Therapeutic monoclonal antibodies in human breast milk: a case study

Elle Ross, Steven E. Robinson, Carol Amato, Colette McMillan, Jay Westcott, Tiffany Wolf and William A. Robinson

Recently, therapeutic monoclonal antibodies have been introduced for the treatment of advanced melanoma and other diseases. It remains unclear whether these drugs can be safely administered to women who are breast feeding because of the potential hazardous side effects for nursing infants. One such therapy for metastatic melanoma is ipilimumab, a human monoclonal antibody that blocks cytotoxic T-lymphocyte-antigen-4, and is the preferred treatment for patients with metastatic melanoma when other molecular therapies are not viable. This study measured ipilimumab levels in the breast milk of a patient undergoing treatment that were enough to raise concerns for a nursing infant exposed to ipilimumab. ***Melanoma Res***

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Melanoma Research Program, School of Medicine, Division of Medical Oncology, Anschutz Medical Campus, University of Colorado, Aurora, Colorado, USA

Correspondence to William A. Robinson, MD, PhD, 12801 E 17th Ave, Mail Stop 8117, Aurora, CO 80045, USA
Tel: +1 303 724 3870; fax: +1 303 724 3889;
e-mail: william.robinson@ucdenver.edu

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Introduction

The human monoclonal antibody, ipilimumab, is a relatively recent treatment for patients with metastatic melanoma and shows promise, with response rates of 20–30% in untreated patients [1,2]. It is one of a variety of monoclonal antibodies used currently for the treatment of diseases including lymphomas, breast cancer, inflammatory bowel disease, multiple sclerosis, and other autoimmune-related diseases [3–5]. In these patient populations, there are a significant number of women who are in their childbearing years and may be breast feeding, raising concerns about the safety of therapeutic monoclonal antibodies used during breast feeding. The guidelines issued with respect to the safety of ipilimumab during breast feeding are cautionary, with physicians advised to either discontinue ipilimumab treatment or to advise the patient to stop nursing [6].

There are reports on the use of human monoclonal antibodies during pregnancy, but information on the safety for breast-feeding infants is very limited. Several studies have examined the levels of therapeutic human monoclonal antibodies in breast milk, including infliximab and adalimumab, and have found detectable levels of these drugs, but there are currently no published data available for ipilimumab [7]. In a 2008 study, the breast milk of female patients being treated with the drug infliximab was assayed, with no infliximab being detected [3]. This prompted guidelines suggesting that breast feeding was safe for the infants [4]. Yet, in a different study, levels of infliximab were detected in the breast milk of patients being treated for inflammatory bowel disease [8]. Further, the antibody adalimumab was found in the breast milk of a patient being treated for Crohn's disease [9]. Whether these discrepancies are the results of structural differences in human monoclonal antibodies or the techniques used to detect their presence remains unknown.

As an estimated one-third of cases of melanoma are diagnosed in women during their childbearing years, coupled with the fact that melanoma is the most common form of cancer for young adults 25–29 years of age, many women will be faced with developing melanoma during and after pregnancy [10]. Therefore, it follows that more pregnant and nursing patients may be advised to undergo treatment with ipilimumab and other new therapeutic monoclonal antibodies. Some of these could have serious consequences for developing infants if present in breast milk and absorbed even in small amounts. The aim of the present study was to investigate the presence of ipilimumab in breast milk to provide data to physicians and nursing mothers with metastatic melanoma who may face the same dilemma.

Case report

A 31-year-old woman presented during her 12th week of pregnancy, with multiple subcutaneous nodules on her chest and abdominal wall, as well as in her right breast. Her history included a resection of a 1.9 mm superficial spreading melanoma on her right lower leg in 2003, for which she had a wide local excision with clear margins and a negative sentinel lymph node biopsy. No other treatment was provided and she was well until 8 years later, in early 2011, when she presented with an isolated right axillary lymph node recurrence. This was removed

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surgically and sent for molecular testing and found to be both *BRAF* and *cKIT* wild type. She was then free of disease for 4 months until 12 weeks into her second pregnancy, when she presented with multiple subcutaneous biopsy-proven metastases and a questionable lesion in L4 seen on PET scan. She was largely asymptomatic and because of her pregnancy was treated only with surgical excision of some painful subcutaneous metastases until an uneventful delivery of a healthy baby girl 25 weeks later and at week 37 of gestation. We then elected to treat her with ipilimumab. Breast feeding her child was very important to her, but we could not find data guiding us toward a decision as to whether this might be safe for the infant. We asked her to postpone breast feeding until after completion of a standard 12-week course of ipilimumab. This was administered at the recommended dosing schedule of 3 mg/kg administered intravenously over a 90-min period once every 3 weeks for a total of four doses. During this time, she continued to lactate and we obtained her consent to collect breast milk and serum before, during, and after two of the infusions to determine ipilimumab levels as described below. The patient showed a partial response to the ipilimumab, resumed breast feeding 3 weeks after the last infusion, and remains alive and clinically well with limited disease at 30 weeks after her last ipilimumab treatment.

Results

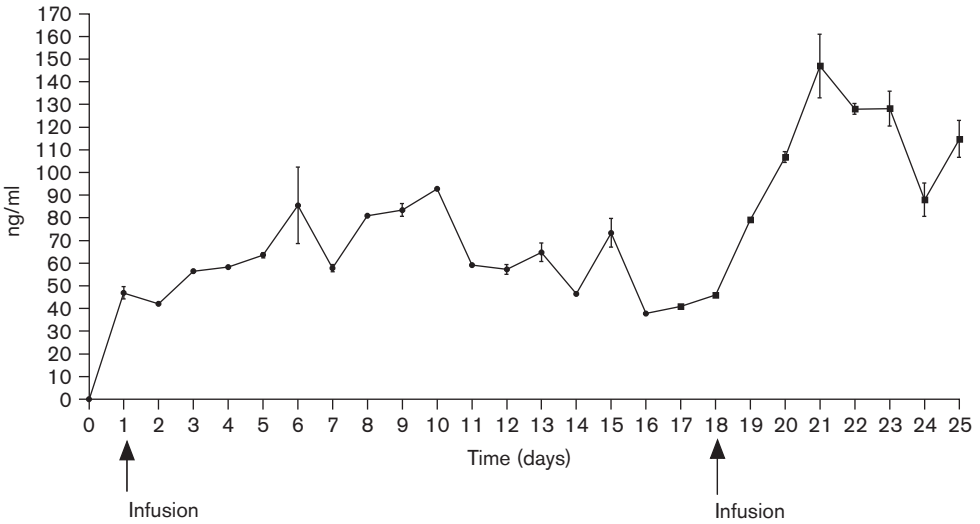
The patient received four infusions of ipilimumab 3 weeks apart. Breast milk and serum were collected before the patient received the drug and at various time points during her treatment. As shown in Fig. 1, the levels of the drug in breast milk increased ~5 days after infusion. Further, over a 25-day period, the data clearly show a cumulative increase from one infusion to the next. The patient did not breast feed during the course of ipilimumab treatment and began breast feeding 21 days past her fourth and final infusion without problems.

The ipilimumab levels in serum and breast milk were measured using a specifically developed ELISA assay (ELISATech, Aurora, Colorado, USA). The plates used in the assay were coated with a CTLA 4 antigen, with the addition of a histidine tag (Acrobiosystems, Bethesda, Maryland, USA), at a concentration of 500 ng/ml. Serial dilutions were prepared to develop a reliable standard curve, with a detection limit of 20 ng/ml. The validity of the measurements in this medium used a spiking experiment whereby the assay was used on breast milk samples obtained before the initiation of ipilimumab. Homogenization of the breast milk samples included several freeze/thaw cycles and vortexing of each sample before assaying.

Figure 2 shows the ipilimumab serum levels at 10 min, 1 h, 2 h, and 7 days after the infusion, along with the corresponding breast milk sample levels. The background before ipilimumab infusion was minimal for the serum sample at 0.6 μg/ml and is shown in the upper plot line with closed squares. Ipilimumab in the serum obtained after treatment began reached a peak of 98 μg/ml 10 min after infusion and then decreased to 35 μg/ml after 7 days. The data from the post-treatment serum are consistent with a previous study by Wolchok *et al.* [11].

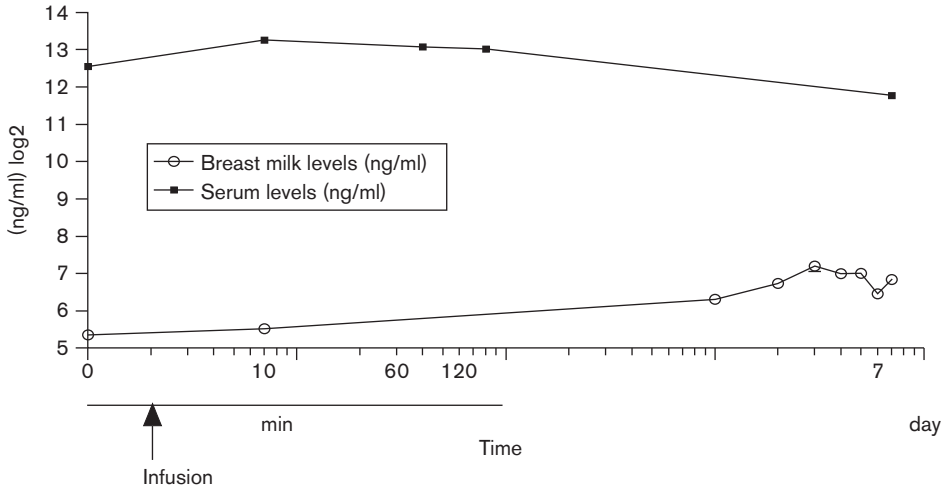
The lower plot line with open circles in Fig. 2 shows that breast milk collected before treatment has undetectable levels of ipilimumab. Moreover, the levels in breast milk were considerably lower than in serum, but easily detectable

Fig. 1



Levels of antibody in breast milk.

Fig. 2



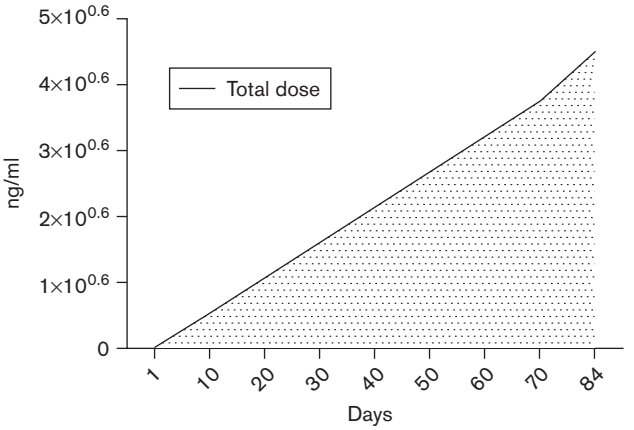
Levels of antibody.

after reaching a peak of 147 ng/ml 4 days after infusion began. It remained detectable at 41 ng/ml 19 days after infusion began. These levels are relatively low, with an average level of ipilimumab in the breast milk of 75.36 ng/ml.

To determine the amount an infant is possibly exposed to during one day's breast feeding, we used our average level of 75.35 ng/ml and then multiplied it by an infant's average intake of breast milk over a 24-h period of ~710 ml, suggesting that the total amount an infant has the potential to be exposed to is ~53 481 ng/day. This indicates that over an 84-day treatment course, 4.5 mg is available for an infant to absorb during the first 4 months of feeding.

Figure 3 shows that the cumulative effect of a postpartum mother breast feeding her infant during a standard 12 weeks of ipilimumab treatment would result in total infant dose exposure of ~4 492 976 ng.

Fig. 3



Infant exposure over time.

Discussion

It is well known that infants absorb antibodies from breast milk, particularly in the first 6 weeks of life, for the development of passive immunity [12,13]. In addition, the gastrointestinal tract of infants, unlike adults, is designed to allow antibody absorption as a means of early protection before developing a fully responsive immune system [13–15]. There are very limited data on the levels of therapeutic monoclonal antibodies in breast milk and their safety during breast feeding. It is quite reasonable that if absorbed by infants, even in small amounts, these agents may be harmful. The protective immunoglobulins in human breast milk are predominantly of the secretory immunoglobulin A class, whereas immunoglobulin G (IgG) antibodies are present at high levels over the first few days postpartum during colostrum production [16]. The average IgG levels are ~42.3 μg/ml in colostrum [17] and subsequently decrease over the following weeks. The current therapeutic antibodies used for metastatic melanoma are of the IgG1 class [18]. Whether these can be absorbed functionally intact by the infant gut has not been determined here, nor in previous studies, but even small amounts could have significant consequences [19]. Ipilimumab's therapeutic effect is a result of activating the immune system, thus allowing a cellular immune response against melanoma cells [1]. As with all such agents, the response is not entirely specific and side effects may be significant including colitis, severe diarrhea, skin rash, arthralgias, and hypophysitis with severe endocrine dysfunction [20]. The safety of ipilimumab in breast milk for feeding infants is undetermined. Further, we show here that, when exposure over time is calculated as in this study, there is concern that infants ingesting even small

doses over time would lead to a cumulatively significant amount of ipilimumab.

Although there is overwhelming evidence supporting the role of breast feeding in protecting children from most immune-mediated diseases [3], the components in breast milk responsible for facilitating this protection are not well defined. Maternal transfer of IgG confers offspring with short-term protective immunity. The primary immunoglobulin isotypes that are used therapeutically include IgG1, IgG2, and IgG4 [19]. Because of their extended half-life in humans of $\sim$20 days, these immunoglobulins bind to the Fc receptor (FcRn) and the longer half-life makes them a good choice for therapeutic use [19]. It is possible that ingested IgG selected by FcRn for absorption from the gut lumen has a higher binding affinity for FcRn systemically compared with an intravenous injection, and thereby has increased protection from catabolism [19]. Previous studies are unclear whether this is the case in the absorption of breast milk as no direct comparison of half-lives has been made. Furthermore, there appears to be a lack of consensus in the field on the half-life of injected IgG, yielding ambiguity in terms of the half-life of absorbed IgG [19].

Since the introduction of new immune therapies such as ipilimumab, female patients have been provided with better treatment options for a number of diseases [1,7]. These new therapies also present new problems with respect to pregnancy, breast feeding, and the adverse effects of human monoclonal antibodies on mothers and infants. Furthermore, scant information is available on the short-term and long-term consequences of treatment with targeted monoclonal antibodies on nursing infants. Our data suggest continued caution because of the potential cumulative toxicity in infants. An additional concern is the physiologic effect of the active drug in the breast milk and its associated risks to the infant; however, no suitable models exist to test physiologic effects.

We should be cautioned by the results of this breast milk analysis, and it warrants further research to maintain the highest standard of clinical care to both patients being treated with a monoclonal antibody and their breast-feeding infants.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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