Preclinical efficacy of Virulizin in human breast, ovarian and prostate tumor models

Caigan Du[a,b], Ningping Feng[a], Hongnan Jin[a], Ming Wang[a], Jim A. Wright[a] and Aiping H. Young[a]

Virulizin is a novel biological response modifier (BRM) approved for the treatment of melanoma and is currently in a phase III clinical trial against advanced pancreatic cancer. The purpose of this study was to define the anti-cancer activity of Virulizin against a number of solid human tumors. The therapeutic effect of Virulizin was evaluated in mouse xenograft models, and the results demonstrate that Virulizin has high efficacy against breast, ovarian and prostate tumor xenografts. Seventy-seven percent inhibition, with an optimal T/C value of 24.8%, was observed in human beast MDA-MB-231 xenografts treated with Virulizin as compared to saline-treated controls ($p=0.0004$). In human ovarian SK-OV-3 tumor xenografts, administration of Virulizin inhibited tumor growth by 77.6% compared to saline controls ($p=0.0439$). Furthermore, high anti-tumor activity was also demonstrated in DU145 and PC-3 prostate tumor xenografts, as indicated by 72.6 and 49.1% suppression of tumor growth (versus saline controls, $p=0.0007$ or $p=0.0049$), respectively. Direct comparisons with the anti-tumor activities of conventional drugs demonstrated that Virulizin has higher or equal efficacy against all four tumors tested. Finally, addition of Virulizin into co-cultures of tumor cells and macrophages stimulated the cytolytic activity of the macrophages against the tumor cells in a dose-dependent manner. This result suggests that stimulation of immune cells is at least part of the anti-tumor mechanism of action of Virulizin. These results clearly demonstrate that Virulizin inhibits the growth of human breast, ovarian and prostate tumors, indicating great potential for expansion of the clinical indications for this novel BRM. *Anti-Cancer Drugs* 14:289–294 © 2003 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2003, **14**:289–294

Keywords: breast cancer, immunotherapy, macrophage, ovary cancer, prostate cancer, Virulizin

[a]R & D Department, Lorus Therapeutics Inc., Toronto, Ontario, Canada.
[b]Present address: Division of Nephrology, Department of Medicine, LHSC, University of Western Ontario, London, Ontario, Canada.

Correspondence to A.H. Young, Lorus Therapeutics Inc., 2 Meridian Road, Toronto, Ontario M9W 4Z7, Canada.
Tel: +1 416 798-1200; fax: +1 416 798-2200;
e-mail: ahyoung@lorusthera.com

Received 13 December 2002 **Accepted** 10 January 2003

Introduction

Virulizin is a novel biological response modifier (BRM) obtained from bovine bile by a standardized extraction process (Lorus Therapeutics, Toronto, Canada). Addition of Virulizin into *in vitro* cultures stimulates human blood monocytes, peritoneal macrophages or alveolar macrophages to mediate a high level of cytotoxicity against tumor cells [1]. In preclinical studies, administration of Virulizin suppressed growth of human pancreatic and melanoma tumor xenografts in mice [2,3], and increased survival time in a murine melanoma model [1]. In a number of phase I/II clinical studies, with over 450 patients, Virulizin demonstrated an excellent safety profile. The data from clinical trials of advanced pancreatic cancer and melanoma showed that patients who received Virulizin treatment survived longer compared to historical controls [1]. Previous studies have focused exclusively on the anti-tumor efficacy of Virulizin against either pancreatic cancer or melanoma. In the current study, the anti-tumor activity of Virulizin was evaluated against human breast, ovary and prostate tumors in the mouse xenograft model.

Materials and methods

Drugs

Virulizin (registered trademark of Lorus Therapeutics) is an aqueous solution obtained from bovine bile by a standardized process involving solvent extraction and heat hydrolysis. The drug contains 5% (w/v) solid material, comprised of inorganic salts (95–99% of the dry weight) and organic compounds of molecular weights below 3000 Da (1–5% of the dry weight). Virulizin is provided as a sterile, injectable formulation. Studies are ongoing to identify all the organic and inorganic components in Virulizin. Doxorubicin was purchased from Pharmacia & Upjohn (Ontario, Canada), taxol (paclitaxel) was from Bristol-Myers Squibb Pharmaceutical (Montreal, Canada), cisplatin was from Faulding (Quebec, Canada) and novantrone (mitoxantrone) was from Wyeth-Ayerst Canada (Montreal, Canada).

Animals and cells

Mice (CD-1 athymic nude or SCID, 6–8 weeks old, female) were purchased from Charles River (Montreal, Canada), and maintained in the animal facility of

0959-4973 © 2003 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000065041.82984.f2

Sunnybrook and Women's College Health Sciences Center (Toronto, Canada). Human tumor cell lines (MDA-MB-231, SK-OV-3, DU145 and PC-3) were from the ATCC (Manassas, VA). Cells were grown in culture media, under conditions recommended in the ATCC technical datasheet. Peritoneal macrophages were isolated from thioglycollate-primed CD-1 mice as described previously [4], and grown in DMEM culture medium (Wisent, Quebec, Canada) supplemented with 10% FBS and 20% Ladmac supernatant [5]. The Ladmac supernatant harvested from Ladmac cell cultures after 5–7 days of incubation provides a source of colony stimulating factor (CSF)-1.

Cytotoxicity assay

Tumor cells were radiolabeled by the addition of 10 μl/ml of [methyl-^{3}H]thymidine (5.0 Ci/mmol;. Amersham Pharmacia Biotech, Quebec, Canada) into the culture medium. After an overnight incubation the labeled cells were washed 4 times with PBS. These target tumor cells (5000 cells/well in a U-bottom 96-well plate) were co-cultured with murine peritoneal macrophages at an effector:target (E/T) cell ratio of 10:1, which was in the plateau range on a curve prepared by varying the E/T ratio from 5:1 to 50:1 (not shown). The co-cultures were grown in DMEM medium supplemented with 10% FBS, 20% Ladmac supernatant and 1 ng/ml recombinant murine interferon-γ (eBioscience, San Diego, CA) in the presence or absence of Virulizin, in a total volume of 200 μl. After 24 h incubation at 37°C, the supernatant (150 μl) was collected and ^{3}H release quantitated using a Beta spectrometer. The specific cytotoxicity was calculated as follows: specific release (%) = $(E-S)/(T-S) \times 100$, in which E is c.p.m. released from target cells in the presence of effector cells, S is c.p.m. released from target cells in the absence of effector cells and T is c.p.m. released from target cells after treatment with 10% sodium dodecylsulfate.

Xenograft tumor model

Before reaching approximately 80% confluence in cell culture medium, tumor cells were harvested and resuspended in sterile PBS. Ten million tumor cells, in 100 μl, were s.c. implanted into the right flank of mice (20–28 g body weight). MDA-MB-231 and SK-OV-3 cells were grown in CD-1 athymic nude mice, while DU145 and PC-3 were implanted in SCID mice. The animals were monitored daily. Treatment started when the tumors reached a volume of 50–100 mm^3. The animals were randomly separated into groups of at eight to 15 animals, so that the mean tumor size distribution was the same in each group. Animals were treated with Virulizin, saline or standard chemotherapeutic drugs until the endpoint of each experiment. The doses and treatment schedules were as described in the text. All animal experimentation was performed following the National Institutes of Health, Sunnybrook and Women College Health Science Center, and Lorus Therapeutics Inc. Animal Care and Use guidelines.

Evaluation of anti-tumor activity

Tumor volume was estimated by caliper measurements, using the formula: length $\times$ width $\times$ height/2 [6]. The efficacy of drug treatment was evaluated based on the following calculations: (i) tumor growth inhibition (T/C), calculated as a percentage of the mean tumor volume of drug-treated (T) versus control (C) groups: T/C (%) = (mean tumor volume of drug-treated group/mean tumor volume of control) $\times$ 100; the optimal value being the minimal T/C ratio which reflects the maximal tumor growth inhibition achieved [7]; (ii) tumor growth delay (T – C), defined as the difference in time for drug-treated (T) and control (C) tumors to reach a given volume (300 mm^3); and (iii) the final tumor weight, determined by the mass of tumor tissue surgically excised from the animal on the last day of the experiment. The percentage of inhibition (%) = (mean tumor weight of controls – mean tumor weight of drug-treated group)/mean tumor weight of controls $\times$ 100. Statistical analyses of the differences in tumor weight between treatment groups were carried out by the Biostatistical Consulting Unit of the Department of Community Health Sciences at the University of Manitoba (Winnipeg, Canada). A p value ≤ 0.05 was considered to be statistically significant.

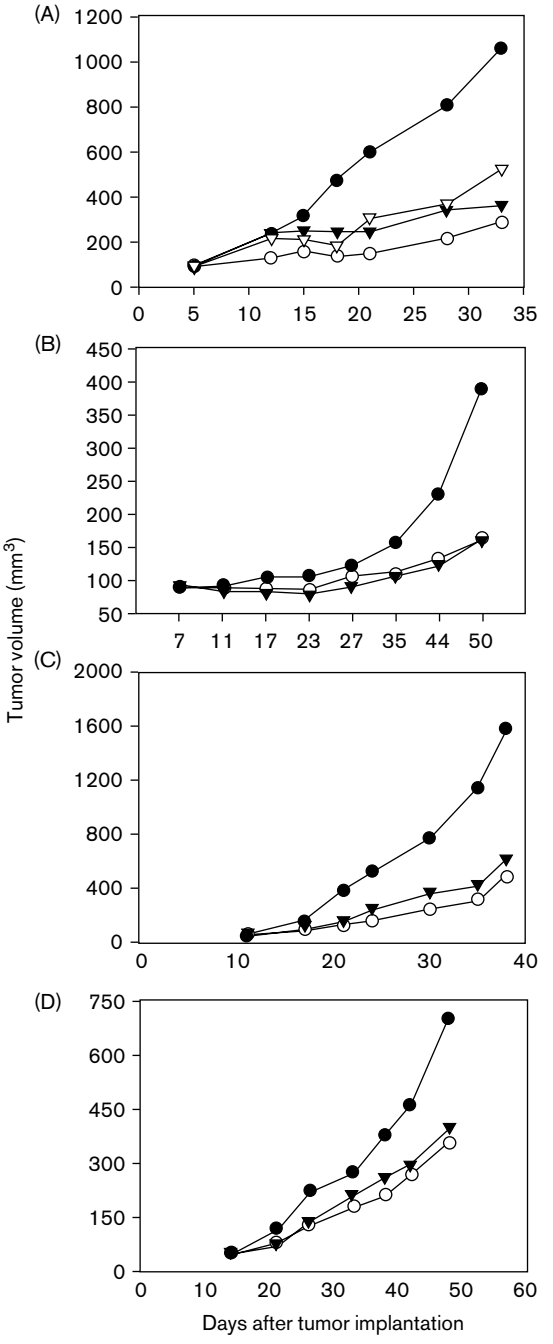
Results

Anti-tumor efficacy of Virulizin in human breast, ovarian and prostate tumor xenograft models

The therapeutic efficacy of Virulizin, as a single agent, against human tumors (MDA-MB-231, SK-OV-3, DU145 and PC-3) xenografted in mice was tested and compared with standard chemotherapeutic drugs. The results are shown in Figure 1 and summarized in Table 1. Administration of Virulizin resulted in a significant delay of MDA-MB-231 breast tumor growth as compared to saline-treated controls (Fig. 1A). This is indicated by a significant optimal T/C value of 24.8% and a T – C value of 20 days. The mean tumor weight of Virulizin-treated animals was decreased by 77% at the endpoint of the experiment as compared to that of saline controls ($p = 0.0004$) (Table 1). In comparison with the tumor growth, inhibition by standard chemotherapeutic drug treatments at an optimal dose, 69.4% of tumor weight reduction by doxorubicin, or 53.2% of tumor weight reduction by taxol, the efficacy of Virulizin was higher than observed in the treatment with either doxorubicin or taxol (Table 1).

In studies with SK-OV-3 ovarian tumor xenografts, Virulizin treatment resulted in a significant delay of tumor growth compared to saline controls (Fig. 1B). This

Fig. 1



Anti-cancer activities of Virulizin and clinical use drugs in a panel of human breast, ovarian and prostate xenografted tumors. Ten million tumor cells were s.c. implanted into each mouse on day 0. The treatment started on: (A) day 5, MDA-MB-231 breast carcinoma; (B) day 7, SK-OV-3 ovary carcinoma; (C) day 12, DU145 prostate carcinoma; and (D) day 14, PC-3 prostate carcinoma. The dosage of each injection and route of administration for each agent were as follows: (solid circles) 0.2 ml saline/day, i.p.; (open circles) 0.2 ml Virulizin/day, i.p.; (triangles) 0.5 mg taxol in 0.1 ml saline (20 mg/kg)/week, i.v.; (open squares) 0.125 mg doxorubicin in 0.1 ml saline (5 mg/kg)/week, i.v.; (solid squares) 0.1 mg cisplatin in 0.1 ml saline (4 mg/kg)/week, i.v.; and (inverted triangles) 0.02 mg novantrone in 0.1 ml saline (0.8 mg/kg)/week, i.v. In each group of treatment, $n = 10$. The data are representative of at least two independent experiments.

was indicated by a T/C value of 41.2%, with a minor regression from day 17 to 23 (Table 1). The mean tumor weight was decreased significantly by 77.6% in the Virulizin-treated mice as compared to saline-treated controls ($p = 0.0118$), while cisplatin treatment demonstrated a similar effect on the mean tumor growth as Virulizin (Fig. 1B) and the decrease in tumor weight was also statistically significant compared to saline-treated animals ($p = 0.0443$) (Table 1). These results demonstrate that administration of Virulizin effectively suppresses the growth of SK-OV-3 ovarian tumors in the xenograft mouse model and that the efficacy of Virulizin is on par with that of cisplatin.

The therapeutic effect of Virulizin on prostate cancer was tested in two prostate tumor models (DU145 and PC-3). In DU145 xenografts (Fig. 1C), Virulizin treatment retarded tumor growth with an optimal T/C value of 26.1% and a T – C value of 16 days, as compared to saline controls (Table 1), indicating a high level of anti-tumor activity against DU145 prostate tumors. The treatment with Virulizin resulted in 72.6% inhibition in mean tumor weight compared to saline ($p = 0.0007$). The anti-tumor activity of Virulizin was slightly higher than the conventional drug novantrone, which gave a 50.8% decrease in mean DU145 tumor weight compared to saline control, a 33.4% T/C value and a T – C value of 8 days. To further examine the efficacy of Virulizin against prostate tumors, Virulizin was tested against PC-3 prostate tumor xenografts (Fig. 1D). Virulizin showed a high level of anti-tumor activity against this tumor as indicated by a 50.9% optimal T/C value and a T – C value of 10 days (Table 1). The mean tumor weight was decreased by 49.1% in Virulizin-treated animals as compared to saline-treated controls ($p = 0.0049$). As compared to the treatment with novantrone, which demonstrated anti-tumor efficacy with an optimal T/C value of 56.8%, a T – C of 8 days and 42.9% decrease in tumor weight in this PC-3 tumor; again, Virulizin demonstrated superior anti-tumor efficacy compared to novantrone.

Stimulation of macrophage cytotoxicity against tumor cells

In order to understand the mechanism of Virulizin anti-tumor action *in vivo*, the cytotoxicity and immunoregulatory functions of Virulizin were examined *in vitro*. No direct cytotoxic effect or growth retardation was observed with addition of Virulizin [up to 5% Virulizin (v/v)] into the culture medium of breast, ovary or prostate tumor cells (data not shown). The potential of Virulizin to stimulate the cytolytic activity of macrophages against tumor cells was evaluated using a co-culture cytotoxicity assay. As indicated in Figure 2, the presence of Virulizin in co-cultures of macrophages with tumor cells stimulated the cytolytic activity of macrophages against all four tumor cell lines. The effect was dose-dependent with a

Table 1 Summary of Virulizin efficacy against xenografted human breast, ovarian and prostatic tumors

Tumor	Drug ($n=10$)	Optimum T/C (%) [day]	T − C (days) or MR [period]	Mean tumor weight (mg)	Inhibition (%)	p value (versus saline)
MDA-MB-231	saline			1649		
	virulizin	24.8 [21]	20	378.8	77	0.0004
	doxorubicin	34.4 [33]	10	505.3	69.4	0.0008
	taxol	38.6 [18]	7	771	53.2	0.0107
SK-OV-3	saline			500		
	virulizin	41.2 [49]	MR [17–23]	112	77.6	0.0118
	cisplatin	41.7 [49]	MR [17–23]	195	61	0.0443
DU145	saline			1401		
	virulizin	26.1 [35]	16	381	72.6	0.0007
	novantrone	33.4 [30]	8	690	50.8	0.0295
PC-3	saline			629		
	virulizin	50.9 [48]	10	320	49.1	0.0049
	novantrone	56.8 [48]	8	359	42.9	0.0141

Body weight loss was not observed in any animal treated with saline or Virulizin alone. The tumor growth delay (T − C) was calculated at mean tumor size of 300 mm^3. MR=minor regression.

maximum increase in cytotoxicity observed for all cultures at a concentration of 2.5% (v/v) Virulizin. Macrophage-mediated cytotoxicity in co-cultures with breast tumor MDA-MB-231 cells was 28.5 ± 4.3% in the absence of Virulizin. This was increased to 38.3 ± 5.1% in the presence of 2.5% (v/v) Virulizin, an increase of 34.4% (Fig. 2A). Similarly, the cytotoxicity in co-cultures with ovarian tumor SK-OV-3 cells, in the presence of 2.5% (v/v) Virulizin, was increased by 72.5% compared to that of cultures in the absence of Virulizin (from 13.8 ± 2.2% in the absence of Virulizin to 23.8 ± 3.7% in the presence of Virulizin) (Fig. 2B). In co-cultures of macrophages with prostate tumor cells, an increase of 32.8% in macrophage-mediated cytotoxicity (from 17.7 ± 1.7% in the absence of Virulizin to 23.5 ± 3.0% in the presence of 2.5% Virulizin) was observed against DU145 prostate tumor cells (Fig. 2C), while macrophage-mediated cytotoxicity increased more than 300%, from 7.3 ± 0.7% (absence of Virulizin) to 24.2 ± 3.9% (2.5% Virulizin), against PC-3 prostate tumor cells (Fig. 2D).

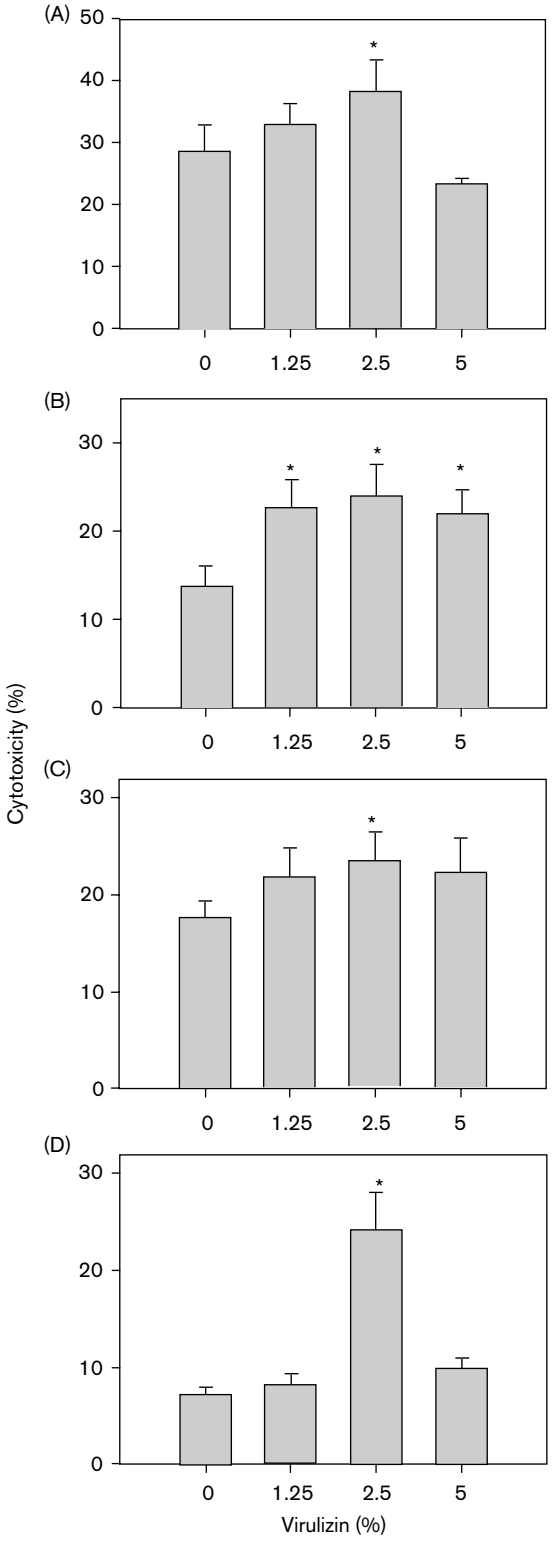
Discussion

The results of previous preclinical studies and phase I/II clinical trials indicated that Virulizin treatment delayed the growth of human pancreatic tumors and melanoma [1–3]. The present study extended the investigation of the anti-tumor efficacy of Virulizin against other types of human tumors. Consistent with previous studies, Virulizin had a high level of anti-tumor activity against human breast, ovarian and prostate tumor xenografts. Furthermore, in comparison with the efficacies of standard chemotherapeutic drugs administered concurrently, the anti-tumor activity of Virulizin was equal or superior to that of doxorubicin, taxol, cisplatin and novantrone. Finally, while Virulizin did not directly affect the growth of tumor cells *in vitro*, it stimulated macrophage-mediated cytotoxicty against all four tumor cell lines tested in the xenograft model.

To date, the mechanism of Virulizin anti-tumor action has not been fully elucidated. Previous studies demonstrated that Virulizin does not act as a direct cytotoxic agent. Addition of Virulizin (up to 50 μl in a total of 200 μl) into cultures of Calu-6 (human non-small cell lung cancer) or PANC-1 (human pancreatic carcinoma) cells did not demonstrate any cytotoxicity against the tumor cells [2]. Rather, Virulizin was found to be a monocyte/macrophage activator, as indicated by its ability to stimulate monocyte/macrophage-mediated tumor cell killing, similar to granulocyte macrophage colony stimulating factor (GM-CSF), but not stimulate human peripheral lymphocyte proliferation or cytotoxicity against tumor cells [1]. Consistent with a role in monocyte activation, the potency of Virulizin is determined by its stimulation of tumor necrosis factor-α production by a human monocytic cell line (U-937). In agreement with previous studies, the data presented here demonstrate that Virulizin does not act as a direct cytotoxic agent against MDA-MB-231, SK-OV-3, DU145 and PC-3 tumor cell lines. Given that Virulizin increased mouse macrophage-mediated cytotoxicity against these human tumor cells *in vitro*, it is reasonable to propose that the anti-tumor activity of Virulizin, observed *in vivo*, is due in part to an immunomodulating activity.

In addition, results from the present study and previous studies [2,3] demonstrate that administration of Virulizin effectively suppresses growth of many different human tumors transplanted in athymic nude or SCID mice, which are immune deficient (T lymphocytes [8,9] or both T and B lymphocytes [10], respectively). Therefore, the anti-tumor efficacy of Virulizin observed in these models is likely due to activation of macrophages. Furthermore, mitogenesis of T/B lymphocytes was enhanced in Virulizin-treated normal mice [2], suggesting that Virulizin activation of macrophages and the subsequent production of cytokines could act as an intermediate to the stimulation of T/B lymphocytes.

Fig. 2



Stimulation of cytotoxicity of peritoneal macrophages by Virulizin against human cancer cell lines: co-cultures of [^{3}H]labeled tumor cells (T: target) with macrophages (E: effector) were incubated at a ratio (E/T) of 10:1 in the absence (vehicle PBS only: control) or presence of Virulizin for 24 h. (A) breast carcinoma MDA-MB-231 cells, (B) ovarian carcinoma SK-OV-3 cells, (C) prostate carcinoma DU145 cells and (D) prostate carcinoma PC-3 cells. *$p < 0.05$ (versus controls) ($n = 6$). The data represent the mean ± SD of representative of three separate experiments.

Activation of the immune response is an attractive approach for the treatment of cancer because of potentially greater specificity and lower toxicity. Earlier research has indicated that residual T cells, NK cells and macrophages contribute to an active immune reaction against human tumor xenografts in mice [11]. In addition, anti-tumor immunity against murine B16 melanoma requires macrophages for either processing of antigen or cytokine production [12]. Moreover, in clinical studies of antibody therapy against tumor cells, macrophages were found to play a role in the mechanism of antibody-mediated tumor killing [13,14]. Taken together these studies suggest that activation of macrophages could be a viable strategy for the development of anti-cancer immunotherapeutics. GM-CSF is a potent activator of the natural cytotoxic activity of macrophages [15]. Dranoff *et al.* were the first to test the anti-tumor efficacy of more than 10 known immune-activating cytokines in a non-immunogenic murine tumor, B16F10 melanoma. Only one cytokine, GM-CSF, stood out as a potent anti-tumor agent against this very aggressive tumor [16]. In clinical trials of patients with prostate cancer, administration of GM-CSF as a single agent [17] or in combination with a bi-specific antibody, MDX-H210 (anti-HER2 × CD64) [18] demonstrated that GM-CSF has anti-tumor activity against advanced prostate cancer. Furthermore, clinical studies showed that the use of GM-CSF, in combination with chemotherapeutic agents, was associated with a substantial response rate in ovarian cancer patients for whom there was a poor prognosis [19,20]. These studies highlight the potential for immunotherapy that acts via activation of macrophages in the treatment of cancer. Similar to GM-CSF, Virulizin was found to stimulate macrophage/monocyte-mediated cytotoxicity against tumor cells, supporting the clinical development of Virulizin as a cell-mediated immunotherapeutic for breast, ovarian and prostate cancers.

In conclusion, human tumor xenograft models are widely used as tumor models in assessment of new therapeutics and improvement of current treatment strategies. Administration of Virulizin showed anti-tumor efficacy in the treatment of human pancreatic cancers and melanoma in previous preclinical studies and phase I/II clinical trials [1–3]. In addition to a recent study, which demonstrated that Virulizin along with OPLA-Pt, a polymer system for releasing high concentrations of cisplatin, significantly decreased the local recurrence of breast cancer after surgery in a murine mammary carcinoma model [21], the results presented here not only support previous findings that suggest Virulizin is a promising new immunotherapeutic agent, but also strongly support the potential for expanding clinical treatment using Virulizin for human breast, ovarian and prostate cancers.

Acknowledgments

We thank Vivian Lee for excellent technical assistance, and Drs Ming-Yu Cao and Aikaterini Vassilakos and Viengthong Lam for critical reading of the manuscript.

References

1 Ferdinandi ES, Braun DP, Liu C, Zee BC, Ely G. Virulizin—a review of its antineoplastic activity. *Exp Opin Invest Drugs* 1999; **8**:1721–1735.
2 Liu C, Ferdinandi ES, Ely G, Joshi SS. Virulizin-2γ, a novel immunotherapeutic agent, in treatment of human pancreatic cancer xenografts. *Int J Oncol* 2000; **16**:1015–1020.
3 Feng N, Jin H, Wang M, Du C, Wright JA, Young AH. Antitumor activity of Virulizin, a novel biological response modifier (BRM), in a panel of human pancreatic cancer and melanoma xenografts. *Cancer Chemother Pharmacol* 2002; in press.
4 Mishell BB, Shiigi SM. *Selected Methods in Cellular Immunology*. San Francisco, CA: Freeman; 1980.
5 Du C, Sriram S. Induction of interleukin-12/p40 by superantigens in macrophages is mediated by activation of nuclear factor-κB. *Cell Immunol* 2000; **199**:50–57.
6 Tomayko MM, Reynolds CP. Determination of subcutaneous tumor size in athymic (nude) mice. *Cancer Chemother Pharmacol* 1989; **24**:148–154.
7 Hendriks HR, Langdon S, Berger DP, Breistol K, Fiebig HH, Fodstad O, *et al.* Comparative antitumor activity of vinblastine-isoleucinate and related vinca alkaloids in human tumor xenografts. *Eur J Cancer* 1992; **28A**:767–773.
8 MacDonald HR, Lees RK. Frequency and specificity of precursor of interleukin 2-producing cells in nude mice. *J Immunol* 1984; **132**:605–610.
9 MacDonald HR, Blanc C, Lees RK, Sordat B. Abnormal distribution of T cell subsets in athymic mice. *J Immunol* 1986; **136**:4337–4339.
10 Bosma GC, Custer RP, Bosma MJ. Severe combined immunodeficiency mutation in the mouse. *Nature* 1983; **302**:527–530.
11 Silobrcic V, Zietman AL, Ramsay JR, Suit HD, Sedlacek RS. Residual immunity of athymic NCr/Sed nude mice and the xenotransplantation of human tumors. *Int J Cancer* 1990; **45**:325–333.
12 Wu TY, Fleischmann Jr WR. Murine B16 melanoma vaccination-induced tumor immunity: identification of specific immune cells and functions involved. *J Interferon Cytokine Res* 2001; **21**:1117–1127.
13 Adams DO, Hall T, Steplewski Z, Koprowski H. Tumors undergoing rejection induced by monoclonal antibodies of the IgG2a isotype contain increased numbers of macrophages activated for a distinctive form of antibody-dependent cytolysis. *Proc Natl Acad Sci USA* 1984; **81**:3506–3510.
14 Shetye J, Frodin JE, Christensson B, Grant C, Jacobsson B, Sundelius S, *et al.* Immunohistochemical monitoring of metastatic colorectal carcinoma in patients treated with monoclonal antibodies (Mad 17-1A). *Cancer Immunol Immunother* 1988; **27**:154–162.
15 Masucci G, Wersall P, Ragnhammar P, Mellstedt H. Granulocyte-monocyte-colony-stimulating factor augments the cytotoxic capacity of lymphocytes and monocytes in antibody dependent cellular cytotoxicity. *Cancer Immunol Immunother* 1989; **29**:288–292.
16 Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, *et al.* Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci USA* 1993; **90**:3539–3543.
17 Small EJ, Reese DM, Um B, Whisenant S, Dixon SC, Figg WD. Therapy of advanced prostate cancer with granulocyte-macrophage colony-stimulating factor. *Clin Cancer Res* 1999; **5**:1738–1744.
18 James ND, Atherton PJ, Jones J, Howie AJ, Tchekmedyian S, Curnow RT. A phase II study of the bispecific antibody MDX-H210 (anti-HER2 × CD64) with GM-CSF in $HER2^+$ advanced prostate cancer. *Br J Cancer* 2001; **85**:152–156.
19 Reed E, Janik J, Bookman MA, Rothenberg M, Smith J, Young RC, *et al.* High-dose carboplatin and recombinant granulocyte macrophage colony-stimulating factor in advanced-stage recurrent ovarian cancer. *J Clin Oncol* 1993; **11**:2118–2126.
20 McClay EF, Bradly PD, Kirmani S, Plaxe SC, Kim S, McClay ME, *et al.* A phase II trial of intraperitoneal high-dose carboplatin and etoposide with granulocyte macrophage-colony stimulating factor support in patients with ovarian carcinoma. *Am J Clin Oncol* 1995; **18**:23–26.
21 Morello E, Dernell WS, Kuntz CA, LaRue SM, Lafferty M, Nelson A, *et al.* Evaluation of cisplatin in combination with a biological response modifier in a murine mammary carcinoma model. *Cancer Invest* 2002; **20**:480–489.