Hyperbaric Oxygen: Does it Have a Cancer Causing or Growth Enhancing Effect?

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I. Introduction:

The first reported concern that hyperbaric oxygen might have cancer growth enhancing effects appeared in a paper by Johnson and Lauchlan in 1966.[1] These authors published their experiences in irradiating 25 patients with Stage III or IV Cervical Cancer utilizing hyperbaric oxygen as a radiosensitizer. The authors reported a more frequent than expected incidence of metastases and a pattern of metastases that appeared to be unusual. One patient is reported to have developed an esophageal metastasis. This first publication was subsequently followed by a number of larger human trials wherein hyperbaric oxygen was used as a radiosensitizer. Additionally, a number of animal trials have been published specifically to address the issue of hyperbaric oxygen's effect on primary tumor and metastatic growth. Several *in vitro* studies are also in the literature which address these concerns. This issue is still of concern to some[2] and is the topic of this paper to be presented at the joint ECHM and ESTRO Consensus Conference on the role of hyperbaric oxygen in the treatment of radiation-induced injury in normal tissues.

Certainly, it is a reasonable concern that a therapeutic modality which is recommended as an adjunct to healing and is administered to promote proliferation of fibroblasts, epithelial cells and blood vessels in a wound could also lead to proliferation of malignant cells and angiogenesis in the tumor as well. The assumption that since cellular and vascular proliferation are promoted by hyperbaric oxygen in a healing wound that it will necessarily have the same effect in a tumor, though understandable at first glance, is also fairly naïve since it fails to recognize the important differences that exist between the complex physiology of wound healing and the equally complex and unique pathophysiologies of malignant transformation, tumor growth and metastases.

I will approach this important discussion by reviewing the published pre-clinical studies (animal and *in vitro*) followed by the results of clinical publications which ultimately are the most important. I will also discuss some of the mechanisms whereby hyperbaric oxygen could be postulated to have malignant growth potentiating effects and hopefully refute such putative effects. I will emphasize the discussion of tumor angiogenesis since recent concerns about the potential for hyperbaric oxygen to enhance malignant growth have been most frequently related to hyperbaric oxygen's effect as an inducer of angiogenesis. Other possible mechanisms of carcinogenesis and malignant growth enhancement will include discussions of a possible direct effect on cancer growth, immune suppression, free radical formation and mutagenesis. The discussion will also deal broadly and simultaneously with the concerns of enhanced carcinogenesis and enhanced metastatic growth although the author recognizes that important differences exist in the pathophysiology of each of these entities.

II. Pre-clinical Studies:

The effect of hyperbaric oxygen on tumor cells in cell culture and tumor growth in animal models have been studied in many publications. In terms of a potential impact on initiating or enhancing malignant growth, these publications can be divided into the following categories: 1. Papers addressing the direct effect of hyperbaric oxygen on cell growth in culture; 2. The effects of hyperbaric oxygen on immune competency; 3. The effects of hyperbaric oxygen on free radical formation; 4. The effects of hyperbaric oxygen on mutagenesis (generally as a result of free radical formation); and 5. Animal models of tumor growth and metastases.

1. Direct Effects on Cells in Cell Culture:

Kalns[3] and associates have reported the effects of hyperbaric oxygen on the growth of two prostate cancer cell lines in cell culture. In this study, both cell lines had their growth suppressed after exposure to 3.0 ATA 100% oxygen for 90 minutes relative to normobaric controls by 8.1% and 2.7% respectively.

Feldmeier[4] and associates in abstract form have reported a dose dependent reduction of numbers of colonies of B16 (amelanotic melanoma) cells grown cell culture by increasing pressures or times of exposure to hyperbaric oxygen. In this study, cells exposed to hyperbaric oxygen were also less likely to adhere to fibronectin substrata suggesting decreased metastatic potential since the ability of cells to adhere to vascular endothelium is a prerequisite for successful metastasis.

The studies cited above demonstrate an inhibitory direct effect on tumor cell growth in cell culture and suggest an effect which may decrease metastatic potential. A caution should properly be applied in interpreting the results of both of these reports in that the oxygen tensions experienced by the cells in culture are much higher than those cancer cells would experience *in vivo* in either an animal model or in human subjects in clinical trials.

2. Hyperbaric Effects on Immune Competence:

Cancer incidence and progression are known to increase in individuals chronically immune suppressed. A number of publications report immune suppression by hyperbaric exposures mostly in animal models and mostly as a result of extreme exposures in terms of pressure and time of exposure. In 1997, Xu[5] and colleagues in a murine trial have shown a decrease of some lymphocyte subpopulations in the spleen and thymus after exposure to hyperbaric oxygen, but no delay in T cell response to Con A was observed. Brenner and associates[6] have recently reported depression of several immune parameters including a weakening of response to

antigens, a slowing of allograft rejection and a weakening of autoimmune response after hyperbaric exposures. They suggest that such effects are offset by acclimatization. Feldmeier and associates[7] have reported no effects on a broad range of immune parameters in healthy human volunteers exposed to a typical course of clinical hyperbaric oxygen.

The above studies do not consistently demonstrate a frequency or degree of immune suppression, which is likely to potentiate malignant growth. The study by Brenner suggests that adaptation does occur in human subjects. Even if prolonged extreme hyperbaric exposures are immune suppressive, it is likely that the intermittent nature of clinical hyperbaric oxygen wherein the patient is only exposed to increased oxygen tensions for 90 to 120 minutes does not overwhelm the influences of the other 22 to 22 and ½ hours that the patient lives at normoxic and normobaric conditions

3. Hyperbaric Oxygen Effects on Free Radical Generation:

Free radicals are known to contribute to the development of a number of degenerative and deleterious conditions including cancer. Several recent studies suggest that exposure to hyperbaric oxygen does not necessarily lead to increased free radical damage. Kaelin and associates[8] have shown a significant increase in the activity of the free radical scavenger superoxide dismutase in animals exposed to hyperbaric oxygen. Zamboni and his collaborators[9] failed to demonstrate signs of increased free radical damage by hyperbaric exposure in an animal model of reperfusion injury. On the other hand, Monstrey et al[10] showed an increase in soft tissue damage in a model of Adriamycin extravasation in animals exposed to hyperbaric oxygen both before and after the extravasation. The authors attribute this additional damage to increased free radical activity Elayan and associates[11] showed no evidence of increased levels of 2,3-dihydroxybenzoic acid (a surrogate measure of free radical generation) in Sprague-Dawley rats exposed to hyperbaric oxygen at 3.0 ATA.

The available scientific information does not conclusively demonstrate an increase in free radical damage induced by hyperbaric oxygen. Again the intermittent nature of the hyperbaric exposure probably reduces the effects of any increase in reactive oxygen species. Adaptive mechanisms, which lead to an increase in free radical scavengers, seem to also reduce the deleterious effects of any additional free radical generation.

4. Mutagenesis and Subsequent Carcinogenesis:

Several authors have voiced concerns about mutagenesis and resultant carcinogenesis caused by free radical generation as a result of hyperbaric oxygen exposure. In 1985, Ceruti[12] discussed the carcinogenic effects of prooxidants including hyperbaric oxygen. This paper presents no first hand evidence of a carcinogenic or mutagenic effect of hyperbaric oxygen but instead discusses the

known effects of oxygen active oxygen species (free radicals) and assumes that hyperbaric oxygen exposure will necessarily result in increased free radical damage including mutation and carcinogenesis. Interestingly, several of the author's key references were not reports of true hyperbaric exposure but instead prolonged exposure to increased concentrations of oxygen at ground level.[13,14] The author extrapolates the results and makes the assumption that such observations would be the case even more so at hyperbaric pressures. Similar reasoning had suggested that hyperbaric oxygen was contraindicated in ischemia-reperfusion injuries since it was assumed that exposure to hyperbaric oxygen under these circumstances would lead to increased free radical generation and resultant damage. Investigators demonstrating beneficial effects of hyperbaric oxygen in iscemia-reperfusion injury including the induction of free radical scavengers as already discussed above have refuted this rationale. A group from the University of Ulm have studied the effects of hyperbaric oxygen on mutations in the leukocytes of healthy human volunteers exposed to 2.5 ATA.[15] In this study and follow on studies, there were no changes seen in levels of 8-Ohguanine (one of the major DNA modifications induced by reactive oxygen species).[15,16] Also no induction of mutations at the HPRT locus were detected. This too is a standard test for mutagenesis. DNA damage was demonstrated by the comet assay and mutations were demonstrated in the mouse lymphoma assay (MLA). The authors suggest that mutations observed due to hyperbaric exposures are clastogenic, i.e. the result of DNA strand breaks.

There is no doubt that reactive oxygen species can under some circumstances cause mutations which may lead to carcinogenesis. The available information cited above does not give consistent evidence for hyperbaric induced mutagenesis. Some *in vitro* studies do show mutagenesis in cells in cell culture. Again we should observe the caution that oxygen levels achievable in cell cultures are much higher than those achievable *in vivo.* Furthermore, *in vitro* studies and *in vivo* studies which only involve a single exposure or a short course of exposure may not allow for the development of protective mechanisms such as the induction of free radical scavengers including superoxide dismutase. Also, as before, the intermittent rather than continuous exposure of patients to hyperbaric oxygen is likely to permit repair of many of the DNA breaks that may result during the clinical hyperbaric treatment. A publication by Bruyninckx and associates[17] in 1978 discusses that oxygen levels that are mutagenic in sensitive cells in cell culture may be physiologic in humans bringing into question cell culture studies that show mutagenesis due to hyperbaric oxygen exposure in terms of their carcinogenesis in human subjects.

5. Animal Studies of Tumor Growth and Metastasis:

In response to Johnson and Lauclan's[1] publication, a number of researchers set out to investigate the effects of hyperbaric oxygen exposure on animals with transplanted, induced or spontaneous tumors. Table 1 lists those animal studies

specifically designed to answer the issue of whether hyperbaric oxygen exposure of these animals led to enhanced growth of the dominant tumor mass or of resultant metastases. The first of these studies was published in 1966 and the last in 2001. A total of 17 publications are briefly summarized in the Table 1.[18-34] Fifteen of the 17 studies show no increase in primary or metastatic growth. Two studies that show evidence of enhanced growth are mixed in their results. The paper by Shewell and Thompson[26] shows an increase in the rate of lung metastases for spontaneous mammary tumors in mice while in the same study transplanted tumors had identical rates of growth and metastases in the control and hyperbaric groups. The increase in incidence of lung metastses in the spontaneous tumor group does not achieve statistical significance. In the paper by McMillan et al[29] with an anthracene induced tumor in a hamster cheek pouch model, animals exposed to hyperbaric oxygen had fewer but larger tumors. In an almost identical model, Marx and Johnson[27] showed a delay in the development of cancers in animals exposed to hyperbaric oxygen. Six of these studies in Table 1 actually show some evidence of decreased tumor growth or metastases in animals exposed to hyperbaric oxygen. Mostly this decrease is seen as a trend and generally not in a statistically or clinically significant fashion. Please note that the Table legend identifies those studies with an enhancing, an inhibitory, a neutral or a mixed result.

Taken on the whole, these animal studies demonstrate no worse than a neutral effect by hyperbaric oxygen on the growth of induced, transplanted and spontaneous tumors and their secondary metastases. It is important to note that a broad range of tumor types and histologies were investigated in these studies. The tumors studied include squamous cell carcinomas, adenocarcinomas (mammary tumors), melanomas, leukemias and sarcomas. Some have suggested that hyperbaric oxygen may stimulate growth in one tumor histology and not another. The consistent results in a broad spectrum of tumor types fails to support this belief.

III. Human Studies:

Fifteen clinical reports are given in Table 2.[1,35-48] These list the publications from which we can analyze the effects of hyperbaric oxygen on recurrence or metastases in patients exposed to hyperbaric oxygen. Twelve of the 15 publications come from studies published to report the efficacy of hyperbaric oxygen as a radio-sensitizer. The study by Van Den Brenk et al[35] compared outcome in a group of head and neck cancer patients radiosensitized by hyperbaric oxygen and compared this to outcome in a historic control group. Also the study by Denham[45] and associates compared patients irradiated under hyperbaric conditions to historic controls. Likewise, the original publication by Johnson and Lauchan[1] was not a controlled trial. The remainder of the radio-sensitization studies were randomized and controlled. These studies were not specifically designed to address the issue of the effect of hyperbaric oxygen on primary growth or metastasis. The focus of our review in table 2 centers on incidence

of metastases and survival of the patients since the control or growth of the primary tumor was impacted by the radiation, which the patient received as the primary endpoint of these studies. Destruction of the primary tumor was consistently improved in the hyperbaric group compared to the air controls. Often, this improvement in local control did not translate into a survival advantage for the patients. Ten of these 12 studies are clearly either neutral or advantageous in terms of patient survival or incidence of metastases. The original paper by Johnson and Lauchan[1] that first voiced concerns of enhanced tumor growth under hyperbaric conditions is refuted by a larger experience in cervical cancer by the same author.[37] The report by Cade[35] and associates is a mixed study wherein the hyperbaric group radio-sensitized for lung cancer had no increased metastases; whereas, the bladder cancer patients receiving hyperbaric oxygen had increased metastases. The patients in the control and hyperbaric groups were not well matched. There were increased numbers of patients in the hyperbaric group with advanced stage and more aggressive histologies. Outcome of treatment for patients with bladder cancer is substantially worse for advanced and poorly differentiated tumors. Most of the trials of hyperbaric radio-sensitization involve patients with squamous cell cancers of the head and neck or cervix. These patients were favored for enrollment into these trials because local control is often tantamount to cure since neither tumor commonly metastasizes until quite late in its course.

The other 3 studies present anecdotal experiences in patients with a history of malignancy who undergo a course of hyperbaric oxygen as treatment for radiation injury or non-healing wounds. One is a report of 3 patients with paralysis secondary to spinal cord injury who had had HBO_2 for pressure ulcers and were found to have urothelial tumors which progressed rapidly after discovery.[45] Two of the 3 patients had indwelling catheters for many years. The authors discuss long term usage of catheters for bladder drainage as a risk factor for urothelial tumors. The authors also report that another 113 patients with spinal cord injury were given hyperbaric oxygen at their facility for various reasons and that none of these patients developed malignancy. Bradfield and associates[47] in 1996 reported 4 head and neck patients with advanced head and neck cancer who were treated with hyperbaric oxygen for radiation injury and had recurrence and rapid progression of their malignancies thereafter. All 4 patients had originally presented with advanced cancers. Two had already had recurrence before hyperbaric oxygen Another patient had his irradiation delayed by 6 months after surgery as a result of pneumonia. Delayed initiation of radiation as an adjunct to surgery is well known to increase the likelihood of recurrence.

Finally, Marx[48] has reported his follow-up of 245 patients who received hyperbaric oxygen for radiation injury. He compares this to another group of 160 patients treated by him for radiation injury but who did not receive hyperbaric treatments. Recurrence was decreased in the hyperbaric group from 19.6% compared to 28% in the non-hyperbaric group.

Those studies listed in Table 2 that report enhanced or accelerated tumor or metastatic growth after hyperbaric oxygen include a total of 72 patients. Those studies which

show a neutral or tumor suppressive effect include more than 3,000 patients. The weight of clinical evidence available to us fails to give convincing support to concerns that hyperbaric oxygen enhances malignant growth.

IV. Angiogenesis

1. Introduction:

The coordinated steps needed for angiogenesis in wound healing and tumor growth are very complex and not yet completely understood. Recent medical discoveries begin to elucidate these very involved processes. This discussion is meant to present a synopsis of the presently understood mechanisms and to consider the effects of hyperbaric oxygen on tumor angiogenesis based on what we know and what we can postulate based on indirect evidence. Before we begin, stop to consider that angiogenesis is not only important in tumor growth and wound healing but also in myocardial ischemia and diabetic retinopathy. There is no ground swell of concern that hyperbaric oxygen is pathologically increasing angiogenesis in diabetic retinopathy or therapeutically enhancing angiogenesis in coronary artery disease. I suggest from the outset that each of these entities represents at least in part a unique pathophysiology with commonalties but also with important differences.

2. A Primer of Tumor Angiogenesis:

Tumor angiogenesis has become a very hot topic in Oncology over the past few years with the somewhat delayed popularization of the work of Judah Folkman, M.D. from Harvard. Since 1971 Dr. Folkman[49] has proposed that tumor angiogenesis plays a key role for tumor growth and metastasis and that anti-angiogenic therapies should be pursued as strategies in the control and treatment of cancers. His work is now widely accepted in principle, and there are currently a number of different anti-angiogenic factors under study in Phase I, II and III clinical trials.[50] These trials are directed at blocking tumor angiogenesis at multiple points along an involved and complex cascade of events that must come together to allow tumor angiogenesis to successfully progress. Without angiogenesis, tumor growth is restricted to 1 to 2 mm^3 and metastases will not grow. (Dr.Folkman[51] has estimated that every endothelial cell supports as many as 100 tumor cells).

3. Steps in the Angiogenesis Process: For tumor angiogenesis to occur a number of coordinated steps must successfully occur.[52]

A. Initially the basement membrane of existing blood vessels must be broken down along with their extracellular matrix. These actions are mediated by a class of enzymes called matrix metalloproteinases (MMP's). This

breakdown of the basement membrane allows new branches to form off an existing blood vessel.

B. Endothelial cells must divide to form vascular tubules branching off from existing blood vessels. This process of endothelial cell division is regulated by a complex balance between growth factors and inhibitory factors. Once these endothelial cells have begun to proliferate, they must come together to form a closed tube.

   i. Over a dozen growth factors have been identified which increase proliferation, survival and motility of endothelial cells. VEGF (Vascular endothelial growth factor) appears to have the most cell specific effect on endothelial cell mitosis. Acidic and basic fibroblast growth factors (aFGF and bFGF), epidermal growth factor (EGF), interleukin-8, and tumor necrosis factor alpha also play a prominent role. Endothelial surface proteins such as $alpha_v$, $beta_3$ integrin and E-selectin increase the motility and survival of endothelial cells.

   ii. Another group of circulating factors has also been identified which inhibit endothelial cell mitosis and motility. These include angiostatin, endostatin, interferons alpha and beta, platelet factor 4 (PF4), and thrombospondin-1. Several antagonists of the matrix metalloproteinases have also been identified. These include TIMP-1, TIMP-2 and TIMP-3 (tissue inhibitors of metalloproteinase).

   iii. A final group of factors regulates the re-establishment of the basement membrane for the newly formed vascular tubules. These are not as well studied but are known to include the angiopoietins (ang-1 and ang-2).

   iv. A group of receptors on the endothelial cells has also been identified with which both the inhibitory and angiogenic factors interact. These also represent potential targets for disruption of angiogenesis.

4. Summary of Tumor Angiogenesis:

   The process of tumor angiogenesis is complex involving multiple discrete steps. Each of these may offer a separate potential strategy for disrupting the complex system of tumor vasculature and thus destroying a tumor or at least inhibiting its growth.

5. Two Compartment Model of a Tumor:

   Dr.Folkman[53] has suggested that in regard to angiogenesis, a tumor can be considered as composed of 2 compartments: 1) The tumor cell compartment and 2) the endothelial cell compartment. Each compartment is highly interdependent and each offers opportunities for therapeutic intervention.

   i. The predominant environment of the tumor cell compartment is hypoxic, acidotic and hypoglycemic. Cancer cells are rapidly dividing and their hypermetabolic activity in a poorly vascularized

region leads to anaerobic glycolysis with glucose depletion and lactic acid production. The elaboration and release of mitogenic growth factors including VEGF and bFGF occurs in this compartment. Hypoxia is known to upregulate the release of VEGF. These growth factors in turn stimulate a rapid proliferation of endothelial cells.

ii. Endothelial cells release growth factors including PDGF, interleukin-6 and IGF-1 (Insulin-like growth factor). These growth factors in turn stimulate proliferation and/or motility of tumor cells.

6. Angiogenesis in Wound Healing: the Role of Oxygen, A Brief Review

Wound healing like tumor angiogenesis requires complex multi-step interactions between cells, growth factors and the extracellular matrix. Angiogenesis is a major component of the wound healing process.[54]

A. The Process of Wound Healing: Dr. Knighton[55] has suggested that the healing wound can also be approached as a 2 Compartment Model:

The wound space is the first compartment and comprises the regulatory compartment. Here, the environment is hypoxic, acidotic, hyperkalemic and hypercarbic. At the edge of the wound near the last perfused capillary, oxygen tensions are in the range of 40mmHg and go to 0 to 15mmHg at the center of the wound. In this hypoxic environment from the regulatory compartment a number of growth factors are elaborated that lead to angiogenesis.

i. These growth factors can be grouped into 3 major categories:
   a. Mitogens which signal cells to proliferate.
   b. Chemoattractants which lead cells including macrophages to migrate.
   c. Transforming growth factors which change the cellular phenotype.

   Many growth factors are both mitogens and chemoattractants.

ii. The mitogens include platelet derived growth factor (PDGF), epidermal growth factor (EGF) and several angiogenesis factors including acidic and basic fibroblast growth factors (aFGF and bFGF). In the wound space compartment, hypoxia and lactic acid stimulate both growth factor production and macrophage migration. In short order after wounding, macrophages are attracted into the wound space where they perform a dual role: 1) They engulf and destroy bacteria and other cellular debris in the wound and 2) They release many growth factors including angiogenesis factors. Just as in tumors, these factors must encourage endothelial cell migration, proliferation and basement membrane matrix production after new vascular tubes are formed.

iii. The chemoattractants include complement C5a which is chemotactic for neutrophils and PDGF which is chemotactic for fibroblasts.

iv. The final group of growth factors are the transforming growth factors. These growth factors are believed to stimulate production of matrix molecules, i.e. collagen and glycosoaminoglycans. In certain concentrations, they may inhibit fibroblast mitoses.

B. The second compartment in the 2 compartment model is the Responder Compartment which is composed of vascularized connective tissues and replaces the wound space as the wound heals**.** Here oxygen plays a crucial role in collagen synthesis, hydroxylation and cross linking. Oxygen is also necessary for epithelization.

7. Oxygen and Tumor Angiogenesis: What We Know and What We Can Surmise

A. Basic Principles: The similarities between tumor angiogenesis and wound healing are striking. Since we actively promote hyperbaric oxygen in part to promote angiogenesis as a component of successful wound healing, should we be concerned that it might also enhance angiogenesis in cancers? Should we refuse to treat a patient with cancer or even a remote history of cancer because we might activate an inactive cancer or its dormant metastases? These are valid questions, and though all of the mechanisms by which hyperbaric oxygen might enhance tumor angiogenesis are not known, the information that is available strongly suggests that hyperbaric oxygen is not likely to enhance tumor angiogenesis. In fact, we do know that tumor cells which grow and survive in hypoxic regions of the tumor are more aggressive, more prone to metastasis and more resistant to treatment. What are the specific considerations?

i. At this point in time, we only partially understand the mechanisms by which angiogenesis is enhanced by oxygen and shut down at the completion of wound healing. Well-oxygenated wounds do not have their healing accelerated by hyperbaric oxygen. The growth of malignancies including angiogenesis continues regardless of oxygen status. In other words, tumor angiogenesis is very different from angiogenesis in normal healing wounds at least in some very important ways.

ii. The intermittency of hyperbaric oxygen which increases oxygen tensions optimally to the range of 30 to 40 mmHg to stimulate collagen synthesis, hydroxylation and cross linking appears to be the key in HBO_2 as an adjuvant to healing in chronic hypoxic wounds. No similar mechanisms have been identified in tumor stroma formation.

iii. Angiogenic growth factors elaborated in the wound require hypoxia and lactic acid.[54] Some have suggested that macrophages, a major source of angiogenic factors in wounds, will continue to

use anaerobic pathways of glycolysis even in the presence of oxygen at least for some time.[54] It is widely accepted that normal levels of oxygen attained once the wound is healed and vascularized are the signal to discontinue further angiogenesis.[54] It is likely that prolonged exposures to hyperbaric oxygen even if tolerable to the patient would have negative effects and ultimately inhibit healing. Consider the following quote from Davis et al[56], 1988:

"**Periodic** elevation of PO_2 in relatively ischemic wounds has powerful effects on wound dynamics both by enhancing leukocyte bacterial killing and by providing fibroblast-collagen support for capillary angiogenesis factor provided by **hypoxic** macrophages during the 20-22 hours a day that wound PO_2 drops to **hypoxic** levels."

B. Angiogenesis in wounds differ from cancers in several ways:

i. A wound necessarily involves negative space. Even in an approximated surgical wound, the healing process must generate new tissue to occupy this negative space. Tumors generally arise in space already occupied by existing tissues and are characterized by invasiveness. For tumors to grow they must release collagenases to dissolve basement membranes and dissolve normal tissue into which the cancer population of cells can invade and proliferate. Tumors are known to co-opt existing vessels and it is likely that they also co-opt pre-existing stroma.[52] This obviates or at least reduces the need to generate collagen and other connective tissues de novo.

ii. The substance of the healed wound, i.e. the supporting connective tissues and the overlying epithelium are unlike malignant tumors in that their continued proliferation past healing is regulated by various feedback signals including contact inhibition.[54] In the healed wound unbridled growth is not supported; whereas, it is the nature of malignant cell division that it does not respond to feedback signals from other cells and tissues and that its growth continues unabated.[53]

iii. Tumor vasculature is not well organized and does not conform to normal patterns (artery-arteriole-capillary-venule-vein).[57] Tumors often contain giant capillaries and arteriovenous shunts without intervening capillaries. Blood sometimes flows from one vein to another. Leaks in these vessels often occur contributing to the well known and frequent phenomenon of peritumoral edema. In other words, tumor angiogenesis does not undergo maturation and integration with pre-existing vasculature in the same fashion as a successfully healed wound.

C. What is known about Tumor Angiogenesis/Growth/Metastasis and Oxygen

i. Hypoxia has been shown to be an intense stimulus for angiogenesis.[54-56]

ii. VEGF (Vascular Endothelial Growth Factor) has its elaboration and release upregulated by hypoxia.[58-62] Numerous publications have demonstrated the increase of VEGF with hypoxia.[58-]VEGF is released by the tumor cell itself.[52]

iii. Interleukin-8 release is increased by hypoxia[63] This phenomenon has been demonstrated in human glioblastoma cells in culture. IL-8 has been shown to have angiogenic properties in this model.

iv. PEDF (Pigment Epithelium Derived Factor) an angiogenic inhibitor is down regulated by hypoxia and upregulated by hyperoxia[64]. This effect was demonstrated in human retinoblastoma cells in culture.

v. Large scale DNA overreplication and gene (oncogene) amplification occurs in hypoxic regions of tumors.[65] The frequency of mutations in tumor cells in hypoxic conditions was five fold those cells cultured in normoxic conditions. Teicher[66] has suggested that the genetic instability demonstrated by tumor cells in hypoxic regions is likely to result in the development of drug resistance.

vi. Hypoxia selects for tumor cells with diminished potential for apoptosis.[67] Apoptosis or programmed cell death is felt to be an important protection against malignancy since malignant cells become immortal and continue to divide indefinitely. Graeber et al[67] have shown that hypoxia causes defects in apoptosis in oncogenically transformed Rat1 fibroblasts grown in tissue culture.

vii. Hypoxic tumors are resistant to radiation and some chemotherapy agents.[66] Since the 1950's we have known that tumors with large populations of hypoxic cells are resistant to cell kill by ionizing radiation.[68] More recent studies have shown that many chemotherapeutic agents have their efficacy reduced in areas of hypoxia. Teicher et al[69] reported that 3 discrete types of chemotherapies exist in regard to their killing of cells related to the oxygen status of those cells. Type 1 agents are those which demonstrate diminished cell kill in regions of hypoxia; Type 2 agents selectively kill hypoxic cells; Type 3 chemotherapies kill cancer cells equally well in hypoxic and normoxic environments. Type 1 drugs include Bleomycin, Procarbazine, Actinomycin-D and Vincristine. Rice et al[70]have reported that hypoxia leads to resistance to Methotrexate by enhancing the frequency of dihydrofolate reductase gene amplification in Chinese hamster ovary cells.

viii. Hypoxia has been shown to predict for tumor aggressiveness and metastatic potential. Hoeckel and associates[71] have shown that patients with cervical cancer with significant regions of hypoxia have decreased survival. Gatenby et al[72] have reported a higher likelihood of metastases in patients with hypoxic squamous cancers. Brizel and his associates[73] reported that patients with larger fractions of hypoxic cells in their soft tissue sarcomas had worse survival and more common metastases than those who had higher oxygen levels in their tumors. For survival the break point was <or> 10 mmHg and for metastases the favorable group had median oxygen values greater than 20 mmHg while the unfavorable group had oxygen levels less than 7.5 mmHg.

8. Summary of Considerations Related to Angiogenesis: Many similarities exist between tumor and wound angiogenesis. Many important differences exist as well. Both require hypoxia for the release of angiogenic growth factors. In wounds, oxygen is needed for its immune effect and for the support it provides for fibroblastic proliferation, collagen release, hydroxylation and cross-linking. Oxygen is also needed for epithelization.[54] Cancers co-opt blood supply initially from surrounding structures and may co-opt stroma as well.[57] Certainly, those who have intensely studied tumor angiogenesis have not identified collagen production or release as part of the complex series of events needed to successfully generate tumor angiogenesis. Epithelial coverage is not a major component of cancer growth though it is vital for wound healing. Often cancers become ulcerated and do not have an epithelial cover. The preponderance of known characteristics of tumors shows with consistency that hypoxic tumor cells elaborate angiogenesis factors, grow more aggressively, throw off more metastases and are subject to decreased apoptosis and increased genetic instability and therefore increased drug resistance. Hypoxic cells are resistant to irradiation and some chemotherapies. Most importantly, the vast majority of published clinical experience and animal studies specifically designed to answer this issue show that neither the primary tumor nor metastatic deposits grow more aggressively when hyperbaric oxygen has been administered.

V. Final Conclusions:

The available published evidence strongly suggests that intermittent hyperbaric oxygen has no enhancing effect of cancer primary or metastatic growth. Likewise, there is no credible evidence that hyperbaric oxygen is an initiator or promotor of cancer de novo. Ample pre-clinical and clinical information have been reviewed. Animal studies specifically designed to study the impact of hyperbaric oxygen on malignant tumor growth and metastasis conducted from 1966 to 2001 fail in an overwhelming fashion to demonstrate a tumor growth enhancing effect. While 3 clinical publications entailing 72 patients suggest a possible cancer or metastases promoting effect, large numbers of mostly controlled studies including over 3,000

patients enrolled in trials designed to investigate hyperbaric oxygen as a radio-sensitizer demonstrate either a neutral or cancer inhibitory effect. Dr. Marx has followed 405 patients treated for delayed radiation injury and observed a decreased incidence of recurrence in those patients treated with hyperbaric oxygen. The possibility that significant immune suppression, free radical induced damage or mutations leading to carcinogenesis is likely to enhance malignant growth in hyperbaric patients is not well supported by the reviewed literature. Finally, contentions that tumor angiogenesis is likely to be promoted by hyperbaric oxygen in the same fashion that angiogenesis is promoted in non-healing hypoxic wounds fail to recognize the unique nature of those processes in these very different physiologic and pathophysiologic systems. Most recent evidence supports the findings that tumors which thrive in hypoxic environments are more prone to a rapid aggressive course including resistance to treatment, increased incidence of metastases, decreased cell death due to apoptosis and a higher likelihood of tumor lethality.

The author proposes that patients for whom hyperbaric oxygen treatments are likely to be useful for the treatment of radiation injuries should not have this therapy denied to them because of unsubstantiated fears that hyperbaric oxygen might cause a higher likelihood of tumor recurrence or metastases.

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Table 1: Animal Studies

Author	YEAR	Details of Report
McCredie, et al[18] ↔	1966	C3HBA mouse mammary tumor; no effect on primary or metastasis.
Suit, et al[19] ↔	1966	Strong A and BDF mouse mammary tumor; no effect on primary or metastasis.
DeCosse, et al[20] ↓	1966	For mouse melanoma decrease in pulmonary metastases; no change in primary growth.
Johnson, et al[21] ↔	1967	Mouse melanoma and leukemia. For melanoma no increase in primary or size or number of metastases For leukemia no decrease in survival.
Dettmer, et al[22] ↓	1968	Rat carcinosarcoma; both primary and metastases decreased in HBO arm.
Evans, et al[23] ↔	1968	Mouse skin cancer; same incidence of lung metastases.
Feder, et al[24] ↔	1968	Implanted rhabdomyosarcoma in mice; metastases identical in HBO group.
Johnson, et al[25] ↔	1971	Transplanted lymphoblastic leukemia; no difference in survival, primary tumor growth or metastases.
Shewell et al[26] ↑ ↔	1980	Two separate studies: Both transplanted and spontaneous murine mammary tumors; for spontaneous tumors 88.8% mets in HBO vs 66.6% in air; otherwise primary tumor and mets in transplanted tumor identical.
Marx, et al[27] ↓	1988	DMBA induced SCCA in hamsters; delayed growth in HBO Group
Frid et al[28] ↔	1989	No increase in growth of transplanted tumor or metastases in transplanted sarcoma and melanoma in murine model
McMillan, et al[29] ↑ ↓	1989	DMBA induced tumors in hamsters; larger but fewer tumors in HBO vs air .
Mestrovic, et al [30] ↓ ↔	1990	Suppression of metastatic tumors in lung after IV injection of anaplastic tumor; no change in growth when transplanted in hind limb
Headley, et al [31] ↔	1991	Human SCCA xenografts in nude mice; no difference in growth
Sklizovic, et al[32] ↔	1993	Human xenotransplants of SCCA in mice; HBO group received 21 treatments; No difference in tumor weight, volume or histology compared to control
Lyden, et al[33] ↔	1997	MCG 101 Sarcoma transplanted in mice; HBO exposed to 2.8 ATA for 9 days; compared to control in HBO group accumulation of cells in S-phase but no change in tumor growth
Takiguchi et al [34] ↓	2001	In sarcomas transplanted into mice growth slightly inhibited by exposure to HBO

Legend :↑ indicates increased growth; ↓ indicates decreased growth; ↔ indicates no effect on growth. If 2 symbols are given, the effect is mixed.

Table 2: Clinical Reports

AUTHOR	YEAR	Details of Report
Johnson, et al[1] ↑	1966	25 patients HBO radiosensitized for cervical cancer showed unusual frequency and pattern of metastases; 30 exposures at 3.0 ATA
Van DenBrenk, et al[35] ↓	1967	85 head and neck patients with historic controls; had statistically significant decrease in metastases in HBO Group; 2-6 exposures at 3.0 ATA
Cade, et al[36] ↔ ↑	1967	Controlled trial of 49 patients with lung CA and 40 patients with bladder CA; Metastases the same in HBO and control for lung but increased in bladder HBO group; not well matched for tumor grade; 40 exposures at 3.0 ATA
Johnson, et al[37] ↔ ↓	1974	Controlled trial of 64 cervical cancer patients; metastases identical in HBO and control groups; 5 yr survival 44% HBO vs 16% control; 25-30 exposures at 3.0 ATA
Henk, et al[38] ↔ ↓	1977	Controlled trial of 276 head and neck cancers; rates of metastasis identical for HBO and air groups; recurrence-free survival better in HBO group; 10 exposures at 3.0 ATA
Henk, et al[39] ↓	1977	Controlled trial of 104 head and neck cancers; disease-free survival statistically improved in HBO patients; 10 exposures at 3.0 ATA
Bennett, et al[40] ↔	1977	Controlled trial of 213 cervical cancers; no increased metastases in HBO group; 10 exposures at 3.0 ATA
Perrins, et al[41] ↔	1978	Controlled trial of 236 bladder cancers; no difference in survival at 4 yrs and no difference in metastases; 6-40 exposures at 3.0 ATA
Watson, et al[42] ↔	1978	Controlled trial of 320 cervical cancers; metastases identical in HBO and control groups; 6-27 exposures at 3.0 ATA
Dische et al[43] ↔	1978	Controlled trial of 1500 patients with head and neck, bladder, bronchus or cervical cancer; No difference in metastases from HBO to control; 6-12 exposures at 3.0 ATA
Brady, et al[44] ↓	1981	Controlled trial of 65 cervical cancers; distant failure higher in control (34%) vs HBO group (16%); 10 exposures at 3.0 ATA
Eltorai, et al[45] ↑	1987	3 anecdotal cases of urothelial cancer patients in patients with chronic spinal cord injury whose cancer progressed rapidly after HBO; 10-20 exposures at 2.0 ATA
Denham, et al[46] ↓	1987	201 patients irradiated for head and neck cancer with hyperbaric radio-sensitization; tumor control and survival better than historic controls
Bradfield, et al[47] ↑	1996	The authors present 4 cases of head and neck cancer which rapidly progressed after HBO exposure; all were advanced; 2 had prior recurrences; 1 had radiation interrupted for 6 wks because of pneumonia
Marx[48] ↓	1999	The author presents 405 patients with head and neck cancer: 245 received HBO for 30 to 40 treatments; 19.6% recurrence in HBO group vs 28% recurrence in non-HBO group

Legend: ↑ indicates increased growth; ↓ indicates decreased growth; ↔ indicates no effect on growth. If 2 symbols are given, the effect is mixed.