis of colorectal neoplasia (cancer, adenomas, or dysplasia). Of these, 183 (40.8 percent) had an adenoma at the index colonoscopy. Information was collected on personal and family history of colonic diseases, cigarette smoking, medication, and vitamin and micronutrient supplement usage on a questionnaire that was completed by the patients before the colonoscopy. Odds ratios were obtained by unconditional logistic regression analysis, adjusting for age and gender, and used adenoma recurrence at index colonoscopy as the outcome. **RESULTS:** The mean interval between colonoscopic examinations was 37 months for the recurrent adenoma group and 38 months for the nonrecurrent group of patients ($P =$ not significant). **In this case-control study we found a protective effect for the use of vitamin supplements in general (any vitamin) on the recurrence of adenomas (odds ratio, 0.41; 95 percent confidence interval, 0.27-0.61).** Specifically, this protective effect was observed for the use of multivitamins (odds ratio, 0.47; 95 percent confidence interval, 0.31-0.72), vitamin E (odds ratio, 0.62; 95 percent confidence interval, 0.39-0.98), and for calcium supplementation (odds ratio, 0.51; 95 percent confidence interval, 0.27-0.96). Nonsignificant protective effects were noted for carotene/vitamin A, vitamin D, and vitamin C. **CONCLUSIONS:** **The use of multivitamins, vitamin E, and**

calcium supplements were found to be associated with a lower incidence of recurrent adenomas in a population of patients with history of previous colonic neoplasia. Prospective, randomized trials are needed to better assess the impact of these agents and to determine whether the use of these supplements is associated with a protective effect against recurrent adenomas.

Lamm, D. L., D. R. Riggs, et al. (1994). "Megadose vitamins in bladder cancer: a double-blind clinical trial." *J Urol* **151**(1): 21-6.

Epidemiological and laboratory studies suggest that vitamin supplements may be helpful in the prevention of some cancers but clinical trials to date have failed to demonstrate protection with naturally occurring vitamins. Without substantiation of the highly touted benefits of vitamins, few physicians who care for cancer patients have recommended their use. **A total of 65 patients with biopsy confirmed transitional cell carcinoma of the bladder enrolled in a randomized comparison of intravesical bacillus Calmette-Guerin (BCG) with or without percutaneous administration was also randomized by closed envelope to therapy with multiple vitamins in the recommended daily allowance (RDA) versus RDA multivitamins plus 40,000 units vitamin A, 100 mg. vitamin B6, 2,000 mg. vitamin C, 400 units vitamin E and 90 mg. zinc.**

The addition of percutaneous BCG did not significantly lessen tumor recurrence but recurrence after 10 months was markedly reduced in patients receiving megadose vitamins. The 5-year estimates of tumor recurrence are 91% in the RDA arm and 41% in the megadose arm ($p = 0.0014$, Mantel-Cox). Overall recurrence was 24 of 30 patients (80%) in the RDA arm and 14 of 35 (40%) in the high dose arm ($p = 0.0011$, 2-tailed Fisher's exact test). **Megadose vitamins A, B6, C and E plus zinc decrease bladder tumor recurrence in patients receiving BCG immunotherapy.** Further research will be required to identify which ingredient(s) provide this protection.

Ades, L., S. Chevret, et al. (2005). "Outcome of acute promyelocytic leukemia treated with all trans retinoic acid and chemotherapy in elderly patients: the European group experience." *Leukemia* **19**(2): 230-3.

We analyzed the outcome of patients aged more than 60 included in a multicenter trial in newly diagnosed acute **promyelocytic leukemia (APL93 trial), which tested the role of early addition of chemotherapy to all trans retinoic acid (ATRA) and of maintenance with ATRA and/or low-dose chemotherapy.** In total, 129/533 (24.2%) patients included in this trial were older than 60. The CR rate was 86% in patients older than 60 as compared to 94.5% in younger patients ($P=0.0014$), due to a higher incidence of early deaths in elderly patients. The 4-year incidence of relapse was 15.6% in adults older than 60 and 23.2% in

younger adults although most elderly patients received less intensive consolidation chemotherapy. However, 18.6% of the patients older than 60 years who achieved CR died in CR, mainly from sepsis during consolidation course or maintenance treatment, as compared to 5.7% of younger adults ($P < 0.001$). Thus, overall 4-year survival of elderly patients was 57.8% as compared to 78% in younger adults ($P < 0.0001$). **APL in elderly patients appears as sensitive to ATRA-Chemotherapy based regimen as in younger adults.** Less favorable outcome is mainly due to an increase of early deaths and to toxicity of consolidation treatment, strongly suggesting a beneficial role for less intensive consolidation chemotherapy and possibly introduction of arsenic derivatives in the treatment of APL in the elderly.

Okuno, M., S. Kojima, et al. (2004). "Retinoids in cancer chemoprevention." Curr Cancer Drug Targets 4(3): 285-98.

We review the therapeutic and preventive applications of a retinoid analog (vitamin A and its derivatives) for human cancers.

Chemoprevention of cancer is an intervention in the carcinogenic process by chemical agents that block or reverse the malignant transformation of cells. Retinoids are prime candidates for cancer chemoprevention since cancer is characterized by abnormal growth with a lack of differentiation, which could be modified by retinoids. **Retinoids exert their biological functions through nuclear receptors, retinoic acid receptor (RAR) and retinoid X receptor (RXR).** 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 malignancy as well as premalignant and malignant lesions in the oral cavity, head and neck, breast, skin and liver.

We particularly focus upon the therapeutic application of all-trans RA (atRA) to acute promyelocytic leukemia (APL) and on the preventive approach to hepatocellular carcinoma (HCC) by a synthetic retinoid analog, acyclic retinoid. In both malignancies, malfunction of retinoid nuclear receptors is closely related to their carcinogenic process. In APL, a chromosomal translocation produces a chimeric protein between RAR alpha and a protein called promyelocyte leukemia protein (PML). PML-RAR alpha works as a dominant negative receptor in the leukemic cells, interfering with the normal function of RAR alpha and/or PML, which in turn results in the arrest of cell maturation at the stage of promyelocytes. **Oral administration of atRA induces differentiation of promyelocytic leukemic cells to mature neutrophils, and leads to a high rates (over 90%) of complete remission. AtRA therapy has become standard in the treatment of APL. In the case of HCC, post-translational modification of RXR by phosphorylation impairs its function, which leads to uncontrolled**

cell growth. Acyclic retinoid suppresses the phosphorylation of RXR alpha, restores its function in the presence of its endogenous ligand, 9-cis RA, and thereby induces apoptosis of the cancer cells. **Acyclic retinoid given orally successfully suppresses the development of second primary tumors in cirrhotic patients who undergo curative removal of preceding HCC.** Eradication of (pre)malignant clones ('clonal deletion') from the liver is suggested as a mechanism of the chemopreventive effect. Further development of more effective retinoids as well as their use in combination with other classes of anticancer agents including immunopreventive drugs like interferons may provide strategies for cancer prevention.

de Botton, S., V. Coiteux, et al. (2004). "Outcome of childhood acute promyelocytic leukemia with all-trans-retinoic acid and chemotherapy." J Clin Oncol **22**(8): 1404-12.

PURPOSE: To determine the results of treatment combining all-trans-retinoic acid (ATRA) and chemotherapy (CT) in childhood acute promyelocytic leukemia (APL). PATIENTS AND METHODS: Children (< 18 years) with newly diagnosed APL were included in the APL93 trial, treated by ATRA followed or combined with daunorubicin-cytarabine, and then randomly assigned between no maintenance, intermittent ATRA, continuous CT, or both. RESULTS: **Of the 576 patients included in APL93 trial, 31 (5%) were children, including 22 girls (71%) and nine boys (29%). Thirty of the children (97%) obtained complete remission (CR).** ATRA syndrome occurred in four children (13%), who all achieved CR, and headaches occurred in 12 children (39%), with signs of pseudotumor cerebri in five children (16%). Seven patients (23%) relapsed. **None of the eight patients who received both ATRA and CT for maintenance relapsed.** All relapsing patients achieved a second CR. Twenty-two patients remained in first CR after 43+ to 96+ months, six remained in second CR after 17+ to 66+ months, and three patients had died. The 5-year event-free survival (EFS), relapse, and overall survival rates were 71%, 27%, and 90%, respectively. No difference between adults and children included in the APL93 trial was seen for CR rate, 5-year relapse rate, EFS, and overall survival, but significantly better survival was seen in children after adjustment on WBC counts ($P = .02$) and incidence of microgranular M3 variant ($P = .04$). CONCLUSION: **ATRA combined with CT for induction and also probably for maintenance provides as favorable results in children with APL as in adults and currently constitutes the reference first-line treatment in both age groups.**

Fenaux, P., C. Chomienne, et al. (2001). "All-trans retinoic acid and chemotherapy in the treatment of acute promyelocytic leukemia." Semin Hematol

38(1): 13-25.

All-trans retinoic acid (ATRA) can induce complete remission (CR) in most patients with acute promyelocytic leukemia (APL) through in vivo differentiation of APL-blasts. However, it cannot eliminate the leukemic clone and must be used in combination with anthracycline-based chemotherapy. Experience accumulated over the last 10 years has clearly shown that the combination of ATRA and chemotherapy gave better survival than chemotherapy alone in newly diagnosed APL because of fewer relapses and a slightly higher CR rate. It is also strongly suggested that maintenance treatment with ATRA, and possibly with low-dose chemotherapy, can further reduce the incidence of relapse. **Overall, more than 90% of patients with newly diagnosed APL can achieved CR, and about 75% can be cured by the combination of ATRA and chemotherapy.** ATRA syndrome remains the major side effect of ATRA treatment, which should be prevented by addition of chemotherapy and/or dexamethasone in case of increasing white blood cell (WBC) counts. Current issues in the treatment of newly diagnosed APL include the role of early addition of chemotherapy to ATRA, whether or not ara-C is useful in combination with anthracycline, and a possible interest of arsenic trioxide during consolidation in patients remaining at relatively high risk of relapse.

Hansen, L. A., C. C. Sigman, et al. (2000). "Retinoids in chemoprevention and differentiation therapy." *Carcinogenesis* **21**(7): 1271-9.

Retinoids are essential for the maintenance of epithelial differentiation. As such, they play a fundamental role in chemoprevention of epithelial carcinogenesis and in differentiation therapy. Physiological retinoic acid is obtained through two oxidation steps from dietary retinol, i.e. retinol-->retinal-->retinoic acid. The latter retinal-->retinoic acid step is irreversible and eventually marks disposal of this essential nutrient, through cytochrome P450-dependent oxidative steps. Mutant mice deficient in aryl hydrocarbon receptor (AHR) accumulate retinyl palmitate, retinol and retinoic acid. This suggests a direct connection between the AHR and retinoid homeostasis. **Retinoids control gene expression through the nuclear retinoic acid receptors (RARs) alpha, beta and gamma and 9-cis-retinoic acid receptors alpha, beta and gamma, which bind with high affinity the natural ligands all-trans-retinoic acid and 9-cis-retinoic acid, respectively. Retinoids are effective chemopreventive agents against skin, head and neck, breast, liver and other forms of cancer. Differentiation therapy of acute promyelocytic leukemia (APL) is based on the ability of retinoic acid to induce differentiation of leukemic promyelocytes.** Patients with relapsed, retinoid-resistant APL are now being treated with arsenic oxide, which results in apoptosis of the leukemic cells. Interestingly, induction of differentiation in promyelocytes and consequent remission of APL

following retinoid therapy depends on expression of a chimeric PML-RAR alpha fusion protein resulting from a t(15;17) chromosomal translocation. This protein functions as a dominant negative against the function of both PML and RARs and its overexpression is able to recreate the phenotypes of the disease in transgenic mice. The development of new, more effective and less toxic retinoids, alone or in combination with other drugs, may provide additional avenues for cancer chemoprevention and differentiation therapy.

Nikolaou, A. C., G. Fountzilas, et al. (1996). "Treatment of unresectable recurrent head and neck carcinoma with 13-cis-retinoic acid and interferon-alpha. A phase II study." *J Laryngol Otol* **110**(9): 857-61.

Sixteen patients with unresectable recurrent head and neck carcinomas were treated with 13-cis-retinoic acid and interferon-alpha. All patients had presented with recurrences after having been treated primarily with surgery and radiotherapy, while **two of them had also received induction chemotherapy.** The site of relapse was strictly locoregional in all cases (only at the primary site in three cases, at the cervical lymph nodes only in four cases and both at the primary site and the neck in the remaining nine cases. Two patients were female, and 14 male, with an age range of 47-72 years (median 61 years). Interferon-alpha was administered subcutaneously at a dose of 3×10^6 IU every second day. **The dose of retinoids was 40 mg per os every day.** The duration of treatment was two to 14 months (median seven months). There were two cases of partial response (tumour regression > 50 per cent), eight cases of stable disease lasting for three to seven months (median four months) and six cases presented with progressive disease. All patients died after a survival of three to 17 months (median 9.5 months). **Toxicity was generally minimal. We believe that the results are not encouraging, but also not disappointing.** The fact that toxicity was indeed mild, with not a single case of life-threatening sequelae even after prolonged administration of the two agents, allows us to conclude that an increase of the dose of IFN-alpha might be more beneficial. Selection of patients with more 'favourable' recurrences will give a better chance to the treatment combination to prove its real efficacy. Larger numbers of patients have to be treated and evaluated before definite conclusions can be reached.

Smith, M. A., D. R. Parkinson, et al. (1992). "Retinoids in cancer therapy." *J Clin Oncol* **10**(5): 839-64.

PURPOSE: Recent reports of the dramatic antitumor effect of all-trans retinoic acid (RA) in patients with acute promyelocytic leukemia (APL) have generated renewed enthusiasm for clinical studies of retinoids for oncologic therapeutic indications. Here we

provide an overview of relevant aspects of retinoid physiology and molecular biology, review preclinical studies indicating antitumor activity for retinoids, and summarize the current status of clinical investigations of retinoid use for the treatment of adult and pediatric tumors. DESIGN: The published literature was reviewed with attention to areas of retinoid research that would shed insight into the oncologic uses of retinoids. RESULTS: Retinoids play critical roles during normal fetal development and induce differentiation (and/or growth inhibition) in a variety of tumor-cell lines. Retinoid effects seem to result from changes in gene expression mediated via specific nuclear receptors (termed retinoic acid receptors, RAR-alpha, -beta, and -gamma), and a specific chromosomal translocation involving the RAR-alpha gene occurs in APL patients. In addition to the very high clinical response rate for RA in patients with APL, significant clinical responses have been observed for patients with cutaneous T-cell malignancies, juvenile chronic myelogenous leukemia, and dermatologic malignancies. Additionally, the combination of 13-cis-retinoic acid (cRA) with interferon alpha (IFN alpha) has produced high objective response rates for patients with squamous cell carcinomas of the head and neck and of the cervix. CONCLUSIONS: **The antitumor activity demonstrated for retinoids (especially RA) alone and in combination with other agents supports the need for targeted phase II trials to define the spectrum of responsive tumors and for laboratory studies to further delineate the biologic mechanisms associated with therapeutic responses.** High priority should then be given to phase III trials to delineate optimal strategies for improving outcome by combining retinoid-based treatments with conventional chemotherapy and radiotherapy regimens.