

Anti-HER2/neu-IFN- α fusion protein: a potent therapeutic for treatment of B cell lymphoma in vivo

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IFN- α is a crucial cytokine in initiating the innate immune response and also demonstrates a wide spectrum of anti-tumor activities. First, IFN- α is an important cytokine in activating dendritic cells, which can potentially initiate an anti-tumor immune response in vivo. Second, it causes cytolysis of some tumor cell lines by activation of the apoptotic pathway. Third, IFN- α enhances the expression of a variety of antigens on the tumor cell surface, including tumor associated antigens and MHC class I, thereby, increasing the immunogenicity of tumor cells. However, clinical use of IFN- α as an anticancer drug is hampered by its short half-life, which, significantly compromises its therapeutic effect. One promising approach to improving the therapeutic index of IFN- α is to use a tumor specific antibody as a targeting vehicle to deliver higher doses of IFN- α to the tumor site; the increased molecule size will also provide IFN- α with a longer half life.

Murine IFN- α was fused to the C terminus of an anti-human HER2/neu IgG3 antibody (anti-hHER2 IgG3-IFN- α). Anti-hHER2 IgG3-IFN- α was expressed efficiently in the murine myeloma cell lines, Sp2/0, and a SDS-PAGE analysis confirmed that proteins of the expected molecular weight were produced. Anti-hHER2 IgG3-IFN- α exhibited high affinity binding to a tumor cell line over-expressing HER2/neu (CT26/HER2), while an IFN- α fused IgG3 antibody with an irrelevant variable region exhibited weak binding to CT26/HER2 through the heparin binding motif of IFN- α . Anti-hHER2 IgG3-IFN- α and IgG3-IFN- α demonstrated a similar ability to protect the VSV sensitive L929 fibroblast cell line from VSV induced cytotoxicity. The potency of anti-hHER2 IgG3-IFN- α and IgG3-IFN- α is 2.48×10^3 U per μ g. In addition, anti-hHER2 IgG3-IFN- α exhibited in vitro cytotoxicity against a B cell lymphoma cell line, 38C13, expressing human HER2/neu. Anti-hHER2 IgG3-IFN- α exhibited a potent cytotoxic activity against 38C13/HER2 ($IC_{50}=10$ pM), while IgG3-IFN- α was 10 fold less cytotoxic (100 pM). Therefore, targeting of IFN- α to tumor cells significantly increases its cytotoxicity in vitro.

Anti-hHER2 IgG3-IFN- α was also effective against 38C13/HER2 in vivo. 6 of 8 mice treated twice with either 5 μ g anti-hHER2 IgG3-IFN- α or IgG3-IFN- α on day 1 and day 3 after tumor challenge were still tumor free after 50 days, while 8 of 8 mice treated with either control medium or anti-hHER2 IgG3 antibody had bulky tumors (1500 mm³ average) after 15 days. Remarkably, all mice (8/8) treated three times with either 5 μ g anti-hHER2 IgG3-IFN- α or IgG3-IFN- α on day 1, day 3 and day 5 after tumor challenge were still tumor free after 50 days. IgG3 fused to IFN- α demonstrated a more potent therapeutic effect against 38C13/HER2 than recombinant IFN- α since 7 of 8 mice treated with 9000U of IgG3-IFN- α were still tumor free on day 15 after tumor challenge while only 1 of 8 mice treated with the same amount of recombinant IFN- α was tumor free. In summary, these results demonstrated that fusion of IFN- α to a tumor specific antibody can significantly increase its cytotoxic activity against B cell lymphoma in vitro and the longer half life may contribute to its enhanced therapeutic effect in vivo. The remarkable therapeutic effect of anti-HER2/neu IgG3-IFN- α may make it a potential therapeutic for B cell lymphoma in clinic.