**BACKGROUND**

The selective destruction of an individual cell or a specific cell type is often desirable in a variety of clinical settings. For example, it is a primary goal of cancer therapy to specifically destroy tumor cells, while leaving healthy cells and tissues intact and undamaged.

An attractive way of achieving this is by inducing an immune response against the tumor, to make immune effector cells such as natural killer (NK) cells or cytotoxic T lymphocytes (CTLs) attack and destroy tumor cells. CTLs constitute the most potent effector cells of the immune system, however they cannot be activated by the effector mechanism mediated by the Fc domain of conventional therapeutic antibodies.

In this regard, bispecific antibodies designed to bind with one “arm” to a surface antigen on target cells, and with the second “arm” to an activating, invariant component of the T cell receptor (TCR) complex, have become of interest in recent years. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell. Hence, the immune response is re-directed to the target cells and is independent of peptide antigen presentation by the target cell or the specificity of the T cell as would be relevant for normal MHC-restricted activation of CTLs. In this context it is crucial that CTLs are only activated when a target cell is presenting the bispecific antibody to them, i.e. the immunological synapse is mimicked. Particularly desirable are bispecific antibodies that do not require lymphocyte preconditioning or co-stimulation in order to elicit efficient lysis of target cells.

CD3 has been extensively explored as drug target. Monoclonal antibodies targeting CD3 have been used as immunosuppressant therapies in autoimmune diseases such as type I diabetes, or in the treatment of transplant rejection. The CD3 antibody muromonab-CD3 (OKT3) was the first monoclonal antibody ever approved for clinical use in humans, in 1985.

A more recent application of CD3 antibodies is in the form of bispecific antibodies, binding CD3 on the one hand and a tumor cell antigen on the other hand. The simultaneous binding of such an antibody to both of its targets will force a temporary interaction between target cell and T cell, causing activation of any cytotoxic T cell and subsequent lysis of the target cell.

FOLR1 is expressed on epithelial tumor cells of various origins, e.g., ovarian cancer, lung cancer, breast cancer, renal cancer, colorectal cancer, endometrial cancer. Several approaches to target FOLR1 with therapeutic antibodies, such as farletuzumab, antibody drug conjugates, or adoptive T cell therapy for imaging of tumors have been described (Kandalaft et al., J Transl Med. 2012 Aug 3;10:157. doi: 10.1186/1479-5876-10-157; van Dam et al., Nat Med. 2011 Sep 18;17(10):1315-9. doi: 10.1038/nm.2472; Cliftonet al., Hum Vaccin. 2011 Feb;7(2):183-90. Epub 2011 Feb 1; Kelemen et al., Int J Cancer. 2006 Jul 15;119(2):243-50; Vaitilingam et al., J Nucl Med. 2012 Jul;53(7); Teng et al., 2012 Aug;9(8):901-8. doi: 10.1517/17425247.2012.694863. Epub 2012 Jun 5. Some attempts have been made to target folate receptor-positive tumors with constructs that target the folate receptor and CD3 (Kranz et al., Proc Natl Acad Sci U S A. Sep 26, 1995; 92(20): 9057–9061; Roy et al., Adv Drug Deliv Rev. 2004 Apr 29;56(8):1219-31; Huiting Cui et al Biol Chem. Aug 17, 2012; 287(34): 28206–28214; Lamers et al., Int. J. Cancer. 60(4):450 (1995); Thompson et al., MAbs. 2009 Jul-Aug;1(4):348-56. Epub 2009 Jul 19; Mezzanzanca et al., Int. J. Cancer, 41, 609–615 (1988). However, the approaches taken so far have many disadvantages. The molecules used thus far could not be readily and reliably produced as they require chemical cross linking. Similarly, hybrid molecules cannot be produced at large scale as human proteins and require the use of rat, murine or other proteins that are highly immunogenic when administered to humans and, thus, of limited therapeutic value. Further, many of the existing molecules retained FcgR binding.

More recently, WO2016/079076 describes T cell activating bispecific antigen binding molecules targeting CD3 and FolR1.

For therapeutic purposes, an important requirement that antibodies have to fulfill is sufficient stability both *in vitro* (for storage of the drug) an *in vivo* (after administration to the patient).

Modifications like asparagine deamidation are typical degradations for recombinant antibodies and can affect both *in vitro* stability and *in vivo* biological functions.

Given the tremendous therapeutic potential of antibodies, particularly bispecific antibodies for the activation of T cells, there is a need for bispecific CD3/FolR1 antibodies with optimized properties.