MultYmab™ Technology

A novel antibody engineering platform to generate multimeric IgG antibodies with potent agonist activities

JN Biosciences
Bridging Science to Life
320 Logue Avenue
Mountain View CA 94043
www.jn-bio.com

***Summary**: JN Biosciences has developed a proprietary antibody engineering platform that enables conversion of an IgG antibody (MultYmab™ technology) to a uniform molecular species of mulitimeric polyvalent form. The modified multimeric IgG antibody (MultYbody™) works as a potent agonist for TNF receptor family members, including death receptors and immune costimulatory molecules. MultYbodies™ against these receptors are applicable for cancer treatment and immunotherapy. JN Biosciences is seeking a partner for further development and clinical application of the MultYmab™ technology.*

***The Company:** JN Biosciences is a biotechnology company based in Mountain View CA. It was founded with the dual intent of generating novel therapeutic antibodies against under-appreciated targets that possess potential therapeutic value and developing innovative antibody engineering technologies to improve the efficacy of therapeutic antibodies.*

Background: IgG antibodies have proven their utility as human therapeutics. More than thirty IgG antibodies have been approved in the U.S. for treatment of various human diseases. The vast majority of these IgG antibodies work as antagonist by blocking the function of their antigens; however, no agonist IgG antibodies capable of triggering receptor-mediated cellular functions have been approved to date. The TNF receptor family of cell surface proteins, including death receptors and immune costimulatory molecules, require homo-trimerization to induce intracelluar signaling. Divalent IgG antibodies cannot trimerize surface proteins alone. Although Fc receptor-bearing cells can crosslink antigen-bound IgG antibodies on the surface of other cells, the high level of circulating IgG antibodies makes this process inefficient *in vivo*. Multimeric IgG antibodies (MultYbodies™) generated by JN Biosciences' proprietary MultYmab™ technology are capable of polymerizing TNF receptor family members on the surface independently, resulting in intracellular signal transduction for apoptosis via death receptors and enhancement of immune responses via costimulatory molecules.

Expression and purification of MultYbody™: An IgG antibody can be converted to the multimeric polyvalent form (MultYbody™) by antibody engineering using JN Biosciences' proprietary MultYmab™ technology. A MultYbody™, which is composed of modified heavy chains and intact light chains, can be expressed in a stable transfectant of CHO-K1 or other mammalian cells, and purified from culture supernatants using standard methods with protein A column chromatography. Fig. 1A shows a typical result of the size analysis of protein A-purified MultYbody™. After an additional fractionation step by gel filtration, the MultYbody™ shows a single dominant peak by size analysis (Fig. 1B).

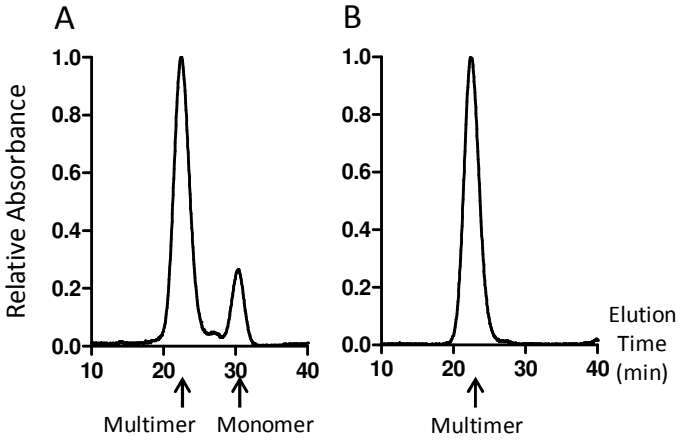

Figure 1. Analysis of MultYbody™ by a size exclusion column A. after protein A purification; B. after gel filtration

Anti-DR5 MultYbody™ for Induction of Apoptosis: MultYbodies™ against death receptors, members of the TNF receptor family, are capable of inducing apoptosis by polymerizing these receptors on the cell surface. An in-house generated humanized IgG1 antibody against death receptor 5 (DR5), which is frequently expressed in cancer cells, was converted to the MultYbody™ form using the MultYmab™ technology. The anti-DR5 MultYbody™ was expressed in CHO-K1 cells and purified by protein A chromatography. The apoptosis-inducing activity of the anti-DR5 MultYbody™ was measured using the DR5-expressing human colon carcinoma cell line COLO 205. The anti-DR5 MultYbody™ potently induced apoptosis of COLO 205 cells with an EC_{50} value of 0.6 ng/ml, whereas the parental anti-DR5 IgG1 antibody showed no apoptosis-inducing activity even at 300 ng/ml (Fig. 2).

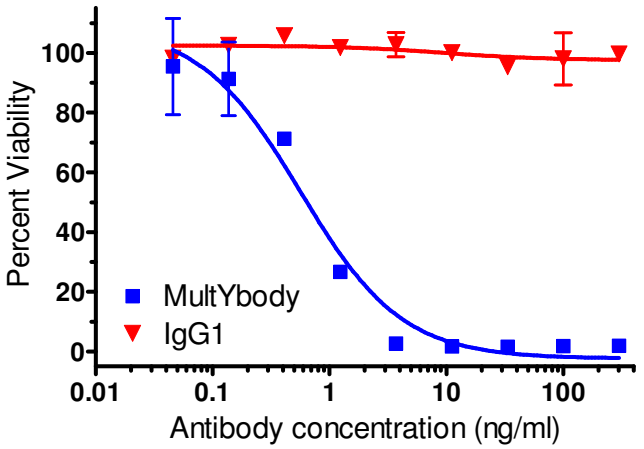

Figure 2. Apoptosis of COLO 205 colon carcinoma cells induced by humanized anti-DR5 antibodies

Anti-CD40 MultYbody™ for Enhancement of Immune Response:. Costimulatory molecules of the TNF receptor family, such as CD40, OX40 and GITR, are positive regulators of immune responses. MultYbodies™ can function as agonists by polymerizing these molecules on the surface of immune cells. The agonist activity of an anti-human CD40 MultYbody™ was demonstrated using the human Burkitt's lymphoma cell line Ramos. Treatment of Ramos cells with the anti-CD40 MultYbody™ strongly induced the expression of CD95, an indication of cell stimulation, while the parental anti-CD40 IgG antibody only marginally increased the expression of CD95 (Fig. 3).

Applications of the MultYmab Technology™: The MultYmab™ technology provides the ability to generate various forms of multimeric antibodies and Fc fusion proteins that have unique functional characteristics. Following are two examples.

Multimeric Fc Fusion Proteins: Fc fusion proteins can be converted to the multimeric form by using the MultYmab™ technology. For instance, multimeric CD70-Fc fusion proteins can cross-link CD27, a TNF receptor family member of costimulatory molecules, on the surface of T cells and enhance antigen-specific immune responses in the body for immunotherapy of cancer and infectious disease.

Bispecific MultYbody™: Single-chain Fv (scFv) IgG antibodies can be expressed in the multimeric form using the MultYmab™ technology. Simultaneous expression of two modified scFv IgG antibodies results in production of bispecific MultYbodies™. Such MultYbodies™ are unique in that both antigens are polymerized on the surface, an activity which traditional IgG-based bispecific antibodies cannot achieve.

Conclusions: JN Biosciences' proprietary MultYmab™ technology makes it possible to generate potent agonist IgG antibodies against death receptors for cancer treatment and constimulatory molecules for immunotherapy. The MultYmab™ technology is also applicable for generation of multimeric Fc fusion proteins and bispecific (and even multispecific) IgG antibodies with agonist or antagonist activities.

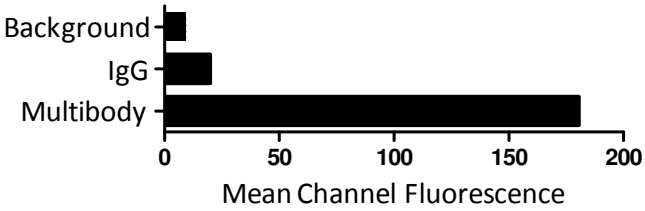

Figure 3. Expression of CD95 on Ramos cells after 48 hr treatment with 1 μg/ml of an anti-CD40 antibody

***The Opportunity**: JN Biosciences is actively seeking partners for further development and clinical application of the MultYmab™ technology. Please send inquiries to: Info@jn-bio.com*