# Mifepristone Treatment Improves Length and Quality of Survival of Mice with Spontaneous Leukemia

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**Abstract.** *Background: Mifepristone was found to suppress expression of the progesterone-induced blocking factor (PIBF). Progesterone-induced blocking factor suppresses natural killer cell activity. The objective of the present study was to determine if treatment of mice with spontaneous murine lymphocyte leukemia with the progesterone receptor antagonist mifepristone could improve length and quality of life. Materials and Methods: Sixty-one mice were gavaged with mifepristone and 33 controls with olive oil. Quality of life was determined by body conditioning score (BCS). Treatment was initiated when the mice were 6 months old. Results: Within 2 weeks of therapy only 11.4% of the mifepristone treated mice died vs. about 50% of controls. The BCS was 5 (highest quality) in 82% of treated mice vs. 11% of controls after 2 weeks of therapy. Conclusion: Mifepristone therapy should be further evaluated for treating leukemia and lymphoma.*

There are data supporting the concept that one of the mechanisms involved in escape from immune surveillance especially by natural killer (NK) cells in normal pregnancy is through the hormone progesterone (P) (1). A 34 kDa protein has been identified in pregnant women which can block NK cell-mediated lysis of K562 tumor cells (2). Because the expression of this protein by $CD8^+$ T-lymphocytes (specifically gamma/delta T-cells) needs P exposure for its expression, it was called the progesterone-induced blocking factor (PIBF) (3).

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There is evidence that PIBF may induce a shift from TH1 to TH2 cytokines (3, 4). Progesterone receptors have not been demonstrated in normal T lymphocytes yet these receptors have been found at a lower density than other P receptor tissues (*e.g.* endometrium) in normal pregnant women (5-7). Liver transplants and blood transfusions have been shown to induce P receptors on these gamma/delta T-cells even in male patients (8). Injection of paternal lymphocytes prior to ovulation has been shown to increase PIBF secretion in mid to late luteal phase in women exposed to embryo transfer (9).

These data have led to the following hypothesis as to at least one way that the fetus escapes immune rejection by NK cells: The fetal semi-allograft induces P receptors in gamma/delta T-cells following trophoblast invasion. The interaction of these receptors with a high concentration of P causes the expression of PIBF by these gamma/delta T-cells with induced P receptors. PIBF is only made at the maternal fetal interface because that is where there is an adequate P concentration. Progesterone receptors in gamma/delta T-cells are made throughout the body but the P level is insufficient to cause PIBF expression by gamma/delta T-cells not at the maternal-fetal interface.

PIBF inhibits NK cell cytological activity at least partially by inhibiting the release of perforin from storage granule of NK cells (10). PIBF also inhibits TH1 cytokines and favors TH2 cytokines thus inhibiting cellular immune response and promoting hormonal response (10). The suppression of the cellular immune system is limited to the maternal-fetal interface and this constitutes selective immune tolerance. Support for this hypothesis was provided by finding in two studies much less PIBF expression in women who eventually lost their pregnancies *vs.* healthy pregnant women (11, 12). However, no such difference was found in aborters *vs.* non-aborters in patients aggressively treated with P (13).

Thus these data are consistent with the hypothesis that many miscarriages are related to immune rejection related to insufficient P secretion. With aggressive P therapy, many of these miscarriages can be avoided and presumably the ones that still occur would be predominantly of a genetic origin, especially accidental aneuploidy.

It has been hypothesized that cancer may use a similar mechanism to pregnancy to escape NK cell immune surveillance (14). It was proposed that some types of cancer may be initiated and continue to evade NK cells by secreting a P-like steroid. This hypothesized steroid interacts with P receptors, 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. Luteinizing hormone (LH) made by the pituitary gland and human chorionic gonadotropin (hCG) hormone made by the placenta are both capable of making P. There are many types of cancer that have been demonstrated to secrete hCG (15, 16).

The possibility thus exists that progesterone could represent a unique molecule which may be needed for growth of the tumor cells by helping the tumor cells evade surveillance by NK cells in the tumor microenvironment 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 progesterone receptors 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. Theoretically this could be achieved by treating with a progesterone receptor antagonist, *e.g.* mifepristone.

According to the hypothesis, the tumor itself should not express the PIBF protein itself but instead from gamma/delta T-cells in the microenvironment of the tumor. One exception could be leukemia and lymphomas since such white blood cells may have the capacity to express PIBF. In fact, 27 human leukemia cell lines were found to express mRNA for *PIBF* (17). There were 10 major cell line lineages evaluated for staining of PIBF proteins and 4 showed expression of the protein (17). In support of the hypothesis, the addition of progesterone to the media upgraded the expression of PIBF protein (17).

Mifepristone (RU486) is a progesterone receptor antagonists that can terminate a first trimester pregnancy with a one time 600 mg dosage (18-20). Adding mifepristone to the media of 3 human leukemia cell lines that expressed PIBF down-regulated PIBF expression by these cell lines (17).

Based on these findings with human leukemia cell lines, a study was initiated to determine if mifepristone could inhibit spontaneous murine leukemia as manifested by increased length and quality of survival.

Table I. *Survival rates following one year of treatment.*

| | 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% |
| | $p$=0.056 | $p$=0.05 |

Table II. *Mean number of days sick (BCS <4) (data presented as mean±standard error, 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) |
| | $p$=0.768 | $p$=0.05 |

## Materials and Methods

AKR/J mice with spontaneous lymphocytic leukemia were used exclusively. Treatment was not initiated until the mice were 6 months old when the cancer was well advanced. Sixty-one mice were gavaged with 0.3 mg of mifepristone in 0.3ml olive oil. The control group consisted of 33 mice gavaged with 0.3ml of olive oil, only. Treatments were given every 2 weeks then increased to once weekly if they were still alive after 10 weeks of treatment. Quality of life was determined by body conditioning score (BCS), which was evaluated using the following criteria: 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 to <4. BCS was determined by members of the vivarium staff. The employees of 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. Any mouse with BCS of 3 or lower for a week or with visible signs of pain or distress, such as difficulty breathing, eating, or moving, was euthanized.

The percentage of deaths (*via* natural expiration or euthanasia) was determined within two weeks of first gavage and within thirty days of the first gavage.

Veterinary care of the animals was provided by the staff under 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 a 24 hour video surveillance, and temperature and humidity controlled rooms. The Vivarium staff provided clean housing on a schedule so as not to interfere with the research. The staff at the Vivarium provided food and water daily or as needed. If the mice were showing any signs of pain or distress they were euthanized immediately in a bell jar with isofluorene by our trained Vivarium staff.

Table III. *Percentage of population (n) with sick days.*

| | Status: Survived | | Status: Expired/Euthanized | |
|---|---|---|---|---|
| | Mifepristone | Olive oil | Mifepristone | Olive oil |
| Sick days | | | | |
| 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) |
| | $p$=0.009 | | $p$=0.164 | |

This work has been approved by the appropriate ethical committee related to the institution in which it was performed and although there were no human subjects to give informed consent to the work, appropriate consideration was given to animal suffering.

## Results

Table I indicates that within the first 2 weeks of the first gavage, nearly half the olive oil controls had expired, while only 11.4% of the experimental group receiving mifepristone had expired ($p$<0.001). There were 5 out of 61 (8.3%) mice in this group still alive 90 days from initiation of therapy, but none of the controls.

After two weeks of treatment 36 of 44 (81.8%) of mice treated with mifepristone had a BCS score of 5 *versus* 2 of 18 (11.1%) of controls ($p$<0.001). After 90 days all 4 surviving mice treated with a mifepristone had a BCS score of 5.

## Discussion

Some tumors, *e.g.* murine hepatoma cells, have been found to have an increased level of glucocorticoid receptors (21). Mifepristone has been able to partially suppress corticosteroid induced growth of hepatoma cells presumably by the fact that not only does it block the progesterone receptor but also the glucocorticoid receptor (21). The mere demonstration that treatment with mifepristone improved survival and quality of life in mice with spontaneous lymphocytic leukemia does not prove that the mechanism of its action was related to the elaborate hypothesized mechanism of suppressing progesterone-induced immunomodulatory proteins commonly expressed in human pregnancies, but rather could be by suppressing glucocorticoid mediated growth of tumor cells by blocking the glucocorticoid receptor. One way to determine more certainly that the mifepristone exerts its beneficial through suppression of the P receptor and not the glucocorticoid receptor would be to substitute other, more potent, P receptor antagonists that have been developed that have little blocking effect on the glucocorticoid receptor.

The proposed mechanism would involve the need for the tumor to actually make progesterone. There have been studies suggesting that various cancer cells secrete beta hCG which biologically is similar to LH (15, 16). LH is required in mammals to make P.

The possibility exists that the efficacy of mifepristone in inhibiting death and improving quality of life in AKR/J mice with spontaneous leukemia could be increased by starting the treatment with mifepristone at an earlier age or at a more frequent treatment interval.

If mifepristone did, in fact, improve the length and quality of life of AKR/J mice by inhibiting PIBF expression, it is not clear if the source of PIBF was the leukemia cells themselves or gamma/delta T-cells in the microenvironment of the tumor cells. Thus if mifepristone proves effective in the treatment of murine leukemia, it is not clear if it would be similarly effective in solid tumors. In other words, the possibility exits that the AKR/leukemia cells may express PIBF protein (although this is not known). Although all 27 human leukemia cell lines expressed mRNA for *PIBF*, only 4 of 10 actually expressed the protein (17). Nevertheless, a higher percentage of leukemia cells may express the protein under *in vivo* conditions.

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