Clinical Trials of Differentiation Therapy of Epithelial Cancers

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The major purpose of this session was to broadly address issues related to the control of human epithelial cancers using differentiation therapy. Two general areas were covered:

1. Prevention of epithelial aerodigestive and respiratory malignancies, and

2. Differentiation of human epithelial cancers in vitro with certain compounds.

These two groups of studies present entirely different issues and will be covered separately.

Compared to therapeutic trials, there are relatively few chemoprevention studies being conducted. The majority of these trials use carotenoids or retinoids as the therapeutic compound. The results from four different types of studies were presented and included:

1. A number of trials using retinoids and carotenoids in the prevention of oral cancer.

2. A randomized trial using 13 cis-retinoic acid (cRA) for the prevention of second malignant tumors in patients with surgically cured head and neck cancers.

3. A randomized study of high-dose vitamin A for adjuvant treatment and prevention of stage I lung cancer.

4. In vitro and clinical studies of Barrett's esophagus.

The first presentation (Garewal and Meyskens, U.S.A.) reviewed studies, including their own, of the relative effectiveness of retinoids and carotenoids in controlling oral leukoplakia. Both types of compounds caused regression of established pre-cancerous lesions, but retinoids produced considerable mucocutaneous toxicity while the use of carotenoids was associated with no side-effects. The relative efficacy of cRA and β-carotene needs to be established in future trials, nevertheless, these studies have convincingly shown that oral pre-cancers can be favorably modulated with two quite different differentiation agents.

The chemoprevention trial by Hong and colleagues (U.S.A.) is of particularly high interest since this study was randomized and showed that the short-term appearance of second malignant tumors in patients with previously treated head and neck cancers could be reduced by 80% with a clinically acceptable dose of cRA. This is the first randomized study in humans examining this issue and a full report was recently published (1). Since toxicity was considerable, the eventual role of cRA for chemoprevention in humans remains to be determined.

The third study tested the efficacy of high dose vitamin A in stage I lung cancer (Pastorino, Italy). Although there was no demonstratable effect on the appearance of second malignancies, there was a statistically significant effect on the total number of failures, including relapses and new primary cancers at any site (DFS at 5 years, 61% vs 48%, $p < 0.05$). The relative lack of side-effects using high dose (300,000/day) retinol palmitate raises the interesting issue of what vitamin A toxicity means. Although not proven, the results of this trial as well as others recently reported suggests that retinol palmitate is less toxic than the retinol form of vitamin A. This is an important consideration since retinol has been - until about 5 years ago - the major form used in the United States, where considerable toxicity has been described.

The last study reported on the use of a metaplastic condition, Barrett's esophagus, for in vitro as well as for clinical studies (Garewal, U.S.A.). The results demonstrated that the use of cells from this preneoplasia could be used for biologic studies. The cultured cells were extensively characterized and were shown to have a trisomy 7 and overexpression of EFGR. This is a particularly useful model to use for clinical chemoprevention trial as the metaplastic lesion can be accurately measured using endoscopy. A phase II trial of cRA in Barrett's esophagus was negative and produced no regressions. Results of these studies have

recently been reported elsewhere (2).

The complexity in interpreting and planning chemoprevention studies was well-delineated in a study by DeLuca (U.S.A.). In their model system (female SENCAR mice, using DMBA as initiator and TPA as promoter) vitamin A depletion resulted in a marked inhibition of skin tumorigenesis. Dietary repletion with retinoids permitted a rapid tumorigenic response. These results are in contrast to other models and reinforce the important general conclusion that dietary depletion of vitamin A may either enhance or inhibit tumor formation depending on the type of epithelium and its differentiation characteristics.

There are a large number of clinical chemoprevention trials underway throughout the world (review, 3). To date only retinoids and carotenoids have been widely used in addition to a number of dietary compounds. There is ample opportunity for study of other differentiation agents as chemoprevention compounds. Studies of the biology and clinical aspects of preneoplasias of various types should be a particularly rich area for investigation as well.

One of us (Frank Meyskens) has published extensively on the effect of retinoids on epithelial malignancies (review, 4, 5). The recently described striking differentiating effect of all trans-retinoic acid (tRA) on promyelocytic cells both in vitro and in clinical trials has renewed interest in retinoids as well as other types of biomodulators as therapeutic agents for epithelial malignancies.

In the first study Greiner et al. (U.S.A.), demonstrated that IFN-γ induced expression of CEA and TAG-12 in colon cell lines. A subsequent trial measured the ability of IFN-γ to increase their antigens in malignant ascites; binding to tumor cells of the relevant antibodies increased from 10% to 90%. These results - as well as other studies - show that tumor antigen expression can be augmented by IFN-γ and other lymphokines, which may be important in an adjuvant setting to increase monoclonal antibody localization to human tumor cell populations.

In a second study (Min Shen, Shanghai), the effect of tRA on the differentiation and proliferation of two human gastric cancer cell lines was measured. A modest effect (50% inhibition) on cellular proliferation on plastic as well as on colony-forming ability in agar was shown. Similar effects were demonstrated on the growth of these tumors in nude mice. In another investigation Tahara and colleagues (Japan) studied the anti-tumor effect of the cyclic AMP analogue 8-Cl-cAMP on human gastric

and esophageal carcinoma cells in vitro. At relatively high concentrations (10μM) 8-C1-AMP markedly inhibited the growth of 6 of 7 gastric and all 3 esophageal cell lines. These results were sufficiently impressive that an in vivo anti-tumor study was done in nude mice and 8-C1-cAMP, infused continually, was found to inhibit growth in the single gastric tumor tested. In a final investigation, Heby and Wallon (Sweden), showed that the ornithine decarboxylase inhibitor 2-difluoromethylornithine induced terminal differentiation in cultures of human teratocarcinoma stem cells, but not in neuroblastoma or promyelocytic leukemia cells, although strong anti-proliferative effects were evident against all three cell types.

These results with various differentiation agents and different tumor cell lines reported in this symposium confirm what is being widely reported. Biomodulators are effective, but highly selective, in controlling tumor growth and in general do so in a cytostatic fashion.

The keys to success will be in finding the correct match of biomodulator/differentiating agent and tumor responsiveness. The initial data also suggests that low doses of combinations of modulators is likely to have a role as well In the design of combination therapies, an understanding of the underlying mechanisms will be important to the conduct of these trials. It is highly likely that tumor therapy will become increasingly specific in the future as selective matches are found between differentiating agents and tumors; the striking specificity of interferon/hairy cell leukemia and tRA/acute promyelocytic leukemia are two cases in point.

References

1. Hong WK, Lippman SM, Itri LM, Karp DD, Lee JS, Byers RM, Schantz SP, Kramer AM, Lotan R, Peters LJ, et al. Prevention of second primary tumors with isotretinoin in squamous-cell carcinoma of the head and neck. New England Journal of Medicine 323(12):795-801, 1990.

2. Garewal HS, Sampliner R. Barrett's esophagus: a model premalignant lesion for adenocarcinoma. Preventive Medicine, 18(5):749-56, 1989.

3. Meyskens FL Jr. Coming of age: chemoprevention of cancer. In: New England Journal of Medicine, September, 1990.

4. Lippman S, Kessler J, Meyskens FL Jr. Retinoids as preventive and therapeutic anticancer agents. Cancer Treat. Rep. Part I, Vol. 71, 4:391-405, 1987. Part II, 5:493-515, 1987.

5. Meyskens JL, Jr. Clinical trials of retinoids as differentiation inducers. In: The Status of Differentiation Therapy in Cancer, Waxman S, Rossi GG, and Takaku E (eds), Raven Press, New York, pp. 349-359, 1987.