



WMIC educational session
Dublin, 2012
Monoclonal antibodies, fragments and peptides

Nick Devoogdt, PhD

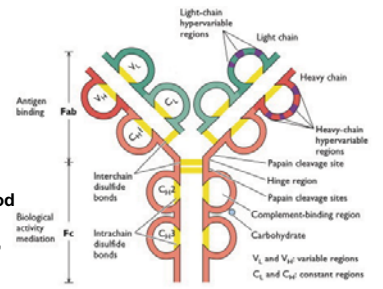


antibody structure

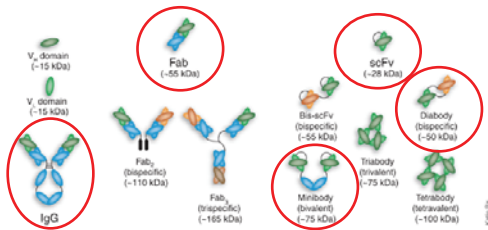
• antigen binding mediated by Fv fragment (V_H/V_L)

• Fc fragment exerts effector functions via interaction with FcγR on MΦs and with complement

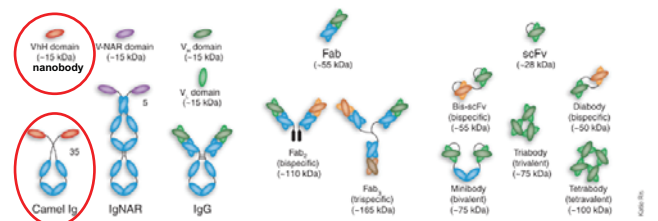
• Fc fragment increases Ig blood half-life by rescuing Igs from default degradation pathways, via interaction with FcRn receptors in liver



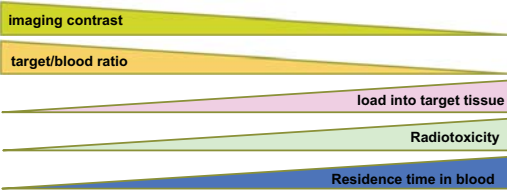
antibody fragments



antibody fragments



effect of residence time in blood



imaging versus therapy

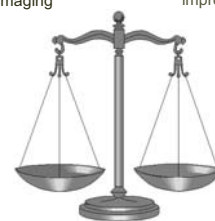
• Reduce Ab size to cut-off below renal clearance
• Contrast = primordial

theragnostic?

• Maintain Ab size above renal clearance cut-off or attach to carrier
• antibody load = primordial

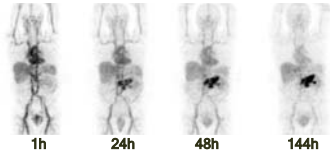
In vivo imaging
Reduce half life of antibody to fasten imaging

Therapy
Increase half life of antibody to improve efficacy



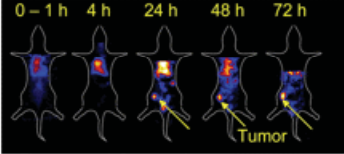
imaging with mAbs

⁸⁹Zr-rituximab (targeting CD20) of intra-abdominal lymphoma



Muyile K, Van Dongen G et al.

¹¹¹In-Antimindin mAb in xenografted mice

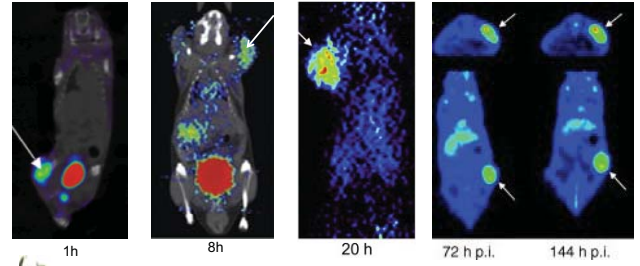


Schneider et al., 2009



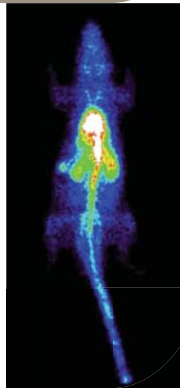
tumor imaging with radio-labeled Abs and fragments

^{99m}Tc-anti-HER2 nanobody Vaneeycken, 2011 ¹²⁴I-Cys-anti-CD20 diabody Olafsen, 2010 ¹²⁴I-anti-HER2 minibody Olafsen, 2010 ⁸⁹Zr-anti-HER2 mAb Munnink, 2010



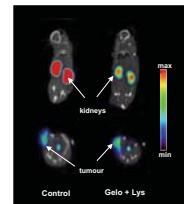
nanobody-tracer pharmacological behavior

- efficient extravasation & tissue penetration
- fast tissue and blood clearance
- low aspecific binding in non-targeted tissues
- efficient filtering via the kidneys into urine
- high kidney retention, which is problematic
 - ✓when imaging close to kidneys or bladder (pancreas, renal metastases, prostate, ...)
 - ✓for dosimetry and renal toxicity reasons

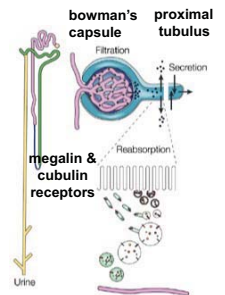


renal re-uptake mechanism

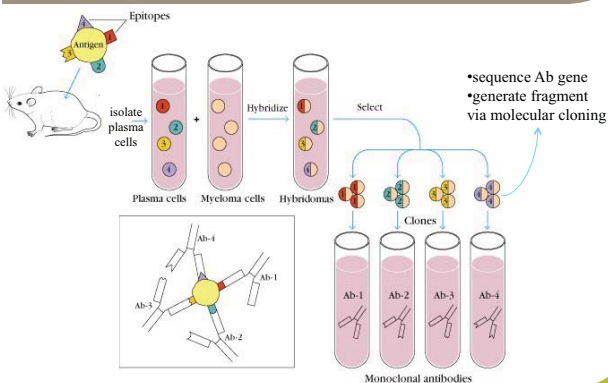
- kidney re-uptake influenced by:
- AA sequence of Ab-fragment (# pos charged residues)
 - method of labeling and radionuclide
 - blockade of megalin/cubulin function



Gainkam et al., Nucl Imaging & Biol 2011



antibody generation



generation of antibody fragment libraries

Starting material:

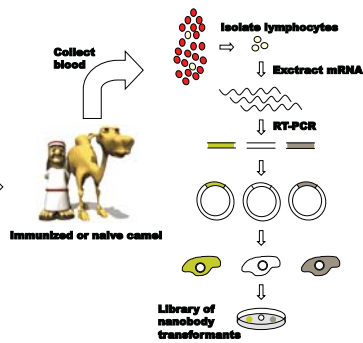
- naive library
- immune library
- synthetic library



generation of antibody fragment libraries

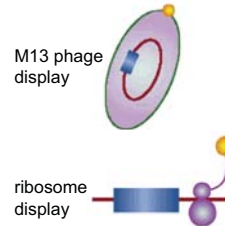
Starting material:

- naive library
- immune library
- synthetic library



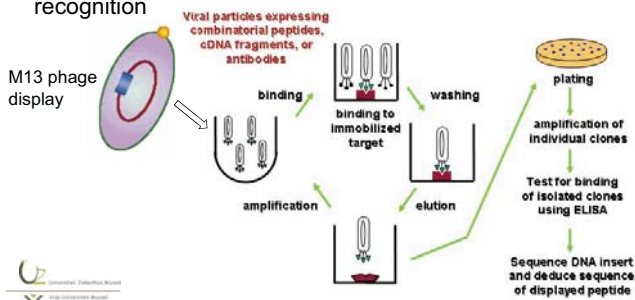
selection of antibody fragments

physical coupling of phenotype (Ab fragment display) to genotype (Ab coding sequence) and selection for antigen recognition



selection of antibody fragments

physical coupling of phenotype (Ab fragment display) to genotype (Ab coding sequence) and selection for antigen recognition



important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility (aggregation scFvs)

important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility
- ease of production and purification (both for preclinical studies and under cGMP conditions)

important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility
- ease of production and purification
- epitope targeted
 - ✓no competition with therapeutic antibody or drug
 - ✓no agonistic or antagonistic activity (toxicity studies!)
 - ✓conserved epitope in order to perform preclinical and clinical imaging with same compound

important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility
- ease of production and purification
- epitope targeted
- immunogenicity (HAGA response)
 - ✓blocks tracer functionality, changes tracer pharmacology
 - ✓flue-like symptoms, anaphylactic shock
 - ✓increasingly important for FDA & EMEA



how to reduce immunogenicity

Factors that influence the immunogenicity of proteins		
Parameter	Increased immunogenicity	Decreased immunogenicity
Size	Large	Small (MW<2000)
Dose	Intermediate	high or low
Route	Subcutaneous > intraperitoneal > intramuscular or intragastric	
Composition	Complex	Simple
Form	Particulate	Soluble
	Denatured	Native
Similarity to self protein	Multiple differences	Few differences
Adjuvants	Slow release	Rapid release
	Bacteria	No bacteria
Interaction with host MHC	Effective	Ineffective

- small tracer, fast excretion
- micro-dosing, avoid >1 injections
- i.v. route
- soluble
- hydrophilic
- humanize
 - ✓veneer
 - ✓CDR grafting

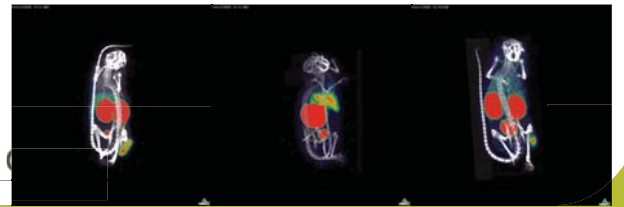
generation humanized nanobody



	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
Human V _H	QVQLVSGGQVQVGGSLRLSCIAS	XYZ...	WRQAPGKGLKLEIVS	XYZ...	RFTISRDNKNTYFLMINLRLEDTAIYYCAA	XYZ...	WGQGTLYVSS
nanobody	QVQLVSGGQVQVGGSLRLSCIAS	XYZ...	WRQAPGQEREIVS	XYZ...	RFTISRDNKNTYFLMINLRLEDTAIYYCAA	XYZ...	WGQGTLYVSS
Humanized nanobody scaffold	QVQLVSGGQVQVGGSLRLSCIAS	XYZ...	WRQAPGQEREIVS	XYZ...	RFTISRDNKNTYFLMINLRLEDTAIYYCAA	XYZ...	WGQGTLYVSS



tumor imaging with grafted nanobodies

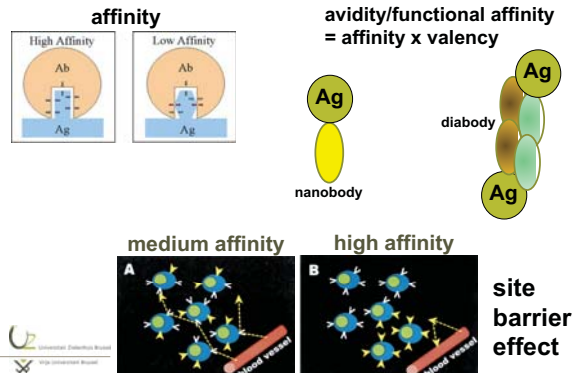


important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility
- ease of production and purification
- epitope targeted
- immunogenicity
- affinity

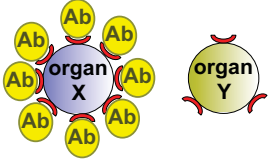


affinity and avidity

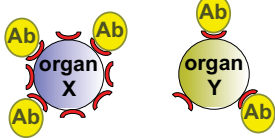


antigenic sink

high affinity Ab



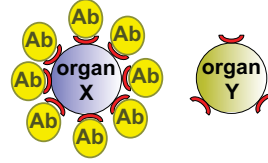
medium affinity Ab



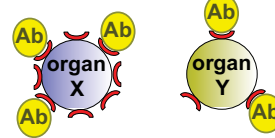
Movahedi et al., Cancer Research, 2012

antigenic sink

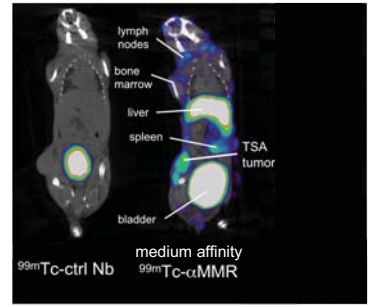
high affinity Ab



medium affinity Ab

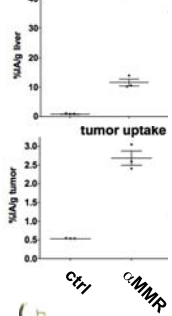


Movahedi et al., Cancer Research, 2012

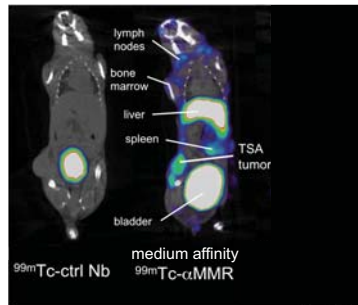
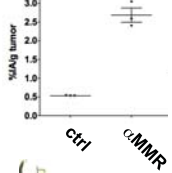


antigenic sink

liver uptake



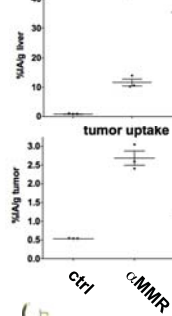
tumor uptake



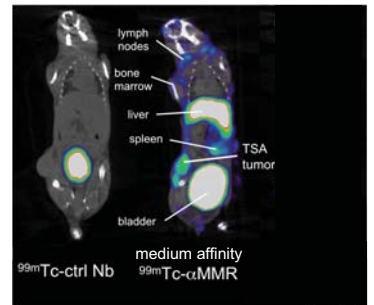
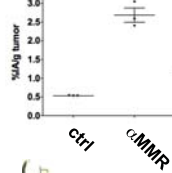
Movahedi et al., Cancer Research, 2012

antigenic sink

liver uptake

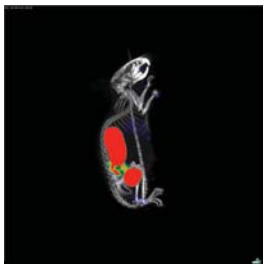


tumor uptake

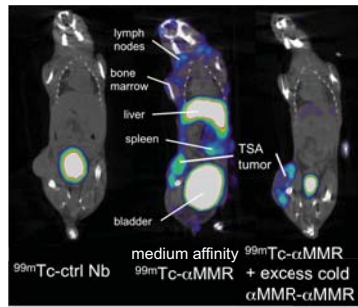


Movahedi et al., Cancer Research, 2012

antigenic sink



$^{99m}\text{Tc-}\alpha\text{MMR}$
+ excess cold $\alpha\text{MMR-}\alpha\text{MMR}$

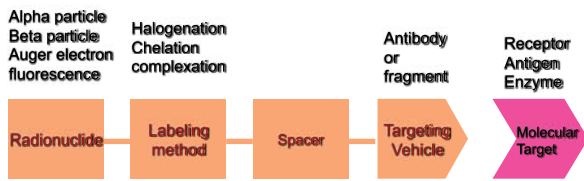


Movahedi et al., Cancer Research, 2012

important characteristics to aim an Ab-tracer for clinical translation

- size
- stability and solubility
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- labeling methods

labeling methods



- SPECT or PET or fluorescence
- radionuclide half-life should fit tracer blood half-life
- stability labeling
- labeling does not destroy Ab functionality
- tracer internalization

conclusion

- discover the power of antibodies and antibody fragments.
- understand the relationship between antibody and fragment structural, biochemical and pharmacokinetic properties.
- learn about the art to generate imaging tracers derived from antibodies and its engineered fragments