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Ontak-like human IL-2 fusion toxin

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ABSTRACT

Ontak® is a FDA-approved diphtheria toxin-based recombinant fusion toxin for treatment of human CD25$^+$ cutaneous T cell lymphoma (CTCL). However, it has been discontinued clinically due to the production issue related to the bacterial expression system with difficult purification. Recently we have developed monovalent and bivalent human IL-2 fusion toxins targeting human CD25$^+$ cells using advanced unique diphtheria toxin resistant yeast *Pichia Pastoris* expression system. *In vitro* efficacy characterization using human CD25$^+$ HUT102/6TG cells demonstrated that both monovalent and bivalent isoforms are potent and the bivalent isoform is approximately two logs more potent than the monovalent isoform. In this study, we further assessed the *in vivo* efficacy of the human IL-2 fusion toxins using human CD25$^+$ HUT102/6TG tumor-bearing *NSG* mouse model. The data demonstrated that both monovalent and bivalent human IL-2 fusion toxins significantly prolonged the survival of the human CD25$^+$ tumor-bearing *NSG* mice in a dose-dependent manner. Then we further assessed the residual tumor cells from the HUT102/6TG tumor-bearing *NSG* mice using the residual tumor cell bearing *NSG* mouse model. The results demonstrated that the residual tumor cells were still sensitive to the continual treatment with the human IL-2 fusion toxin. This yeast-expressed human IL-2 fusion toxin will be a promising candidate to replace the clinically discontinued Ontak®.

1. Introduction

Cutaneous T-cell lymphomas (CTCL) are extranodal non-Hodgkin's lymphomas characterized by skin lesions infiltrated with malignant CD4$^+$ or CD8$^+$ T lymphocytes. Mycosis fungoides and Sezary syndrome are the two most common forms of CTCL (Prince et al., 2010). Overexpression of the IL-2 receptor, CD25, is a prominent molecular characteristic of CTCL. Ontak® (denileukin diftitox, DAB389IL-2, Eisai Medical Research, Inc.) is a genetically engineered monovalent human IL-2 fusion toxin expressed in *Escherichia coli* which has been approved by the Food and Drug Administration (FDA) for clinical treatment of patients with persistent and recurrent human CD25$^+$ CTCL. The overall response rates of Ontak® range from 30% to 50%, and are even higher (63% and 48%, for early and late stage CTCL, respectively) in patients treated less heavily with other modalities (Prince et al., 2010). Of particular note is the large prolongation (>2 years) of progression-free survival by Ontak®. In addition, treatment significantly improves the patient quality of life (Duvic et al., 2002). However, Ontak® was discontinued clinically due to the production issues related to the *E. coli* expression system with difficult purification. Recently we have developed monovalent and bivalent human IL-2 fusion toxins using an advanced unique diphtheria toxin resistant yeast *Pichia Pastoris* expression system (Fig. 1) and characterized their efficacy *in vitro* (Peraino et al., 2014). In this study, we further assessed the *in vivo* efficacy of the yeast-produced human IL-2 fusion toxins in human CD25$^+$ CTCL tumor-bearing *NSG* mouse model. We also assessed the residual tumor cells from the HUT102/6TG tumor-bearing *NSG* mice using the residual tumor cell bearing *NSG* mouse model.

2. Materials and methods

2.1. Tumor cells and antibodies

Human CD25$^+$ T-cell lymphoma cell line HUT 102/6TG (Williams et al., 1990) was kindly provided by Dr. Robert Harrison, Anjin Group, Inc., Boston, MA. The HUT 102/6TG tumor cells were cultured in RPMI 1640 media supplemented with 12% fetal bovine serum, 10 mM hepes (*N*-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid), 1× nonessential amino acids, 1 mM sodium pyruvate, 2 mM glutamine,

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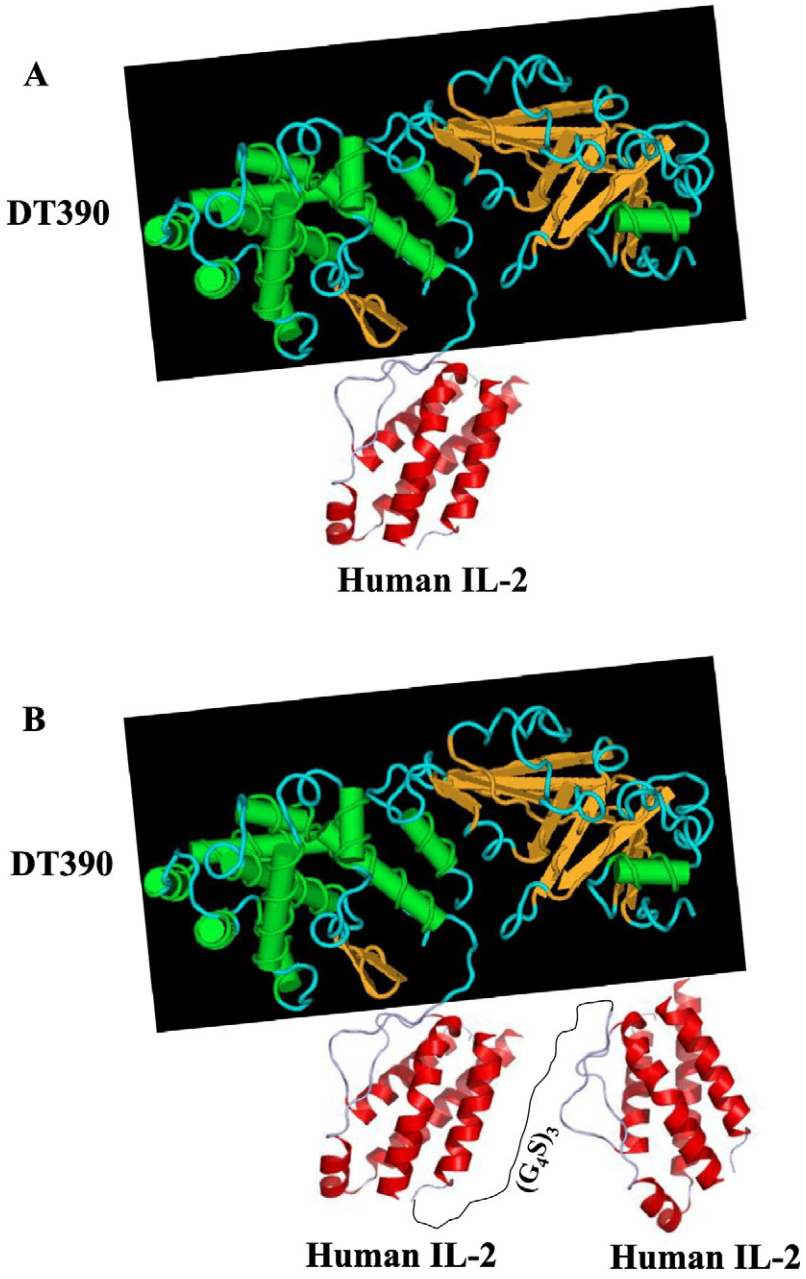

Fig. 1. Schematic diagram of the human IL-2 fusion toxins. **A)** Monovalent human IL-2 fusion toxin. **B)** Bivalent human IL-2 fusion toxin. The two human IL-2 were linked by a $(G_4S)_3$ linker.

and 2.5×10^{-5} M 2-mercaptoethanol. The HUT 102/6TG tumor cells were washed twice with 50 mL of the above RPMI1640 media by centrifugation at 558 x *g*, 25 °C for 5 min. The HUT 102/ 6TG cells were then diluted to 5×10^{7} cells/mL for injection. FITC-anti-human CD4 mAb (clone# SK-3, cat# 344604, BioLegend) and APC-anti-human CD25 mAb (clone# M-A251, cat# 555434, BD) were used for the flow cytometry analysis. Flow cytometry binding analysis, K_D analysis, and tumor cell viability analysis following the incubation with the human IL-2 fusion toxin were performed as described previously (Wang et al., 2015; Peraino et al., 2013a).

2.2. In vitro efficacy analysis

In vitro efficacy of the human IL-2 fusion toxin to the residual tumor cells was assessed using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, cat# G7571) as described previously (Zheng et al., 2017).

2.3. In vivo efficacy study

NOD/SCID human IL-2 receptor γ−/− (*NSG*) mice were purchased from Jackson laboratories and bred in Massachusetts General Hospital (MGH) rodent barrier facilities. The *in vivo* experiments were approved by the Institutional Animal Care and Use Committee (*IACUC*) of MGH. MGH is an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) recognized institution.

The immune deficient *NSG* mice were randomly divided into following groups: 1) vehicle ($n = 7$); 2) monovalent human IL-2 fusion toxin at 3 μg/kg ($n = 7$); 3) monovalent human IL-2 fusion toxin at 30 μg/kg ($n = 7$); 4) monovalent human IL-2 fusion toxin at 100 μg/kg ($n = 7$); 5) bivalent human IL-2 fusion toxin at 3 μg/kg ($n = 7$); 6) bivalent human IL-2 fusion toxin at 30 μg/kg ($n = 7$); 7) bivalent human IL-2 fusion toxin at 100 μg/kg ($n = 7$). All animals were injected IV with 10 million of human $CD25^{+}$ CTCL HUT102/6TG cells *via* the tail vein. Human IL-2 fusion toxin was injected IP daily for 10 consecutive days