

# **Epithelial Ovarian Cancer – Adjuvant Measures with Potential for Slowing Its Spread and Boosting or Restoring its Chemosensitivity**

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Although epithelial ovarian cancer (EOC) - constituting about 90% of all ovarian cancers – is not one of the most common malignancies, it accounts for more deaths than any other gynecological cancer. This reflects the fact that, by the time it is detected, it has usually spread beyond the point where it could be surgically curable. Post-surgically, EOC is often responsive to chemotherapy drugs such as platins and taxanes; but such therapy is rarely curative, as EOC tends to evolve resistance to these agents. Hence, adjuvant measures which could enhance or restore the responsiveness of EOC to chemotherapy drugs would be of great value. Additionally, measures for slowing the growth and spread of EOC in the intervals between chemotherapy sessions would also be worthwhile. A review of the current literature suggests that the following measures *may* have practical potential in these regards. Each discussion is preceded by a less technical summary in italics.

Please note however, that none of these measures are yet “proven” in the sense of having shown clear efficacy in formal clinical studies; most oncologists will currently be somewhat skeptical of them, and may even discourage their use. Nonetheless, all “proven” therapies were once unproven, and cancer patients who want to achieve optimal therapeutic outcomes quite reasonably may wish to avail themselves of safe and feasible strategies which show considerable promise in light of pre-clinical or epidemiological data. It has been difficult to get funding for sophisticated clinical studies with many of the agents discussed here because they are nutraceuticals or off-patent drugs that no pharmaceutical company could profit from; priority is given to clinical research on new drugs with unknown long-term toxicities that will cost a fortune if ever approved. In contrast, the off-patent drugs discussed here have well known side effect profiles, are typically safe in defined dose ranges, are not very expensive, and can legally be prescribed for off-label (not officially approved) uses by physicians who wish to do so. Note however that a physician’s prescription will be required for use of these agents.

Evidently, it is neither practical nor desirable to implement all of these measures simultaneously. Table 1 provides suggestions regarding the priority with which these measures can be introduced, and categorizes them with respect to their likely utility as adjuvants to chemotherapy, and/or their potential for use as long-term therapies to slow cancer growth and spread. Those given priority 1 are, in the main, nutraceuticals which your physician is unlikely to object to.

**Fasting for 2 days prior to, and during, chemotherapy** – *Recent pilot research from the University of Southern California suggests that fasting for two days prior to a chemotherapy session, and during the day that chemotherapy is administered, may selectively spare normal tissues from damage and alleviate side effects such as nausea, fatigue, and GI upset, while not impairing, or even amplifying, the cancer-killing efficacy of the chemo. Normal tissues react to fasting by boosting their antioxidant and stress response mechanisms – whereas persistent growth factor activity blunts this protective response in cancer cells. Moreover, by decreasing activity of the important growth factor IGF-I in tumor tissue, fasting can be expected to render cancer cells more sensitive to platin chemotherapy drugs, a standard component of firstline chemotherapy for EOC.*

**Table 1 - EOC Treatment Strategies**

This table categorizes the suggested adjuvant measures with respect to their likely utility as adjuvants to chemotherapy, their potential as long-term therapies for growth control, and recommended priority of use. Priority 1 therapies are suggested as first-line measures; priority 2 or 3 measures can be considered for future use if chemoresistant cancer eventually develops. Asterisked agents require a prescription and/or are relatively expensive.

	Chemo Adjuvant	Growth Control	Priority	Adjunctive Therapy
Fasting	X		1	
Metformin	X	X	1*	
Green Tea	X	X	1	
Melatonin	X	X	1	
Aspirin		X	1	
Prudent Diet		X	1	
Vitamin D		X	1	
Spirulina		X	1	
Soy Isoflavones		X	1	
DIM	X	X	1	
Naltrexone		X	1*	
GcMAF		X	1*	
I.V. Ascorbate	X		2*	X
Tocotrienols	X	X	2	
Salsalate \	X	X	2*	
Imatinib	X		2*	
Valproate/Hydralazine	X		2*	
Nelfinavir	X		2*	
Hydroxychloroquine	X		2*	
Itraconazole	X	X	2*	
Met Cyclophosphamide		X	2*	

Ambrisentan

X

X

3\*

A key intent of this strategy is to boost responsiveness to platin drugs by minimizing IGF-I activity in cancer, while improving tolerance to chemotherapy. A high proportion of EOCs express the IGF-I receptor as well as IGF-I and/or IGF-II, and up-regulation of IGF-I receptor signaling often plays a key role in the evolution of resistance to cisplatin in ovarian cancer.<sup>1-5</sup> Sensitivity of diverse ovarian cancer cell lines to cisplatin correlates inversely with mRNA expression of IGF-I, and exposure of these lines to exogenous IGF-I exacerbates chemoresistance; conversely, agents which inhibit function of the IGF-I receptor boost sensitivity of these cell lines to cisplatin. Fasting for 24-48 hours not only modestly lowers plasma IGF-1, but also increases plasma levels of IGFBP-1 – a functional antagonist of both IGF-I and IGF-II<sup>6</sup> – by an order of magnitude;<sup>7</sup> this IGFBP-1 potentially can inhibit not only plasma IGF-I, but also the IGF of autocrine origin within ovarian tumors. Hence, a short-term fast can be expected to restore or boost the responsiveness of many ovarian cancers to platins by suppressing IGF-I receptor signaling with the cancer. Moreover, such fasting may selectively protect healthy tissues from the cytotoxicity of chemotherapy drugs; in a pilot clinical trial, fasting for 48 hours prior to chemotherapy alleviated side effects such as fatigue, weakness, and gastrointestinal distress.<sup>8-10</sup> Fasting metabolism evokes protective stress resistance mechanisms in normal cells, but does so less readily in cancer cells, owing to their constitutive growth factor activity.

**Metformin or Berberine** – *The diabetes drug metformin is gaining the attention of oncologists as a potentially valuable adjuvant to chemotherapy and an aid to cancer control. Studies reveal that the incidence of EOC is lower in diabetics using metformin than in diabetics using other drugs for blood sugar control. And another recent study concluded that, in diabetics who already have EOC, those using metformin have a greatly superior survival in comparison to those who don't; in fact, their survival is better than that of non-diabetics. In mice implanted with human EOC, metformin treatment slows growth of the cancer and potentiates the efficacy of the chemo drug cisplatin. Metformin's utility as an adjuvant to chemotherapy reflects the fact that it can kill or impede the formation of cancer stem cells, which are typically resistant to chemotherapy and often give rise to cancer recurrence. The nutraceutical berberine has anti-diabetic properties similar to those of metformin, and seems likely to share metformin's potential for cancer control. Although metformin is thought of as a diabetes drug, it can be safely used by non-diabetics (unlike some diabetes medications) because it won't unduly lower blood sugar; its efficacy in cancer reflects its ability to activate a key enzyme which it activates in diabetics and non-diabetics alike. Oncologists are unlikely to object to the use of metformin with chemotherapy because they have all treated diabetics with cancer who were using this drug at the time – and studies are now showing that these patients tend to do better.*

Diabetics who use metformin may be at lower risk for EOC than other diabetics.<sup>11</sup> Moreover, in diabetics who already have EOC, metformin use is associated with much greater survival than use of other medications; indeed, diabetics who use metformin are at lower risk for this cancer than are non-diabetics.<sup>12</sup> Metformin has also been shown to retard the growth of EOC in vitro and in rodents, and to potentiate the cytotoxic response to cisplatin *in vivo*.<sup>13-16</sup> Metformin aids durable response to chemotherapy in many cancers by suppressing the formation or survival of cancer stem cells – a phenomenon that has now been demonstrated in human ovarian cancer.<sup>16-21</sup> Since AMPK is the chief target of metformin's clinical efficacy, it is likely that the nutraceutical berberine, which likewise activates this enzyme,<sup>22</sup> will have comparable efficacy, and, indeed, it inhibits the proliferation of various

EOC cell lines.<sup>23</sup> However, metformin's ability to slow EOC proliferation in vitro may not be entirely dependent on AMPK activation – so it might be prudent to use metformin if feasible.<sup>13</sup>

**Green Tea/Green Tea Polyphenols** – *In China, where many people drink green tea regularly, studies have found that green tea drinkers are less likely to develop EOC; moreover, in EOC patients, prolonged survival is much more common in green tea drinkers. This apparent protection may reflect that fact that a key compound in green tea, EGCG, undermines the ability of ovarian cancer cells to make a growth factor, endothelin, that in many such cancers boosts their proliferation and renders them harder to kill with chemo. In mice implanted with EOC, simply replacing their drinking water with green tea was associated with a 60% reduction in cancer growth. Drinking several cups of green tea daily, and/or taking several capsules of green tea polyphenols, may represent a simple, safe, and inexpensive way to improve control of EOC.*

Epigallocatechin gallate (EGCG), in concentrations as low as 1  $\mu$ M has been shown to suppress expression of both endothelin and the A-type endothelin receptor in ovarian cancer.<sup>24, 25</sup> Autocrine endothelin activity supports aggressive behavior and chemoresistance in a high proportion of EOCs.<sup>26-31</sup> In nude mice injected with a human EOC, tumor size at 60 days was 60% lower in mice drinking green tea (12 g/L) in place of drinking water.<sup>25</sup> In a Chinese epidemiological study which followed up EOC patients about 4 years after original diagnosis, 78% of patients who had drunk green tea since their diagnosis were still alive, as opposed to 48% of those who had not (adjusted hazard ratio 0.55); furthermore, chance for survival varied directly with the amount of green tea used.<sup>32</sup> Regular green tea use is also associated with reduced risk for this cancer.<sup>33, 34</sup>

**Melatonin** – *Melatonin, a hormone produced by the brain's pineal gland, has demonstrated a remarkable ability to mitigate the side effects of chemotherapy and increase survival when employed as an adjuvant the standard care of a wide range of cancers. Most of the randomized clinical studies demonstrating these benefits were done by a clinical group in Milan over the last two decades; the patients receiving melatonin were asked to take a 20 mg dose every night before bedtime. This regimen is inexpensive and, aside from morning drowsiness in some patients, is devoid of side effects. When patients took metformin in conjunction with chemotherapy, they were less likely to experience fatigue, nausea, nerve damage, or dangerous depressions of blood levels of white cells or platelets (clotting cells). Long-term use of metformin was also associated with lesser risk for the muscle wasting syndrome known as cachexia. Whether used in conjunction with chemotherapy or not, patients receiving melatonin tended to achieve longer survivals than those not receiving it. The benefits of melatonin are seen in a wide range of cancers, and likely reflect melatonin's ability to support the activity of immune cells that play a key role in cancer control. In some cancers, melatonin may also inhibit the new blood vessel growth required to sustain the growth of tumors. Melatonin's favorable impact on the side effects of chemotherapy reflects, in part, its ability to boost the production of bioactive factors in the bone marrow that help marrow cells withstand chemotherapeutic assault. Although few of these studies have specifically targeted patients with EOC, there is good reason to suspect that melatonin will be as helpful in therapy of ovarian cancer as it is in a wide range of other cancers. Melatonin is sold as a nutraceutical, and does not require a prescription.*

Melatonin, a hormone produced nocturnally by the pineal gland, plays a role in promoting the circadian rhythms of the body, and also exerts a range of antioxidant, immunomodulatory, and marrow-protective

effects via cell surface and nuclear receptors.<sup>35-37</sup> Over the last 2 decades, oncologists in Milan have conducted a number of clinical studies, often randomized, of the impact of bedtime melatonin administration (typically 20 mg) on cancer control.<sup>38-42</sup> Melatonin has been tested in the context of chemotherapy regimens, but it has also been studied in patients with advanced cancer for whom chemo was no longer deemed appropriate, either used alone or in conjunction with other immunoadjuvants. Recent meta-analyses of randomized trials with melatonin in clinical cancer therapy, focusing primarily on these Milanese studies, have concluded that, as an adjuvant to chemotherapy, melatonin use is associated with a higher incidence of responses to chemo, as well as increased survival. Moreover, common side effects of chemotherapy, such as leucopenia, thrombocytopenia, hypotension, fatigue, neurotoxicity, and nausea and vomiting were less frequent in the patients treated with melatonin.<sup>43, 44</sup> A notable survival advantage was also seen in patients with advanced cancer receiving melatonin as an adjuvant to usual supportive care. Moreover, patients on long-term melatonin therapy appear to be at lower risk for cancer cachexia.<sup>45, 46</sup> Nocturnal melatonin administration is generally well tolerated, albeit morning drowsiness is reported by some subjects and might in some instances warrant a reduction in dose.

Despite scattered reports that melatonin can slow the proliferation of certain cancer cell lines – including some ovarian cancer cells lines<sup>47, 48</sup> - melatonin does not appear to have a uniform effect in this regard, and there is little evidence that it can act directly on cancer cells to potentiate their response to cytotoxic agents. It therefore seems likely that its remarkably consistent positive impact on survival in randomized trials is primarily reflective of melatonin's immunosupportive activity. Melatonin can act directly on NK cells and Th1 lymphocytes to boost their effector capacities, which play a key role in immune rejection of cancer.<sup>49</sup> In one large randomized trial enrolling patients with advanced cancer, melatonin alone achieved a notable survival advantage, but survival was even greater in patients who in addition to melatonin received repeated subcutaneous injections of the immunostimulant cytokine IL-2.<sup>50</sup> There is also recent suggestive evidence that melatonin might exert an anti-angiogenic effect on hypoxic tumor regions by suppressing activation of hypoxia-inducible factor-1; the antioxidant impact of melatonin on some cancer cells may impede hypoxic activation of HIF-1.<sup>51, 52</sup> Indeed, a reduction of serum VEGF levels has been reported in non-progressing cancer patients treated with melatonin.<sup>39</sup> The antioxidant effect of melatonin on healthy tissues<sup>35</sup> might also rationalize its favorable impact on certain side effects of chemotherapy. Melatonin's impact on cachexia might reflect its antioxidant effect, and possibly a modulatory effect on production of pro-cachectic cytokines.<sup>45</sup>

In the bone marrow, melatonin provokes the release of IL-4 and certain opioids from Th2 lymphocytes; these agonists, in turn, induce increased production of granulocyte/macrophage colony stimulating factor (GM-CSF) by marrow stromal cells.<sup>37, 53, 54</sup> GM-CSF protects marrow cells from the apoptosis induced by cytotoxic drugs, and could also be expected to aid repopulation of the marrow after chemotherapy. This mechanism likely accounts for the favorable effect of melatonin on risk for leucopenia following chemotherapy. The basis for melatonin's remarkable ability to prevent thrombocytopenia in chemotherapy patients is unclear, but it appears to be reflect a trophic effect on bone marrow megakaryocytes, as nocturnal melatonin has been found useful in treating thrombocytopenia stemming from other causes.<sup>55, 56</sup>

Although the Milanese studies have not been analyzed to determine the specific impact of melatonin on clinical EOC, it stands to reason that, inasmuch as the main benefits of melatonin are not mediated by

direct effects on cancer cells, but rather by immunosupportive and tissue-protective effects, and are observed in a wide range of clinical cancers, its impact on EOC would likely be analogous to its favorable impact on other cancers. In a small pilot study enrolling 12 patients with advanced chemoresistant ovarian cancer, melatonin administration plus repeated s.c. doses of IL-2 led to 2 partial responses and disease stabilization in 5 patients.<sup>57</sup> Because of its low cost, convenience, good tolerability, and broad range of favorable effects – boosting immune capacities, mitigating the toxicities of chemotherapy, reducing risk for cachexia, and above all enhancing survival - bedtime melatonin may represent an exceptionally worthwhile adjuvant for management of EOC.

**Low-dose daily Aspirin** – *A number of controlled clinical trials have evaluated the impact of daily low-dose aspirin (81-325 mg daily) on risk for heart attack or stroke. Recent analysis of these studies reveals that, in participants who developed cancers known as adenocarcinomas – including EOC – during the studies, those who were taking low-dose aspirin had improved survival and were at lower risk for metastases. Likely, this reflects aspirin’s ability to stabilize blood cells known as platelets that often play a role in the establishment of metastases. However, this stabilizing impact on platelets can also increase bleeding risk in susceptible people, so consult your doctor before starting a low-dose aspirin regimen.*

A recent meta-analysis concludes that, in controlled studies evaluating the utility of aspirin for prevention of vascular accidents, patients who developed adenocarcinomas (including EOC) during these studies had notably improved survival if they were taking low-dose aspirin (81-325 mg daily); they were also less likely to develop metastases. This effect seems likely to reflect an anti-metastatic benefit of platelet stabilization.<sup>58</sup>

**Dietary measures** – *Several studies have examined the impact of habitual diet on survival in patients with EOC. By and large, these studies conclude that diets high in foods rich in saturated fats – notably red meats and full-fat dairy products – are associated with decreased survival, whereas patients whose diets are rich in yellow and cruciferous vegetables tend to survive longer. “Mediterranean” or plant-based diets high in these vegetables may therefore be a smart bet for patients with EOC.*

Diets low in red meats and dairy products, and high in yellow and cruciferous vegetables, have been associated with greater survival in EOC patients.<sup>59</sup> Diets high in saturated fat likewise have been associated with increased risk for this cancer, and EOC risk was significantly reduced in women randomized to a low-fat diet (20% of calories) in the Womens’ Health Initiative Dietary Modification Randomized Controlled Trial.<sup>60-63</sup>

**Vitamin D** – *The activated form of vitamin D can slow the proliferation of some EOCs. Little information is currently available regarding the impact of vitamin D status on survival in EOC. However, one study found that survival in EOC tended to be superior at lower latitudes where the skin’s year-round exposure to UV light tends to be higher; UV catalyzes the production of vitamin D in skin, so vitamin D status is usually better in people at low latitudes. Since there is no reason to suspect that good vitamin D status would have any adverse effect on patients with EOC, it might be prudent for such patients to insure good vitamin D status by taking 4,000-8,000 IU of supplemental vitamin D daily.*

Activated vitamin D (calcitriol) has the potential to slow the growth of some EOCs.<sup>64,65</sup> Attempts to correlate estimates of vitamin D status with EOC risk have yielded inconsistent results, but a recent meta-analysis observes a non-significant trend associating good status with lower risk.<sup>66</sup> The impact of vitamin

D on clinical course in pre-existing EOC has not been reported, although prognosis may be better at lower latitudes with good ultraviolet exposure.<sup>67</sup>

**Spirulina** – *There is recent evidence that a modest level of oxidative stress, produced by an enzyme complex known as “NADPH oxidase”, boosts the proliferation and aggressiveness of many OECs. The remarkable antioxidant activity of the food algae spirulina has recently been traced to the ability of a key component of this food to potently inhibit NADPH oxidase activity. Spirulina also has cell membrane components with immunostimulant activity. It is therefore reasonable to suspect that an ample daily intake of spirulina – perhaps 1-2 tablespoons daily- may aid the control of EOC. However, since induction of oxidative stress plays a key role in the killing mechanism of chemo drugs such as the platins, it may be unwise to ingest spirulina while undergoing chemotherapy.*

There is recent evidence that NADPH oxidase is constitutively active in many EOCs, and that the resulting mild oxidative stress promotes proliferation and survival in these cells; this may reflect the ability of hydrogen peroxide to reversibly inhibit tyrosine phosphatases which antagonize tyrosine kinase growth factor activities.<sup>68, 69</sup> Phycocyanobilin, which constitutes about 0.6% of the dry weight of spirulina, can act as a potent inhibitor of some NADPH oxidase complexes; this may rationalize the remarkable antioxidant and anti-inflammatory effects of dietary spirulina observed in many rodent studies.<sup>70</sup> The immunostimulant impact of spirulina’s cell wall polysaccharides also has the potential to aid cancer control.<sup>71</sup> Extrapolations from rodent studies suggest that human may need to take 1-2 tablespoons (15-30 g) of spirulina daily to achieve the potent antioxidant benefits observed in such studies.<sup>70</sup> It may not be wise to use spirulina concurrently with certain chemotherapy drugs, such as the paclitaxel used in treatment of EOC, whose killing mechanisms are dependent on induction of oxidative stress.<sup>72</sup> However, using it at other times may have the potential to slow cancer spread.

**Soy foods/Phytoestrogens** – *In East Asia, where consumption of soy products is prominent, risk for EOC has been reported to be about 40% lower in people eating high levels of soy, as opposed to those eating low levels. This apparent protection might reflect the fact that soy phytoestrogens (isoflavones) are selectively capable of activating the beta form of the estrogen receptor at blood concentrations that are achievable with soy-rich diets. The beta estrogen receptor has been shown to exert anti-proliferative effects on ovarian cancers. Although the impact of soy-rich diets on survival in EOC patients has not yet been studied, one study has reported that physiologically relevant levels of soy isoflavones can slow the growth an EOC cell line. So it is conceivable that some patients with EOC could be benefited by a diet high in soy products, and/or a soy isoflavone supplement.*

In Asian epidemiology, high habitual intakes of soy and soy phytoestrogens are associated with a marked reduction in risk for EOC.<sup>73-75</sup> There are no published studies pertaining to the effects of soy on pre-existing EOC, aside from one suggestive case report.<sup>76</sup> Physiologically achievable concentrations of soy isoflavones ( $10^{-10}$  –  $10^{-8}$  M) are reported to inhibit proliferation in an EOC cell line, possibly via selective activation of estrogen receptor- $\beta$ .<sup>77, 78</sup> ER $\beta$  can indeed exert antiproliferative effects in EOC, although it has not yet been clarified whether ER $\beta$  ligands amplify this effect.<sup>79-82</sup> ER $\beta$  expression tends to be lost as EOC evolves to a more aggressive form, and this loss correlates with poorer prognosis.<sup>83-85</sup>

**Diindolylmethane** – *The compound diindolylmethane (DIM) forms spontaneously in the stomach whenever people eat cruciferous vegetables such as broccoli or Brussels sprouts. In cancer cells lines and in rodent studies, DIM has exerted a wide range of effects that potentially can aid cancer control. In*

*particular, in EOC cell lines, modest concentrations of DIM can block the activation of Stat3, a factor which tends to be chronically activated in EOCs, and that boosts cells growth while promoting chemoresistance. Indeed, administration of DIM to mice bearing a human EOC has been reported to slow the growth of the cancer and to boost its responsiveness to the chemo drug cisplatin. Pilot clinical studies in prostate cancer indicate that a dose of 300 mg DIM twice daily is well tolerated, and there were hints that this may be a clinically useful dose. Many preparations of DIM are poorly absorbed; the BioResponse brand is being employed in clinical studies, and appears to have adequate absorption. In EOC, DIM may have the potential both to amplify the cancer killing achieved by chemotherapy, and to slow cancer growth and spread.*

Diindolylmethane is formed spontaneously in the stomach via a condensation reaction when cruciferous vegetables are ingested. This compound is now available as a nutraceutical, and has the potential to suppress activation of JAK2 and Stat3 in EOC; administered orally to mice, it can slow the growth of human EOC xenografts and boost their responsiveness to cisplatin.<sup>86, 87</sup> Stat3 is constitutively active and promotes chemoresistance and aggressiveness in a high proportion of EOCs.<sup>88-93</sup> Diindolylmethane, in a Phase I clinical trial, has been well tolerated in a dose of 300 mg twice daily,<sup>94</sup> a dose of at least this magnitude may be needed to suppress Stat3 activation.

**Low-Dose Naltrexone** - *Naltrexone, a drug which blocks the activity of hormones known as opioids, is FDA-approved for treatment of alcohol dependence. Surprisingly, when a very low dose of naltrexone (3-4.5 mg – the regular therapeutic dose is 50 mg) is administered just before bedtime, the body compensates by markedly boosting its nightly production of opioids. By the morning and throughout the next day, there is a net **increase** in opioid activity because the small dose of naltrexone administered the previous night has been rapidly metabolized. Numerous studies at Penn State University have revealed that a particular opioid – known as met-enkephalin – acts on almost all cancer cells to slow their growth. Hence, low daily doses of naltrexone have been shown to slow the growth of cancers in mice. Moreover, in over twenty years of clinical experience, Dr. Bernard Bihari judged that low-dose naltrexone aided cancer control in a high proportion of his cancer patients. Since opioids also have anti-inflammatory and analgesic effects, low-dose naltrexone is now being employed to treat a range of additional disorders. Low-dose naltrexone is virtually free of side effects, quite inexpensive, and in two decades of use has proved to be quite safe – which isn't surprising, as the dose involved is less than a tenth as high as that approved for long-term treatment of alcoholism; it can legally be prescribed by physicians for off-label use in cancer. Since it does not appear to boost the cell kill achieved by concurrent chemotherapy, it may be prudent to avoid its use during chemotherapy so as to eliminate any risk that it might somehow interfere with chemotherapy's efficacy. It cannot be used by patients who require opiate medications for pain control. Its neglect by orthodox medicine reflects the fact that no pharmaceutical company will fund the elaborate clinical studies required to conclusively prove the efficacy of this off-patent drug in cancer therapy.*

During 30 years of research, Zagon and colleagues have demonstrated that a high proportion of human cancers, including ovarian cancer cell lines, secrete met-enkephalin – a.k.a. opioid growth factor (OPG) – and also express an OPG receptor that responds to autogenous or systemic OPG by slowing cellular proliferation; this anti-proliferative effect appears to be mediated by p16 and/or p21, and is not associated with increased apoptosis.<sup>95-100</sup> Moreover, repeated short-term exposure (e.g. 6 hours every 24 hours) of ovarian cancer cell lines to opioid antagonists such as naltrexone or naloxone leads to a marked post-

transcriptional up-regulation of the expression of both OPG and the OPG receptor, with the net result that cell proliferation is slowed.<sup>97, 98</sup> Importantly, when nude mice bearing the SKOV-3 human ovarian cancer received daily i.p. injections of low-dose naltrexone (0.1 mg/kg), intended to be active for only several hours, growth of the tumor was suppressed by about 40%.<sup>98</sup> In mice receiving this regimen in conjunction with weekly injections of either taxol or cisplatin, the growth-inhibitory impact of cisplatin, but not taxol, was additive to that of the naltrexone. In vitro, intermittent exposure to naltrexone did not enhance the apoptotic response to either of these cytotoxins. Zagon also demonstrated that OPG receptor plays a key role in modulating SKOV-3 growth; SKOV-3 cells in which OPG expression had been permanently knocked down by transfection with shRNA proliferated more slowly in mice.<sup>101</sup> The response of ovarian cancer in nude mice to low-dose daily naltrexone is quite comparable to the responses which Zagon reported some years previously for a neuroblastoma and a colon cancer; more recently, they have reported similar findings with a head and neck squamous cell carcinoma.<sup>102-105</sup>

Zagon's work dovetails nicely with the clinical experience of Dr. Bernard Bihari.<sup>106</sup> In the mid-1980s, he and his colleagues discovered that nightly administration of 1.5-4.5 mg naltrexone led to a marked upregulation of systemic endorphin production; on theoretical grounds, he suspected that this could benefit the immune status of AIDS patients, whom he commenced to treat with this regimen. When a friend with lymphoma suggested that this treatment be tried on her, Bihari, mindful of Zagon's recent report that low-dose naltrexone could slow neuroblastoma growth in mice, agreed to cooperate, and the patient subsequently achieved a complete remission. Bihari then implemented this therapy in a number of additional cancer patients. As of 2004, Bihari had treated 450 cancer patients with low-dose naltrexone, and claimed that in about 20% of these cases – including 4 cases of ovarian cancer - marked remission had been achieved that could not credibly be attributed to other concurrent treatments. He also judged that an even higher percentage of patients were achieving worthwhile suppression of tumor growth with this regimen. Sadly, Bihari was never able to formally publish his observations in the medical literature. However, Berkson has published several provocative case histories of patients with pancreatic adenocarcinoma who achieved marked tumor regression while receiving low-dose naltrexone and intravenous lipoic acid.<sup>107, 108</sup> He has also reported a remission in a case of B cell lymphoma treated with low-dose naloxone alone.<sup>109</sup> Moreover, encouraging responses to low-dose naltrexone – with no significant toxicity – have been reported in pilot clinical studies in Crohn's disease and multiple sclerosis.<sup>110-113</sup> These clinical observations, in conjunction with Zagon's research in cell cultures and mice, suggest that low-dose naltrexone may have some utility for slowing or reversing growth of clinical ovarian cancer. There is no reason to suspect that this would have a chemopotentiating effect. Nonetheless, the anti-proliferative impact of low-dose naltrexone might have the potential to induce cancer regression in cancers with a sufficiently high spontaneous rate of cell death from apoptosis or necrosis. Since the approved prescription form of naltrexone is 50 mg, the small doses of naltrexone required for cancer therapy must be prepared by a compounding pharmacist.

**Macrophage Activating Factor (GcMAF)** – *This is a factor produced naturally in inflamed tissues that markedly activates a type of immune cell, the macrophage, that when optimally stimulated can work to promote cancer rejection. A high proportion of cancers secrete an enzyme, nagalase, which can prevent generation of GcMAF within the immediate environment of the tumor, thereby helping the tumor to escape immune rejection. For this reason, it has been proposed that regular injections of GcMAF may do an “end-run” around this evasive mechanism, so that the full anti-cancer efficacy of macrophages can be restored. Three provocative clinical studies have evaluated this agent in patients with cancers of the*

*breast, colon, or prostate who were undergoing surgery at a time when overt metastases were not present. GcMAF therapy - 100 ng injected subcutaneously once per week – was initiated immediately following surgery. The authors of these studies interpreted their data as indicating that, in patients in whom small residual nests of tumor cells remained after surgery – presenting a risk for tumor recurrence – the administration of GcMAF enabled the immune system to kill off these remaining cells, effectively making the surgery curative. Many cancer experts have not taken these reports very seriously, as a non-traditional tumor marker was used to assess cancer status (nagalase), and no placebo control was employed. Nonetheless, injectible GcMAF is now available via the internet from several suppliers, and some cancer patients are choosing to use it, as it appears to be safe and is not as expensive as some new cancer drugs (about \$500 per month). This does not require a prescription, as it is not a drug. The subcutaneous injections of GcMAF are analogous to insulin injections, and can be done by a doctor or by the patient. The use of GcMAF as an immunostimulant in more advanced cancer is also feasible, although no published studies have evaluated the results of such therapy. (Note – neither you nor your physician should confuse GcMAF with GM-CSF – granulocyte-macrophage colony stimulating factor – which is sometimes used to stimulate the bone marrow after chemotherapy.)*

Over the last two decades, Yamamoto and colleagues have characterized a natural bioactive factor, known as macrophage activating factor, or GcMAF, that is produced in inflamed tissues and markedly boosts the activity of macrophages and (presumably) dendritic cells.<sup>114-117</sup> He has further shown that a very high proportion of cancers secrete an enzyme, N-acetylgalactosaminidase (called “nagalase” for short) that can prevent the generation of GcMAF within the microenvironment of the tumor, and hence can suppress the contribution of macrophages and dendritic cells to tumor rejection.<sup>116, 118</sup> These findings motivated him to propose injections of pre-formed GcMAF as an immunostimulant strategy in cancer therapy. Several published studies employing immunocompetent tumor-bearing mice demonstrate that this strategy does have the potential to slow tumor growth.<sup>119, 120</sup> Yamamoto has also proposed that serum nagalase can be used as a tumor marker, as he finds that its level is considerably elevated in patients with cancer, as opposed to healthy controls, and that relatively few other medical conditions (certain viral infections, including HIV, and SLE) are associated with elevated nagalase.<sup>118, 121-123</sup> Several independent groups have confirmed that many cancers secrete nagalase.<sup>124-127</sup>

More recently, Yamamoto has published three pilot clinical studies which have excited considerable interest.<sup>128-130</sup> These studies focused on patients with early stage cancers of the breast, colon, or prostate who had just received potentially curative surgery, but who were at risk for cancer recurrence. In each of these studies, post-surgical patients were identified who had no radiologically-demonstrable metastases, but whose nagalase levels remained elevated for a number of days after surgery, and hence were judged to harbor residual cancer cells. (Anemic patients were excluded from this group, as erythropoietin therapy was not permitted during this trial.) These patients then were treated with a subcutaneous injection of 100 ng GcMAF, once weekly, for a number of months. Yamamoto charted the serum nagalase levels of these patients during this therapy. In every patient, the level of nagalase gradually declined, until, several months later, it fell to healthy baseline levels. Yamamoto notes that, several years later, all of these patients are clinically cancer-free. These findings suggest that, in patients who have small nests of viable cancer cells remaining surgery, prompt therapy with GcMAF has the potential to enable the immune system to eliminate these cells, in effect rendering surgery curative.

No pharmaceutical company has shown interest in developing GcMAF as a drug, as it is non-patentable. Yamamoto is currently working to develop a synthetic analog of GcMAF that may be a more viable alternative for pharmaceutical development. In the interim, several chemical companies have manufactured it, and are selling it via the internet in injectible form, without official drug approval. Allegedly, it is very safe when administered as 100 ng s.c. injections once weekly; the only side effects reported are occasional transient flu-like episodes, consistent with increased macrophage secretion of cytokines. The first commercial source of GcMAF (others are arising) can be accessed through this website: <http://www.gcmf.eu/info/>. It currently costs about \$500 per month to administer one 100 ng GcMAF injection per week. Although GcMAF is not an approved drug, we are informed that least a hundred medical clinics throughout the world are using it therapeutically, primarily for cancer.

GcMAF also appears to have anti-angiogenic potential if administered on a daily basis. This was originally reported by Yamamoto in an *in vitro* study, and subsequently confirmed in by two independent groups in nude mice bearing human cancers.<sup>131-135</sup> (The “father of anti-angiogenesis”, Dr. Judah Folkman, was co-author of one of these studies.)<sup>132</sup> These findings in mice are provocative, as tumor growth is virtually halted with daily injections of GcMAF. So far, there have been no reports of clinical efforts to replicate these findings in cancer patients, and, at its current cost, daily administration of GcMAF would be a pricey proposition.

GcMAF is accorded a priority 1 in Table 1 because of its putative potential for increasing the chance that initial surgery for stage II/III OEC will be curative, if GcMAF is implemented soon after (or presumably soon before) surgery. Its use in patients with advanced cancer would be more speculative.

**Intravenous Ascorbate** – *Although vitamin C (ascorbate) is usually thought of as an antioxidant, in high concentrations achievable only by high-dose intravenous infusions, it can act as a pro-oxidant, triggering the increased production of hydrogen peroxide in tissues. This proves harmless to normal tissues, as their cells contain adequate levels of antioxidant enzymes. However, many cancers make increased amounts of superoxide, a pro-oxidant compound that renders them selectively susceptible to damage by hydrogen peroxide. Hence, high intravenous doses of ascorbate have the potential to generate severe levels of oxidative stress in many tumors, resulting in the death of some of the cancer cells. Daily injections of ascorbate have been shown to slow cancer growth in mice bearing a human EOC. Moreover, the oxidative stress imposed by intravenous ascorbate in susceptible tumors can potentiate the killing activity of some chemotherapy drugs – hence, intravenous ascorbate merits study as an adjuvant to chemotherapy. Indeed, a phase I study evaluating high-dose intravenous ascorbate, used in conjunction with standard firstline chemotherapy for EOC, and as a monotherapy following the conclusion of this chemotherapy, has been conducted at the University of Kansas, and its results should soon be reported. Many physicians practicing “integrative” medicine now offer intravenous vitamin C protocols for cancer patients.*

Although ascorbate (vitamin C) functions physiologically within cells as an antioxidant, in millimolar concentrations it can generate superoxide by transferring an electron to molecular oxygen. When ascorbate is administered in high doses intravenously, transition metals in the extracellular space catalyze this transfer, leading to the production of superoxide which in turn is rapidly dismutated to form hydrogen peroxide.<sup>136</sup> This hydrogen peroxide is not toxic to normal cells, as they express adequate levels of the antioxidant enzyme catalase, but many cancer cells either have low catalase activity, or make increase

amounts of superoxide via NADPH oxidase - likely because a modest chronic level of oxidative stress boosts their proliferation and survival.<sup>137</sup> Within cancer cells, superoxide can interact with hydrogen peroxide to generate the deadly hydroxyl radical, in a reaction catalyzed by free transition metals.<sup>138, 139</sup> Hence, many cancers are relatively defenseless when exposed to millimolar concentrations of ascorbate.<sup>140</sup> One recent study found that, in two out of three human ovarian cancer cell lines, low millimolar concentrations of ascorbate – a concentration readily achievable by i.v administration of high doses in vivo<sup>141</sup> – killed 50% or more of the cells,<sup>142</sup> this may reflect the high NADPH oxidase activity of many ovarian cancers.<sup>68</sup> It is therefore thought that intravenous ascorbate therapy may have the potential to aid control of some ovarian cancers, particularly those with limited antioxidant defenses. Indeed, repeated intraperitoneal injections of ascorbate (4g/kg) were shown to retard the growth of a human ovarian cancer in nude mice.<sup>142</sup> There is also preliminary evidence that concurrent exposure to millimolar ascorbate can increase the killing efficacy of certain cytotoxic chemotherapy drugs; it will be interesting to explore the utility of i.v. ascorbate as an adjuvant to chemotherapy.<sup>139, 143</sup> The mechanism by which ascorbate kills cancer cells involves autophagy, and does not depend on apoptosis – hence, ascorbate might prove valuable in the treatment of some chemoresistant cancers that have a diminished capacity for apoptosis.<sup>144</sup> As demonstrated by Belgian researchers, concurrent infusion of menadione (vitamin K3) has the potential to amplify the efficacy of ascorbate therapy by expediting the transfer of electrons from ascorbate to molecular oxygen.<sup>145, 146</sup> A controlled clinical trial examining the utility of repeated infusions of high-dose ascorbate as an adjuvant to standard chemotherapy for ovarian cancer has been in progress at the University of Kansas; the results of this trial may be available soon. Although the first formal phase I study of ascorbate monotherapy in various advanced malignancies failed to observe objective responses, it should be borne in mind that, in mouse studies, ascorbate has slowed cancer growth rather than eradicated tumors.<sup>147</sup> Many practitioners of “alternative” medicine, in the U.S. and elsewhere, currently offer intravenous ascorbate therapy to cancer patients, and a protocol incorporating intravenous menadione is in use at Oasis of Hope Hospital in Tijuana.

**Tocotrienols** – *Tocotrienols are variant forms of vitamin E, richly supplied by palm or rice oils. In studies with various cancer cell lines – which unfortunately did not examine EOCs – the gamma-form of tocotrienol was shown to suppress activation of the Stat3 factor. As noted above in our discussion of DIM, activated Stat3 is found in a high proportion of aggressive EOCs, and tends to boost the cancer’s proliferation while making it harder to kill with chemo. Commercial tocotrienol supplements feature primarily the delta form of this nutrient, but it is reasonable to suspect that this shares the protective properties of the gamma form. If so, high intakes of supplemental tocotrienols may be useful as adjuvants to chemotherapy in EOC, and may also slow cancer spread. The dose required to achieve this benefit, however, is not clear; as much as 1 g of tocotrienols daily might be needed to replicate the cancer-retardant benefits reported in some mouse studies, and such high supplemental intakes of tocotrienols have not yet been assessed in published clinical studies.*

Two recent studies indicate that  $\gamma$ -tocotrienol can decrease Stat3 activation in a range of cancer cell lines – albeit ovarian cancers were not tested.<sup>148, 149</sup> This effect resulted from up-regulation of the tyrosine phosphatase SHP-1, which can reverse the activating tyrosine phosphorylation of JAK2 and Stat3. If this property is shared by the more common  $\delta$ -tocotrienol, as seems likely, then commercially available mixed tocotrienol preparations might be clinically useful for suppressing Stat3 activation in cancers. Extrapolating from the mouse studies in which oral tocotrienols inhibited cancer growth, an intake of 1

gram or more daily might be required for useful efficacy; such high doses have not yet been examined in clinical trials.

**Salsalate** – *Salsalate, a safe drug which has been used for decades to treat rheumatoid arthritis, breaks down spontaneously in the GI tract to release salicylic acid, a natural compound with anti-inflammatory activity. A sufficient concentration of salicylic acid can inhibit the activation of a cellular factor known as NF-kappaB. Like Stat3, NF-kappaB is chronically active in many EOCs, and renders cancer cells both more aggressive and harder to kill with chemotherapy. Hence, salsalate, administered at a dose of 1.5-2.25 g twice daily, may have the potential to make chemotherapy more effective and slow cancer spread in many patients with EOC. Although salsalate is far safer than NSAID drugs such as ibuprofen (it doesn't increase risk for GI bleeding), high doses can cause ear problems – ringing in the ears, or mild hearing loss – in susceptible people; fortunately, these effects are readily and rapidly reversible if the drug is discontinued or its dose reduced. Most people can tolerate 1.5 g twice daily without problems.*

This affordable drug, which breaks down in the GI tract to yield the venerable anti-inflammatory agent salicylic acid, has the potential to offset chemoresistance by suppressing activation of NF-kappaB; constitutive activation of this factor mediates chemoresistance and aggressive behavior in many advanced chemoresistant EOCs.<sup>150-156</sup> Salicylate achieves this by binding to and blocking the activation of IkappaB kinase- $\beta$ , a key upstream mediator of NF-kappaB activation. Hence, salsalate may often be useful as an adjuvant to chemotherapy in EOC, and may also be used following chemotherapy to slow cancer spread. However, there is one report that in early EOC NF-kappaB may actually have an anti-proliferative, tumor suppressor effect, so it isn't clear whether salsalate should be used in the initial treatment of EOC.<sup>155</sup> Unlike NSAIDs, salsalate is a safe drug that does not increase risk for GI bleeds; it can however cause fully reversible ototoxicity (tinnitus, mild hearing loss ) if too high a dose is used. Most people tolerate 1.5g twice daily without problems; a dose as high as 2.25 g twice daily can be employed in patients who aren't prone to salicylate-induced ototoxicity.

**Imatinib** – *It is now increasingly accepted that malignant tumors harbor a small fraction of cells known as cancer stem cells that tend to be highly resistant to chemotherapy and radiotherapy, and have a greater potential for giving rise to cancer recurrences or new metastases than other cells in the tumor. The survival and proliferation of these cells is thought to be largely responsible for the recurrence of EOC that usually occurs after initial chemotherapy has succeeded in killing off the bulk of the cancer cells. Recent studies on EOC stem cells indicates that they are prone to express the c-Kit growth factor receptor as well as the natural molecule that activates it; moreover, inhibition of c-Kit activity tends to dramatically boost the sensitivity to EOC stem cells to the cytotoxicity of the first-line chemo drugs cisplatin and paclitaxel. The drug imatinib (a.k.a. Gleevec), originally approved to treat a type of leukemia, can effectively inhibit c-Kit in clinically feasible concentrations, and has been reported render EOC stem cells much more sensitive to cisplatin and paclitaxel. Hence, although the bulk of EOC cancer cells do not express c-Kit and are not influenced by imatinib, the inclusion of imatinib as an adjuvant to chemotherapy might increase the chance that the highly dangerous stem cell population will be markedly reduced or eliminated. Unfortunately, imatinib is currently a very expensive drug – about \$150 a tablet - and its off-label use would be costly; however, some patients might be able to afford to take it for a few days prior to and during their chemotherapy sessions – and some may be able to obtain less expensive generic imatinib from India.*

There is a growing consensus that a small population of cells within a malignant tumor, known as cancer stem cells, tend to be unusually resistant to chemo- and radiotherapy, and also have a greater capacity than other cells to give rise to recurrences or new metastases.<sup>157</sup> In cancers such as EOC that tend to respond well to initial chemotherapy, surviving stem cells are thought to be largely responsible for the relapses which usually occur. Efforts to define the characteristic of stem cells in EOC are therefore underway. Several studies indicate that these stem cells tend to express the tyrosine kinase receptor c-Kit, as well as its natural ligand, stem cell factor; thus, autocrine activation of c-Kit promotes proliferation and survival in these cells.<sup>158-162</sup> Moreover, a recent study provides evidence that c-Kit activation is largely responsible for the poor sensitivity of EOC stem cells to the first-line drugs used in EOC chemotherapy, carboplatin and paclitaxel.<sup>161</sup> This effect results, at least in good part, from a signaling pathway in which the level of beta-catenin is enhanced via Akt-mediated inhibition of glycogen synthase kinase-3; this beta-catenin then interacts with the transcription factor TCF to induce expression of ABCG2, one of the ATP-binding cassette transporters that efficiently extrudes a range of chemotherapeutic agents from cells, and hence promote multi-drug resistance.<sup>161</sup> Independent evidence suggests that ABCG2 activity is indeed a key determinant of progression-free survival in EOC patients receiving chemotherapy.<sup>163</sup> Fortunately, clinically feasible levels of the drug imatinib (Gleevec) can effectively inhibit c-Kit, and imatinib not only inhibits the proliferation (and sphere-forming capacity) of EOC stem cells, but also substantially restores their sensitivity to carboplatin and paclitaxel.<sup>161</sup> Hence, although imatinib has little impact on the growth or chemosensitivity of the bulk of EOC cells in a tumor – imatinib has not proved useful as a monotherapy in EOC<sup>164, 165</sup> - its inclusion in a chemotherapy protocol may considerably enhance the capacity of the chemotherapy to eliminate dangerous stem cells. This effect is analogous to that of metformin, and it will be interesting to learn how metformin and imatinib might interact in EOC stem cells.<sup>16</sup> Imatinib is currently a very expensive “designer drug” originally approved for use in chronic myelogenous leukemia; while its continuous off-label use would be prohibitively expensive for most patients, using it for several days prior to and during chemotherapy sessions might be within the means of some. The dose shown to be effective for inhibiting c-Kit-dependent GIST tumors is 400 mg once or twice daily.<sup>166</sup> Combining imatinib with taxane drugs in EOC appears to be reasonably safe, although it can't be expected to achieve objective responses if the bulk of the cancer cells are resistant to these agents.<sup>167, 168</sup>

**Valproate/Hydralazine** - *Heritable changes in the structure of DNA and proteins associated with it are usually responsible for the fact that cancers once responsive to a given chemotherapy drug often lose their sensitivity to it over the course of time. Fortunately, a high proportion of these changes – known as “epigenetic” because they don't involve permanent mutations of DNA structure – are potentially reversible. Oncologists in Mexico City have demonstrated that a combo of two old drugs – valproate, long used to treat epilepsy, and hydralazine, a high blood pressure medication – can work as a “tag team” to reverse such changes. In a remarkable clinical trial, they recruited a number of patients with cancer – including 7 with ovarian cancer – who were no longer responding to the chemo drugs that had once been effective for them. After giving them a week's treatment with valproate/hydralazine, they gave these patients another course of the drug to which they had lost sensitivity. Remarkably, a majority of the patients – including all of those with ovarian cancer – once again showed response to that drug (tumor regression or a failure of the cancer to grow for several months). The work of these researchers has been so impressive that this drug combination has not achieved approval as a cancer drug in Mexico (under the name Transcrip). Fortunately, valproate and hydralazine are available as single drugs in the U.S.*

*and elsewhere, and they are not exorbitantly expensive. The chief side effect associated with this strategy is that valproate tends to make people a bit drowsy; however, this should not be an insuperable problem if these drugs are only used for limited periods prior to chemotherapy. Mouse studies also show that long-term administration of valproate has the potential to slow the growth of some EOCs – so this might be an option in patients who tolerate this drug well.*

There is evidence that acquired resistance to chemotherapeutic agents most commonly results, not from mutations or deletions in DNA, but from potentially reversible epigenetic changes in the genome.<sup>169</sup> By preventing or reversing epigenetic modifications which inhibit gene expression, hydralazine – which can function as a DNA methylase inhibitor – and valproate – which can inhibit class I histone deacetylases – have the potential to restore responsiveness of the cancer to chemotherapy drugs to which the cancer has evolved resistance.<sup>170, 171</sup> Duenas-Gonzalez and colleagues evaluated this drug combination in 15 cancer patients – including 7 with ovarian cancer – whose cancers were progressing on chemotherapies to which they formerly had been responsive. Hydralazine and valproate were administered to these patients for a week prior to resuming chemotherapy with the same agents they had received previously. The majority of these treated patients – including all of the ovarian cancer patients – showed renewed responsiveness, as their tumors either regressed or failed to progress for several months.<sup>172</sup> Aside from some drowsiness induced by valproate, the valproate/hydralazine treatment was well tolerated. In vitro, valproate has been reported to enhance or restore the sensitivity of a range of ovarian cancer cell lines to cisplatin.<sup>173</sup> Class I histone deacetylases are overexpressed in ovarian cancers relative to normal ovarian tissue; reducing their expression suppresses their proliferation in vitro.<sup>174</sup> Valproate slows proliferation and promotes apoptosis in a range of ovarian cancer cell lines; safe doses can impede the growth of ovarian cancer in nude mice.<sup>174</sup> Other available agents which can inhibit class I histone deacetylases in clinically feasible concentrations include vorinostat, phenylbutyrate, and tributyrin.<sup>175, 176</sup> The former two are vastly more expensive than valproate, and hence aren't feasible for use in off-label therapy; the food additive tributyrin – effectively a “time-release” source of the rapidly metabolized histone deacetylase inhibitor butyrate – is not currently marketed directly to consumers, and must be ingested in very high doses to achieve sustained inhibition of histone deacetylases.

**Nelfinavir** – *Nelfinavir is a so-called “protease inhibitor” drug employed in the treatment of HIV infections. But recent evidence suggests that it may have important potential as an anti-cancer drug. Via a complex mechanism, it tends to decrease the activity of an enzyme, Akt, whose activity is increased in many EOCs and tends to make these cancers more chemoresistant and aggressive. Phase I clinical trials in other cancers indicate that, at a dose of 1250 mg twice daily, it is well tolerated and renders some cancers more sensitive to radiotherapy; there is reason to suspect that it likewise might boost response to chemotherapy. Nelfinavir, while not cheap, is not so crushingly expensive as most new cancer drugs, so its use as an adjuvant to chemotherapy would be feasible for many people. There is reason to suspect that its efficacy for cancer control might be potentiated if combined with the drug hydroxychloroquine, as discussed below.*

This protease inhibitor, used for treatment of HIV infection, has interesting potential as an anti-cancer agent.<sup>177, 178</sup> Within a concentration range achieved in plasma by high-dose but well-tolerated dosage schedules employed in HIV therapy – 4-9  $\mu\text{M}$ <sup>179</sup> – nelfinavir functions as a proteasome inhibitor.<sup>180, 181</sup> Like other HIV protease inhibitors it has the potential to inhibit certain metalloproteinases that promote tumor invasiveness and angiogenesis.<sup>182, 183</sup> Computational analysis suggests that it may also modestly

inhibit a range of kinases that often contribute to cancer aggressiveness, including Akt, IGF-1R, and EGFR.<sup>184, 185</sup> Clinically feasible concentrations of nelfinavir slow the proliferation of various ovarian cancer cell lines in vitro; the closely related agent saquinavir likewise has this effect.<sup>186, 187</sup> A downstream effect of nelfinavir's inhibition of proteasomal activity is endoplasmic reticulum stress, which induces the unfolded protein response and autophagy.<sup>181, 188</sup> The unfolded protein response, in turn, is often associated with a suppression of Akt phosphorylation, likely reflecting upregulation of phosphatase activity targeting Akt.<sup>181, 189, 190</sup> Indeed, in HIV patients using nelfinavir, decreased phosphorylation of Akt in peripheral blood mononuclear cells was observed.<sup>191</sup> This effect is of considerable interest, in light of the fact that increased activating phosphorylation of Akt is commonly seen in ovarian cancers, and mediates chemoresistance to platin drugs and paclitaxel by inhibiting apoptosis through a broad range of coordinated actions.<sup>192-199</sup> Hence, co-administration of nelfinavir, via its impact on Akt, may have the potential to improve the responsiveness of many ovarian cancers to chemotherapy. So far, little research has evaluated the impact of nelfinavir on chemosensitivity, but pre-clinical studies as well as pilot clinical studies indicate that nelfinavir can provide useful potentiation of radiotherapy in various cancers – likely reflecting a key role for Akt in radioresistance.<sup>200, 201</sup> On the other hand, the tendency of nelfinavir to provoke autophagy is not necessarily favorable to the success of chemotherapy, as autophagy often enables cancer cells exposed to cytotoxic agents to avoid death by apoptosis.<sup>202, 203</sup> (Similar logic may apply to metformin, which likewise promotes autophagy.<sup>204, 205</sup>) Hence, it would be interesting to determine whether co-administration of a clinical autophagy inhibitor such as hydroxychloroquine (see below) might boost the chemosensitizing benefit of nelfinavir. A dose schedule of 1250 mg nelfinavir twice daily was well tolerated in a phase I trial evaluating its utility as a radiosensitizing agent in locally advanced pancreatic cancer; the favorable clinical outcomes observed suggest that it may indeed be useful for this purpose.<sup>201</sup> The same dose schedule was associated with promising efficacy in a phase I trial of nelfinavir as an adjuvant to chemoradiotherapy in non-resectable non-small cell lung cancer.<sup>206</sup> In the management of ovarian cancer, nelfinavir may have potential both as a chemosensitizer and as an agent that can be used chronically to impede the growth and spread of cancer. A phase I study of nelfinavir as a monotherapy in a range of advanced solid tumors is currently in progress. Although the drug bortezomib also functions as a proteasome inhibitor, its outrageous cost and greater toxicity gives nelfinavir a great practical advantage in this regard.

**Chloroquine/Hydroxychloroquine** – *In many cancers, chemotherapy evokes a response known as “autophagy” (self-eating) in cancer cells, in which the cells digest their internal constituents at an accelerated rate. Surprising, this autophagic response often helps the cells to survive the chemotherapy. Therefore, drugs capable of inhibiting autophagy are now being studied as adjuvants to chemotherapy. The drug hydroxychloroquine, long used to treat malaria, is known to suppress autophagy, and a number of pilot clinical trials are now evaluating it as an adjuvant to chemotherapy. A phase I study has concluded that a dose of 400-600 mg daily is tolerated reasonably well. However, when used in conjunction with cytotoxic chemotherapy, it appears to potentiate the toxicity of chemotherapy to bone marrow; doses in excess of 400 mg daily may be inadvisable when used with chemotherapy.*

The anti-malarial drugs chloroquine and hydroxychloroquine are currently the only clinically approved drugs with practical clinical potential for direct inhibition of autophagy.<sup>207</sup> Cytotoxic chemotherapy commonly provokes autophagy in cancer cells; for reasons which remain to be clarified, this autophagic response often shields the cancer from apoptosis, aiding its survival.<sup>202, 203</sup> Hence, in many though not all studies, agents which suppress autophagy tend to potentiate cancer chemosensitivity. With respect to

ovarian cancer, there are several reports that autophagy inhibitors, including chloroquine, can boost the cell kill achieved with cisplatin.<sup>208, 209</sup> This phenomenon is particularly interesting in light of the fact that some potential adjuvants for chemotherapy, such as metformin, nelfinavir, or a preliminary fast, also tend to induce autophagy; it is reasonable to suspect that concurrent inhibition of autophagy would potentiate their utility in this regard, and indeed there are now several reports that the anti-proliferative impact of metformin or nelfinavir on several cancer cell lines is enhanced by autophagy inhibitors.<sup>205, 210, 211</sup> Phase I studies evaluating hydroxychloroquine as an adjuvant to chemotherapy in various cancers are in progress; an intake of 400–600 mg daily appears to be well tolerated and to achieve plasma concentrations sufficient for partial inhibition of autophagy; however, higher doses may notably potentiate the myelosuppressive impact of chemotherapy.<sup>212</sup> A double-blind trial of chloroquine (150 mg daily) as an adjuvant to radiotherapy and carmustine chemotherapy of glioblastoma observed a median survival of 24 months in the chloroquine group as opposed to 11 months in the control group; this difference missed statistical significance owing to the small number of patients enrolled.<sup>211</sup> The subsequent clinical experience of this group tends to bear out this trend.<sup>213</sup> When chloroquine or hydroxychloroquine are employed as adjuvants to chemotherapy, their use should be initiated in considerable advance of chemotherapy, as these drugs accumulate gradually, and weeks may be required for equilibrium blood levels to be reached.<sup>214</sup>

**Itraconazole** – *In many EOCs, a signaling pathway known as “hedgehog” is highly active, and this promotes chemoresistance and aggressive growth. Although new cancer drugs which directly target this pathway are under development, it has recently been discovered that an approved antifungal drug, itraconazole, can also inhibit this pathway, in concentrations which appear to be clinically feasible. Remarkably, itraconazole also has the potential to retard tumor growth by inhibiting angiogenesis, the process whereby new blood vessels grow into a tumor, aiding its growth and spread. At a dose of 300 mg twice daily, this well tolerated drug has shown a growth-retardant impact on prostate cancer in phase II clinical trial. Itraconazole may therefore have potential both as a chemotherapy adjuvant and as a cancer growth retardant in EOC. However, it should not be used in combination with a class of chemo drugs known as “vinca alkaloids”, as it can potentiate their toxicity.*

A number of studies indicate that the hedgehog signaling pathway is up-regulated in a high proportion of ovarian cancers, often reflecting a low expression of Patched relative to that of Smoothed; this increased hedgehog signaling promotes increased proliferation, chemoresistance, and stem cell properties.<sup>215-221</sup> Hence, the hedgehog-inhibitory drug cyclopamine tends to suppress proliferation, anoikis resistance, and stem cell behavior in various ovarian cancer cell lines, both in vitro and in vivo. The drug vismodegib is been developed as a clinical hedgehog pathway inhibitor, and is in phase II trials, but its approval is likely years away.<sup>222</sup> It is therefore intriguing that the available anti-fungal drug itraconazole has been found to inhibit hedgehog signaling via interaction with the Smoothed receptor; moreover, this effect can be achieved with clinically relevant levels.<sup>223</sup> Thus, itraconazole inhibits the growth of a hedgehog-dependent medulloblastoma in nude mice. Moreover, itraconazole also exerts an anti-angiogenic effect, at least in part because it blocks trafficking of the VEGF2 receptor to the endothelial cell membrane.<sup>224-226</sup> This anti-angiogenic effect can be evoked in vivo, and suppresses the growth of a non-small cell lung cancer in nude mice.<sup>225</sup> A phase II study of itraconazole in prostate cancer patients has demonstrated a favorable impact on PSA levels when this drug is administered in a dose of 300 mg twice daily.<sup>227</sup> Conceivably, this regimen could aid control of EOC by suppressing both hedgehog signaling and angiogenesis. It should be noted, however, that itraconazole potentiates the

toxicity of vinca alkaloid drugs, and so should not be used in conjunction with chemotherapy regimens including these.<sup>228, 229</sup>

**Metronomic Cyclophosphamide** – *Low daily doses – 50 mg daily – of the old and not-too-expensive chemotherapy drug cyclophosphamide have been found to slow the development of new blood vessels by selectively killing the endothelial cells which form these new blood vessels; it therefore impedes the angiogenic process required for tumor growth. Remarkably, such low doses of cyclophosphamide are too low to kill the cancer directly or cause notable side effects. Hence, this type of low-dose daily chemotherapy – known as “metronomic” because the daily dosing mimics the monotonous beat of a metronome – can slow cancer growth through an anti-angiogenic effect. The utility of this strategy in breast cancer control has been well documented, but unfortunately clinical trials examining its impact on EOC have not yet been conducted. However, one encouraging case report indicated that, in a patient with EOC unresponsive to the standard chemotherapies, the cancer failed to progress for over 5 years while the patients received metronomic cyclophosphamide. This was probably an unusual response, but it does suggest that this approach might notably help some patients.*

Since the primary target of metronomic cyclophosphamide is the endothelial cell, its anti-angiogenic and therapeutic utility should to some extent be independent of tumor type.<sup>230, 231</sup> This strategy may also aid immune scavenging of cancer by selectively killing or disabling Treg cells; this benefit may however be transitory.<sup>232, 233</sup> In breast cancer, metronomic cyclophosphamide (50 mg daily), sometimes in conjunction with low-dose methotrexate, has shown a favorable impact on progression-free survival.<sup>234, 235</sup> Formal trials of metronomic cyclophosphamide alone in ovarian cancer have not been conducted, though a case history has been published in which a patient resistant to standard chemotherapy achieved 65 months of progression-free survival while using this therapy.<sup>236</sup> Several groups are however studying the combination of metronomic cyclophosphamide with bevacizumab (Avastin); objective response is observed in at least a quarter of patients, and progression-free survival averages about 6 months.<sup>237-240</sup> Since Avastin is extremely expensive and can have significant toxicity, it would be useful to know to what degree it potentiates response to metronomic cyclophosphamide. In patients with advanced ovarian cancer, Avastin alone has increased progression-free survival by about 2 months, while failing to influence overall survival.<sup>240</sup> The anti-angiogenic agent sunitinib is also being evaluated in ovarian cancer, and may have modest efficacy; however, it would be too expensive for use in EOC until and unless it achieves clinical approval for this purpose.<sup>241</sup>

**Ambrisentan** - *We have noted that the remarkable impact of green tea on survival in EOC may reflect its ability to block the impact of the hormone endothelin on this cancer. A drug which inhibits endothelin receptors, known as ambrisentan, has recently been approved for treatment of a condition known as pulmonary hypertension. Hence, it has been suggested that ambrisentan might prove to be a good adjuvant for chemotherapy and cancer control in EOC. The drawback with this sensible idea is that ambrisentan, like many new drugs for rare conditions, is extremely expensive; until such time as it achieves approval as a treatment for EOC, insurance or Medicare will not cover its cost.*

This is an approved drug for pulmonary hypertension that works by inhibiting the endothelin type-A receptor; it therefore has been suggested that it may have potential in the therapy of EOC.<sup>242</sup> Although well tolerated, its considerable drawback is that it costs over \$150 a day. Conceivably, it could be used for just a few days at a time as a chemosensitizing agent.

**A Postscript on PARP Inhibitors** – *A new category of drug – known as “PARP inhibitors” – is showing encouraging activity in phase II clinical studies targeting EOC. This strategy is rooted in the fact that a significant proportion of women with EOC have inherited a mutation in one of two genes – BRCA1/2 – that greatly increases their risk for EOC. Moreover, even in EOC patients who lack these mutations, their cancers are fairly likely to carry such mutations. Cells with defective function of BRCA1/2 are selectively susceptible to being killed by PARP inhibitors. Initial clinical trials with the PARP-inhibitory drug olaparib in EOC patients have found that this drug produces tumor shrinkage in about 40% of patients who have inherited BRCA1/2 mutations, and about a quarter of patients who haven’t. Nausea and fatigue are the chief side effects reported with this drug, and are usually mild. It seems likely, based on findings published so far, that olaparib will gain approval for use in EOC within the next several years. Until then, some patients should be able to receive it by enrolling in formal clinical trials with this agent.*

A novel category of drug – known as “PARP inhibitors” because they potently inhibit the enzyme poly(ADP-ribose) polymerase – has shown highly promising activity in EOC in phase II clinical trials. The basis for the efficacy of these agents is as follows: women with a germline mutations in the genes BRCA1 or BRCA2 – not uncommon in the Ashkenazi Jewish population – are at greatly increased risk for ovarian or breast cancer. Also, in a relatively high proportion of ovarian cancers, BRCA1/2 activity is deficient, even in women without germline mutations in these genes. The enzyme PARP plays a crucial role in the repair of single-strand breaks in DNA, which can occur spontaneously during the cell cycle. If PARP activity is inhibited, single strand breaks often become double-strand breaks that potentially threaten cell viability. Fortunately, our cells have strategies for repairing double-strand breaks – but one of the most important of these is dependent on the activity of BRCA1/2. Hence, PARP inhibitors can be selectively lethal to cells which lack effective BRCA1/2 activity – a characteristic of a high proportion of ovarian cancers.<sup>243</sup> Two phase II clinical studies with the PARP-inhibitory drug olaparib in EOC patients who either carry or do not carry germline mutations in BRCA1/2 have been completed.<sup>244, 245</sup> Among women carrying such mutations, 33-40% achieved objective response with 400 mg olaparib daily; reported side effects – primarily nausea and fatigue – were common but rarely severe. In women not carrying germline BRCA mutations, about a quarter responded to olaparib with an objective response. These initial findings are encouraging, and if they are confirmed in phase III studies, clinical approval of olaparib for use in EOC can be anticipated. Until its formal approval, some EOC patients may be able to receive it by enrolling in formal investigative protocols.

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