Reply to the Letter to the Editor by Bara, et al

In Response: We welcome the interest of Drs. Bara and Forgue-Lafitte in our publication (1). There are two issues that we will address. The first and more clinically relevant is that the PAM4-reactive epitope seems to be a highly specific biomarker for pancreatic adenocarcinoma. We have presented considerable data, including immunohistochemical studies of tissue specimens presented in this and prior publications (1, 2), as well as results from immunoassays of patient sera (3), to show that monoclonal antibody (mAb) PAM4 is reactive with a biomarker, the presence of which provides high diagnostic likelihood of pancreatic neoplasia. Furthermore, we determined that PAM4, although not reactive with normal adult pancreas or active pancreatitis, is reactive with the earliest stages of neoplastic progression within the pancreas, PanIN-1 and IPMN, and remains at high levels of expression throughout the progression to invasive pancreatic adenocarcinoma (1). This specificity of PAM4 serves as the basis for studying the diagnostic value of PAM4 imaging (4–6) and its therapeutic efficacy as a radiolabeled antibody (7).

The second issue concerns the nature of the epitope and antigen against which PAM4 is reactive; that is, whether the antibody is reactive with MUC1, MUC5ac, or perhaps another mucin altogether. Our initial studies on characterization of PAM4 examined a set of MUC1 gene-transfected cells (8), generously provided by Dr. M.A. Hollingsworth (University of Nebraska Medical Center, Omaha, NE). Neither the parent Panc1 cells nor the Panc1 cells transfected with control vectors (no insert and reversed insert controls) had detectable levels of PAM4-reactive mucins by enzyme immunoassay of supernatants; however, both the 30 tandem repeat (30TR) and 42TR MUC1 gene-transfected cells were highly reactive with PAM4. Recently, we reexamined these cells with similar results. Briefly, cells were grown in T75 flasks until they reached ~80% to 90% confluency (~4-5 days from initial seeding). At this time, the spent medium was collected, centrifuged at high speed, and used for quantitation of PAM4-reactive mucin by enzyme immunoassay. The cells were also collected and counted. Both the Panc1 parent cell line originating from Dr. Hollingsworth and a separate Panc1 cell line recently obtained from the American Type Culture Collection, as well as the vector control, produced low but detectable quantities of PAM4-reactive mucin (0.87 ± 0.17, 0.54 ± 0.17, and 0.02 ± 0.02 μg/mL/10⁶ cells, respectively), whereas the 30TR MUC1 gene-transfected cells produced 14.17 ± 2.22 μg/mL/10⁶ cells (P < 0.0003 or better for comparison of 30TR MUC1 gene-transfected medium compared with all other samples). It should be noted that the current enzyme immunoassay (3) provides higher sensitivity than the assay used for the prior studies. Certainly, it is possible that expression of MUC1 up-regulated expression of another mucin that contained the PAM4-specific epitope, or in some manner affected exposure of the PAM4 epitope that was already being synthesized. It is also conceivable that during cell selection with genetin, clones were selected that, in addition to high MUC1 expression, also showed increased expression of another mucin, such as MUC5ac. Although several counter-arguments can be made, based on the data from gene transfection studies, as well as physicochemical data presented in our original report on the generation of PAM4 (2), the information available at the time strongly supported the view that the PAM4 epitope is present on MUC1.

Drs. Bara and Forgue-Lafitte reference several publications, in particular the TD-4 Workshop (1997) of ISOBM (9), to indicate that MUC1, as detected by several different mAbs, is located in epithelial cell membranes both in normal tissues and in early PanIN lesions, and that its presence in the cytoplasm within PanIN-3 and invasive neoplasia is regarded as a biomarker of malignancy. Such mAbs may react with different MUC1 epitopes (or cross-react with other mucins) that may or may not be expressed by individual tissue specimens. In fact, the cited reference used only a single specimen of ileum and breast along with two specimens of colon to perform the immunohistochemical studies. Although Drs. Bara and Forgue-Lafitte are correct that many of the anti-MUC1 mAbs showed membrane staining of these normal tissues, it is also true that many of the mAbs were unreactive with one or more of the tissues, with the well-studied anti-MUC1 mAb, SM3 (10), being negative with all of the tissues examined. Mucins are complex molecules that exhibit high levels of microheterogeneity (e.g., vntr, gene-splice variants, glycosylation status). Thus, many anti-MUC1 (or other mucin) antibodies identify cell surface, membrane, and/or cytoplasmic labeling of the respective mucin species, based on the biosynthetic mechanisms operative within the individual tissue specimen. PAM4 is reactive with both cytoplasmic and/or cell surface (apparent membrane) mucin, dependent on the individual tissue specimen, as was the MA5 anti-MUC1 antibody that was used as a control but that recognizes a different epitope.

With more recent data demonstrating the PAM4 epitope within normal gastric mucosa and the distribution of PAM4 within the cancer precursor lesions, we specifically made note of the similarity to MUC5ac (1). We are in agreement that PAM4 should be tested for immunoreactivity with recombinant MUC5ac apomucin as well as other recombinant mucin structures, and this is the focus of ongoing studies. Aside from such further characterizations, the principal interest is how to best apply PAM4 to the improved early detection and therapy of pancreatic carcinoma.

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Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
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