Antibody engineering - Part 5
Antibody expression and clinical application
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Antibody expression

Several expression systems are available for the production of antibody and antibody fragments including bacteria, yeast, plants, insect cells, and mammalian cells.

Each has advantages, potential applications, and limitations.

- Bacterial expression
- Expression in eucariotic microorganisms (S. Cerevisiae)
- Insect cell expression (Baculovirus vectors)
- Plant expression
- Mammalian cell expression

Bacterial expression

Bacteria cannot assemble whole glycosylated antibodies but they are very suitable hosts for the production of antibody fragments.

Yeast expression

Complete antibodies have been expressed successfully in yeast, but they contain high-mannose, multiple-branched oligosaccharides and were shown to be defective in effector functions such as complement-mediated lysis.
Insect cell expression

Antibodies produced in insect cells via baculovirus vectors also contain carbohydrate structures very different from those produced by mammalian cells.

Mammalian cell expression

Intact, effector function-competent antibodies have been successfully expressed in myeloma cells and also in nonlymphoid mammalian cells, which possess the mechanisms required for correct immunoglobulin assembly, posttranslational modification, and secretion
Mammalian cell expression

- Secreted antibody production
- Intracellular expression of antibody fragments
- Surface expression of antibody fragments
Intracellular expression of antibody fragments

- Eukaryotic cells can produce antibodies that function intracellularly.

- Such intracellular antibodies (intrabodies) represent a powerful and promising approach to modulate the function of selected intracellular gene products in higher organisms (phenotypic knock-out) or to protect the cell from infectious agents (intracellular immunization).

Antibody constructs in cancer therapy

Mechanisms operating with therapeutic mAbs

Enlarged text:

- **Targeting**: mAb crosslinking
- **Blocking**: mAb to trigger tyrosine phosphorylation of intracellular proteins
- **Signalling**: Crosslinked by Fc receptor-bearing effector cells

**Diagram notes**:
- Red: Soluble factor receptor
- Pink: Crosslinked receptor
- Green: Target-molecule for Ab-attack
- Blue: Target-molecule for reactive cell
- Black: mAb
- Red: Soluble factor
- Green: Fc receptor
- Blue: Target-molecule ligand

**Text content**:

- crossing of the B-cell receptor (BCR) with Ab results in growth
- Such results were consistent with mAb to trigger tyrosine phosphorylation of intracellular proteins
- Patient's anti-Id mAb and the ability of that between the therapeutic efficacy of a patient
- which showed a strong positive correlation for such activity came from anti-Id studies, inhibitory Fc receptor, Fc bodies can be increased strikingly if the
- Inhibitory Fc indicates an important potential role for mour therapy; and second, because they expressing effectors in mAb-mediated tu-
- significant for two reasons: first, because they by genetic knockout. These results are sig-
- that the enhanced effect in Fc lymphatic leukaemia in the USA, is a potent
- Application for the treatment of chronic
- currently involved in a Biological Licence
- of mAb targeting. Campath1H, which is
- crucial to its therapeutic success, and that its
- shown that the isotype of Campath 1H is
- anti-Id-induced dormancy still express surface Id, but often show
- in vitro
- generally felt to operate via this mechanism
- direct to the soluble factor itself or to the factor's receptor. Similarly, mAbs can prevent cell–cell
- therapeutic mAbs preventing access of a growth factor, cytokine, or other soluble mediator by binding
- function is highly efficient and can operate at a number of levels. Thus, the ‘blocking’ section shows
- success of mAbs in the treatment of a range of diseases (Table 1) demonstrates that this blocking
- ally been seen as glycoproteins that protect the body by blocking invasion by microbes. The current
- potential mechanisms have been identified that allow mAbs to operate
- in vitro
- in vivo
- Fig. 2.
Biological properties of solid tumors

- Solid tumors differ from normal tissue with regard to vasculature, interstitial fluid pressure, cell density, tissue structure and composition, and extracellular matrix (ECM) components.

- Interstitial fluid pressure is elevated uniformly throughout a tumor and drops precipitously to normal values in the tumor’s periphery or in the immediately surrounding tissue.

- Macromolecules must diffuse against this pressure gradient to penetrate tumors.

Antibodies attack tumors by 3 general mechanisms

1. opsonization, which triggers killing by immune cells

2. modification of innate biological processes such as growth and apoptosis

3. delivery of a cytotoxic payload such as a chemotherapy drug, catalytic toxin, radioisotope or enzyme
Definition of the concept ‘tumor target zone’ based on pharmacokinetic and antigen-binding properties of monovalent and multivalent antibodies.

Schematic diagram displaying blood clearance (orange), tumor retention (blue) and tumor penetration (green) estimates of monovalent and multivalent antibodies. Small monovalent antibodies show great tumor penetration, but fast off-rates and blood clearance, large multivalent antibodies show high tumor retention times, but long serum half-lives and poor tumor penetration.

In designing antitumor constructs, a suitable balance must be found between properties that promote tumor penetration and those that promote tumor retention.

The optimal binding affinity balances 2 goals:

- sufficiently rapid diffusion to enable penetration into the core of the tumor
- sufficiently long retention to enable signaling inhibition, internalization, or other events required for a pharmacodynamic effect

Ab characteristics that influence tumor distribution

- A major determinant of speed of diffusion through tumors is molecular size: scFv fragments diffuse approximately 6 times faster than IgG, due to their smaller size and other factors.

- Molecular charge and shape also affect tumor distribution.

- Affinity for the target antigen (Ag) is an important variable affecting tumor distribution.
mAbs in the potentiation of immune responses

Fig. 3.

Tumour processing
- MHC II priming
- MHC I X-priming

Stimulatory mAb
- (e.g., a-CD137
- a-CTLA4
- a-BAT)

CTL

Co-stimulation
- (e.g., CD28–B7)

Cytokines
- (e.g., IL-12)

APC

T-cell-based response

Stimulatory mAb
- (e.g., a-CD40
- a-RANK
- a-Flt3)

MHC I

Tumour antigen

Tumour

MHC II

Therapeutic mAb metrics

Cumulative number of human mAbs entering clinical study between 1985 and 2008

Approval phases for the 23 products were affected by whether the candidate received a priority or a standard review by the FDA (Table 1). An application is assigned a priority review when the candidate might, if approved, be a safe and effective therapy where none currently exists, or provide significant improvement in disease treatment. If the candidate does not meet either of these criteria, then the application is assigned a standard review. The FDA's current performance goal for priority reviews is 6 months for a first action for 90% of the applications submitted in any given fiscal year. The first action is not necessarily an approval and 10% of the reviews might take longer than 6 months even when the first action is an approval.

Clinical phases were calculated for the 23 currently marketed mAbs that were approved after 1996. The mean clinical phase for these products was 85.7 months (Table 2). The broad range (37.3–140.3 months) was likely due to the variety of indications studied, which included solid and hematological cancers, disorders of the immune system, infectious disease and eye and bone diseases. Eleven of the 23 products (48%) were first approved as treatments for immunological indications, including psoriasis, asthma, Crohn disease, multiple sclerosis, rheumatoid arthritis (RA), paroxysmal nocturnal hemoglobinuria and prevention of acute kidney rejection. A common feature of these conditions is the need for immune system modulation. The mean clinical phase for the 11 immunological mAbs was 85.6 months and the range was 59.2–113.2 months. Nine of the 23 products (39%) were approved for cancer. These mAbs were first approved as treatments for non-Hodgkin lymphoma, chronic lymphocytic leukemia, breast cancer and colorectal cancer. The mean clinical phase for the nine products was 91.0 months and the range was 50.5–140.3 months.

Only three of the 23 products were approved for indications other than immunological diseases or cancer. Palivizumab (MedImmune) was approved for prevention of respiratory syncytial virus (RSV) infection, ranibizumab (Genentech) was approved for treatment of patients with neovascular age-related macular degeneration and denosumab (Genentech) was approved for postmenopausal osteoporosis. Clinical phases cannot be given for the individual products because the information is proprietary.

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<tr>
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*Products approved between January 1997 and July 2010. FDA, US Food and Drug Administration; mAbs, monoclonal antibodies.
A total of 28 mAb therapeutic products have been approved by the United States Food and Drug Administration (US FDA) and 4 are undergoing regulatory review.

Global sales exceeded US $1 billion in 2009 for each of at least nine mAb products.
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Table 2. Mean (median) clinical and FDA approval phases for 23 therapeutic monoclonal antibodies

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<thead>
<tr>
<th>INN (trade name)</th>
<th>Description, target</th>
<th>Therapeutic category</th>
<th>Year (review status) of first FDA approval</th>
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<tbody>
<tr>
<td>Muromonab-CD3 (Orthoclone OKT3)</td>
<td>Murine, IgG2α, anti-CD3</td>
<td>Immunological</td>
<td>1986* (NA)</td>
</tr>
<tr>
<td>Abciximab (ReoPro)</td>
<td>Chimeric, IgG1κ Fab, anti-GP1b/IIα</td>
<td>Hemostasis</td>
<td>1994 (P)</td>
</tr>
<tr>
<td>Rituximab (Rituxan)</td>
<td>Chimeric, IgG1κ, anti-CD20</td>
<td>Cancer</td>
<td>1997 (P)</td>
</tr>
<tr>
<td>Daclizumab (Zenapax)</td>
<td>Humanized, IgG1κ, anti-CD25</td>
<td>Immunological</td>
<td>1997* (P)</td>
</tr>
<tr>
<td>Basiliximab (Simulect)</td>
<td>Chimeric, IgG1κ, anti-CD25</td>
<td>Immunological</td>
<td>1998 (P)</td>
</tr>
<tr>
<td>Palivizumab (Synagis)</td>
<td>Humanized, IgG1κ, anti-RSV</td>
<td>Anti-infective</td>
<td>1998* (P)</td>
</tr>
<tr>
<td>Infliximab (Remicade)</td>
<td>Chimeric, IgG1κ, anti-TNF</td>
<td>Immunological</td>
<td>1998 (P)</td>
</tr>
<tr>
<td>Trastuzumab (Herceptin)</td>
<td>Humanized, IgG1κ, anti-HER2</td>
<td>Cancer</td>
<td>1998 (P)</td>
</tr>
<tr>
<td>Gemtuzumab ozogamicin (Mylotarg)</td>
<td>Humanized, IgG4κ, anti-CD3; toxin</td>
<td>Cancer</td>
<td>2000* # (P)</td>
</tr>
<tr>
<td>Alemtuzumab (Campath)</td>
<td>Humanized, IgG1κ, anti-CD52</td>
<td>Cancer</td>
<td>2000 (P)</td>
</tr>
<tr>
<td>Ibritumomab tiuxetan (Zevalin)</td>
<td>Murine, IgG1κ, anti-CD20; Y-90</td>
<td>Cancer</td>
<td>2002 (P)</td>
</tr>
<tr>
<td>Adalimumab (Humira)</td>
<td>Human, IgG1κ, anti-TNF</td>
<td>Immunological</td>
<td>2002 (S)</td>
</tr>
<tr>
<td>Omalizumab (Xolair)</td>
<td>Humanized, IgG1κ, anti-IgE</td>
<td>Immunological</td>
<td>2003* (S)</td>
</tr>
<tr>
<td>Tositumomab-I131 (Bexxar)</td>
<td>Murine, IgG2αλ, anti-CD20; I-131</td>
<td>Cancer</td>
<td>2003 (P)</td>
</tr>
<tr>
<td>Efalizumab (Raptiva)</td>
<td>Humanized, IgG1κ, anti-CD11a</td>
<td>Immunological</td>
<td>2003* # (S)</td>
</tr>
<tr>
<td>Cetuximab (Erbitux)</td>
<td>Chimeric, IgG1κ, anti-EGF receptor</td>
<td>Cancer</td>
<td>2004 (P)</td>
</tr>
<tr>
<td>Bevacizumab (Avastin)</td>
<td>Humanized, IgG1κ, anti-VEGF</td>
<td>Cancer</td>
<td>2004 (P)</td>
</tr>
<tr>
<td>Natalizumab (Tysabri)</td>
<td>Humanized, IgG4κ, anti-α4β1-integrins</td>
<td>Immunological</td>
<td>2004 (P)</td>
</tr>
<tr>
<td>Ranibizumab (Lucentis)</td>
<td>Humanized, IgG1κ Fab, anti-VEGF</td>
<td>Ophthalmic</td>
<td>2006 (P)</td>
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<tr>
<td>Panitumumab (Vectibix)</td>
<td>Human, IgG2κ, anti-EGF receptor</td>
<td>Cancer</td>
<td>2006# (P)</td>
</tr>
<tr>
<td>Eculizumab (Soliris)</td>
<td>Humanized, IgG2/4κ, anti-C5</td>
<td>Immunological</td>
<td>2007# (P)</td>
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<tr>
<td>Certolizumab pegol (Cimzia)</td>
<td>Humanized, Fab’, anti-TNF; PEG</td>
<td>Immunological</td>
<td>2008# (S)</td>
</tr>
<tr>
<td>Golimumab (Simponi)</td>
<td>Human, IgG1κ, anti-TNF</td>
<td>Immunological</td>
<td>2009 (S)</td>
</tr>
<tr>
<td>Canakinumab (Ilaris)</td>
<td>Human IgG1κ, anti-IL1β</td>
<td>Immunological</td>
<td>2009# (P)</td>
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<tr>
<td>Ustekinumab (Stelara)</td>
<td>Human IgG1κ, anti-IL12/23</td>
<td>Immunological</td>
<td>2009# (S)</td>
</tr>
<tr>
<td>Ofatumumab (Arzerra)</td>
<td>Human IgG1κ, anti-CD20</td>
<td>Cancer</td>
<td>2009# (P)</td>
</tr>
<tr>
<td>Tocilizumab (Actemra)</td>
<td>Humanized IgG1κ, anti-IL6 receptor</td>
<td>Immunological</td>
<td>2010# (S)</td>
</tr>
<tr>
<td>Denosumab (Prolia)</td>
<td>Human IgG2, anti-RANK-L</td>
<td>Bone disorders</td>
<td>2010# (S)</td>
</tr>
<tr>
<td>Motavizumab</td>
<td>Humanized IgG1κ, anti-RSV</td>
<td>Anti-infective</td>
<td>Pending (S)</td>
</tr>
<tr>
<td>Raxibacumab</td>
<td>Human IgG1, anti- B. anthrasis PA</td>
<td>Anti-toxin</td>
<td>Pending (P)</td>
</tr>
<tr>
<td>Belimumab</td>
<td>Human IgG1κ, anti-BLyS</td>
<td>Immunological</td>
<td>Pending (P)</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>Human IgG1κ, anti-CTLA-4</td>
<td>Cancer</td>
<td>Pending (P)</td>
</tr>
</tbody>
</table>
Of the 28 products, the protein sequences of 3 (11%) were murine-derived, 5 (18%) were chimeric, 13 (46%) were humanized and 7 (25%) were fully human and derived from either a transgenic mouse or phage display platform.
Percentage of four types of mAbs in clinical development during the periods 1990-1999 and 2000-2008.
Transition rates between clinical phases for human mAbs

Therapeutic antibody fragment metrics
Phase of clinical development for 54 antibody fragment therapeutics

Distribution of fragment type across developmental categories

Molecular classes of fragment therapeutics in preclinical research and clinical development - Fab

PC, preclinical; App, approved.
Molecular classes of fragment therapeutics in preclinical research and clinical development - scFv

PC, preclinical; App, approved; tascFv, tandem single chain variable fragment; Bis, bispecific

Molecular classes of fragment therapeutics in preclinical research and clinical development - 3rd generation (3G)

PC, preclinical; App, approved; 3G, third generation.

The approved products accounted for ~$20 billion of healthcare spending in 2006, and the market is growing at ~14% per year.

Eight mAbs had sales >$1 billion in 2007, with Rituxan (rituximab; Genentech, S. San Francisco, CA, USA) being the bestseller, with sales of $5.2 billion. 

Cost of therapeutic mAbs (Italy)

€ 650 per year of Denosumab (post-menopausal osteoporosis)

€ 550,000 per year of Eculizumab (paroxysmal nocturnal hemoglobinuria, PNH)

€ 7,500 to 13,000 per year Tocilizumab and Rituximab (Rheumatoid Arthritis)

€ 130,000 for Brentuximab vedotin, 1 year of treatment for a 70 kg patient (anti-neoplastic mAbs)

Contrast relatively to the intermediate clearing (I253A and H310A) and the fast clearing (H310A/H435Q) scFv-Fc fragments. The blood clearance of the scFv-Fc H310A/H435Q, also referred to as scFv-Fc double mutant (scFv-Fc DM), is shown in Fig. 2A. This fragment exhibits longer persistence in the circulation than the minibody which results in higher tumor uptake as seen in Fig. 2B. The PET images of 124I- and 64Cu-DOTA-scFv-Fc also show high, specific tumor uptake in xenografts (Fig. 5A). The pharmacokinetics of radioiodinated versus radiometal-labeled I253A, H310A and H310A/H435Q scFv-Fc variants were further evaluated in xenografted mice to predict their therapeutic potential.

Tumor uptakes were inversely related to blood clearance, and hepatic radiometal activity correlated with the blood clearance rate of the fragment; that is faster clearance resulted in higher activity. On the basis of the biodistribution data with 125I and 111In, it was predicted that the fast clearing scFv-Fc DM would be able to deliver /H110227000 cGy to the tumor, with favorable tumor to liver and kidney ratios when radiolabeled with 131I, whereas for 90Y therapy, a low-activity antibody would be the protein of choice as the liver or kidney activities would be lower.

The scFv-Fc DM fragment has also been evaluated in other tumor models. An anti-HER2 scFv-Fc DM fragment was evaluated by PET in xenografted mice after conjugation with DOTA and radiolabeling with 64Cu. The scFv-Fc DM fragment exhibited over 2-fold improved tumor targeting and reduced kidney activity relatively to the anti-HER2 minibody fragment, which was probably due to its slightly longer residence time in the blood. When this fragment was labeled with 124I however, very low activity was observed in the tumor. In another recent study, an anti-CD20 scFv-Fc DM fragment with IgG4 Fc was evaluated in tumor bearing mice after radioiodination with 124I (Fig. 5B). Although rapid tumor localization was observed, the activities in the tumor and the blood were 2-fold lower than of the minibody. Such difference was not observed with the anti-CEA (Fig. 2A) and anti-HER2 fragments. Thus, it appears to be related to the change of IgG Fc isotype. Intact human IgG4 has been reported to clear faster than human IgG1 in both humans and mice.

107,108 Consistent with this, a lower background activity of the radioiodinated anti-CD20 scFv-Fc DM fragment than that of the anti-CEA scFv-Fc DM at 18-20 hours p.i. was observed. Thus, the introduced mutations in IgG4 Fc may accelerate the scFv-Fc fragment to clear faster than the minibody.

Figure 5B also shows the images obtained with 64Cu-DOTA scFv-Fc DM fragment. A rapid method to generate scFv-Fc fragments targeting endothelial cell from binders isolated from phage display libraries and in vivo evaluation by SPECT have been described.

109 Using this approach, 3 antibodies were identified that, when converted to scFv-Fc using mouse IgG1 Fc, targeted the lungs in rats with uptakes of 30%-40% ID/g at 1 and 2 hours p.i. Because the blood activities were less than 2% ID/g, high-contrast images were achieved early.

Impact of Antibody Modifications on Imaging

From the numerous studies done with the different engineered antibody fragments, there are several factors, such as antibody constructs for imaging.

The discipline of molecular imaging is one of the most rapidly growing areas of science

- Molecular imaging offers the ability to visualize, characterize, and measure processes on molecular and cellular levels noninvasively in living systems.
- The key players for obtaining this information are the molecular imaging agent (probe or tracer) and the target, which can be intracellular or cell surface proteins.
- Radiolabeled probes for SPECT (Single-Photon Emission Computed Tomography) or PET (Positron Emission Tomography), offer visualization of physiological and biochemical changes.
- In contrast, conventional imaging modalities (radiography, ultrasonography, CT, and magnetic resonance imaging (MRI)) offer visualization of nonspecific changes related to morphology.

After the optimal antibody-based vector has been generated, they can be tagged with radionuclides (PET, SPECT), magnetic nanoparticles (MRI), or fluorescence/bioluminescence (optical) probes that enable a variety of imaging modalities.
scFv limitations in molecular imaging

- The major limitations of scFv molecules are low functional affinity and short in vivo half-life due to their valency and small size, respectively.

- Consequently, rapid dissociation from the target antigen due to monovalent binding will result in modest retention time in the target and potentially poor image quality.
%ID/g = percentage of injected dose per gram

nuclide for same day imaging.

suggests that the diabody is compatible with a short-lived radio-
with clearance predominantly through the kidneys. This study

firefly luciferase being the most commonly used for imag-
enzyme. Luciferases are mainly used as reporter genes, with

process called bioluminescence) are known as luciferases.

172

mice.

Clear delineation of the positive tumor was obtained as
negative C6 rat glioma tumors (arrowhead) are seen in all the images. Renal clearance is evident at 4 hours p.i. with

18

F-labeled diabodies, which is expected for a fragment of <60 kDa in size. High-contrast imaging is achieved with

124

I-labeled diabody at 20 hour p.i. due to almost complete elimination of any background activity, and a PET/computed
tomography overlaid image is shown for anatomical reference. (B) Bioluminescence imaging of mice injected with diabody-
Renilla (Db-RLuc8) and -Gaussia (Db-GLΔ15) luciferase fusion proteins at 4 hours p.i. The control mouse injected with
Rluc8 only is shown. Specific targeting to the positive tumor is seen with both fusion proteins.

Figure 3 Small animal positron emission tomography (PET) (A) and optical imaging (B) of anti-CEA T84.66 diabody in
LS174T xenograft-bearing mice.\textsuperscript{75-78} (A) Coronal PET images of mice injected with diabody after radiolabeling with
different positron emitting radionuclides. Specific localization to the positive tumor (arrow) and no localization to the
negative C6 rat glioma tumors (arrowhead) are seen in all the images. Renal clearance is evident at 4 hours p.i. with
\(^{64}\text{Cu}\) and \(^{18}\text{F}\)-labeled diabodies, which is expected for a fragment of <60 kDa in size. High-contrast imaging is achieved with
\(^{124}\text{I}\)-labeled diabody at 20 hour p.i. due to almost complete elimination of any background activity, and a PET/computed
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agent with rapid high-level accumulation of activity in xenografts and rapid disappearance from the circulation.

Se-rial small animal PET imaging with 64Cu-DOTA-minibody demonstrated rapid and specific localization to the positive tumor with increasing accumulation of activity over time (from 2 to 24 hours p.i.) that was 20% ID/g at 24 hours with a tumor-to-soft-tissue ratio of 6.

As expected, high persistent activity in the liver was also seen with activity moving into the GI-tract at the late time-point. PET images with 124I-minibody produced excellent images at 18 hours p.i., with tumor uptakes of 21% ID/g and tumor-to-background ratio of 26.2.

The high ratio is due to the rapid clearance from the blood combined with fast metabolism and clearance of radioiodine from nontargeted tissues. In comparison with FDG scan, both radiometal labeled and radioiodinated minibody produced am u c hc l e a n e ra n dh i g h e rc o n t r a ti m a g e .

Minibodies have also been produced against HER2 and PSCA expressed in breast and prostate cancers.

Although both anti-HER2 and anti PSCA minibodies demonstrated similar pharmacokinetics to the anti-CEA minibody and localized specifically to tumors, the accumulation in tumors was considerably lower. Activity in the tumor was about 5% ID/g at 12-24 hours with anti-HER2 131I-10H8 minibody with tumor-to-blood ratios of 1.1 at 12 hours that increased to 3 at 48 hours.

This lower activity was explained by internalization of the minibody on binding, followed by metabolism and rapid excretion of radiolabel from the cells.

Biodistribution with anti-HER2 111In-DOTA-10H.8 minibody showed hepatic clearance is evident with the 64Cu-DOTA minibodies as expected for a fragment of >60 kDa in size.


Figure 4 Small animal PET imaging of xenograft-bearing mice injected with 124I-labeled (top) and 64Cu-DOTA mini-bodies (bottom) against different cell surface antigens. Minibodies against CEA (T84.66 Mb), CD20 (Rx. Mb), HER2 (Herc. Mb) were radiolabeled and imaged with both radionuclides. The anti-HER2 C6.5Mb and anti-PSCA 2B3 Mb were radiolabeled with 124I only. All 124I-labeled minibodies, except the 124I-Herc. Mb, produce high-contrast images at 18-21 hours p.i. Less contrast is achieved with 64Cu-DOTA minibodies due to high-background activity. Hepatic clearance is evident with the 64Cu-DOTA minibodies as expected for a fragment of >60 kDa in size.
A rapid method to generate scFv-Fc fragments targeting CEA (A) and CD20 (B).\textsuperscript{99,105} The background activity of \textsuperscript{124}I-anti-CD20 scFv-Fc with IgG4 Fc is less (1.9\% ID/g at 21 hours p.i.)\textsuperscript{99} than that seen with \textsuperscript{124}I-anti-CEA scFv-Fc with IgG1 Fc region (6.9\% ID/g at 12 hours and 2.8\% ID/g at 24 hours p.i.),\textsuperscript{19} suggesting a more rapid clearance rate of this anti-CD20 fragment. This is consistent with published observation of the different blood clearances of human IgG1 and IgG4 isotypes. Again, \textsuperscript{64}Cu-labeled fragments produce less contrast than \textsuperscript{124}I-labeled fragments, and hepatic clearance is evident.

Figure 5 Small animal PET imaging of xenograft-bearing mice injected with \textsuperscript{64}Cu and/or \textsuperscript{124}I-labeled scFv-Fc double mutant (DM) fragments targeting CEA (A) and CD20 (B).\textsuperscript{99,105} The background activity of \textsuperscript{124}I-anti-CD20 scFv-Fc with IgG4 Fc is less (1.9\% ID/g at 21 hours p.i.)\textsuperscript{99} than that seen with \textsuperscript{124}I-anti-CEA scFv-Fc with IgG1 Fc region (6.9\% ID/g at 12 hours and 2.8\% ID/g at 24 hours p.i.),\textsuperscript{19} suggesting a more rapid clearance rate of this anti-CD20 fragment. This is consistent with published observation of the different blood clearances of human IgG1 and IgG4 isotypes. Again, \textsuperscript{64}Cu-labeled fragments produce less contrast than \textsuperscript{124}I-labeled fragments, and hepatic clearance is evident.

Factors that influence recombinant Ab pharmacokinetics

- There are several factors, such as the molecular weight, Fc domains, valency, and specificity that will influence targeting and pharmacokinetics.
- Fragments above 60 kDa will clear through the liver, whereas those below this size will clear through the kidneys.
- Presence of Fc domains will increase the serum residence time, and increase the exposure time to the target.
- However, for imaging purposes, a more rapid clearance from the blood is warranted to increase contrast and sensitivity.
- Difficulties in localizing and accumulating activity in target tissue are related to specificity and avidity.
- In addition, the number of target antigen per cell and whether it internalizes or not will affect accumulation in target tissue.