The high surface expression of the NAD+-metabolizing ectoenzyme CD38 is considered the phenotypic hallmark of plasma cells which has propelled its intense investigation as a target for antibody-dependent cellular cytotoxicity [1]. The resulting anti-CD38 antibodies that may elicit immune-mediated elimination of CD38-expressing cells (mostly through antibody-dependent cellular cytotoxicity (ADCC)), frequently show considerable epitope overlapping with commercially available monoclonal mouse anti-CD38 antibodies, limiting the study of surface CD38 expression at the single cell level in the presence of ADCC-inducing conventional antibodies.

Nanobodies, derived from heavy-chain antibodies that naturally occur in llamas and other camelids, can circumvent these undesired interferences. Nanobodies are single variable domain antibody fragments (VHH) [2,3] that often expose a long complementarity-determining region 3 (CDR3). This feature allows them to recognize hidden epitopes, e.g. in molecular cavities, that are inaccessible to the CDRs in conventional analytical and ADCC-inducing antibodies [4].

JK36 is an anti-CD38 nanobody recognizing a cryptic epitope not masked by anti-CD38 therapies, opening new avenues in multiple myeloma clinical research [5].
Material & Methods

Like all nanobodies, JK36 has unique structural and functional properties. Compared to mouse or human immunoglobulins, the most variable portion of the nanobody, the Complementarity-Determining Region 3 (CDR3), has an extended loop, which allows JK36 to reach a cryptic CD38 epitope not accessible to conventional antibodies (Figure 1).

Results

The Rabbit-JK36 (Rb-JK36) construct was compared to conventional mouse antibodies clones LS198-4-3 and T16. As shown in Figure 3, staining patterns in normal whole blood with the three clones are similar. As expected, the Alexa Fluor 700 conjugate is less bright than the APC-Alexa Fluor 700 conjugate and brighter compared to FITC.

When the same staining is performed on whole blood upon incubation with Daratumumab, a therapeutic humanized anti-CD38 mouse antibody, LS198-4-3 and T16 partially or completely fail to label the CD38 epitope, now masked with Daratumumab. In contrast, the Rb-JK36 staining pattern and intensity remains unchanged, confirming that Rb-JK36 binds to an epitope which is not masked by Daratumumab.

Figure 1: Schematic structure of human and camelid antibodies and VHH domain [6]

Figure 2: F/P ratio is increased with the Rabbit Fc-JK36 construct compared to the sole nanobody
Figure 3: Staining pattern of the Rabbit Fc-JK36 construct compared to mouse LS198 and T16 on a normal whole blood before (top) and after incubation with Daratumumab (bottom) using Kaluza software on Navios cytometer.

These data demonstrate that the Rb-JK36 construct is a useful alternative to conventional mouse antibodies to gate plasma cells by flow cytometry (Figure 4), allowing researchers to identify and study plasma cells in the presence of anti-CD38 biologics.

Figure 4: Use of the Rabbit Fc-JK36 construct as a plasma cell gating marker compared to mouse LS198 on a Multiple Myeloma blood sample before (top) and after incubation with Daratumumab (bottom).

In addition to Alexa Fluor 700, the Rabbit Fc-JK36 can be conjugated to various Alexa Fluor dyes, providing flexibility in panels design (Figure 5).
Beyond rescuing the detection of surface-expressed CD38 in the presence of anti-CD38 ADCC-inducing conventional antibodies, the simultaneous assessment of CD38 expression and CD38 occupation at the single cell level may grant valuable mechanistic insight and support in the development of working hypotheses for the escape of plasma cells (Figure 6).

**Figure 5:** Example of staining pattern of the Rabbit Fc-JK36 conjugated to Alexa Fluor 488, 647 and 700 dyes (left to right) on whole blood and bone marrow samples from healthy donors.

**Figure 6:** The simultaneous assessment of receptor expression with JK36 and occupancy with a conventional analytical antibody allows for correlation of CD38 expression density and CD38 occupancy at the single cell level.
**Discussion**

Nanobodies have unique properties with impressive advantages for analytical and therapeutic applications. The first nanobody-based therapy was approved by the US FDA in early 2019 (Caplacizumab from Sanofi Genzyme [7]) and joins many targeted biologics already on the market. With the directly fluorescently labeled Rb-JK36 construct, Beckman Coulter advances cellular analysis to overcome the limitations of epitope masking with targeted biologics. This nanobody provides opportunity to laboratories to more accurately monitor response to anti-CD38 therapies, improving patient care.

**References**
