Mifepristone Causing Complete Remission of Rapidly Advancing Leukemia with Measurement of Progesterone-induced Blocking Factor

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Abstract. *Background: Mifepristone has been demonstrated to cause palliation from murine and human cancer, even in cancers not known to be positive for expression of progesterone receptors. The aim of the present study was to determine if rapidly advancing chronic lymphocytic leukemia responds to mifepristone therapy, and if so, is this effect related to increased expression of the progesterone-induced blocking factor? Case Report: An 81-year-old woman with chronic lymphocytic leukemia whose condition progressed to the acute rapidly progressing stage agreed to be exclusively treated orally with 200 mg mifepristone daily. Results: The patient showed a dramatic improvement after a short exposure time to mifepristone. Complete remission has persisted so far for 12 months on exclusive mifepristone therapy. Her PIBF levels were normal before mifepristone therapy and did not change after treatment. Conclusion: Mifepristone can provide marked improvement of human leukemia even in the absence of increased serum PIBF levels.*

Mifepristone, a progesterone receptor antagonist drug has been demonstrated to have therapeutic benefit for some benign and malignant tumors known to be progesterone receptor-positive (1-6). Mifepristone has also been shown to provide palliative benefit for a wide variety of murine and human cancer types not known to be progesterone receptor-positive (7-11).

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It has been hypothesized that the beneficial effect of mifepristone for progesterone receptor-negative malignant tumors may be by suppressing the production of an immunomodulatory protein, up-regulated by exposure to progesterone, known as the progesterone-induced blocking factor (PIBF) (12, 13). Circulating PIBF protein that is up-regulated by exposure of gamma/delta T-cells to progesterone has been shown to block natural killer (NK) cell-mediated lysis of k562 tumor cells *in vitro* mostly by stabilizing perforin granules and thus inhibiting their release from the large storage granules in NK cells (14, 15).

Early studies of PIBF were thwarted by the fact that the PIBF protein was not pure, hence an enzyme-linked immunosorbent assay (ELISA) nor other sensitive tests, *e.g.* western blot were practical. Although an ELISA test was described in 1989, using a polyclonal antibody, we were not able to confirm its accuracy (16). Instead of our early studies of detecting PIBF shortly after implantation or during the early first trimester of pregnancy were performed using an immunocytochemical technique that detected the percentage of circulating lymphocytes expressing PIBF protein (17, 18).

With the purification of the PIBF protein with recombinant DNA technology, a novel monoclonal antibody against PIBF was developed (19). As mentioned, one of the theories of how PIBF can aid malignant tumors to escape immune surveillance is that the tumor influences gamma/delta T-cells in the tumor microenvironment to secrete PIBF, which in turn inhibits NK cell cytolytic activity in the tumor microenvironment. If this were so, PIBF should be detected in the serum of patients with malignant tumors with a sensitive ELISA similar to sensitive assays that detect it in the serum shortly after a person is exposed to progesterone (20).

There are at least two forms of PIBF. One is a 90-kDa molecule that has a nuclear location on the centrosome (21). This is the dominant form present in most rapidly-growing

cells, as evidenced by western blot analysis using PIBF specific antibodies (21, 22). Exon 1-5 and 17-18 transcript encoding for a 35 kDa protein has been identified (21). The deletion observed in this transcript preserves the open reading frame of the full-length PIBF protein. Translation of the transcript results in a 35-kDa isoform of PIBF containing the *N*-terminal 223 and *C*-terminal 75 amino acids (21). The *PIBF* gene has been identified on chromosome 13 in the vicinity of breast cancer antigen-1 (*BRCA1*) and *BRCA2*, which are breast cancer susceptibility genes. Variations in other centrosome proteins, *e.g.* p53 are associated with increased risk of cancer (23, 24). RNA expression analysis of several tumor cell lines, some of which are positive for progesterone receptors and some not, have been performed (22). These studies showed PIBF to be overexpressed in highly proliferating cells and to be associated with the centrosome (22).

Studies performed in our own laboratory showed that all 29 human leukemia cell lines tested expressed a considerable amount of mRNA for *PIBF* (25). Furthermore, 4 out of 10 leukemia cell lines tested with the immunocytochemical technique detected leukemia cells that expressed the PIBF protein (25). Even more interestingly, adding progesterone to the cell culture media up-regulated PIBF expression, whereas adding the progesterone receptor antagonist mifepristone down-regulated PIBF expression (25). The question arises as to whether only 4 out of the 10 human leukemia cell lines actually expressed the PIBF protein or the immunocytochemical technique lacks sensitivity to detect the secretion by the other six cell lines.

Immunofluorescence microassay demonstrated a 35-kDa form of PIBF localized to the cytoplasm of tumor cells (21). Thus, an alternate hypothesis is that PIBF protects tumor cells from NK cell attack by its intra-cytoplasmic location. Mifepristone may negate the immunprotective effect of PIBF in the tumor cell *per se*, allowing direct destruction by NK cells rather than by its action on gamma/delta T-cells in the tumor microenvironment (12, 13).

The aim of the present study was to help answer the question as to whether the intra-cytoplasmic presence of PIBF (or possibly even its nuclear centrosomal position) protects the tumor cell from immunosurveillance and thus whether evidence of tumor regression or clinical improvement could be obtained by treating with mifepristone even without the demonstration of increased serum PIBF. A direct effect of mifepristone on tumor cells would be ascertained if one can be sure that the benefit was not related to reduction of NK cell cytolytic activity in the tumor microenvironment. Now that a very sensitive ELISA has been developed, if one could demonstrate no increase in the serum level of PIBF in a given patient with cancer, a positive response to mifepristone therapy in progesterone receptor-negative cancer would be the result of action specifically against the tumor and not indirectly by changing NK cell activity in the tumor microenvironment. In other words, the gamma/delta T-cells in the tumor microenvironment would have to secrete PIBF in order for it to reach NK cells and thus be detected in serum. In contrast, the intra-cytoplasmic PIBF found in cancer cells and other highly proliferative cells would remain in those cells and not be secreted and thus serum PIBF levels would not increase.

Case Report

The subject of this study is an 81-year-old woman who sought treatment with mifepristone for chronic lymphocytic leukemia which had progressed to a highly symptomatic acute phase. Because of a nearly fatal complication of treatment with oral chlorambucil (marked hyponatrinia from the syndrome of inappropriate anti-diuretic hormone), she refused other chemotherapy when informed of the potential side-effects. She agreed, however, to mifepristone because of its lack of side-effects at the 200-mg dosage.

Serum was obtained prior to initiation of mifepristone at 200 mg per day to measure PIBF by ELISA and this was repeated after one month of treatment.

Methodology of PIBF assay. A non-commercial ELISA was used to measure PIBF in serum. Serum specimens were stored at −20˚C. Fifty microliters of recombinant PIBF standard (Cusabio, Wuhan, China) was added to each pre-coated goat anti-rabbit antibody well in duplicate. The concentrations of the PIBF standard were 0, 3.2, 11.2, 40, 160, and 802 ng/ml. The patient's serum was then added to each well. Fifty microliters of horseradish peroxidase-conjugated PIBF antigen was then added to each well except the zero standard before adding anti-PIBF IgG antibody to each well. The microtiter plate was then incubated in the dark for 1 h at 37˚C. After 1 h, the wells were washed with PBS and decanted three times. Fifty microliters of carbamide peroxide and 50 μl of tetramethyl-benzidine were added. The microtiter trays were then incubated in the dark at 37˚C for 15 min then 50 μl of stop solution were added (main component is H_2S0_4). The plates were read within 10 min using a microplate reader at 450 nm. The results were calculated using a four-parameter logistic curve fit.

Two months after the start of mifepristone treatment, a repeat computed tomographic (CT) scan of the lungs was performed.

Informed consent from the patient for the off-label use of mifepristone therapy was obtained, as well as permission to obtain serum to measure the PIBF protein with the realization that this is a research tool and is non-commercial and no clinical decisions were to be based on the results of this test.

The PIBF level before and after mifepristone therapy was 34.9 ng/ml and 48.3 ng/ml, respectively. These levels are not

higher than those found in most women without cancer not exposed to progesterone (20).

Despite failure to detect increased levels of circulating PIBF, the patient exhibited a dramatic improvement in her condition. Prior to treatment, her white blood count was 28×10^6/μl and her platelet count was 40×10^6/μl. After one month of therapy, the white blood cell count decreased to 8×10^6/μl and the platelet count increased to 240×10^6/μl.

Clinically, she showed marked improvement. She had been very weak and had shortness of breath and chronic cough. CT scan of the lungs showed several pulmonary nodules which were consistent with either her leukemia or with primary lung cancer (radiologist and oncologists favored the latter). Re-assessment after one month of treatment revealed marked improvement in her strength and respiratory symptoms. A repeat CT scan after two months on therapy showed the pulmonary nodules to have completely disappeared.

She has been asymptomatic now for eight months of taking 200 mg mifepristone orally per day.

Discussion

Mifepristone blocks not only progesterone receptors but also glucocorticoid receptors (26-28). Thus, one could argue that its beneficial effect was possibly from blocking glucocorticoid receptors rather than progesterone receptors. However, this is unlikely because her energy markedly improved and if there was adrenal insufficiency, weakness is always present. Furthermore, glucocorticoids generally help treat lymphoid tumors, hence blocking glucocorticoid receptors would not seem likely to improve leukemia. The possible confounding effect on the glucocorticoid receptor could be eliminated if in another case similar benefits are demonstrated using other progesterone receptor antagonist, *e.g.*, ullipristal tht has little effect on the glucocorticoid receptor.

The fact that this dramatic response to therapy occurred in the absence of increased serum PIBF suggests that intra-cytoplasmic presence of PIBF may serve as an immunoprotective mechanism. This excellent response also suggests that progesterone plays a role in converting the parental 90-kDa compound to the 34-36 kDa split variant product found in the cytoplasm. Of course in this study, we did not measure the PIBF in the cytoplasm so this conclusion is speculative.

There is the possibility that in some circumstances, malignant tumors can also direct gamma/delta T-cells in the tumor microenvironment to express PIBF and thus have a local inactivating effect on NK cells. Thus, it would be interesting to continue measuring PIBF in the sera of patients with a wide variety of cancer types to determine if PIBF can be a marker to determine dosage of therapy. Unfortunately, at doses above 200 mg, mifepristone has too many anti-glucocorticoid side-effects but other progesterone receptor antagonists could prove efficacious.

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