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*Editorial*

# Single-Domain Antibodies—Biology, Engineering And Emerging Applications.

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The basic characteristics of sdAbs were covered in one original study. In order to comprehend the perhaps different mechanisms of sdAbs' recognition of antigens, Gordon et al. analyzed the structures of 345 sdAb:antigen complexes with 892 conventional antibody:antigen complexes in the largest study of this kind to date. The analysis's findings are consistent with previous research in that sdAbs' paratopes are smaller than those of conventional antibodies; however, there were no discernible variations in the size (measured in residues), amino acid composition, or accessibility of the epitopes that sdAbs target. This seeming paradox can be explained by the fact that a longer complementarity-determining region 3 (CDR3) loop contributes more interactions per residue within smaller sdAb paratopes.

One original study looked into a novel method for finding camelid sdAb. Matsuda et al. created a predictive algorithm to find antigen-specific sdAbs without in vitro screening by using longitudinal sequencing and phylogenetic analysis of the peripheral sdAb repertoire, despite the fact that many groups have incorporated highthroughput sequencing of antibody repertoires into already-existing discovery pipelines that assess the antigen reactivity of individual clones in vitro. The accumulation of somatic hypermutations and high turnover rates within clonal families during the affinity maturation process serve as the foundation for discovering antigen-specific sdAbs. However, initial analysis of antigen-specific sdAbs recovered with this approach revealed inconsistent binding results across tests.

Although concurrent immune responses against pathogens and other non-immunizing antigens would be expected to complicate predictions, the positive overall findings suggest that antigen-specific sdAbs may eventually be accurately identified through peripheral blood repertoire sequencing after immunization. By attaching to serum albumin, sdAbs—or even smaller antibody-derived fragments—can increase the serum persistence of biologics, according to two original research studies. From the repertory of a llama inoculated with dog and horse serum albumin, Harmsen et al. extracted and described sdAbs. In contrast to other attempts in this area, the sdAbs bind different animal species' albumins.

cow ultralong CDRH3s, or "knob domains," were discovered by Adams et al. to promote autonomous high-affinity binding to human or mouse serum albumin when the parental cow antibody is not present. Recombinant introduction of these albumin-specific knob domains into the VH framework region 3 D-E loop (also referred to as the "CDR4 loop") of aTNF-specific Fab or chemical conjugation to an IL-17 inhibitory peptide, which results in dual antigen recognition, are two possible approaches. In the former instance, mice showed a prolongation of the half-life of the bispecific anti-TNF Fabearing the anti-mouse serum albumin knob domain. These findings support the usefulness of bovine knob domains as a distinct class of antigen recognition units and show that adding a 4-5 kDa polypeptide produced from an antibody can enhance serum albumin recognition and prolong its half-life.

Utilizing sdAbs' high affinity binding and quick clearance from circulation, four unique research publications concentrate on their use as non-invasive imaging tracers. Benloucif et al. produced llama anti-MSLN sdAbs that do not compete with amatuximab or MUC16 for MSLN binding, and they assessed their capacity to identify MSLN expression using PET/CT (68Ga labeling) or fluorescence (ATTO 647N labeling). The resultant tracers are consistent with monitoring of existing therapy and shown preferential uptake in tumors expressing high amounts of MSLN. Zeven et al. identified new llama anti-TIGIT sdAbs and created a scFv based on vibostolimab. They then tagged

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these molecules with 99mTc and used SPECT/CT imaging to assess their capacity to identify TIGIT expression. Although the scFv binds to TIGIT-expressing PBMCs more strongly,
The improved stability and/or tissue penetration of the sdAbs may have contributed to their superior in vivo tumor labeling. Novel alpaca sdAbs against SIRPa, some of which disrupt the CD47-SIRPa interaction, are described by Wagner et al. PET/MR was utilized to visualize tumor infiltration by myeloid cells using one of the non-blocking sdAbs that was 64Cu labeled. It is possible to anticipate theranostic uses for these sdAbs by altering the radioisotope.
The majority of in vivo imaging tracers use a single label that is either radioactive or fluorescent. Declerck et al. conjugated 99mTc and IRDye800CW site-specifically to C-terminal His6 and Cys tags, respectively, to create bimodal anti-uPAR sdAb tracers. The drawbacks of each method, such as the imprecision of gamma probing for intraoperative decision making and the restricted tissue penetration of fluorescent signals, may be addressed by combining fluorescence and SPECT/CT imaging.
One brief study report, two reviews, and two original research publications examine the use of sdAbs in the diagnosis and management of infections, mainly SARS-CoV-2. A thorough analysis of recent research employing shark VNARs as antiviral agents against SARS-CoV-2 is provided by Cabanillas-Bernal et al. De Greve and Fioravanti, meanwhile, conduct a thorough analysis of the larger body of research on camelid sdAbs for the treatment of microbiological illnesses caused by bacteria and viruses, a diverse and dynamic subject. SARS-CoV-2 diagnostic assays based on sdAb are described in one original research paper and one brief research report. Using all recombinant reagents, Segoviade los Santos et al. created a diagnostic luciferase assay in which antigen is collected, streptavidin-coated plates are loaded with a biotinylated nucleocapsid-specific sdAb, and a second noncompetitive sdAb coupled to NanoLuc is utilized to detect the bound antigen. 144 clinical samples from 2022, when Omicron (B.1.1.529) was the predominant variation in Uruguay, were used to validate the assay. A Luminex MagPlex test was created by Goldman et al., wherein SpyTag nucleocapsid-specific sdAb is loaded onto SpyCatcher-coated magnetic beads, antigen is caught, and bound antigen is identified using a second non-competitive reporter sdAb that has been biotinylated. The directed (as opposed to randomly adsorbed) sdAb matrices are essential for both investigations' higher assay sensitivity.
The difficult task of creating antibodies that can precisely identify certain protein conformational states was addressed in one original scientific study. Zupancic et al. used MACS- and FACS-based selection to find llama sdAbs from yeast-displayed libraries that selectively target aggregated (fibrillar) tau over soluble monomeric tau. These sdAbs may find use in the diagnosis or treatment of neurodegenerative illnesses because they were able to identify tau aggregation in brain samples from transgenic mice and individuals with tauopathies.

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