

# A liquid cartilage extract as an *adjuvant* to conventional treatment

for the maintenance of a healthy angiogenic balance as well as  
the support of healthy hematocrit level and red blood cell count

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## A New Perspective on Cancer

The way we think of a disease has profound implications in terms of its treatment. The concept of cancer as a monolithic terminal illness raised the hope of “winning the war on cancer”. This idea has led to the development of successive generations of weapons used at their maximum tolerated dose. While the strategy appears useful in prolonging survival, complete remission remains a rare event. This is because even if only one single enemy is spared, it can still raise an army of new, now resistant, cancer cells that can strike again in a fatal breaking wave.

As the long hoped for idea of a universal cure for cancer fades away, a new vision of multidisciplinary complementary approaches emerges and opens the door to mixed protocols that combine alternative therapies with more conventional cancer treatments.

## Conventional Cancer Therapies

Cancer treatment is traditionally based on the triad of surgery, radiotherapy and chemotherapy. New approaches, such as antiangiogenic therapy, are also being developed. Each modality comes with its own subset of strengths and limitations.

### Surgery

Surgery, in the form of biopsy, is commonly used for cancer diagnosis and staging of tumors. Surgery serves therapeutic purposes as well and has found applications for localized accessible tumors. An early stage primary tumor or a resectable secondary tumor can be removed in the hopes of eradicating all cancer cells. Even when total resection is not possible, surgery can still be used to reduce the tumor burden prior to systemic or radiation therapy. Surgery is also increasingly used post radiation/chemo in an effort to spare the targeted organ as much as possible. Surgery may be recommended in the context of palliative care for the relief of pain resulting from the compression of normal tissue by a tumor.

However, as with any surgery, there are risks of infection, bleeding, and painful or unsightly scars, as well as anesthesia-related complications (clots, strokes, pneumonia and kidney failure). A surgery side effect of major concern to cancer patients is that the removal of a primary tumor is sometimes associated with early relapse and spreading that is believed to be related to an angiogenic surge. The concomitant removal of inhibitors or the release of growth factor stimulators of angiogenesis can activate dormant distant micrometastasis. This was demonstrated in an animal model of lung metastasis (O'Reilly *et al.*, 1994) and also suggested for a subset of premenopausal node-positive breast cancer patients (Retsky *et al.*, 2004).

### Radiotherapy

Radiotherapy – or radiation therapy – can help with both the diagnosis and treatment of cancer, or serve in palliative care for the relief of symptoms. For therapeutic purposes, radiotherapy uses focused beams of ionizing radiation composed of photons (x-rays, gamma rays) or particles (electrons, protons, neutrons) to attack cancer cells. High energy rays break chemical bounds within cells to generate unstable free radicals that can disrupt DNA structures. Cells are most sensitive when in their mitosis phase, making rapidly dividing cancer cells highly vulnerable. The damage caused to their genetic material forces the affected cells to stop repli-

*Angiogenesis is the process by which new blood vessels are formed from post-capillary venules and grow to supply oxygen and vital nutrients to cells. Tumor cells need angiogenesis to grow beyond a critical volume, and can use the newly formed vessels to escape from the primary tumor and colonize distant organs.*

*Free radicals are molecules that have lost an electron due to the splitting of a molecular bond. Oxygen radicals such as superoxide ( $\cdot\text{O}_2^-$ ) and hydroxyl radical ( $\cdot\text{OH}$ ) are short-lived but highly reactive molecules that, in an act of desperation to restore their integrity, steal electrons from surrounding molecules.*

cating and triggers apoptosis (programmed cell death). Radiotherapy is used to treat localized solid tumors, such as cancers of the skin, tongue, larynx, brain, breast, uterine cervix, prostate, or head and neck. For reasons not fully understood, leukemia and lymphoma are also responsive *in vivo*. Radiotherapy can be used before surgery to shrink tumors or after as adjuvant therapy. Mixed protocols with concomitant chemotherapy are increasingly being developed.

Although better equipped than tumor cells with repair mechanisms, normal cells with a rapid turnover (skin, stomach, intestine, hair and progenitor blood cells in the marrow) also run the risk of being damaged. Skin burns, hyperpigmentation, nausea, vomiting, diarrhea, alopecia, tiredness and anemia are therefore common side effects of radiotherapy. Anemia is a particularly insidious side effect. Since radiotherapy relies on the formation of reactive oxygen species (ROS) for cytotoxicity, the oxygen deprivation that occurs with anemia adversely affects cell radiosensitivity, reducing treatment effectiveness (Kumar, 2000). On the other hand, ROS generation is a known trigger of angiogenesis (Maulik and Das, 2002), a fact that may be linked to the observation that radiotherapy sometimes accelerates metastatic growth (Camphausen *et al.*, 2001). Can we solve this apparent conundrum?

### Chemotherapy

Chemotherapy refers to the use of chemical agents (drugs) to treat any disease. In the treatment of cancer, chemotherapy is used either alone or in combination with other therapies to kill or control cancer cells. Unlike surgery and radiotherapy, chemotherapy is a systemic treatment that can reach even disseminated cells. Numerous cancer drugs have been developed over recent years. Standard cancer drugs, taking advantage of the high division rate of cancer cells, work by preventing

them from entering or completing cell division, eventually killing cells. Newer drugs are also being developed that try to target specific signaling pathways known to be defective in cancer, in an effort to revert cancer cells to a normal phenotype.

Attacking cancer with cytotoxic drugs when cells are busy replicating, and therefore off guard, is usually a successful first-line strategy.

Unfortunately, cancer cells have a high mutation index and chances are that a few of them will develop ways to resist the attack and mount multidrug resistance for a deadly comeback. For this reason, conventional chemotherapy for cancer tends to be quite aggressive, with the highest tolerated dose being administered in multiple rounds of treatments, broken by recovery periods to limit neurotoxicity and damage to proliferating cells in healthy tissue. Anemia, nausea and fatigue are nevertheless frequent complications of chemotherapy. For instance, the incidence of anemia can be as high as 90% with some chemotherapeutic regimens (Groopman and Itri, 1999), a problem that needs to be addressed since anemia can interfere with drug efficacy and have a negative effect on the clinical outcome (Caro *et al.*, 2001).

### A Newer Approach to Cancer Treatment

#### Antiangiogenic therapy

Back in the 1970s, Dr. Judah Folkman hypothesized a link between angiogenesis and tumor growth, on the basis of his own experience as a surgeon. His premise was that cancer cells could not grow beyond a critical volume without somehow forcing the development of new blood vessels to feed them with oxygen and nutrients. He argued that blocking angiogenesis would “starve cancer cells to death”. His idea was initially received with a great deal of skepticism by the scientific and medical community, but a fortuitous observation soon confirmed the potential of antiangiogenic therapy. In Dr. Folkman's laboratory at Harvard Medical School, an ordinary unsolicited contamination of a cell culture dish plate with a fungus was preventing endothelial cells from growing. Most researchers would simply have discarded what appeared to be just another aborted experiment, but not in this laboratory! Instead, Dr. Folkman and his team went on to purify, from that mold, what would eventually become TNP-470, the first angiogenesis inhibitor to enter clinical trials. Early on, neurotoxicity stood in the way of the development of TNP-470 as a cancer drug. Nonetheless, the angiogenesis seed had begun to germinate in the minds of many researchers worldwide. Soon, promising pre-clinical studies with various angiogenesis inhibitors were prompting considerable excitement, and by the end of the 1990s, more than 60 antiangiogenic drugs had entered clinical trials. But when they were initially tested on humans – generally in monotherapy for advanced cancers that had not responded to conventional therapies – most of the results were quite disappointing, forcing researchers to rethink their clinical strategy.

*Discovery of the first chemotherapeutic anticancer agent was accidental. When mustard gas was used as a chemical warfare agent during World Wars I and II, the development of low white blood cell counts in exposed military personnel revealed the potential of this agent to interfere with other rapidly growing cells, such as tumor cells.*

### *Milestones in antiangiogenesis research*

- 1971** - Dr. Judah Folkman develops the notion that tumors depend on angiogenesis for their growth.
- 1989** - Napoleone Ferrara identifies a vascular endothelial growth factor (VEGF) as the main angiogenic factor.
- 1992** - The first antiangiogenic drug (TNP-470, a fumagillin derivative) enters clinical trials.
- 1999** - Massive wave of antiangiogenic drugs in clinical trials.
- 2004** - Avastin is the first antiangiogenic drug to gain FDA approval for first-line metastatic colorectal cancer treatment in combination with chemotherapy.

Thanks to a series of clinical observations (see window below), a more realistic picture of the potential of antiangiogenic therapy as a cancer treatment is now emerging. With these clinical facts, it is now possible to put antiangiogenic therapy into perspective. Angiogenesis is indeed part of the malignant process, occurs early in the tumor development phase, and is not limited to solid tumors. In contrast to more conventional therapies, antiangiogenesis is not aimed at killing cancer cells but rather at keeping them in check, with chronic treatment starting as early as possible. A good antiangiogenic agent should therefore be exempt from toxicity and allow for the maintenance of good QOL. Preferably, antiangiogenic therapy should be administered in combination with other therapies. By pruning non-mature vessels, antiangiogenic agents can improve the delivery of therapeutic agents to tumors (Jain, 2001). Normalizing vessels with antiangiogenic agents may also serve to radiosensitize tumors by optimizing their oxygenation while at the same time blocking the provascular effects of radiation (the conundrum is solved!). Antiangiogenic therapy has a promising future as adjuvant therapy to control residual disease and metastatic growth following surgery, radiotherapy or chemotherapy.

So far, only one antiangiogenic drug has reached the market: Avastin was approved by the FDA in February 2004 for first-line treatment of metastatic colorectal cancer in combination with chemotherapy. Avastin, which was developed by Genentech, is a recombinant humanized monoclonal antibody that binds to vascular endothelial growth factor (VEGF), preventing it from binding to the VEGF receptors on endothelial cells. Interfering with VEGF signaling stops angiogenesis. Longer survival (20.3 vs 15.6 months) and better tumor control (10.6 vs 6.2 months) in the context of colorectal cancer were the clinical benefits that allowed Avastin to be approved as a cancer drug. The rela-

tive success of Avastin has validated the antiangiogenic approach to cancer treatment. Nevertheless, this drug has three major drawbacks: it is injectable, is prohibitively expensive, and has side-effects such as hypertension and increased blood clot risk. It may therefore not be appropriate for long term use.

There are natural alternatives for blocking angiogenesis. Green tea (polyphenols), turmeric (curcumin), soy (genistein), tomatoes (lycopenes), cherries (perillyl alcohol), flaxseed (lignans), garlic (diallyl sulfide), selenium, vitamin E, spinach (lutein) and cartilage extract are all natural sources of antiangiogenic molecules (Losso, 2002) that are presently under evaluation in clinical trials on cancer patients. The clinical evaluation of liquid cartilage extract (LCE) is the most advanced (Bukowski, 2003; Dredge, 2004). Accumulated data support its use in conjunction with conventional cancer therapies. Interestingly, recent additional characterization of the product has highlighted its potential to enhance blood parameters in cancer patients, adding to its attractiveness as an adjuvant in cancer treatment.

### *Clinical observations with angiogenesis inhibitors*

- antiangiogenic agents are generally well tolerated
- antiangiogenic therapy is not very good at shrinking tumors but can delay progression and prolong survival
- patients do not develop drug resistance to antiangiogenic agents
- maximum tolerated doses may not be required for optimal biological effects
- non-solid tumors also respond to antiangiogenic therapy
- less advanced cancers respond better
- antiangiogenic therapy normalizes tumor vessels and reduces interstitial pressure in the vicinity of tumors
- antiangiogenic therapy is more effective in combination with other therapies

### *LCE as a support to conventional cancer therapies* *Angiogenesis inhibition*

LCE simultaneously targets many different aspects of angiogenesis. As demonstrated *in vitro*, LCE interferes with VEGF binding and signaling, inhibits matrix metalloproteinase (MMP) activity involved in tumor invasiveness, stimulates apoptosis of endothelial cells, and promotes angiostatin (an endogenous angiogenesis inhibitor) production in the vicinity of tumors.

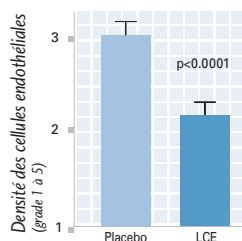
*In vivo*, the antiangiogenic efficacy of LCE was tested in the Matrigel model. Matrigel consists of a solubilized basement membrane preparation containing proangiogenic factors. When introduced subcutaneously in animals, Matrigel solidifies to form a plug that stimulates its colonization by new vessels. Following treatment, the implant can be recovered and examined histologically to determine the extent to which blood vessels have entered it. As evidenced in Figure 1, orally administered LCE reduced vascularization in a subcutaneous Matrigel implant in healthy mice (Dupont *et al.*, 2002).

**Figure 1:** LCE (right panel) inhibits angiogenesis in the Matrigel model.



To assess the bioavailability of LCE in humans, a randomized double-blind placebo controlled clinical trial involving twenty-nine healthy volunteers was designed. The rationale for this trial was based on the ability of endothelial cells to invade a tiny polymer insert (polyvinyl alcohol) when placed under the skin. The higher the number of endothelial cells within the insert, the stronger the angiogenic response. When oral administration of a product inhibits the invasion of the insert by endothelial cells, it provides an irrefutable proof of its bioavailability and a good indication of its antiangiogenic potential. Results from this study are shown in figure 2. Daily oral administration of LCE resulted in a statistically significant reduction of endothelial cell density compared to placebo (Berbari *et al.*, 2000).

**Figure 2: Bioavailability and antiangiogenic effects of LCE in humans.** Oral administration of the liquid cartilage extract to human volunteers reduced endothelial cell density within a subcutaneous polymer insert, as revealed by immunostaining techniques



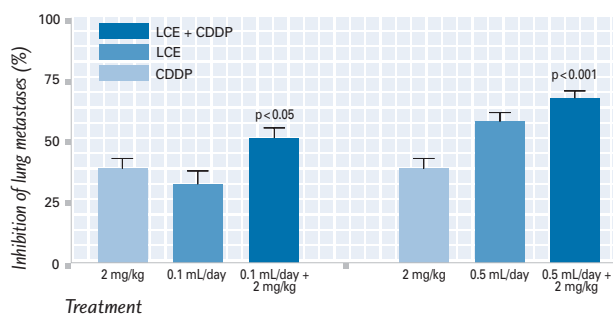
The therapeutic potential of LCE was further investigated clinically in oncology for both solid (breast, lung, prostate and kidney) and non-solid (multiple myeloma) tumors, in dermatology for psoriasis, and in ophthalmology

for macular degeneration. The safety profile of LCE was established through several Phase I/II studies and encouraging results have led to Phase III trials in renal cell carcinoma (RCC) and non-small cell lung cancer (NSCLC). In the RCC study, a significant survival advantage (26.3 versus 12.6 months) was observed for those receiving LCE among a preplanned group (38 patients) with clear cell histology, one metastatic site and an ECOG=0 (indicative of fairly good general condition otherwise). The NSCLC trial is still recruiting.

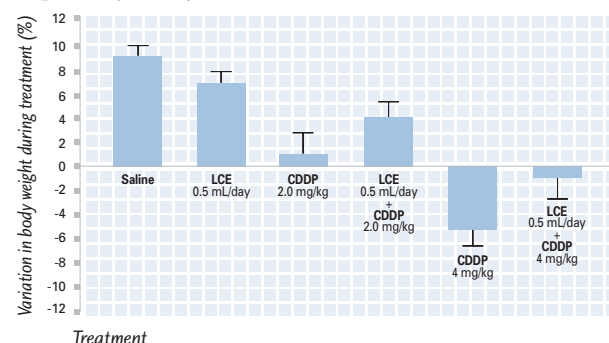
### Synergism with conventional therapies

Interestingly, in animal models, LCE exhibited an additional antitumor effect when administered orally in combination with a suboptimal dose of cisplatin (2 mg/kg) in a mouse model of Lewis lung carcinoma, which is characterized by a large number of lung metastases (Dupont *et al.*, 2002). The combination improved the therapeutic index by increasing anti-metastatic efficacy over either treatment alone (Fig. 2a) and by protecting against cisplatin-induced toxic side effects, including weight loss (Fig. 2b). These findings highlight the potential therapeutic benefits that may be derived from the co-administration of LCE and chemotherapy to cancer patients.

**Figure 2a:** LCE synergizes with cisplatin (CDDP) treatment in inhibiting lung metastases in a mouse LLC model.



**Figure 2b:** LCE attenuates the cachectic effect of cisplatin (CDDP) treatment in a mouse LLC model.



### Maintenance of healthy red blood cell levels

The first insights into the potential of LCE as a natural product to sustain blood parameters in the course of cancer treatment came from an animal study. In a murine LLC model, erythrocyte, hemoglobin and hematocrit values—normally affected by an optimal dose of cisplatin treatment—were normalized when LCE was given in combination with the drug (Table I).

**Table I:** LCE protects blood parameters in cisplatin-treated (CDDP) mice in a model of lung cancer (LLC)

Treatment	*Erythrocytes ( $10^{12}$ cells/L)	*Hemoglobin (g/dL)	*Hematocrit (%)
Normal values for mice	7.9–10.1	11.0–14.5	37–46
Saline (control)	8.6 ± 1.0	13.4 ± 1.8	41 ± 5
LCE (0.5 mL/day)	8.3 ± 0.9	12.9 ± 1.6	40 ± 5
CDDP (4 mg/kg)	6.8 ± 1.8	10.7 ± 3.2	33 ± 9
LCE + CDDP	7.9 ± 1.5	12.7 ± 2.2	39 ± 7

\* Mean and standard deviation from at least 9 animals

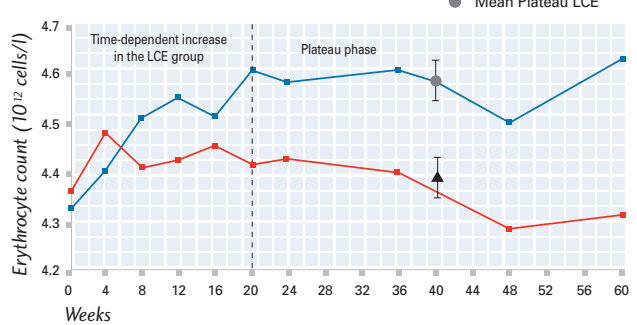
These results were corroborated by a retrospective analysis of the blood parameters of the 305 patients with late-stage refractory RCC who participated in the recently closed randomized double-blind placebo-controlled phase III study. While at baseline the whole cohort of 305 patients had a tendency toward lower than normal blood parameters (normal range given in Table II), generally speaking, in patients receiving LCE, the red blood cell (RBC) count went up to 5.4% (from 4.330 to 4.582  $\times 10^{12}$  cells/L,  $p=0.008$ ) and the hematocrit values went up 3.9% (from 38.1% to 40.2%,  $p=0.001$ ), with hemoglobin values showing a similar trend ( $p=0.10$ ). Blood parameters reached a plateau, close to normal levels, at week 20 and remained stable for the rest of the study. Those of the placebo group remained unchanged (Fig. 3a and 3b).

**Table II:** Normal range for blood parameters in men and women

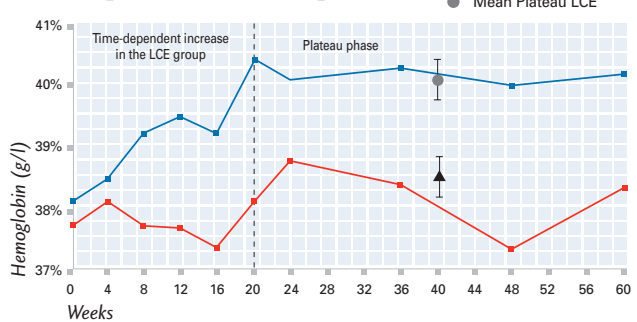
Red blood cell count	Normal range for men	Normal range for women
Erythrocytes ( $\times 10^{12}$ cells/L)	4.7 - 6.1	4.2 - 5.4
Hematocrit (%)	41 - 50	36 - 44
Hemoglobin (g/dL)	14 - 17	12 - 15

Source: Medline Plus, US National Library of Medicine

**Figure 3a:** Mean erythrocyte count for RCC patients on LCE or placebo



**Figure 3b:** Mean hematocrit values for RCC patients on LCE or placebo



A drop in blood parameters leads to anemia, a condition that often complicates cancer, either as a direct effect of the neoplasm or indirectly as a result of its treatment. The overall incidence of anemia is close to 60% among the cancer population but only about 40% of these patients receive supportive therapy to correct their hemoglobin levels, and when they do, it is generally not until these levels have dropped to 8–10 g/dL (Jakel, 2003). This is a greatly underestimated problem with major consequences. Cancer-related anemia is associated with intense debilitating fatigue, which has a significant effect on patient QOL. Due to this intense fatigue, it is often necessary to postpone treatments, increase the intervals between treatments or cut dosages. The general tissue hypoxia that results from anemia interferes with the effectiveness of radiation therapy and chemotherapy. Tissue hypoxia also drives angiogenesis, which further sustains cancer growth. The presence of anemia in cancer patients has been reported to increase the relative risk of death by 65% (Caro *et al.*, 2001). For patients with moderate anemia, the risk of dying decreases 0.76 times with each increment of 1g/dL in hemoglobin lowers the risk of death 0.76 times (Balducci, 2003), strongly supporting the need to address anemia as part of any cancer protocol.

## Conclusion

We are moving toward more comprehensive, chronic treatment of cancer; it is important to consider the inclusion of supportive alternative therapies in conventional protocols. Natural antiangiogenic agents have a great deal of potential as adjuvant therapy in patients at high risk of relapse, or as chronic therapy for remission maintenance in the metastatic setting. Natural products should be developed to support patient QOL during and after treatment. Addressing anemia in cancer patients deserves additional attention since it can impact greatly on patient survival.

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