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## Anti-CA19-9 Diabody as a PET Imaging Probe for Pancreas Cancer

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***Background.*** **Intact antibodies are poor imaging agents due to a long serum half-life (10–20 d) preventing adequate contrast between the tumor and surrounding blood. Smaller engineered antibody fragments overcome this problem by exhibiting shorter serum half-lives (4–20 h).The diabody (55 kDa) is the smallest antibody fragment, which retains the bivalency of the intact antibody. Our goal was to develop and characterize the anti-CA19-9 diabody fragment and determine its ability to provide antigen specific imaging of pancreas cancer.**

***Methods.*** **The diabody DNA construct was created by isolation of the variable region genes of the intact anti-CA19-9 antibody. Diabody expression was carried out in NS0 cells and purified using HPLC from supernatant. Specific antigen binding was confirmed with flow cytometry and immunofluorescence. Radiolabeled diabody was injected into mice harboring an antigen positive xenograft (BxPC3 or Capan-2) and a negative xenograft (MiaPaca-2). MicroCT and MicroPET were performed at successive time intervals after injection. Radioactivity was measured in blood and tumor to provide objective confirmation of the microPET images.**

***Results.*** **Immunofluorescence and flow cytometry showed specific binding of the anti-CA19-9 diabody. Pancreas xenograft imaging of BxPC3/MiaPaca-2 and Capan-2/MiaPaca-2 models with the anti-CA19-9 diabody demonstrated an average tumor:blood ratio of 5.0 and 2.0, respectively, and an average positive:negative tumor ratio of 11 and 6, respectively. With respect to the tumor:blood ratio, these data indicate five times and two times more radioactivity in the tumor than in the blood yielding adequate contrast between tumor tissue and background (i.e., blood) to create the representative microPET images.**

***Conclusions.*** **We successfully engineered a functional diabody against CA19-9, a tumor antigen present on the vast majority of pancreas cancers. Additionally, we demonstrate high contrast antigen specific microPET imaging of pancreas cancer in xenograft models.** Published by Elsevier Inc.

***Key Words:*** **pancreas cancer; PET imaging; tumor targeting; CA19-9; antibody; diabody.**

### INTRODUCTION

Pancreatic cancer is considered one of the most lethal cancers where incidence approximates mortality [1]. Symptoms that might suggest pancreatic cancer occur late in the disease process and are usually vague. Because of this, most patients diagnosed with pancreatic cancer have metastatic disease at presentation. Patient outcomes are poor with actual 5-y survival of approximately 5% [1, 2]. Additionally, those patients who undergo surgical resection for local disease often recur due to unrecognized micrometastatic foci of disease such that overall 5-y survival for this group is only 25% [3–5]. In other words, three of every four patients with pancreatic cancer who are initially diagnosed with localized disease by our current imaging modalities actually have systemic disease. These data indicate the need for novel strategies aimed at improving our ability to image cancer and develop targeted therapies to treat disseminated disease.

Monoclonal antibodies and radioimmunopharmaceuticals represent one of the fastest growing classes of drugs for targeting cancer. They offer high specificity for cancer antigens differentially expressed on cancer cells. Recently, antibodies have been radiolabeled and

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used for molecular imaging of cancer with positron emission tomography (PET). The most common use of PET scanning using fluorodeoxyglucose (FDG) exploits the tumor cells' dependence on glycolysis (Warburg effect) and resulting increased uptake of glucose. Although FDG-PET has been proven valuable for a variety of cancers, some tumors such as pancreatic cancer do not routinely exhibit a significant increase in uptake of glucose to differentiate reliably from normal pancreas and liver [6–10]. Thus, it is limited by a lack of sensitivity to distinguish small lesions for pancreatic cancer diagnosis [6–10]. ImmunoPET or antibody based PET imaging takes advantage of the high specificity of an antibody for its cancer antigen and can be more specific and sensitive in targeting cancer. It has been estimated that PET technology has the potential to provide highly sensitive molecular imaging based on its ability to detect nanomolar to subnanomolar ($10^{-9}$–$10^{-11}$M) concentrations of radiolabeled imaging agents and thus should provide a major improvement to our current anatomic imaging modalities such as CT or MRI [11].

Although favorable in terms of stability, affinity, and specificity, full-sized antibodies used for imaging are hampered by their long serum half-lives causing an increased "background" nonspecific imaging signal. The most common approach to circumvent these issues is the creation by recombinant techniques of domain-deleted antibodies that have decreased half-lives (Fig. 1A). For imaging purposes, the optimal fragment will have fast clearance from the circulation while possessing quick targeting and persistent binding to the tumor to create the greatest signal differential. The single-chain Fv (scFv; 27 kDa) fragment has shown extremely rapid tumor and serum kinetics in clinical studies due to its small size; yet, its monovalency may be a limiting factor in its affinity to and persistence within the tumor [12]. The multivalent fragments such as diabodies (dimers of scFvs, 55 kDa) or minibodies [(scFv-$C_H3)_2$ fusion proteins, 80 kDa] have shown promise as *in vivo* imaging agents in preclinical studies likely secondary to their bivalency and short half-lives [13–15]. In particular, Williams *et al.* showed that the diabody would be the optimal same-day imaging agent compared with the intact, $F(ab')_2$, and minibody [15].

The most highly expressed pancreas cancer antigen is carbohydrate antigen (CA) 19-9. This epitope is a glycosylation product added to membrane and secreted proteins. It is known to be presented in high copy number on approximately 90% of all pancreas cancers and widely used as a serum marker of pancreas cancer [16–20]. CA19-9 was originally identified by Dr. Koprowski through preferential binding of a monoclonal mouse antibody 1116-NS-19-97 raised against colon cancer cells [21, 22]. This tumor antigen is absent to minimally present on normal pancreas epithelial cells apical membrane

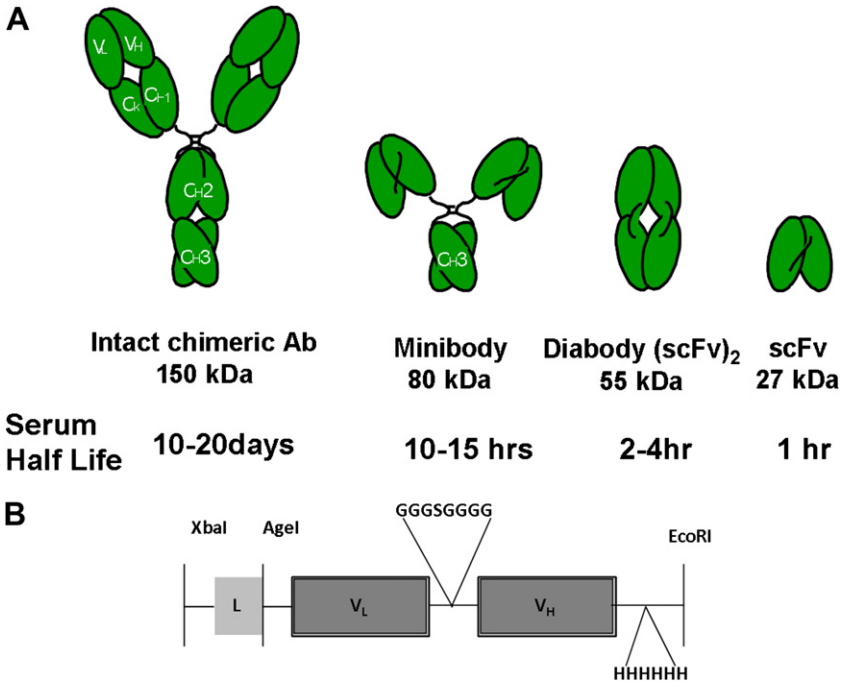


**FIG. 1.** (A) Schematic representation of an intact antibody and engineered antibody fragments with the generic half-life beneath each fragment. As size decreases, so too does the half-life of the fragment as indicated by the half-lives listed. (B) DNA construct of the anti-CA19-9 diabody showing restriction sites, a leader sequence for mammalian expression (L), variable light and variable heavy chain DNA (VL and VH), a linker sequence of eight amino acids between the VL and VH chains, and a 6×Histidine tag at the C-terminus for purification. (Color version of figure is available online.)