

Mifepristone Treatment Improves Length and Quality of Survival of Mice with Spontaneous Lung Cancer

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Abstract. *Background:* There are data showing that human leukemia cell lines have mRNA for an immunomodulatory protein found in normal pregnancy that is stimulated by progesterone. One of the functions of this progesterone-induced blocking factor (PIBF) is to suppress natural killer cell activity. Mifepristone has not only been shown to down-regulate PIBF expression by human leukemic cell lines but has also been shown to prolong and improve the length of life of mice with spontaneous leukemia. *Materials and Methods:* Mifepristone at 0.3 mg was gavaged three times weekly from 8 weeks vs. olive oil in the controls. *Results:* The survival at one year for mice treated with mifepristone was 57.6% vs. 26.6% for controls ($p=0.056$). There were 66.7% of mice treated with mifepristone with no sick days whereas there was not one control mouse that did not become sick within the first year. The mean number of days sick was 11.6 for mifepristone vs. 57.6 days for controls ($p=0.05$) and 66.7% of the survival group had no sick days vs. none of the controls. *Conclusion:* These data suggest a possible novel cancer therapy using progesterone receptor antagonist drugs even in tumors not known to have progesterone receptors to try to change the tumor microenvironment and re-activate suppressed natural killer cells.

In order for cancer to grow, proliferate, and spread, the production of unique molecules that allow growth, angiogenesis, invasion and escape from immune surveillance is required. The hope in thwarting the progression of cancer is to find unique molecules that are needed for the

accomplishment of these goals but not for the growth of normal cells making up the various organs of the body.

The immune system works to inhibit cancer through a combination of processes that involve adaptive and innate immunity. In addition, the immune system works to rid the body of transformed cells through a process called immunoediting. This process involves three main steps: elimination, equilibration (immunosurveillance), and escape (1). The two main types of cells that tumors must elude are cytotoxic T cells and natural killer (NK) cells.

Similarly to cancer cells the fetus needs to escape immunosurveillance. The fetus seems to be particularly sensitive to attack by NK cells since they are the predominant cell invading the spontaneously aborted fetus. To maintain a pregnancy, all mammals require progesterone secretion throughout the entire pregnancy (2). At least one of the important functions of progesterone is to allow the expression of an immunomodulatory protein by gamma/delta T-cells that have had progesterone receptor (PR) induction by the allogeneic stimulus of the fetus. This immunomodulatory protein is called the progesterone induced blocking factor (3). Spontaneously aborted pregnancies are associated with low levels of PIBF expression (3, 4). Since PIBF inhibits NK cell cytolytic activity (at least partially by inhibiting the release of perforin from storage granules of NK cells), the loss of normal pregnancy with low progesterone levels may well be related to NK cell immune destruction of the fetus because of a deficiency in PIBF expression by the mother (5-7).

It has been hypothesized that cancer may use a similar mechanism to that in pregnancy to escape NK cell immune surveillance (8). It was proposed that some types of cancer may be initiated and continue to evade NK cells by secreting a progesterone-like steroid. This steroid interacts with PR, which have been induced on gamma/delta T-cells by the allogeneic stimulus of the tumor cells, and leads to PIBF protein expression by the gamma/delta T-cells (8). Luteinizing

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hormone made by the pituitary gland and human chorionic gonadotropin (hCG) hormone made by the placenta are both capable of making progesterone. There are data suggesting that many, if not most, tumors make hCG, and this may provide the means to produce progesterone (9-11).

The possibility thus exists that progesterone could represent a unique molecule which may be needed for the growth of tumor cells, by helping them to evade surveillance by NK cells, but which is not needed for the growth of normal cells. We therefore hypothesized that if one could inhibit the attachment of the progesterone secreted by the tumor cells to PRs induced in gamma/delta T-cells by the allogeneic stimulus of the tumor, one could inhibit the expression of PIBF from these gamma/delta T-cells and thus remove the block from NK cell cytolysis of the tumor cells (8). Theoretically this could be achieved by treatment with a PR antagonist *e.g.* mifepristone (12-14).

Mifepristone has been shown to improve quality and length of survival in AKR/J mice with spontaneous leukemia (15). Since mifepristone was also shown to suppress PIBF expression in four human leukemic cell lines that are found to secrete PIBF, it is possible that mifepristone would only prove effective for white blood cell cancer and not solid tumors, at least not those without PRs (16). Thus, the present study was initiated to determine if mifepristone could improve the length and quality of life in mice with a high frequency of lung tumors similar to AKR/J mice with leukemia. Since lung cancer is not known to be hormonally sensitive and not possessing PRs, any positive effect on improving survival would be assumed to be related to its effect on the tumor microenvironment.

Materials and Methods

The strain of mouse used was A/J which are mice with a high rate of spontaneous lung cancer. They were 8 weeks of age, and were inbred; males and females were housed in cages with 3-4 mice per cage. In the instance that the mice appeared to be fighting, mice were placed into separate cages to avoid injury. The mice were divided evenly, half of the group receiving mifepristone and the other half receiving olive oil (control group). The mice were divided at random by the Vivarium staff according to their sex.

Veterinary care of the animals was provided by the University of Medicine and Dentistry of New Jersey Medical Research Vivarium staff, which is under the jurisdiction of a licensed veterinarian and an Animal Care and Use Committee. The mice were monitored daily by the Vivarium staff, where there is 24 hour video surveillance, and temperature and humidity controlled rooms. If the mice showed any signs of pain or distress they were euthanized immediately in a bell jar with isofluorane by the Vivarium staff.

The mice were gavaged three times weekly with 0.3 mg of mifepristone in 0.3 ml of olive oil and the control mice were given 0.3 ml of olive oil by gastric gavage. The mice were observed daily and given a body conditioning score (BCS) of 1-5, 1 being the lowest score, most sick, and 5 being the highest score.

Table I. Survival rates following one year of treatment (mean±SE).

	Natural expiration and euthanization	Natural expiration only
Mifepristone	57.6%±9.6%	67.4%±8.9%
Olive oil	26.6%±8.4%	27.0%±8.5%
<i>p</i> -value log-rank test	0.056	0.008

Table II. Mean number of days sick (BCS<4) (mean±SE).

	Expired/Euthanized	Survived
Mifepristone	4.7±3.3 (n=24)	11.6±5.0 (n=15)
Olive oil	6.6±5.7 (n=23)	57.6±19.3 (n=8)
<i>p</i> -value	0.768	0.05

The BCS was assigned 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.

A mouse was considered sick if the BCS fell below 4. BCS was determined by members of the Vivarium staff. The Vivarium staff were not involved in the treatment of the mice and thus they did not know which mice received mifepristone *vs.* olive oil, nor did the staff know the desired outcome of the experiment.

The Vivarium staff also checked on the mice daily for any signs of pain or distress. Close observation for anemia, loss of condition, loss of weight and difficulty in breathing were made. Euthanization criteria were followed to ensure the mice would not suffer. The appearance of a tumor greater than 1.5 cm in diameter or a tumor that interfered with walking, urinating, or defecating were cause for performing euthanization. A tumor that fit the above criteria, or any signs of pain or distress in the mice would result in a BCS of 3 or lower and the mice would be euthanized.

A survival rate comparison was made between those mice gavaged with 0.3 mg of mifepristone (n=39) 3 times per week starting at 8 weeks of life *vs.* the control group (n=31) that was similarly gavaged with 0.3 ml of olive oil. The survival rates were determined by the Kaplan-Meier estimates of survival rate at the end of the first year of life. A comparison of the survival rate in each group was performed using the log rank test. The time until death was defined as the number of days from the first treatment until the mice were euthanized or expired naturally. Results were considered statistically significant when $p \leq 0.05$.

This work has been approved by the appropriate ethical committee related to the institution in which it was performed. There were no human subjects to give informed consent to the work. However, as mentioned extreme consideration was taken to prevent suffering of mice and the Guidelines of the University Animal Care Committee was precisely followed.

Table III. Percent of population (n) with sick days.

Sick days	Survived		Expired/Euthanized	
	Mifepristone	Olive oil	Mifepristone	Olive oil
None	66.7% (n=10)	0.0% (n=0)	37.5% (n=9)	65.2% (n=15)
1-14	6.7% (n=1)	25.0% (n=2)	54.2% (n=13)	30.4% (n=7)
>14	26.7% (n=4)	75.0% (n=6)	8.3% (n=2)	4.3% (n=1)
p-value	0.009		0.164	

Results

The Kaplan-Meier estimate of the survival rate following one year of treatment was 57.6% (standard error, SE 9.6%) for the 39 mifepristone treated mice *vs.* 26.6% (SE 8.4%) for the 31 olive oil controls. The time until death was defined as the number of days from the first treatment until the mouse was euthanized or expired naturally. The rates were statistically significantly different (log rank test, $p=0.056$) (Table I). The Kaplan Meier estimate of survival rate following one year of treatment was 67.4% (SE 8.9%) for those mice treated with mifepristone *vs.* 27.0% (SE 8.5%) for those treated with olive oil. The rates were statistically significant (log rank test, $p=0.008$) (Table I).

The mean number of days sick (BCS<4) in those mice that were euthanized or expired did not differ (Table II). However, the mean number of days sick for those mice who survived was significantly greater for the olive oil control than for those treated with mifepristone (Table II). Thus mifepristone improved the quality as well as the length of life.

Breaking down the survival group further showed that 66.7% of the mifepristone group had no sick days *vs.* 0% for the controls, as illustrated in Table III.

The protective effect of mifepristone was not any better in female *vs.* male mice.

Discussion

The data show that treating mice inbred to develop a high likelihood of lung cancer with mifepristone can significantly improve their length and quality of survival.

An elaborate hypothesis was established proposing that a PR antagonist may help to halt the progression of certain types of cancer by suppressing the production of PIBF from gamma/delta T-cells in the microenvironment of the tumor cells which abrogates PIBF induced inhibition of local NK cell attack against tumor cells. The marked improvement of the length and quality of survival of the mice in this study, however, does not definitely prove the hypothesis.

This model of lung cancer was purposely chosen because there have been no data to date that lung cancer is hormonally sensitive especially to progesterone. There has been a recent report of suppression of murine breast cancer with mifepristone (17) but the hypothesized mechanism was based on inhibition of PRs on the tumor itself which was considered an important growth factor for the tumor.

We presented data at the 2006 Meeting of the American Association for Cancer Research showing similar improved length and quality of survival in mice with a high frequency of spontaneous leukemia treated similarly with mifepristone (15). However since the PR and subsequent expression of PIBF involves a lymphoid cell (the gamma/delta T-cell), it was not clear whether the benefit of mifepristone would only be effective for lymphoid cancer. These data involving a solid tumor suggests that this PR antagonist therapy could be extended to solid tumors and may work by changing the tumor microenvironment and removing a blocking factor which had been inhibiting normal NK cell immune surveillance.

Hopefully the murine models will lead to future human trials with mifepristone and other PR antagonists in patients with various types of cancer, either by themselves or in conjunction with other chemotherapy.

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