Progesterone Receptor Antagonist Therapy Has Therapeutic Potential Even in Cancer Restricted to Males as Evidenced from Murine Testicular and Prostate Cancer Studies

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Abstract. *Background: Treatment with the progesterone receptor antagonist mifepristone has been shown to improve the length and quality of life in mice with spontaneous leukemia, breast cancer, and lung cancer. The present study evaluated the efficacy of mifepristone therapy in murine tumors restricted to males, i.e. testicular and prostate cancer. Materials and Methods: Eight-week-old mice with a strong predisposition to testicular or prostate cancer were gavaged with mifepristone. Olive oil was used in place of mifepristone in order to provide a control. Survival rates and body conditioning scores were compared after one year of treatment. Results: Non-significant trends in survival rates were found in both types of murine cancers. Mifepristone significantly reduced the number of sick days in mice with testicular cancer. There was a significant reduction of adverse events (i.e. a tumor >1 cm or bleeding from the penis) in those with prostate cancer treated with mifepristone. Conclusion: These data support the hypothesis that various cancers may utilize a mechanism that is present in normal pregnancy that involves secretion of a progesterone-induced protein that blocks natural killer cell activity. The hypothesis that the cancer cells have the capacity to direct local progesterone production is supported by demonstrating the benefit of a progesterone receptor antagonist in tumors restricted to males.*

A hypothesis has been presented previously that various cancers may 'borrow' a mechanism that is active in normal pregnancy to escape immune surveillance especially by natural killer cells, which could lead to a novel type of immunotherapy of cancer (1, 2). Support for this hypothesis was provided by a study showing that a large variety of human leukemia cell lines express messenger RNA for this unique pregnancy protein called the progesterone-induced blocking factor (PIBF) (3). Furthermore, the progesterone receptor antagonist mifepristone was found to down-regulate the expression of PIBF protein by some of these leukemia cell lines (3). As an extension of these cell line studies, mifepristone has been used to treat *in vivo* AKR/J mice with spontaneous lymphocytic leukemia and improved survival and quality of life was noted (4).

A dramatic improvement in mice with progesterone receptor-positive breast cancer was noted after mifepristone therapy and the beneficial effect was attributed to its blockage of the growth stimulus provided by progesterone attaching to its receptor (5). However, the hypothesized ubiquitous use of mifepristone for treating various types of cancer is not based on the presence of the progesterone receptors in the tumor cells, but the abrogation of PIBF expression by gamma/delta T-cells in the tumor microenvironment that have had *de novo* progesterone receptors induced by exposure to the allogeneic stimulus of the tumor (1, 2). Indeed, gavaging mifepristone was found to improve length and quality of life in mice with spontaneous lung cancer not known to be progesterone receptor positive (6).

According to the hypothesis, the beneficial effect of the mifepristone was its inhibition of the progesterone receptor induced on the gamma/delta T-cells in the tumor microenvironment, which in turn suppressed PIBF protein expression and allowed natural killer cells to attack the lung cancer cells (6).

Both male and female mice with lung cancer have been shown to respond to mifepristone therapy (6). The purpose of the present study was to determine if treatment with mifepristone is able to provide a similar beneficial effect in mice with spontaneous cancer types that are only present within the male species. If such benefit can be found, it would further support the hypothesis that cancer cells

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directly are capable of producing or directing the secretion of progesterone rather than the ovaries which are the normal organs producing progesterone (1, 2, 7-9). Thus, the present study evaluated the efficacy of mifepristone therapy on prolonging length and quality of life in mice with spontaneous testicular or prostate cancer.

Materials and Methods

Thirty C57BL/6 mice with a strong predisposition for prostate cancer and 50 129P3/J mice with a strong predisposition for testicular cancer were assigned at 6 weeks to be gavaged three times per week with either mifepristone 0.3 mg in 3 ml olive oil or 3 ml olive oil only (controls). Treatment began at eight weeks of age. Quality of life was measured three ways: the number of days sick with a body conditioning score (BCS) <4, tumor >1 cm diameter, and bleeding from the penis. The BCS was defined as follows: BCS 5: mouse obese and bones cannot be felt; BCS 4: mouse is well fleshed and bones are barely felt; BCS 3: the mouse is in suboptimal condition, bones are palpable but not prominent (divided into 3+ and 3–); BCS 2: mouse is becoming thin and bones are prominent; BCS 1: mouse is emaciated, skeletal structure extremely prominent, little or no flesh cover.

The BCS of the mice were determined daily by vivarium rather than research staff since. the vivarium staff were not aware as to which animals received mifepristone or not. Members of the vivarium staff euthanized any mice with BCS <3. If the mouse had a tumor >1 cm or was bleeding from the penis then this was considered an adverse event.

Survival was defined as mice that were still alive at the end of one year of treatment. The survival analysis considered death as either expired or euthanized. The time until death was defined as the number of days from first treatment until the mouse was euthanized or expired naturally. Mice alive at the end of the study were considered censored. Kaplan-Meier estimates at the end of the year were computed using SPSS V16.0 for Windows (SPSS, Inc. Chicago, IL, USA) for each treatment. Log-rank tests were used to compare the survival rates by treatment.

Results

Prostate cancer. All 15 mice assigned to mifepristone were alive at eight weeks when treatment began but there were only 12 controls that survived. Though 6/15 of mifepristone-treated mice were still alive at the end of one year of treatment *vs*. only 2/12 of the controls, the Kaplan-Meier estimate of survival rate was not statistically significant (log-rank test, p=0.620), likely due to the small sample size. The mean number of days sick (BCS <4) was 59+3.6 days for mifepristone-treated mice *vs*. 42+2.5 for the controls (t-test, p-value, 0.694). The distribution of number of sick days was skewed to the right. Although a few mice were sick for a maximum of 46 days, over half of the mice did not exhibit any signs of illness. Thus the median number of sick days was 0 for the treated group (0-46) and 0 (0-23) for controls (Mann-Whitney, p=0.943).

No adverse events (tumor >1 cm in diameter or bleeding from penis occurred in 11 out of 15 mifepristone-treated mice but in only 3 out of 12 controls (Fisher's exact test, p=0.03).

Testicular cancer. Twenty-three mice assigned to mifepristone were alive compared to 22 controls by the time of treatment at 8 weeks of age. After 1 year of treatment animals were alive as follows: mifepristone, 20/23 (one natural death, two euthanized); controls, 15/22 (two natural deaths, five euthanized). The Kaplan-Meier estimate of survival rate following one year of treatment for mifepristone-treated mice was 87.0% (standard error, SE 7.0%) *vs*. 66.3% (SE 12.6%) for controls. There was insufficient power to demonstrate statistical significance (log-rank test, p=0.128).

The mean number of days sick (BCS <4) were 8.4±5.5 with mifepristone therapy *vs*. 33.0±9.4 for controls (p=0.031). The median number of days sick were 0 (0-128) for mifepristone treatment *vs*. 14 (0-149) for controls (Mann-Whitney, p=0.005). No adverse events were found in 21 out of 23 mifepristone-treated mice *vs*. 18 out of 22 controls (p=0.6, chi-square analysis).

Discussion

The theory for using mifepristone to treat prostate and testicular cancer is not based on any evidence that these tumors are progesterone receptor-positive. Instead the theory is that all or most tumors may evade natural killer cell surveillance, similar to the pregnant state, by stimulating gamma/delta T-cells in the tumor microenvironment to express an immunomodulatory protein that is progesterone dependent.

In fact, it has previously been demonstrated that 27 different human leukemia cell lines have mRNA for PIBF and that mifepristone can down-regulate their secretion of PIBF (3). White blood cell cancers may indeed have the capacity to develop progesterone receptors when exposed to an allogeneic stimulus and then actually secrete PIBF protein, and thus suppress local NK cell activity.

Solid tumors, *i.e*., prostate and testicular cancer, would not be likely to develop progesterone receptors or secrete PIBF protein. Instead the tumor cells may actually be able to secrete human chorionic gonadotropin which may allow the cancer cells to produce a high local concentration of progestins (1, 2, 7-9).

The allogeneic stimulus of the tumor cells causes the induction of progesterone receptors in gamma/delta T-cells in the tumor microenvironment. The progesterone secreted by the tumor cells attaches to the progesterone receptor and subsequently stimulates PIBF protein expression, which suppresses NK cell activity similar to how NK cell activity is suppressed in the vicinity of the fetus (1, 2, 10-16). Thus the use of a progesterone receptor antagonist would theoretically prevent the progesterone or progesterone-like substance made by the tumor from interacting with the P receptor on the gamma/delta T cells induced *de novo* by the allogeneic stimulus of foreign tumor antigens and thus inhibit PIBF expression.

Even if this elaborate hypothesized mechanism is not operational, the data are consistent with other studies showing an improvement of length and quality of life in other spontaneous murine cancer models, *e.g.*, leukemia, breast, and lung (4-6). Anecdotally it has proven effective in advanced metastatic human cancers not known to be progesterone receptor-positive (17, 18). Although in the case of prostate cancer there was no improvement in the BCS relative to the controls, there were significantly smaller tumors and less total adverse events in the mifepristone-treated mice.

The data from the current study demonstrate efficacy in improving length and quality of survival in spontaneous murine cancer models. Demonstrations that mifepristone suppresses tumors in cancers that are specific to males further supports the concept that the tumor cells themselves can direct or secrete progesterone and further supports the concept of the induction of progesterone receptors in gamma/delta T-cells by the allogeneic stimulus of the tumor cells. Hopefully these studies will lead to larger controlled studies in humans.

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