Bovine ultralong CDR3 antibodies have a unique “stalk and knob” structure in which two antiparallel β-strands support a disulfide bonded knob protruding out of the antibody surface and forms a mini antigen binding domain (Figure 1A).

Interleukin (IL) 15 stimulates the proliferation and cytotoxicity of cytotoxic T lymphocytes and nature killer (NK) cells, similar to the action of IL2, and thus is an immunotherapeutic candidate for cancer treatment.

IL15 may be a better candidate drug than IL2 because it doesn’t cause vascular leak syndrome or stimulate regulatory T cells.

However, IL15 is difficult to express as a stable soluble protein and has a short half-life in vitro and in vivo.

To solve the problem, we designed a chimeric IL15 ultralong bovine antibody fusion by replacing the knob of the bovine antibody BLV1H12 with IL15 (Figure 1A).

BLV1H12_IL15 (B15) functions the same as IL15 in vitro signaling assays but can be easily produced in mammalian cells and with increased stability.

IL15 needs its high affinity receptor α (IL15Rα) for increased signaling potency, while B15 mixed with IL15Rα to increase its binding to the IL2/15Rβ and γc. IL15 was mixed with IL15Rα at 4°C overnight to achieve full signaling potency, while B15 mixed with IL15Rα right before the assay. As B15 is bivalent, only half molar concentration of B15 was used compared to IL15 monomer.

In vivo pharmacokinetic assay to test their half life.

In vivo assay to test their antitumor responses in the tumor microenvironment in mice.

In vivo assay to test their antitumor responses in the tumor microenvironment in mice.

Figure 3. In vitro STAT5 signaling assays indicated that B15 could associate with 15Rα much faster than IL15 monomer. As there is no IL15Rα subunit expressed in the HEK-Blue IL2 reporter cells, IL15RαFc (R&D Systems) was mixed with IL15 (or B15) to increase its binding to the IL2/15Rβ and γc. IL15 was mixed with IL15Rα at 4°C overnight to achieve full signaling potency, while B15 mixed with IL15Rα right before the assay. As B15 is bivalent, only half molar concentration of B15 was used compared to IL15 monomer.

Figure 4. B15 variants expressed with IL15Rα sushi domain achieved the same signaling potency as premixed B15 and IL15RαFc, which all are much better than B15 without IL15Rα subunit.

Figure 5. All B15 variants could expand NK92 cells, although to a lesser extent than either the IL2 or IL15 monomer. Half molar concentration of B15 was used compared to the IL2 or IL15 monomer.

Figure 6. IL15Rα sushi domain improved B15 ability to expand NK92 cells.