Imaging of pancreatic adenocarcinoma with radiolabeled monoclonal antibodies

G. Mariani
Nuclear Medicine Service, DIMI, University of Genoa Medical School, Genoa, Italy.

Summary
This review focuses on the potential of immunoscintigraphy with radiolabeled monoclonal antibodies in patients with pancreatic cancer. The general limitations of tumor immunoscintigraphy are particularly important in the case of pancreatic cancer, because of the particular anatomic location, that is in close proximity with sites of physiologic high accumulation of radioactivity during such examinations (bone marrow in the spine, liver, spleen, and most of all kidneys).
Thus, the role of immunoscintigraphy in patients with pancreatic cancer appears limited (except in peritoneal carcinosis), or not fully explored given the small cumulative number of patients evaluated so far with such scintigraphic procedure.
Perspectives for development of the radiolabeled monoclonal antibody technology in patients with pancreatic cancer might be seen in the field of radioimmunotherapy and possibly radioimmuno-guided surgery.

Key words: immunoscintigraphy, monoclonal antibodies, pancreatic adenocarcinoma, radioimmunotherapy, tumor-associated antigens

Background
Every year there are about 27,000 new cases of pancreatic cancer and about 25,900 deaths due to this cancer in the USA [1]. Most pancreatic cancers arise from the exocrine component of this organ, mainly in the secretion compartment (mucinous carcinomas or adenocarcinomas deriving from the cuboidal epithelium of the pancreatic duct or from acinar cells), with a minor fraction originating in the excretion compartment (carcinoma of the ampulla of Vater). Pancreatic tumors originating from the endocrine component (such as the so-called APUD-cell tumors, also defined as neuroendocrine tumors) represent a neoplastic entity with pathologic features as well as clinical pattern clearly distinct from exocrine-derived pancreatic cancers; therefore, they will not be considered in this review. On the other hand, scintigraphic imaging of these tumors is based on radiopharmaceuticals with a specific ligand-receptor type rather than an antigen-antibody type interaction with the tumor cells.
Although pancreatic cancer accounts for about 2% only of all malignant tumors, thus ranking as the eleventh leading cancer, it still represents the fifth most common cause of death from cancer, with similar epidemiology in the USA and in Western Europe [2]. Such poor prognosis reflects the fact that primary diagnosis of pancreatic cancer is usually quite late in the natural history of the disease, so that 90% of ductal pancreatic carcinomas cannot be resected at the time of diagnosis. This is due in general to the lack of obvious, specific clinical signs and symptoms, particularly if the tumor is located in the body or tail; on the other hand, even if tumors of the head and periampullary region usually induce earlier signs (because of possible extrahepatic biliary obstruction), their long-term prognosis is only marginally better than that of cancers located in the body or tail [2].
Surgery alone is seldom curative and neoplastic disease most frequently recurs either locally (in up to 85% of patients) or metastatic to the liver (in 50%-70% of patients treated with potentially curative combined chemoradiation and surgery). Despite recent progress in the surgical management of patients with pancreatic cancer, more aggressive versus more conservative approaches do not warrant improved long-term prognosis, so that the overall 5-year survival rate for this cancer still remains between 3%-5%, the lowest for all cancers [3].
While sequential multimodality imaging combining ultrasonography, CT (preferably spiral CT) and MRI provide a definite diagnosis of pancreatic cancer in a relatively high fraction of patients (especially in the more advanced stages of the disease) [4,5], not rarely this approach remains nondiagnostic, so that more or less invasive procedures are necessary, such as endoscopic ultrasonography, angiography, CT-guided biopsy/aspiration, endoscopic retrograde cholangiopancreatography, and laparoscopy. Moreover, while ultrasound and especially CT provide important anatomic structure-related information that are particularly useful for planning the most adequate surgical approach, these imaging techniques perform poorly both for staging early metastatic disease to regional lymph nodes and for detecting local recurrences (such as peritoneal carcinosis).

Biologic markers
The fact that timely diagnosis of pancreatic cancer and its locoregional recurrences remains elusive in most of the patients explains the efforts devoted to the search of clues leading to early identification of the disease. This process also concerns non-imaging modalities, such as humoral signs of neoplastic disease.
While cancers originating from the neuroendocrine components of pancreas are characterized by specific products to be found in the serum (peptide hormones such as insulin, glucagon, gastrin, VIP-like peptides, or biologically
active amines such as serotonin), of particular interest to this review are investigations concerning the so-called tumor-associated antigens, a broad spectrum of proteins (mostly glycoproteins belonging to the mucin family) that, when assayed in serum or other biological fluids, can be used as a parameter of neoplastic growth and extension.

Though generally nonspecific for pancreatic cancer (thus being unreliable for diagnosis when used by themselves), these serum markers can be utilized in confirming a diagnosis of pancreatic cancer and, in some instances, in distinguishing benign from malignant pancreatic disease. Moreover, they are most useful in the follow-up of patients (as an early sign of tumor recurrence, without however any localizing properties) as well as in monitoring the response of cancer to treatment.

The advent of hybridoma technology, therefore the availability of monoclonal antibodies (MoAbs), has provided a simple means of identifying new tumor-associated antigens, or better characterizing already known markers. Concerning in particular pancreatic cancer, several tens of circulating proteins have been described as possible tumor markers (not only in serum, but sometimes also in the bile and/or in fluid aspirated from pancreatic cysts), more or less specific for pancreatic adenocarcinoma.

A tentative list in this field should start with some classical nonspecific markers widely used especially for tumors of the gastrointestinal tract, such as CEA, AFP, TPA/TPS, TAG-72/CA72-4, beta-hCG, CA125, CA15-3, ferritin. On the other hand, other markers have been developed and proposed with particular attention to pancreatic cancer, such as: CA19-9, CA50, CA124, CA-195, CAM17.1, CAR-3, DU-PAN-2, FAP (feto-acinar pancreatic protein), KL-6, NCA (non-cross-reacting antigen), PaA, PaA-15, PC-AA (pancreatic cancer-associated antigen), PNA-binding glycoprotein, POA (pancreatic oncofetal antigen), p53-autoantibodies, RA96, SC6, sialyl-SSEA-1, sIL2r and sIL6r (soluble interleukin-2 and 6 receptor), SLEX, Span-1, ST-439, STN (sialyl-Tn antigen), TATI (tumor-associated trypsin inhibitor), 2H6, 90K, 47D10.

Most of these code denominations derive from MoAb-defined antigens whose actual structure is unknown at the time of developing the MoAb itself (which is usually raised against a crude tumor extract). Moreover, in some instances different MoAbs and different code names/assay systems simply refer to different epitopes on the same antigen molecule (usually of the mucin family).

Out of this legion of tumor markers, whose general validity in the clinical setting can easily be questioned, at least some tumor markers have indeed proved to be reliable indicators for pancreatic cancer. This is the case for CA19-9 (perhaps the most reliable marker for pancreatic cancer, at least in Lewis antigen-positive patients), CA50, sialyl-SSEA-1, DU-PAN-2, and CA242; it has been suggested that the CA19-9, CA50 and CA242 MoAbs recognize different antigenic determinants coexpressed to variable extents on the same macromolecular complex.

Monoclonal antibodies and imaging

Identification of antigen-antibody systems by MoAb technology in the field of pancreatic adenocarcinoma has allowed to develop not only novel assays for tumor markers to be evaluated in the serum, but also radiolabeled MoAbs to be tested for immunoscintigraphy of pancreatic cancer. This has been the case for at least some of the tumor markers listed above, such as CEA (various MoAbs), TAG-72 (B72.3 or newer generation MoAbs), CA19-9 (OC19-9 MoAb), CAR-3 (AR-3 MoAb), and DU-PAN-2. Additional monoclonal preparations (intact MoAbs or their fragments) have been proposed for imaging pancreatic cancer in vivo, after testing expression of the corresponding antigen in vitro (by immunohistochemistry techniques).

In principle, the idea of targeting tumors with radiolabeled MoAbs administered in vivo for either imaging purposes or radioimmunotherapy purposes is simple and straightforward, based as it is on the exclusive specificity of each MoAb-antigen system [6-8]. Some additional basic requirements to be fulfilled for successful immunoscintigraphy include: 1) expression of the antigen at the surface of a large fraction of tumor cells, with high density of antigen molecules/cell (coupled with absent expression on non-tumor cells), 2) high affinity/avidity of the specific MoAb-antigen system, 3) absent cross-reactivity of the MoAb with non-tumor cells, 4) adequate blood supply to the tumor, 5) adequate capillary permeability to macromolecules, 6) stability of the radiolabel attached to the MoAb (a gamma-emitter suitable for scintigraphic imaging), and 7) fast plasma clearance of the radiolabeled MoAb un-bound to the tumor (so to allow for high tumor-to-background ratios, adequate for scintigraphic imaging to be reached early enough after in vivo administration).

The importance the first factor outlined above (level of antigen expression on the tumor cell surface) is elucidated in Figure 1, which depicts the immunohistochemistry pattern observed with two MoAbs that have been proposed as in vitro and in vivo markers of pancreatic cancer [9-11].

**Limitations of immunoscintigraphy**

It should be pointed out that all of the above requirements cannot always be fulfilled at the same time when performing tumor immunoscintigraphy. Furthermore, since the MoAbs most widely employed for tumor immunoscintigraphy are of murine origin, some nonspecific uptake of the radiolabeled preparation in the reticulo-endothelial system (therefore in bone marrow, liver and spleen) is unavoidable. This reduces the tumor/background ratios and therefore represents a confounding factor in the interpretation of scintigraphic images, especially in the abdominal area. Moreover, the fraction of administered radiolabeled MoAb that actually localizes at tumor sites is quite low, in the order of 0.01% of the injected dose/gram of tumor at most.

The use of fragments (F(ab')2 or monovalent Fab) rather than the intact MoAb as the radio-labeled immunoreagent overcomes some but not all of the above limitations. In particular, MoAb fragments exhibit much faster clearance overcomes some but not all of the above limitations. In particular, MoAb fragments exhibit much faster clearance
immunoscintigraphy (or radio-immunotherapy) in patients with pancreatic cancer makes it therefore difficult to obtain satisfactory immunoscintigraphic images in the case of pancreatic cancer, when close proximity of the renal area with the pancreatic region leads to a site of very high nonspecific accumulation of radioactivity. Close proximity of the renal area with the pancreatic region makes it therefore difficult to obtain satisfactory immunoscintigraphic images in the case of pancreatic cancer, when employing both planar and (though to a lesser extent) tomographic imaging (SPECT).

**Immunoscintigraphy of pancreatic cancer**

All the above considerations explain why there are relatively few investigations describing specifically the use of immunoscintigraphy (or radio-immunotherapy) in patients with pancreatic cancer [12-17]. Altogether these reports account for only 32 patients, an exceedingly low number of investigations which, although inadequate to draw any definitive conclusion, nevertheless lead the authors of each study to conclude that immunoscintigraphy with radiolabeled MoAbs so far appears to be unreliable for the primary diagnosis of pancreatic cancer.

It should however be emphasized that the most promising results in this field were obtained in patients with peritoneal carcinosis, a condition in which other imaging modalities (ultrasonography, CT, MRI) frequently yield nondiagnostic results. Still, the obvious limitations mentioned above in the number of patients with pancreatic cancer evaluated by immunoscintigraphy greatly hamper any conclusion on this subject.

Despite the rather disappointing results so far obtained with immunoscintigraphy in patients with pancreatic cancer (with the exception mentioned above for peritoneal carcinosis), the high interest in this field is witnessed by the number of investigations concerning the development of tumor immunoscintigraphy in animal models of experimental human pancreatic carcinoma xenografts [18-23].

**Concurrent nuclear imaging procedures, and perspectives of immunoscintigraphy in patients with pancreatic cancer**

In the last 4-5 years there have been no major breakthroughs concerning immunoscintigraphy of pancreatic cancer in the clinical setting. This is perhaps due to both the important limitations mentioned above for this particular application of immunoscintigraphy and to the high cost of developing a MoAb immunoreagent for commercial purposes. These two factors combined justify the opportunity for commercial companies to focus their efforts in the development of MoAb tracers suitable for wide clinical applications, such as in patients with colorectal, lung, breast, and prostate cancers, as well as in patients with lymphomas. Thus, it is conceivable that the role of immunoscintigraphy by itself in pancreatic cancers will remain quite limited also in the near future; except perhaps for the identification of peritoneal carcinosis. On the other hand, the role of this scintigraphic procedure in discriminating post-therapy fibrosis/scar from tumor recurrence in patients already treated for pancreatic cancer (a diagnostic problem not always solved by ultrasonography and CT/MRI) remains to be fully explored with controlled clinical studies.

It is also worthwhile to emphasize that, at present, nuclear oncology is being dominated by the development of Positron Emission Tomography (PET) as the emerging technology in this field [24]. Although still scarcely available to wide clinical use in some countries because of relatively high installation and operation costs, PET offers a unique combination of satisfactory resolution nuclear imaging with exquisite functional tissue characterization. The oncological applications of PET now mostly rely on the rather well characterized "metabolic probe" represented by 18F-deoxyglucose, based on the high glycolytic rate of many tumors, linked in turn to oncogene expression and corresponding to tumor growth rates [25-28]. Nevertheless, other tracers of tumor cell metabolism (such as protein synthesis and DNA synthesis) are being made more widely available. These features almost totally overcome an
important limitation encountered by tumor immunoscintigraphy, that is some heterogeneity in antigen expression by tumor cells, which may be variously displayed by different groups of cells in the same tumor lesion, as well as by different tumor sites in the same patient (see Figure 1).

As a matter of fact, the more recent nuclear medicine investigations on pancreatic adenocarcinoma concern almost totally PET applications in the diagnosis, staging and follow-up of this cancer, showing the excellent performances of such technique, also in comparison with other imaging modalities, also in this tumor [29-35].

It should however not be neglected that one of the ultimate goals of in vivo tumor targeting with MoAbs is represented by the possibility of cancer treatment [36], achieved usually by labeling the MoAb tracers with beta-emitting radioisotopes (such as $^{131}$I, $^{90}$Y, etc.). In such case, tumor immunoscintigraphy would simply serve as a preliminary test for selecting patients to be treated with radioimmunotherapy, as also for estimating the radiosodimetric burden to tumor lesions.

In this regard, immunoscintigraphy of pancreatic cancer, particularly in patients with recurrent tumor such as peritoneal carcinosis might resume a definite role in planning radioimmunotherapy for these patients. The possible use of labeled MoAbs for radioimmunoguided surgery of locally recurring pancreatic cancer might also be worthwhile exploring, in a manner similar as already done for colorectal cancer.

References

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Correspondence to:
Giuliano Mariani, M.D.
Nuclear Medicine Service, DIMI
Viale Benedetto XV, n. 6
I-16132 Genoa
Italy