Supplementary information

Small but mighty: The versatility of nanobodies in modern medicine

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Supplementary Figures

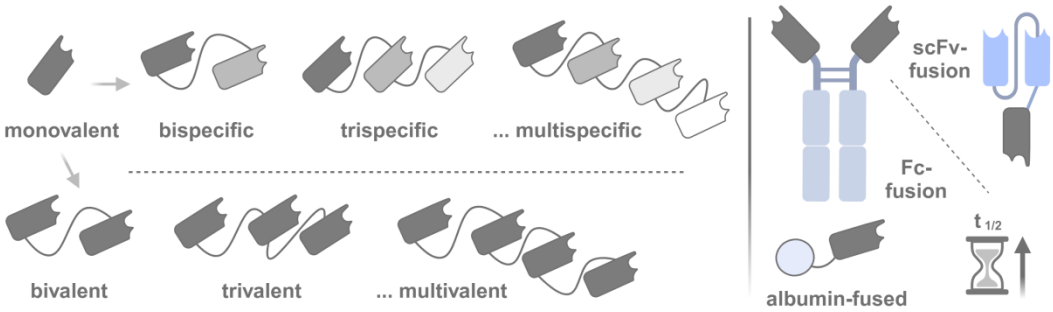

Figure S1. Common nanobody engineering strategies. Left: Schematic illustration of bispecific, trispecific, and multispecific nanobody constructs. Right: Examples of nanobody fusion proteins, including Fc-fusion nanobodies and albumin-fused nanobodies for half-life extension. See main text for details. Created with Biorender.com.

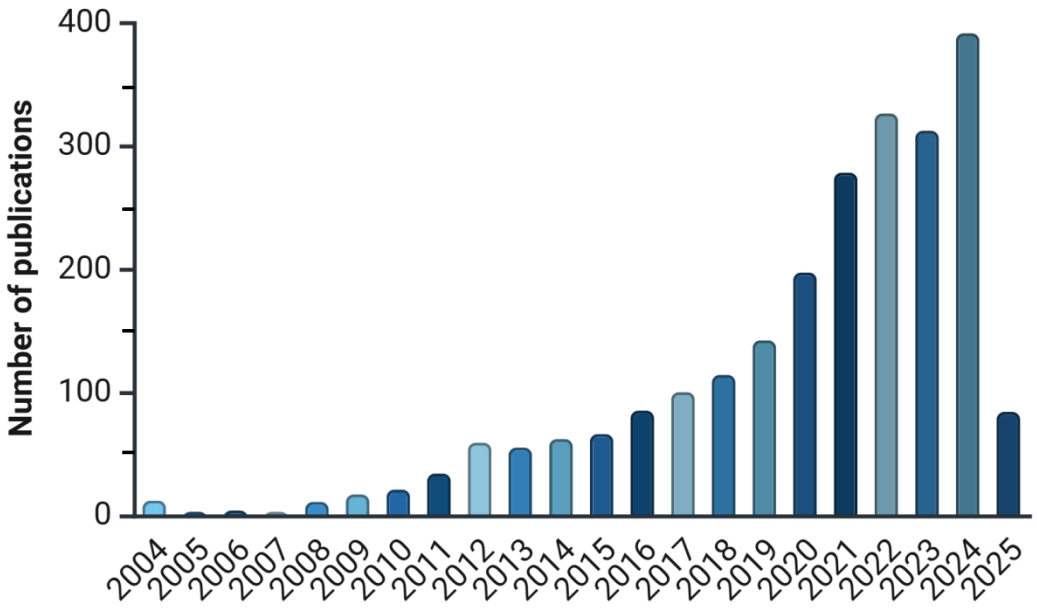

Figure S2. Interest of the scientific community in nanobody-based research is constantly increasing since 2004. The number of publications per year is shown. Data was obtained from PubMed applying the search query: ((nanobody[Title]) OR (nanobodies[Title])). Created with Biorender.com.

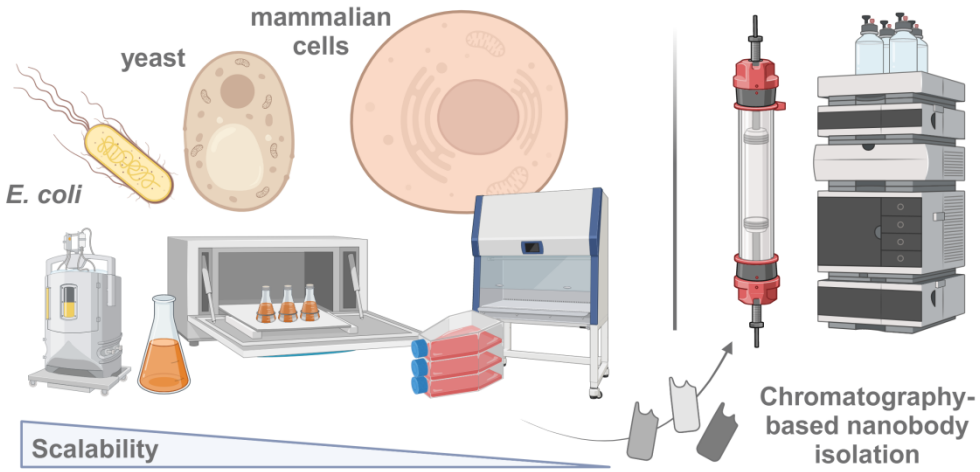

Figure S3. Manufacturing and scalability of nanobody therapeutics. Different host organisms, such as E. coli, yeast, and mammalian (e.g. Chinese hamster ovary, CHO) cells can be applied for nanobody production differing in their scalability, cost-effectiveness and presence of post-translational modifications. Purification steps include chromatography-based nanobody isolation. See main text for details. Created with Biorender.com.

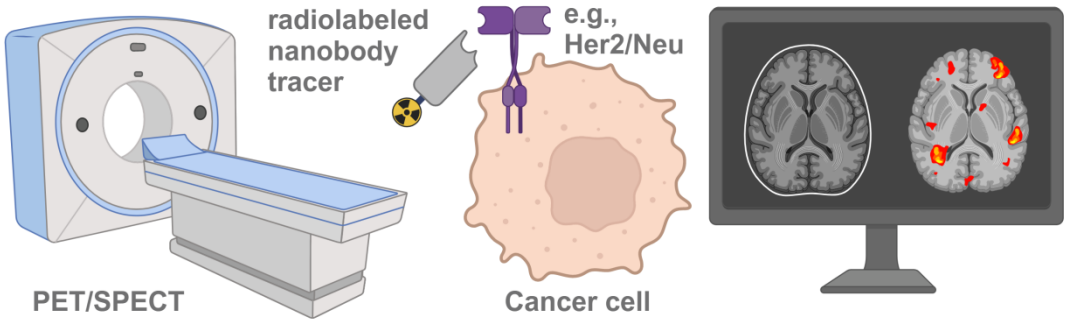

Figure S4. Nanobody PET/SPECT imaging for cancer diagnostics. Radiolabeled nanobody tracers can bind to tumor markers (e.g., HER2/Neu). See main text for details. Created with Biorender.com.

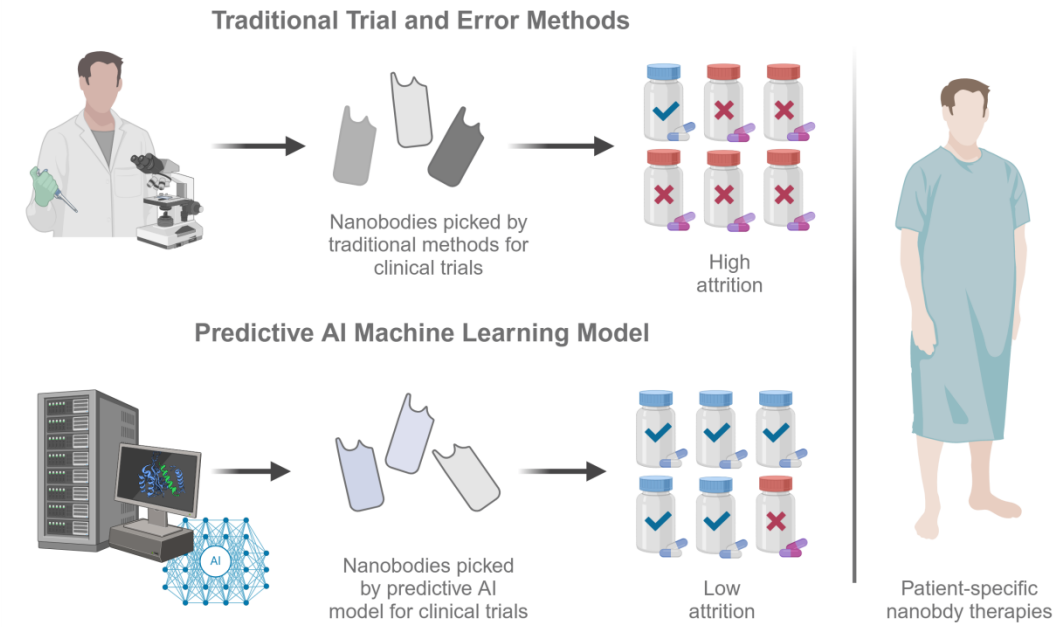

Figure S5. AI-driven nanobody discovery and personalized nanobody therapeutics. In contrast to traditional 'trial and error'-based testing of therapeutically effective nanobodies, machine learning algorithms have the potential to optimize nanobody selection. Thus, AI-driven approaches can be applied to develop patient-specific nanobody therapies based on previous biomarker profiling. See main text for details. Created with Biorender.com.

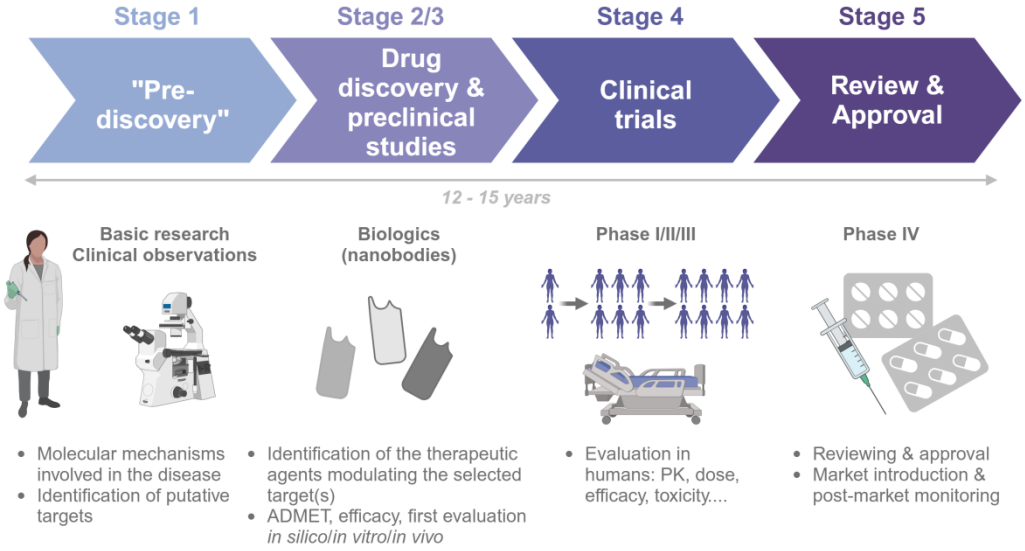

Figure S6. Pipeline of nanobody drug development. After pre-discovery and basic research, a potential nanobody-based drug has to overcome several challenges during pre-clinical studies, clinical studies (phase I-III), and the final approval followed by market introduction and commercialization. See main text for details. Created with Biorender.com.

Supplementary Tables

Table S1. Engineered nanobody formats and their applications.[39-41]*

Nanobody Format	Description	Applications
Monomeric Nanobody	Single VHH domain	Basic binding and inhibition
Multispecific Nanobody	Two or more VHH domains targeting different epitopes	Enhanced specificity, dual-targeting therapies
Nanobody-Fc Fusion	Nanobody fused to an Fc domain for increased half-life	Cancer immunotherapy, autoimmunity
Cell-Penetrating Nanobody	Engineered for intracellular target engagement	Targeting cytosolic proteins, neurodegeneration
Nanobody-PROTACs	Nanobody fused to a degrader molecule	Targeted protein degradation (TPD)

*See main manuscript text for details and references.

Table S2. Comparison of nanobodies vs. conventional antibodies.[4]*

Feature	Nanobody (VHH)	Monoclonal Antibody (IgG)	Single-chain Variable Fragment (scFv)
Size (kDa)	~ 15	~ 150	~ 25-30
Stability	High	Moderate	Moderate
Tissue Penetration	Excellent	Limited	Good
Production System	Bacteria, yeast	Mammalian cells	Bacteria, yeast
Half-life in Blood	Short	Long	Moderate
Immunogenicity	Low	Higher (Fc region)	Low

*See main manuscript text for details and references.

Table S3. Advantages of nanobody-based imaging agents vs. conventional imaging antibodies.[84]*

Feature	Nanobody Imaging Agents	Conventional Imaging Antibodies
Target Specificity	High	High
Tissue Penetration	Excellent	Limited due to size
Clearance Rate	Fast (reduces background noise)	Slow (prolonged circulation)
Radiolabeling Feasibility	Easy	More complex
Clinical Translation	Rapid	Slower

*See main manuscript text for details and references.

Table S4. List of registered clinical studies obtained from ClinicalTrials.gov with search queries: title/acronym “nanobody” and “nanobodies” (Mar, 28th, 2025).*

NCT Number	Study Title	Study Status	Conditions	Interventions	Country	Study type	Group
NCT03881761	CD19/20 Bispecific Nanobody-derived CAR-T Cells in B Cell Lymphoma	UNKNOWN	B-Cell Lymphoma Stage I|Refractory|Relapsed	BIOLOGICAL: CD19/CD20 bispecific CAR-T cells	China	INTERVENTIONAL	Cancer
NCT05803746	PD-L2 Targeting Nanobody Radiotracer for PET Imaging of Solid Tumor	UNKNOWN	Lung Cancer|Colorectal Cancer	DRUG: 18F-FDG	China	INTERVENTIONAL	Cancer
NCT05156515	PD-L1 Targeting Nanobody Probe for PET Imaging of Solid Tumor	UNKNOWN	Lung Cancer|Melanoma|PD-L1|PET/CT	DIAGNOSTIC_TEST: 68Ga-THP-APN09	China	INTERVENTIONAL	Cancer
NCT06503107	Nanobody-based Biepitope CAR-T Cells Targeting BCMA in the Treatment of R/RMM	RECRUITING	Multiple Myeloma	DRUG: Nanobody-based biepitope BCMA-targeting CAR-T cells	China	INTERVENTIONAL	Cancer
NCT06880913	Nanobody-Based CD19/CD22 Tandem Dual CAR-T Therapy for R/R B-ALL	RECRUITING	Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	BIOLOGICAL: Nanobody-Based CD19/CD22 Tandem Dual CAR-T	China	INTERVENTIONAL	Cancer
NCT06874946	Nanobody-Based Anti-CD5 CAR-T for Relapsed/Refractory T-ALL/LBL	RECRUITING	Precursor T-Cell Lymphoblastic Leukemia-Lymphoma	BIOLOGICAL: CD5-targeted CAR-T cells	China	INTERVENTIONAL	Cancer
NCT03664661	BCMA-CAR-T in Relapsed/Refractory Multiple Myeloma	UNKNOWN	Relapsed/Refractory Myeloma	DRUG: BCMA nanobody CAR-T cells	China	INTERVENTIONAL	Cancer
NCT06646952	CD147 Targeting Nanobody Probe for PET Imaging in Solid Tumors	RECRUITING	Solid Tumor	DRUG: 18F-FDG	China	OBSERVATIONAL	Cancer
NCT05436093	CLDN18.2 Targeting Nanobody Probe for PET Imaging in Solid Tumors	RECRUITING	Solid Tumor	DRUG: 18F-FDG	China	INTERVENTIONAL	Cancer
NCT04489862; NCT05373147; NCT04503980	a-PD1-MSLN-CAR T Cells for the Treatment of MSLN-positive Advanced Solid Tumors	UNKNOWN	Different solid tumors	BIOLOGICAL: MSLN-CAR T Cells Secreting PD-1 Nanobodies	China	INTERVENTIONAL	Cancer
NCT05296772	Phase 1 First-in-human Study of JS014	RECRUITING	Neoplasm Malignant|Neoplasm, Experimental|Solid Tumor, Adult|Lymphoma	BIOLOGICAL: JS014, Interleukin 21 and humanized anti-human serum albumin VHH antibody|BIOLOGICAL: Pembrolizumab - anti-PD-1 antibody	Taiwan	INTERVENTIONAL	Cancer
NCT03224702	First-in-Human Trial of Anti-ADAMTS-5 Nanobody in Healthy Volunteers	COMPLETED	Healthy	DRUG: M6495|DRUG: Placebo	Denmark	INTERVENTIONAL	Healthy
NCT01374503	First in Man Study of ALX-0651, a Nanobody Inhibiting CXCR4	TERMINATED	Healthy Volunteers	BIOLOGICAL: ALX-0651|BIOLOGICAL: Placebo,Ablynx	Netherlands	INTERVENTIONAL	Healthy
NCT05849922	A Study to Test the Efficacy and Safety of SAR442970 in Adults with Hidradenitis Suppurativa	COMPLETED	Hidradenitis Suppurativa	DRUG: SAR442970|DRUG: Placebo	US	INTERVENTIONAL	Skin

NCT02156466	Multiple Ascending Dose Trial of MSB0010841 (Anti-IL17A/F Nanobody) in Psoriasis Subjects	COMPLETED	Psoriasis	DRUG: MSB0010841|DRUG: MSB0010841|DRUG: MSB0010841|DRUG: MSB0010841|DRUG: Placebo	Germany	INTERVENTIONAL	Skin
NCT03583346	Multiple Ascending Doses (MAD) of Anti-A Disintegrin and Metalloproteinase With Thrombospondin Motifs-5 (Anti-ADAMTS-5) Nanobody in Participants With Knee Osteoarthritis (OA)	COMPLETED	Osteoarthritis, Knee	DRUG: M6495|DRUG: Placebo	Denmark	INTERVENTIONAL	Bone
NCT01284569	Study to Assess Safety and Efficacy of Anti-Interleukin 6-receptor (IL6R) Nanobody in Rheumatoid Arthritis (RA) Patients	COMPLETED	Rheumatoid Arthritis	BIOLOGICAL: ALX-0061|BIOLOGICAL: Placebo	Czechia, Hungary	INTERVENTIONAL	Inflammatory
NCT06812988	Treatment of Type 1 Diabetes With Anti-OX40L Bispecific With Anti-TNF Activity In a Single Nanobody Molecule	RECRUITING	Type 1 Diabetes Mellitus	DRUG: SAR442970|DRUG: Placebo	Canada, Chile	INTERVENTIONAL	Diabetes
NCT01151423	Study to Assess Efficacy and Safety of Anti-von Willebrand Factor (vWF) Nanobody in Patients With Acquired Thrombotic Thrombocytopenic Purpura (aTTP)	COMPLETED	Acquired Thrombotic Thrombocytopenic Purpura	BIOLOGICAL: Caplacizumab|BIOLOGICAL: Placebo	US	INTERVENTIONAL	others

*See main manuscript text for details and references.

Table S5. FDA-approved and clinically investigated nanobody-based therapeutics obtained from https://www.fda.gov/.*

Nanobody Drug	Target	Indication	Mechanism of Action	Approval Status
Caplacizumab	von Willebrand Factor (vWF)	Thrombotic thrombocytopenic purpura (TTP)	Inhibits vWF-mediated platelet aggregation	FDA & EMA Approved
ALX-0171	RSV (Respiratory Syncytial Virus)	Respiratory Infections	Neutralizes viral fusion protein	Clinical Trials
Ablynx (Various)	TNFα, IL-6, etc.	Autoimmune Diseases	Immune Modulation	Preclinical/Clinical
Nb-based CAR-T	CD19, BCMA	Cancer	Nanobody-derived CAR-T cell therapy	Preclinical

*See main manuscript text for details and references.

Table S6. Current challenges and future directions for nanobody therapeutics.*

Challenge	Current Limitations	Future Innovations
Intracellular Delivery	Limited cytosolic penetration	Cell-penetrating nanobody strategies (CPPs, nanoparticles)
Half-Life Extension	Rapid renal clearance	Albumin fusion, PEGylation, Fc-fusion nanobodies
Large-Scale Production	Scalability in yeast/mammalian cells	Optimized bacterial/yeast production pipelines
Regulatory Approval	Few approved nanobody drugs	Expanding clinical trials and regulatory guidelines
Personalized Therapy	Not yet widely used	AI-driven nanobody design for patient-specific treatments

*See main manuscript text for details and references.