Anti-Cancer Drugs 1996, 7, pp. 800–804

Case report

Prolonged stabilization of progressive squamous cell cancer of the cervix with interferon-α and 13-*cis*-retinoic acid: report of two cases and review of the literature

Surintip Piamsomboon, Wichai Termrungruanglert,[1] Andrzej P Kudelka,[2] Creighton L Edwards,[2] Ralph S Freedman,[2] Clifton F Mountain,[2] Luis Delclos,[2] Matti S Aapro,[3] Louis DeCaro,[4] Joseph Bianca,[5] Claire F Verschraegen[4] and John J Kavanagh[4]

Siriraj Hospital, Mahidol University, Bangkok, Thailand. [1]Chulalongkorn University Hospital, Bangkok, Thailand. [2]The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Box 39, Houston, TX 77030, USA. Tel: (+1) 713 792-7959; Fax: (+1) 713 745-1541. [3]European Institute of Oncology, Milan, Italy. [4] St Luke's Hospital and Texas Heart Institute, Houston, TX 77030, USA. [5]Diagnostic Clinic of Longview, Longview, TX 75605, USA.

Recurrent and metastatic cervical carcinoma has very poor prognosis, mainly because there is no effective systemic therapy which would increase the duration of survival. Biologic agents have recently been found to have activity in cervical carcinoma. The combination of interferon (IFN)-α and 13-*cis*-retinoic acid has additive and synergistic antitumor activity. Both have antiviral, immunoregulatory and antiangiogenic properties, and are known to modulate malignant cell differentiation and proliferation. We report two patients with recurrent squamous cell carcinoma (SCC) of the cervix who had small-volume progressive metastatic disease, and were treated with a combination of IFN-α and 13-*cis*-retinoic acid. The first patient had pelvic lymph node metastases and the other had lung metastases. The previously progressive diseases remained stable for a prolonged period of time, 3 and 4 years, with a good quality of life. These cases suggest the possibility of using IFN-α and 13-*cis*-retinoic acid as a treatment for small-volume residual disease or as postinduction therapy in patients at high risk for disease recurrence.

Key words: 13-*cis*-retinoic acid, cervix, interferon-α, squamous cell carcinoma.

Introduction

Recurrent and metastatic cervical cancer that cannot be managed by surgery or irradiation remains a grave problem. Currently, systemic therapy with cytotoxic agents is used for palliation but does not prolong survival. Many clinical trials of single-agent or combination chemotherapy have resulted in objective responses.[1–3] However, the incorporation of current chemotherapy with radiation or surgery has not prolonged the duration of survival and significant side effects have been noted.[4–9] These observations suggest the need for a different and hopefully more effective approach.

The combination of retinoic acid and interferon (IFN)-α had additive and synergistic antitumor activity.[10–14] The antiproliferative and differentiating effects have been demonstrated in many *in vitro* and *in vivo* systems. This novel treatment has shown activity in hematologic malignancies[15–17] and solid tumors.[18–20] The combination of 13-*cis*-retinoic acid and IFN-α was used in heavily pretreated advanced inoperable squamous cell cancer (SCC) of the skin with an objective response rate of 68%[18] and in previously untreated locally advanced SCC of the cervix with a 50% response rate.[19]

We report two patients with recurrent SCC of the cervix who had small-volume progressive disease. In each patient there was concern that their diseases would begin to progress rapidly. Accordingly, the combination of 13-*cis*-retinoic acid and IFN-α was initiated in the hope of delaying the further progression of their diseases. The first patient had pelvic lymph node metastases and the other had lung metastases. Disease in both patients remained stable for a prolonged period of time.

Correspondence to AP Kudelka

Case 1

A 35-year-old woman, gravida 4, para 3, abortion 1, was referred to the University of Texas MD Anderson Cancer Center in 1990 because of intermittent vaginal bleeding during pregnancy that was thought to be secondary to placenta previa. Two months after delivery she had heavy bleeding from the uterus. The uterine curettage revealed secretory endometrium and fragments of infiltrating poorly differentiated squamous cell carcinoma, possibly of cervical origin. Initial pelvic evaluation at MD Anderson Cancer Center revealed an enlarged cervix, 4 cm in diameter, with a hard consistency. There was a friable circumferential lesion around the external os and an irregular nodule infiltrating into the submucosa of the posterior fornix. Bimanual pelvic examination revealed no parametrial extension. General physical examination was unremarkable. There was no lymphadenopathy. Findings from metastatic survey, including cystoscopy and proctoscopy; chest X-ray; lymphangiography; i.v. pyelography and blood chemistries were unremarkable except for a hemoglobin of 9.6 g/dl. The patient was diagnosed as having invasive SCC of the cervix stage IIA. She received radiotherapy treatment consisting of 40 Gy, in 20 fractions over 4 weeks, of external-beam radiation with 18 MeV photons to the whole pelvis and two intracavitary utero-vaginal cesium insertions (48 h each), and an additional 10 Gy of external-beam radiation to the left hemipelvis. The pelvic tumor showed complete resolution. In July 1991, she had symptoms of left ureteral obstruction and a local recurrence of cervical cancer was suspected. The cervix and parametrial areas appeared to be normal on pelvic examination. Fine needle biopsies of the cervix, the parametrium and the uterosacral ligament revealed no cancer. A computerized tomography (CT) scan of the abdomen and pelvis showed evidence of extrinsic compression of the left ureter but no definite mass. Because of persistent pelvic pain on her left side, the patient underwent an exploratory laparotomy to relieve the obstructed ureter. The left pelvic lymph nodes were enlarged. The biopsy findings confirmed metastatic SCC in the left external iliac nodes. After an uncomplicated postoperative period she was considered for further therapy to delay the symptomatic recurrence of her disease. As of August 1991 she had been treated with 3×10^6 U of IFN-α2a s.c. once a day plus 40 mg of 13-*cis*-retinoic acid taken orally twice a day (approximately 1.5 mg/kg). The side effects of dry skin and dry conjunctivae were ameliorated with a skin lotion and carboxymethylcellulose eye drops, respectively. The patient did well and the disease remained stable for 48 months as confirmed by the absence of pain and magnetic resonance images of the pelvis. In September 1995, she developed increasing and uncontrolled pain in the left pelvic area. Pelvic examination and magnetic resonance images did not reveal any lesions. Nevertheless, because of subjective evidence of progressive disease, this treatment regimen was discontinued and the patient was started on carboplatin with a resultant rapid amelioration of pain.

Case 2

A 44-year-old woman presented to her physician in April 1983 with a history of a clear vaginal discharge and postcoital bleeding of 1 year duration. On pelvic examination a cervical ulceration was found and findings from a Papanicolaou's smear were reported as class II. She received no treatment. Six months later she had severe and persistent pelvic pain on her right side. An i.v. pyelogram showed right hydronephrosis. Findings from a repeat Papanicolaou's smear were reported as class V. A cervical biopsy revealed invasive SCC. She was referred to the University of Texas MD Anderson Cancer Center and was found to have an enlarged cervix, 4.5 cm in diameter. There was a small exophytic friable mass in the center of the cervix. Biopsies revealed nonkeratinizing SCC. The vaginal mucosa was normal. The bimanual and rectovaginal examinations revealed an irregular mass that extended to the right parametrium but was not fixed to the right pelvic wall. The left parametrium was free of disease. There was no lymphadenopathy and the remainder of the physical examination was unremarkable. Findings from metastatic evaluation, including chest X-ray, lymphangiography and blood chemistries including measurements of the serum levels of blood urea nitrogen and creatinine were within normal range. The final diagnosis was SCC of the cervix stage IIIB. She received radiation therapy which consisted of initial transvaginal irradiation 5 Gy given twice and then whole-pelvic irradiation at a total dose of 40 Gy given in 20 fractions over 4 weeks. She tolerated the radiotherapy quite well. She continued irradiation with intracavitary utero-vaginal brachytherapy. The intracavitary radioactive cesium sources were placed with an afterloading uterine tandem and colpostats. She received three intracavitary treatments with a total dose of ~200 mgh equivalent of radium. The treatment ended in January 1984 and resulted in a complete remission

of the pelvic tumor. The cervix remained irregular but had no sign of residual tumor. The right parametrium became densely fibrotic. The patient also had subjective improvement in symptoms with tremendously reduced right-side pelvic pain. A follow-up i.v. pyelogram showed reduced right-side hydronephrosis.

The disease remained in remission for more than 5 years when, in October 1989, a surveillance chest X-ray showed pulmonary metastases. CT scan of the chest revealed bilateral, multiple pulmonary nodules ranging from 0.8 to 1.5 cm in size. She had no evidence of pelvic recurrence on pelvic examination or on magnetic resonance images of the pelvis. There was no lymphadenopathy. Findings on a CT scan of the brain, a bone scan and the blood workup were unremarkable. In view of the absence of extrapulmonary disease, she underwent right thoracotomy with multiple wedge resections of the metastatic nodules. The planned bilateral thoracotomy was completed 2 months later with surgery on the left lung. Microscopic examination of all resected tissues revealed metastatic poorly differentiated SCC consistent with the patient's primary cervical cancer. The postoperative period was uneventful. She did well for almost 20 months, when enlarging multiple pulmonary nodules were detected in her right lung. A repeat right thoracotomy with excision of the multiple metastatic nodules was performed without complication.

In April 1992, 9 months after the last thoracotomy, a CT scan of the chest showed new multiple nodules in both lungs. There was no evidence of recurrent tumor in the pelvis or any other site. Because this recurrence was early after the last thoracotomy, systemic therapy was considered. She was treated with 13-*cis*-retinoic acid (1 mg/kg/day) orally plus IFN-α2a at 3×10^6 U s.c. 3 days a week. The metastatic nodules in both lungs remained stable for almost 3 years. However, eventually the nodules increased in size and this therapy was stopped. The remarkable side effects had been fatigue, cheilitis and conjunctivitis. Despite the side effects, the patient had continued her full-time employment as a physician and her avocations of skiing and hiking in the mountains.

In November 1995, she began experimental therapy with the angiogenesis inhibitor TNP-470.

Discussion

The synergistic antitumor activity of the combination of retinoic acid and IFN-α may be due to the altered expression of various gene products. For example, IFN increases the expression of protein kinase R (PKR) and 2′,5′-oligoadenylate synthetase (2′-5′AS) which is a family of enzyme isoforms. The major function of PKR is believed to be the regulation of the cellular and viral protein synthetic rate.[21–23] The enzyme 2′-5′AS plays a role in the antiviral and antiproliferative actions of IFNs.[24–27] IFN was also found to induce tumor suppressor gene products, such as the retinoblastoma gene product (pRb).[28] Furthermore, IFN-α has been shown to downregulate the expression of angiogene inducers, basic fibroblast growth factor and IL-8, and therefore to reduce the vascularity of tumors[29,30] in pre-clinical models.

Retinoic acid potentiates the antiproliferative actions of IFNs by enhancing cell differentiation.[13,14] The other possible mechanism of tumor suppression is the ability of retinoids to inhibit the expression of the stromolysin gene in tumor cells. Stromolysin degrades the stromal tissue and thus promotes tumor invasion and metastasis.[31,32] Moreover, retinoic acid has been shown to induce transplanted human squamous cancer cell lines to switch from an angiogenic phenotype to a non-angiogenic one by inducing the secretion of an angiogenesis inhibitor.[30] The combination of IFN-α and retinoic acid was synergistic in inhibiting neovascularization in experimental models.[30] Interestingly, IFN and retinoic acid suppress the growth of human papilloma virus type 16 (HPV-16) immortalized cervical epithelial cells; however, only IFN suppressed the level of HPV-16 E6 and E7 mRNA.[38] Because HPV-16 E6 and E7 proteins bind and inactivate p53 and pRb their reduced levels may be one of the mechanisms of IFNs action against HPV-related cervical neoplasia.

In SCC of the skin, major responses were achieved with 13-*cis*-retinoic acid alone,[45,46] but the most interesting results were reported with the combination of 13-*cis*-retinoic acid and IFN-α. Thirty-two patients with heavily pretreated advanced inoperable SCC of the skin were treated.[18] Among 28 patients in whom disease could be evaluated the objective response rate was 68%, with disease in six patients completely responding. Lippman *et al.*[19] then conducted a trial in 32 untreated patients with locally advanced SCC of the cervix (stage IB to IVA). Seventeen patients had bulky tumors at least 10 cm in diameter. Patients received an oral dose of 1 mg/kg/day of 13-*cis*-retinoic acid combined with 6×10^6 U of IFN-α injected s.c. daily for at least 2 months. The response rate was 50% with disease in 9% (two patients) completely responding. Clinical response resulted in reduced pain and bleeding.

Toxicity was well tolerated, manifesting as fatigue and mild cheilitis. This activity was confirmed in another study with a 42% response rate (8% complete).[20]

Murad *et al.*[17] explored the activity of this regimen in 18 patients who had recurrent cervical cancer. The overall response rate in previously irradiated but chemotherapy-naive patients was 17% (two of 12 patients) with all responses achieved within a prior radiotherapy field. There was no objective response among the six patients in whom previous salvage chemotherapy had failed. Another study confirmed the lack of response with this combination in patients with recurrent disease refractory to chemotherapy and radiotherapy.[18] Despite its different antitumor mechanism, this combination may be cross-resistant to chemotherapy.

Conclusion

Current and future trials will help to determine the role of combining biologic agents with conventional therapy as primary or palliative treatment in recurrent cervical cancer. The combination of IFN-α and 13-*cis*-retinoic acid may have a role as treatment for small-volume residual disease or as postinduction therapy in patients at high risk for disease recurrence.

References

1. Stornes I, Mejlholm I, Jakobsen A. A phase II trial of ifosfamide, 5-fluorouracil and leucovorin in recurrent uterine cervical cancer. *Gynecol Oncol* 1994; **55**: 123–5.
2. Murad AM, Triginelli SA, Ribalta JC. Phase II trial of bleomycin, ifosfamide, and carboplatin in metastatic cervical cancer. *J Clin Oncol* 1994; **12**: 55–9.
3. Singhal RM, Jindel R, Gupta AK. Bleomycin, cisplatinum and ifosfamide infusion chemotherapy in advanced/recurrent cancer of cervix. *Indian J Cancer* 1993; **30**: 158–63.
4. Souhami L, Gil RA, Allan SE, *et al.* A randomized trial of chemotherapy followed by pelvic radiation therapy in stage IIIB carcinoma of the cervix. *J Clin Oncol* 1991; **9**: 970–7.
5. Chauvergne J, Rohart J, Heron JF, *et al.* Essai randomise de chimiotherapie initiale dans 151 carcinomes du col uterin localement etendus. *Bull Cancer (Paris)* 1990; **77**: 1007–24.
6. Tattersall MHN, Ramirez CC, Coppelson M. A randomized trial comparing platinum-based chemotherapy followed by radiotherapy vs radiotherapy alone in patients with locally advanced cervical cancer. *Int J Gynecol Cancer* 1992; **2**: 244–351.
7. Tattersall MHN, Lorvidhaya V, Vootiprux V, *et al.* Randomized trial of epirubicin and cisplatin chemotherapy followed by pelvic irradiation in locally advanced cervical cancer. *J Clin Oncol* 1995; **13**: 444–51.
8. Sardi J, Sananes C, Giaroli A, *et al.* The results of a prospective randomized trial with neoadjuvant chemotherapy in stage IB bulky squamous cell carcinoma of the cervix. *Gynecol Oncol* 1993; **49**: 156–65.
9. Tattersall MHN, Ramirez C, Coppelson M. A randomized trial of adjuvant chemotherapy after radical hysterectomy in stage IB–IIA cervical cancer patients with pelvic lymph node metastases. *Gynecol Oncol* 1992; **46**: 175–81.
10. Bandopadhyay SK, Kumar R, Rubin BY, *et al.* Interferon-α inducible gene expression in HL-60 cells: effects of the state of differentiation. *Cell Growth Different* 1992; **3**: 369–75.
11. Schwartz E, Nilson L. Activation of 2′-5′ oligoadenylate synthetase activity on induction of HL-60 leukemia cell differentiation. *Mol Cell Biol* 1989; **9**: 3897–903.
12. Kalvakolanu DVR, Sen GC. Differentiation-dependent activation of interferon-stimulated gene factors and transcription factor NF-κB. *Proc Natl Acad Sci USA* 1993; **90**: 3167–71.
13. Kalvakolanu D, Mannino S, Lindner DJ, *et al.* Synergistic modulation of IFN-stimulated gene expression by retinoic acid in embryonal carcinoma and breast tumor cells. *Proc Am Ass Cancer Res* 1994; **35**: 310 (abstr 1843).
14. Higuchi T, Hannigan G, Malkin D, *et al.* Enhancement by retinoic acid and dibutyryl cyclic adenosine 3′-5′-monophosphate of the differentiation and gene expression of human neuroblastoma cells induced by interferon. *Cancer Res* 1991; **51**: 3958–64.
15. Castaigne S, Chomienne C, Daniel MT, *et al.* All-*trans*-retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. *Blood* 1990; **76**: 1704–9.
16. Chen ZX, Xue YQ, Zhang R, *et al.* A clinical and experimental study on all-*trans*-retinoic acid-treated acute promyelocytic leukemia patients. *Blood* 1991; **78**: 1413–9.
17. Warrell RP Jr, Frankel SR, Miller WH Jr, *et al.* Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans*-retinoic acid). *N Engl J Med* 1991; **324**: 1385–93.
18. Lippman SM, Parkinson DR, Itri LM, Weber RS, *et al.* 13-*cis*-retinoic acid and interferon α-2a: effective combination therapy for advanced squamous cell carcinoma of the skin. *J Natl Cancer Inst* 1992; **84**: 235–41.
19. Lippman SM, Kavanagh JJ, Paredes-Espinoza M, *et al.* 13-*cis*-retinoic acid plus interferon α-2a in locally advanced squamous cell carcinoma of the cervix. *J Natl Cancer Inst* 1993; **85**: 499–500.
20. Lippman SM, Kavanagh JJ, Paredes M, *et al.* 13-*cis*-retinoic acid (13 cRA), interferon α-2a (IFN-α2a) and radiotherapy for locally advanced cancer of the cervix. *Proc Am Soc Clin Oncol* 1993; **12**: 257 (abstr).
21. Hovanessian AG. The double-stranded RNA-activated protein kinase induced by interferon: dsRNA-PK. *J Interferon Res* 1989; **9**: 641–7.
22. Hovanessian A. Interferon-induced and double-stranded RNA-activated enzymes: a specific protein

kinase and 2′-5′ oligoadenylate synthetases. *J Interferon Res* 1991; **11**: 199–205.
23. Meurs EF, Watanabey Y, Kadereit S, *et al.* Constitutive expression of human double-stranded RNA-activated p68 kinase in murine cells mediates phosphorylation of eukatyotic initiation factor 2 and partial resistance to encephalomyocarditis virus growth. *J Virol* 1992; **66**: 5804–14.
24. Saunders M, Gewert D, Tugwell M, *et al.* Human 2′-5′ A synthetase: characterization of a novel cDNA and corresponding gene structure. *EMBO J* 1985; **4**: 1761–8.
25. Silverman RH, Krause D, Jacobsen H, *et al.* 2′-5′ A dependent RNase levels vary with interferon treatment, growth rate and cell differentiation. In: Demaeyer F, Schellekens H, eds. *The biology of interferon system.* Amsterdam: Elsevier 1983: 189–200.
26. Krause D, Silverman RH, Jacobsen H, *et al.* Regulation of ppp(A2′p)n-dependent RNase levels during interferon treatments and cell differentiation. *Eur J Biochem* 1985; **146**: 611–8.
27. Hassel BA, Zhou A, Sotomayor C, *et al.* A dominant negative mutant of 2′-5′A-dependent RNase suppresses antiproliferative and antiviral effects of interferon. *EMBO J* 1993; **12**: 3297–305.
28. Kumar R, Atlas I. Interferon induces the expression of retinoblastoma gene product in human Burkitt's lymphoma Daudi cells: role in growth regulation. *Proc Natl Acad Sci USA* 1992; **89**: 6599–603.
29. Dinney PN, Eve BY, Fidler IJ. Inhibition of basic FGF expression by human transitional cell carcinoma following exposure to interferon-α. *Proc Am Ass Cancer Res* 1996; **37**: 56.
30. Lingen MW, Polverini PJ, Bouck NP. Synergy between retinoic acid and interferon-α in inhibiting angiogenesis induced by head and neck squamous cell carcinoma. *Proc Am Ass Cancer Res* 1996; **37**: 60.
31. Nicholson RC, Mader S, Nagpal S, *et al.* Negative of the rat stromolysin gene promoter by retinoic acid is mediated by an AP1 site. *EMBO J* 1990; **9**: L4443–54.
32. Schule P, Rangarajan P, Yang N, *et al.* Retinoic acid is a negative regulator of AP-1 responsive genes. *Proc Natl Acad Sci USA* 1991; **88**: 6092–6.
33. Grant S, Bhalla K, Weinstein IB, Pestka S, *et al.* Recombinant human interferon sensitized resistant myeloid leukemic cells to induction of terminal differentiation. *Biochem Biophys Res Commun* 1985; **130**: 379–88.
34. Hemmi N, Breitman T. Combinations of recombinant human interferons and retinoic acid synergistically induce differentiation of the human promyelocytic leukemia cell line HL-60. *Blood* 1987; **69**: 501–7.
35. Peck R, Bollag W. Potentiation of retinoic induced differentiation of HL-60 and U 937 cell lines by cytokines. *Eur J Cancer* 1991; **27**: 53–7.
36. Marth C, Daxenbichler G, Dapunt O. Synergistic antiproliferative effect of human recombinant interferons and retinoic acid in breast cancer cells. *J Natl Cancer Inst* 1986; **77**: 1197–202.
37. Lancillotti F, Affabris E, Fiorucci G, *et al.* Antiproliferative effects of retinoic acid (RA) and recombinant interferon α-2b on human cervix carcinoma cells. In: Fisher PB, Scott RE, eds. *Molecular and cellular differentiation.* Boca Raton, FL: CRC Press 1993: 464.
38. Agarwal C, Hembree JR, Rorke EA, *et al.* Interferon and retinoic acid suppress the growth of human papilloma virus type 16 immortalized cervical epithelial cells, only interferon suppresses the level of the human papilloma virus transforming oncogenes. *Cancer Res* 1994; **54**: 2108–12.
39. Kessler JF, Meyskens FL Jr, Levine N, *et al.* Treatment of cutaneous T-cell lymphoma (mycosis fungoides) with 13-*cis*-retinoic acid. *Lancet* 1983; **i**: 1345–7.
40. Kessler JF, Jones SE, Levine N, *et al.* Isotretinoin and cutaneous helper T-cell lymphoma (mycosis fungoides). *Arch Dermatol* 1987; **23**: 201–4.
41. Sporn MB, Squire RA, Brown CC, *et al.* 13-*cis*-retinoic acid: inhibition of bladder carcinogenesis in the rat. *Science* 1977; **195**: 487–9.
42. Studer UE, Biedermann C, Chollet D, *et al.* Prevention of recurrent superficial bladder tumors by oral etretinate. *J Urol* 1984; **131**: 47–9.
43. Alfthan O, Jaunhiainen K, Kangas L, *et al.* Superficial urinary bladder carcinoma: chemotherapy, immunotherapy, retinoids and prevention of relapses. *Int J Immunol* 1986; **11**: 5–17.
44. von Roenn J, von Gunten C, Mullane M, *et al.* All-*trans*-retinoic acid (ATRA) in the treatment of AIDS-related Kaposi's sarcoma: a phase II Illinois Cancer Center study. *Proc Am Soc Clin Oncol* 1993; **12**: 51 (abstr 6).
45. Meyskens FL Jr, Gilmartin E, Alberts DS, *et al.* Activity of isotretinoin against squamous cell cancers and preneoplastic lesions. *Cancer Treat Rep* 1982; **66**: 1315–9.
46. Lippman SM, Meyskens FL. Treatment of advanced squamous cell carcinoma of the skin with isotretinoin. *Ann Intern Med* 1987; **107**: 499–502.
47. Murad AM, Oliveira M, Saldanha TM. Phase II trial of isotretinoin and interferon α-2a in the treatment of advanced recurrent cervical carcinoma. *Int J Gynecol Cancer* 1994; **4**: 414–8.
48. Hallum AV, Alberts DS, Lippman SM, *et al.* Phase II study of 13-*cis*-retinoic acid plus interferon-alpha 2a in heavily pretreated squamous carcinoma of the cervix. *Gynecol Oncol* 1995; **56**: 382–6.

(Received 9 July 1996; accepted 5 August 1996)