Biologic Response Modifiers in Gynecologic Malignancies

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Biological therapy is currently being investigated in the treatment of a number of malignancies. The hypothesis for the use of this therapeutic modality involves an attempt to stimulate an already existent but perhaps suboptimal immune response to foreign protein, including tumor. Immuno-logic therapy appears to work best against small-volume disease, as indicated from animal studies. This condition is potentially achievable in advanced ovarian cancer, where surgery is capable of producing multi-log reductions in tumor mass, and thus immunotherapy may be an option in this disease. The attraction of biologic therapy in patients with ovarian cancer is the potential to treat relatively localized but often chemotherapy-resistant disease. In cervical cancer, the rationale for the use of interferon is somewhat different in that this disease may be a manifestation of a virally induced proliferative lesion. Thus, the antiviral properties of interferon are being investigated in both limited and advanced cervical cancer. Both of these hypotheses have pre-clinical data to support them. This paper presents the pre-clinical and clinical work currently available for consideration of future use.

OVARIAN CARCINOMA

Introduction

Despite objective response rates of 70–80 percent to combination chemotherapy, advanced ovarian carcinoma has proven a frustrating disease to treat, because of the rapid emergence of primary drug resistance and associated cross-resistance [1]. This resistance remains a major clinical problem in spite of aggressive multimodality therapy, regional dose intensification with intraperitoneal therapy, and high-dose systemic therapy with autologous bone marrow rescue. Therefore, new approaches to therapy are being sought, including an investigation of the activity of biologics.

Since ovarian cancer remains confined to the peritoneal cavity for most of the natural history of the disease, the concept of intraperitoneal therapy is attractive. While intraperitoneal (IP) chemotherapy has been explored with some success, more recently interest has turned to the study of biologic agents. One hypothesis is that biological therapy stimulates an already existent but perhaps suboptimal immune response to the presence of tumor by using significantly larger amounts of biological agents than are produced naturally. Two approaches being investigated in ovarian

Abbreviations: BRMP: Biologic Response Modifier Program CIN: cervical intraepithelial neoplasia DNA: deoxyribonucleic acid ECOG: Eastern Cooperative Oncology Group HPV: human papilloma virus IFN: interferon IL-2: interleukin-2 IP: intraperitoneal IU: international units LAK: lymphokine activated killer cell LGL: large granular lymphocyte MU: million units NCI: National Cancer Institute NIH: National Institutes of Health NK: natural killer cells PBL: peripheral blood lymphocytes TAL: tumor-associated lymphocyte T-cell: thymus-derived lymphocyte

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carcinoma are the use of cytokines alone and cytokines in conjunction with their effector cells, which are presumed to mediate immune destruction of tumors.

The human stem cell assay provided initial clues that biologicals may have activity against ovarian carcinoma [2,3], leading to investigation of biological therapy, particularly interferons (IFNs), which stimulate natural killer (NK) cells in vitro and in vivo. Investigators have reported diminished NK cell activity in the peritoneal cavity of women with ovarian carcinoma previously treated with chemotherapy [4]. Therefore, the potential for immune stimulation by administration of interferon is appealing. Initial studies with IP administration of Corynebacterium parvum also gave clues to the potential for an immune approach, produced intense peritonitis, possibly leading to localized immune stimulation, and resulting in true measurable responses [5].

Interferons (IFNs)

These polypeptide products of activated lymphoid cells are the prototype of biologic response modifiers and induce a wide range of physiologic changes [6]. Studies of interferons initially utilized a partially purified substance that contained a mixture of cytokines, but the substances now used are highly purified products of recombinant DNA technology.

There are three distinct antigenic types of interferon, designated α, β, and γ. Interferon-α, usually thought of as leukocyte IFN, is the substance most widely used in clinical trials. IFN-β, derived from fibroblasts, has properties similar to IFN-α, including binding to the same receptor. IFN-γ, often called immune interferon, is derived from T lymphocytes and is quite different from the other two interferons. For example, while both IFN-α and IFN-β are both strong inducers of NK activity, IFN-γ appears to stimulate monocytes and macrophages selectively.

Natural Killer (NK) Cells

NK cells are a subpopulation of normal lymphoid cells with spontaneous cytotoxic reactivity against a variety of tumor cells; NK cells can be rapidly augmented by in vivo or in vitro administration of interferon [7]. There is increasing evidence that NK cells may mediate the natural immune response in vivo against tumor cells and may play an important role in immune surveillance [7,8]. NK cells are included in a morphologically identifiable subpopulation of cells, large granular lymphocytes (LGLs), which comprise approximately 5 percent of the blood peripheral mononuclear leukocytes. Therefore, LGLs would be a logical choice as effector cells for adoptive cell transfer. In addition, when radiolabeled LGLs and thymus-derived lymphocyte (T-cell) populations were administered intraperitoneally, less than 20 percent of the cells were found to migrate from the peritoneal cavity within 24 hours after inoculation [9].

Clinical Trials with Interferons in Ovarian Cancer

Initial clinical trials employed systemic administration of interferon. Einhorn et al. demonstrated evidence of clinical activity when daily intramuscular purified alpha interferon, 3 million units (MU)/day, was given to five patients with advanced ovarian carcinoma [10]. Ascitic fluid production ceased in two patients with ascites, and both remained stable for more than one year. A third patient had a partial response (50 percent or greater reduction in tumor) lasting two months. Also of importance, the
administration of interferon was followed by an increase in natural killer cell activity in peripheral blood lymphocytes, suggesting enhancement of immune activity. Subsequently, others have investigated IP interferon based on the premise discussed earlier, that relatively refractory residual disease remains in the peritoneal cavity.

IP Alpha Interferon (IFN-α)

Berek et al. have reported a nicely designed study in which 14 patients with persistent ovarian cancer limited to the peritoneal cavity and verified at second-look surgery were treated with IP recombinant alpha interferon [11]. Both the clinical and immunological parameters were followed during the course of treatment. Prior therapy included one to three surgeries, as well as a median of nine cycles of combination chemotherapy. Two patients had previously received pelvic irradiation.

Patients received IP interferon administered in 250 ml normal saline following instillation of 1,750 ml of dialysis solution via a Tenckhoff catheter. Ten patients were treated on a weekly dose-escalation schedule of $5 \times 10^6$ units, $10 \times 10^6$ units, $20 \times 10^6$ units, then $50 \times 10^6$ units weekly thereafter. A second dose schedule attempted $50 \times 10^6$ units three times a week, but toxicity precluded more than weekly administration of this dose.

Eleven patients were evaluated surgically for clinical response, and three were evaluated clinically only. Four patients (36 percent) demonstrated pathologically documented complete response, and one patient a partial response. The remaining six patients, evaluated surgically, showed progressive disease.

All of the five responders had minimal residual tumor, less than 5 mm prior to beginning IP interferon, whereas four patients with disease greater than 5 mm failed to respond. The duration of response was reported as 5–14+ months. Thus, tumor burden prior to initiation of therapy may well be a factor in response. Of the three patients evaluated clinically only, one had a complete clinical response with total resolution of ascites, while the others were stable or progressed.

Toxicity with this approach was consistent with that seen after systemic administration of α-interferon, including fatigue, chills, anorexia, and headaches. One-quarter of patients experienced local complaints of nausea/vomiting, diarrhea, and abdominal pain, probably related to receiving IP therapy. Hematologic toxicity also occurred in this group of heavily pre-treated patients but was primarily a mild to moderate anemia and a mild decrease in white blood cells (2,000/µl).

Concomitant immunologic studies were performed, including lymphocyte count in both the peripheral blood and peritoneal washings, and NK activity was assessed in both blood and peritoneal fluid. These studies showed a decrease in peripheral lymphocytes during each cycle, with a subsequent return to baseline. In the peritoneal fluid a consistent increase, nearly a doubling, of mononuclear cells was seen after treatment. NK activity was significantly augmented in the peritoneal fluid, although not in the peripheral blood. Boosting of NK activity did not uniformly correlate with response, however.

Thus,alpha interferon appears to have activity in maximally resected ovarian carcinoma. There is also evidence that interferons may act synergistically with cytotoxic chemotherapeutic agents in vitro and this finding has potential for clinical investigation.
Beta Interferon (IFN-β)

At least one study of IFN-β administered by the IP route to ovarian cancer patients has been reported [14]. Eight patients with chemotherapy-resistant, advanced intra-peritoneal disease (seven had ascites and five had measurable masses) received $3 \times 10^6$ units of IFN-β twice weekly.

Seven of eight had ascites prior to starting therapy and four of seven had total cessation of ascites with IP IFN-β therapy. The ascites-free intervals were six weeks, eight weeks, ten months, and 15 months. The last patient maintained stable disease for 15 months and survived 24+ months from time on study. Toxicities were similar to those seen with α-interferon, except that there was no hematologic toxicity on this schedule and dose. An increase in NK activity of the intraperitoneal lymphocytes was observed in two patients, with no effect on peripheral blood lymphocytes.

Apart from the palliative effect on ascites, there were only two patients with prolonged stable disease. Nevertheless, in this group of patients with large-bulk disease, this result would appear to demonstrate activity. Further studies in minimal-bulk disease are warranted.

Gamma Interferon (IFN-γ)

There are in vitro data demonstrating anti-tumor efficacy of IFN-γ against ovarian cancer tumor cells. Cytotoxicity studies of treatment with both IFN-γ alone and with the addition of IFN-γ-treated macrophages showed anti-tumor activity [15].

Clinical studies are quite preliminary, demonstrating that this drug can be administered safely by this route (IP). Markman et al. have reported a phase I, dose-escalation study of 27 patients with refractory ovarian cancer treated with weekly IP IFN-γ [16,17]. The doses ranged from 0.1 to $8.0 \times 10^6$ u/m². Intraperitoneal peak levels of IFN-γ were 150–200 times higher than levels in the systemic circulation. All patients in this study demonstrated progressive disease, including five patients evaluated surgically. Of the 27 patients, six were considered to have minimal disease at the time of IP treatment. Toxicity in this dose range seemed less than that described with IP IFN-α.

Thus, IFN-γ in one phase I study did not demonstrate the level of activity observed in single-institution studies with IP IFN-α or IFN-β in IP ovarian cancer; however, the biologically active dose may not have been established. In addition, IFN-α may potentiate the effects of IFN-γ so that combinations of biologics or combinations of biologics with chemotherapy remain possibilities open for further investigation.

In summary, interferons administered intraperitoneally in patients with minimal residual IP ovarian cancer have demonstrated benefit. Further optimization and exploration of such therapy is warranted.

Interleukin-2 (IL-2)

The interleukins are part of a family of proteins known as cytokines, produced by lymphocytes and macrophages, which stimulate interactions between the various types of mononuclear white blood cells and potentiate a variety of intercellular activities. Interleukin-2, originally described as T-cell growth factor [18], stimulates the immune responsiveness of a variety of populations of lymphocytes, including natural killer (NK) cells [19], T-cytotoxic cells [20], and the lymphocytes that mediate lymphokine activated killer (LAK) activity [21]. Interleukin-2 is being actively investigated as a
stimulator and potentiator of these killer lymphocytes that have cytotoxic activity against tumor cells and is being utilized as the stimulating substance in adoptive immunotherapy. In adoptive immunotherapy, the approach is the initial in vivo stimulation of lymphocyte activation with IL-2, followed by in vitro expansion and further activation of these cells, followed by re-infusion of the activated lymphocytes with additional IL-2 [22,23]. The expectation is that such activated killer cells will be selectively cytotoxic to tumor cells.

**LAK Cells or LAK Activity**

Lympahkine activated killer cell activity is a functional definition of the activity of what may be a heterogeneous population of lymphocytes capable of antitumor cytotoxicity [21]. By definition, LAK cells are capable of lysing fresh tumor targets, including targets derived from ovarian ascites, and they can lyse target cell lines which are normally resistant to NK cells. Thus, LAK cells have properties different from T-cytotoxic cells or NK cells, but all of these killer cells respond to IL-2 and are probably closely related [24]. LAK cells are stimulated by IL-2. NK cells are also stimulated by IL-2 and by interferon.

**Model for the Study of New Therapy in Ovarian Carcinoma**

Animal models relevant to the study of ovarian carcinoma have been recently improved with the development of the National Institutes of Health NIH:OVCAR-3 cell line [25,26]. This line was derived from a patient with malignant ascites, who was clinically resistant to chemotherapy. The cell line has the capacity to grow in tissue culture and as a xenograft in nude mice. This tumor expresses estrogen and androgen receptors and CA-125. It also has karyotypic abnormalities characteristic of ovarian carcinoma (changes in ploidy) and those possibly related to drug-resistant cells (double minute chromosomes and homogeneous staining regions).

A specific subpopulation of this human ovarian tumor cell line is capable of inducing ascites and intraperitoneal malignancy in nude mice [26]. This subpopulation maintains all of the identifying characteristics of the original tumor and is being used widely to study the issues of drug resistance and the impact of hormonal therapy on ovarian carcinoma.

This model provides characteristics of ovarian carcinoma which have not been demonstrated by other cell systems: (a) histopathology and embryology consistent with human ovarian cancer, (b) a reproducible pattern of metastases which parallels human disease, (c) drug sensitivity profiles similar to that of human ovarian cancer, (d) immunological and biological properties consistent with human ovarian cancer, and (e) suitable markers to follow response to treatment.

The pattern of metastases and the human derivation of this subpopulation model make this cell line useful for evaluation of experimental therapy, such as activated natural killer cells or T lymphocytes, mullerian regression substance, bacterial toxins, and monoclonal antibodies conjugated to toxic substances.

**Animal Studies of Adoptive Immunotherapy in Ovarian Cancer**

Ortaldo et al., using the modification of NIH:OVCAR-3 described earlier as a xenograft in nude mice, studied the efficacy of adoptive immunotherapy in ovarian carcinoma [23]. This group studied activated lymphocytes stimulated by interleukin-2 and monocytes stimulated by gamma interferon. Data from this group have shown that
80 percent of radiolabeled large granular lymphocytes and T cells remain in the peritoneal cavity within 24 hours after inoculation [9].

Animals that were given injections of five doses of untreated LGL with or without IL-2 simultaneously demonstrated no significant increase in mean or median survival time. Animals that were given multiple injections of both IL-2 and IL-2 pre-treated LGL, however, demonstrated a significant increase in mean survival time.

Because these results were achieved with, presumably, NK cells, although the requirement for pre-activation with IL-2 for cytotoxicity defines LAK activity of these lymphocytes, other effector cells were specifically evaluated. In a manner similar to that of LGL, when T cells were stimulated with IL-2, cytotoxic activity was induced, and there was a significant extension in mean and median survival of the subset of animals in which the lymphocytes were pre-exposed to IL-2 and had continued exposure to IL-2 following re-infusion into the peritoneal cavity. Injection of IL-2 alone induced no significant extension of survival time.

Similar treatment with monocytes activated with gamma interferon demonstrated only a small but insignificant improvement in the mean and median survival time, suggesting that this cytokine effector system is not as important in the clinical improvement.

Thus, activation with IL-2 of a mixed population of effector cells, including NK and T-cytotoxic cells, produces functionally defined LAK activity, which is cytotoxic to tumor in this murine model of ovarian cancer. Significant survival benefit was noted in these animals.

**Human Clinical Results of Adoptive Immunotherapy in Ovarian Carcinoma**

To explore the potential for site-directed adoptive immunotherapy, Allavena et al. have demonstrated that the peripheral blood lymphocytes (PBLs) and the tumor-associated lymphocytes (TALs) of patients with advanced ovarian cancer and ascites are activated after culture with IL-2 [27,28]. Cytotoxicity was demonstrated against autologous ovarian tumor cells as well as NK-sensitive (K562) and NK-resistant (Daudi) cell targets. Thus, LAK activity could be induced after culture with IL-2. The TALs in particular had poor cytotoxic activity prior to culture with IL-2 and demonstrated markedly improved cytotoxicity after culture.

The intraperitoneal route of therapy is attractive in ovarian carcinoma and the surgical oncology group at the National Cancer Institute (NCI) has utilized this route in the administration of IL-2 in a number of different diseases [29]. One patient with ovarian carcinoma metastatic to liver, without ascites, was treated among a group of seven patients (three colon, three melanoma). She received 1 to 2, 10,000 u/kg every eight hours for five days, resulting in significant toxicity requiring ventilatory support, but without response to anti-tumor therapy. In general, however, among the other patients treated, toxicity from IP administration of IL-2 was no greater than with systemic administration. Interestingly, one patient with melanoma metastatic to lungs who received IP IL-2 had a dramatic response of lung metastases, verifying that this route of administration is feasible in humans.

West et al. have recently published their experience with continuous infusion IL-2 and LAK cell adoptive immunotherapy [30]. One patient with ovarian carcinoma was among their 48 patients treated with systemic IL-2. This patient had a bulky abdominal wall mass and responded to IL-2/LAK with a 50 percent reduction in tumor, which lasted two months.
A phase I pilot study of IP recombinant IL-2 was reported in abstract form with IP IL-2 administered three times per week, in escalating doses [31]. There was considerable patient-to-patient variability in the clearance of IL-2 from the peritoneal cavity. Augmentation of peripheral blood NK activity was seen in three of three patients, and augmentation of LAK activity in peritoneal lymphocytes was seen in two of three patients. Peripheral LAK activity was generated in three of three patients. Although measurable response was not a goal of this feasibility study, one of two patients demonstrated a marked decrease in CA-125 levels with this therapy.

Thus, there are substantial preliminary data to suggest that biological therapy has a role in the treatment of ovarian carcinoma, particularly in patients with minimal residual disease. The caveats are that these are small numbers of patients, often highly selected, and these data are quite preliminary. In addition, there has been no prospective comparison to the natural history of patients with minimal disease to demonstrate improved survival. Further investigation of adoptive immunotherapy is currently under way at a number of centers, including the National Cancer Institute-Biologic Response Modifier Program (NCI-BRMP) at Frederick and BRMP-sponsored phase I–II clinical trials at other institutions, and we look forward to reports of their results.

A POTENTIAL ROLE FOR RECOMBINANT ALPHA INTERFERON AGAINST HUMAN CERVICAL CANCER

Despite large-scale programs for early detection and readily available treatments for local disease, approximately 7,000 women die of carcinoma of the cervix yearly [31]. Factors in local failure include the uncertainties of lymphatic drainage; unfavorable anatomic factors, such as a narrow (senile) vagina; unfavorable biologic factors, such as bulky disease; and faulty management of the primary lesion or locoregional lymph nodes [32]. Once the primary tumor has advanced beyond the true pelvis, the prognosis for the patient is poor; currently available cytotoxic agents, whether given individually [33,34] or in combination [35], rarely result in prolongation of survival beyond two years. Thus, alternative therapeutic modalities are worth investigating in patients with disseminated disease.

Epidemiologic observations clearly indicate that cervical carcinoma has many of the attributes of a communicable disease [36]. Viruses are the leading contenders for a major role in the induction of cervical neoplasia, and, of these, the human papillomaviruses (HPV) are the most likely carcinogens [37]. HPV is a double-stranded deoxyribonucleic acid (DNA) virus which produces local infections of the epithelial cells of the skin and mucus membranes [38]. It has recently been appreciated that many of the dysplastic lesions of the cervix in fact represent infections with human papillomaviruses [39–41]. Morphologic evidence for the involvement of HPV in pre-cancerous lesions of the cervix includes the presence of koilocytic and warty atypia in cervical biopsies and cytologic smears with dysplasia [40], the demonstration of viral antigens in these tissues by immunocytochemical techniques [42], and the identification of viral particles by electron microscopy [43]. In fact, HPV DNA sequences have been identified in the majority of both pre-malignant and malignant cervical tissues [44–47]. In addition, two animal papillomaviruses, Shope papillomavirus and Bovine papillomavirus, are known to induce, in their natural host, papillomas which may eventually become carcinomas, particularly in association with mutagenic agents (reviewed in [48]). Molecular hybridization studies have amply documented
the presence of HPV DNA within pre-cancerous lesions, pre-invasive lesions, and frank carcinomas of the cervix [49–51].

Frankly invasive carcinoma of the cervix is virtually if not always preceded by either dysplasia or a benign, but pre-malignant neoplastic change; hence the diagnosis and management of pre-clinical carcinoma of the cervix has attracted much interest [52]. The presence of classic venereal warts (condyloma acuminata) in the cervix has been well known for years [41,53]. The link between these benign lesions and both cervical dysplasia and malignant transformation was not well characterized until 1976, when two previously unrecognized forms of cervical condylomas were discovered [54,55]. These flat and inverted (endophytic) lesions closely mimicked cervical dysplasia, carcinoma in situ and invasive squamous cell carcinoma [40,56].

Subsequently, abundant evidence has developed implicating condylomata acuminata as a pre-malignant condition [40,41,56–58]. There is also a well-documented association between this pre-malignant lesion and HPV. Gissmann examined 63 typical lesions, employing a DNA probe for HPV6 and 11, and found a total of 86 percent were positive for the viral genome [44,45]. Schinella et al. [59] found HPV6a DNA fragments and HPV antigen in condylomata which underwent malignant transformation. Electron micrographic examination of condyloma cells has revealed large numbers of HPV particles [60].

There are, also, now well-established links between cervical HPV infections and other pre-malignant or dysplastic conditions, including cervical intraepithelial neoplasia (CIN) [61–64], vaginal in situ carcinoma [65,66], and vulvar intraepithelial neoplasia [67,68]. These data, along with epidemiologic evidence indicating that both HPV infections of the genital tract and cervical neoplasia have similar risk factors, including peak incidence in young, sexually promiscuous women with poor sexual hygiene, now suggest that cervical cancer might reasonably be considered a sexually transmitted disease [69,70].

Human papillomavirus-associated lesions appear to be exquisitely sensitive to treatment with the antiviral, antineoplastic agent interferon. Olsen et al. have reported that, among 34 patients with refractory condyloma acuminata treated in a randomized, double-blind trial, 17 (50 percent) had a response to human lymphoblastoid interferon alpha (3 MU/m² by intramuscular injection daily for two weeks, then three times weekly for four weeks) [71]. In a study by Friedman-Kien et al., patients with condylomata treated with intralesional “ultra-pure” IFN-alpha achieved complete clearing of their lesions, and a sixth achieved a partial response [72]. In a larger multicenter trial of intralesional therapy, 100,000 international units (IU) was significantly better than placebo in reducing the size of the condylomata, with 53 percent of IFN-treated patients achieving complete clearing of lesions [73].

Furthermore, among 13 patients with CIN and vulvar intraepithelial neoplasia associated with HPV infection who were treated with intralesional human fibroblast interferon, seven complete and two partial responses were observed by DePalo et al. [74]. Likewise, intralesional interferon alpha or beta produced complete remission in eight of 12 patients with CIN, while interferon gel produced complete remission in two of eight with CIN [75].

Thus, in light of the fact that cervical carcinoma is closely associated with HPV and that pre-malignant lesions of the female genital tract appear to be exquisitely sensitive to interferon treatment, it is rational to consider interferon therapy in the treatment of disseminated cervical carcinoma.
In one early study, patients with early-stage cervical carcinoma were treated with crude human leukocyte interferon at a dose of only 10,000–50,000 units daily [76]. In addition, in 6/15 patients, interferon was applied topically only. The typical tumor regressed to one-third of its size, with histologic changes consistent with tumor regression. While rigid criteria for tumor response were not applied, the investigators describe an “excellent” and “very good” response noted in 11/15 patients. The Eastern Cooperative Oncology Group (ECOG) is currently undertaking a phase II trial of recombinant alpha-2b-interferon in patients with disseminated cervical carcinoma.

With the characterization of over 30 types of HPV, sensitive probes employing DNA hybridization techniques are now available to detect the virus and its transcripts in tumor specimens [77]. HPV genome has been isolated from cervicovaginal lavage specimens from patients with dysplastic and neoplastic lesions and detected by Southern blot analysis [78]. Thus, accurate correlations can now be made between the presence, type, and expression of viral genetic material in human tumors and response of those tumors to antiviral agents. The ECOG study will correlate response to therapy using interferon with presence of virus and viral subtypes in both primary and, when feasible, metastatic disease. The results of this study will, it is hoped, help to elucidate the mechanism of antitumor activity of alpha interferon in this disease.

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