Stability of the recombinant anti-erbB2 scFv-Fc-interleukin-2 fusion protein and its inhibition of HER2-overexpressing tumor cells

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Abstract. The anti-erbB2 scFv-Fc-IL-2 fusion protein (HFI) is the basis for development of a novel targeted anticancer drug, in particular for the treatment of HER2-positive cancer patients. HFI was fused with the anti-erbB2 antibody and human IL-2 by genetic engineering technology and by antibody targeting characteristics of HFI. IL-2 was recruited to target cells to block HER2 signaling, inhibit or kill tumor cells, improve the immune capacity, reduce the dose of antibody and IL-2 synergy. In order to analyse HFI drug ability, HFI plasmid stability was verified by HFI expression of the trend of volume changes. Additionally, HFI could easily precipitate and had progressive characteristics and thus, the buffer system of the additive phosphate-citric acid buffer, arginine, Triton X-100 or Tween-80, the establishment of a microfiltration, ion exchange, affinity chromatography and gel filtration chromatography-based purification process were explored. HFI samples were obtained according to the requirements of purity, activity and homogeneity. In vivo, HFI significantly delayed HER2 overexpression of non-small cell lung cancer (Calu-3) in human non-small cell lung cancer xenografts in nude mice, and the inhibition rate was more than 60% (P<0.05) in the group treated with 1 mg/kg the HFI dose; HFI significantly inhibited HER2 expression of breast cancer (FVB/neu) transgenic mouse tumor growth in 1 mg/kg of the HFI dose group, and in the following treatment the 400 mm³ tumors disappeared completely. Combined with other HFI test data analysis, HFI not only has good prospects, but also laid the foundation for the development of antibody-cytokine fusion protein-like drugs.

Introduction

The HER2 (ErbB2/neu) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor. ErbB2 is usually expressed at a very low level in adult tissues, but it is overexpressed in a variety of human tumors, such as breast cancer (25-30%), ovarian cancer (25-32%), lung cancer (30-35%), and primary renal cell carcinoma (30-40%) (1-4).

A recombinant humanized monoclonal antibody, Herceptin (trastuzumab) has shown good response in clinical trials, in the treatment of HER2-overexpressing metastatic breast cancers (5-8). The objective response rates of Herceptin monotherapy were lower than its combination therapies. Current treatment regimens which combine Herceptin with the taxane, paclitaxel or docetaxel have increased the response rates, time to progression, and survival (9,10). The humanized Herceptin suppressed the growth of HER2-overexpressing tumor cells by inhibiting HER2-driven signaling, inhibiting VEGF secretion or activity and mediating antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (11-14), but the humanized Herceptin did not induce apoptosis when it is used alone.

Interleukin-2 (IL-2) is an immunoregulatory protein with a wide range of immune effects. IL-2 exerts its biological effects by binding with the IL-2 receptor on the surface of
target cells. The activities of IL-2 include stimulating T cells to proliferate and become cytotoxic, augment NK cytotoxicity, enhancing macrophages cytotoxicity, giving rise to lymphokine-activated killer (LAK) cells (15-20). In tumor therapy, IL-2 has already shown some efficacy in the treatment of metastatic melanoma, kidney cancer or non-Hodgkin's lymphoma when used alone or combined with other anticancer drugs. However, the antitumor efficacy of IL-2 is often associated with unacceptable toxicity. HFI ensures both the prevention of the systemic side-effects and the locally restricted enrichment activity to the tumor sites with decreased IL-2 toxicity (21-23).

Despite the success of the Herceptin in clinical trials for the treatment of ErbB2-expressing metastatic breast cancer, the fusion of IL-2 to an ErbB2-specific antibody may enhance its antitumor efficacy. Firstly, the presence of the antibody variable region should target the tumor cells, allowing for higher doses of IL-2 at HER2 antigen located in tumor tissue, where they elicit an immune response and thus destroy tumors. Secondly, IL-2 is unstable in vivo and it will be quickly eliminated by the kidney due to limiting quantities of the protein. The half-life of IL-2 may be extended by MAb-conjugation due to both the size and stability of the MAb. Thirdly, an antibody-IL2 fusion protein may increase immune access to the antibody's target ligand by increasing permeability of blood vessels near the tumor nidus (24-30). In preliminary studies, it was observed that the HFI expression of clonal cells strained between 60-80 mg/l; the in vitro experiments proved that the HFI contained dual activity of antibodies and IL-2; the in vivo tests showed that the HFI inhibited HER2 expression of ovarian cancer; in (SKOV3) transgenic mouse tumor growth, the HFI antibody ADCC activity and IL-2-mediated killing activity of effector cells; to stimulate effector cells to secrete perforin, and the adhesion factor. However, many aspects of HFI has not been verified, including whether the cell lines is stable, whether the HFI purification is feasible over the following sophisticated purification processes, including microfiltration, ion-exchange, affinity and molecular sieve chromatography.

**Enzyme-linked immunosorbent assay (ELISA).** Quantification of the HFI was assayed by ELISA. Goat anti-human (100 µl) IgG (1:200 dilution) per well was coated onto 96-well ELISA plates overnight at 4°C. After washing the plates, serial dilution of the samples, which were maintained in PBS with 3% bovine serum albumin (BSA) were added to the plates and incubated at 37°C for 2 h. Then after washing the plates, 100 µl per well of horse-radish peroxidase-conjugated goat anti-human IgG (1:1000 dilution) was bound and incubated at 37°C for 1 h. Color was generated by adding peroxidase substrate and absorbance was measured on a plate reader at 492 nm. Dilutions of the HFI, for which the concentration was calibrated by the Kjeldahl method and the Lowry method, were used to generate a standard curve to estimate the concentration of unknown samples.

**Bioactivity assay.** The cytokine activity of the HFI was determined by IL-2 dependent T cell proliferation assay. Recombinant human IL-2 (rhIL-2) standard was obtained from National Institute for the control of Pharmaceutical and Biological products. The mouse CTLL-2 cell line expressing high-affinity IL-2 receptor was used to determine the IL-2 bioactivity by the T cell proliferation assay. Serially diluted samples and IL-2 standards were incubated with 4x10⁴ cells per well in triplicate in 96-well flat bottom plates for 24 h at 37°C in a humidified atmosphere of 5% CO₂, MTT (5 mg/ml) reagent was added 4 h before the end of culture, and then cells were lysed with 10% SDS and 50% N,N-dimethyl formamide, pH was maintained at 7.2. The OD values were read at 570 nm. The mouse CTLL-2 cell was provided by Professor N. Guo (Institute of Basic Medical Sciences, China).

**Antigen binding assay.** About 1x10⁶ cells per well were incubated with varying concentrations of the HFI or the same molar concentrations of the different proteins in a final volume of 0.2 ml. After incubation on ice for 30 min, cells were washed twice with PBS-BSA, and a fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG antibody (Beijing Dingguo Biotechnology Co. Ltd.) was added. Incubation was continued for an additional 30 min on ice. After two washes with PBS-BSA, the cells were analyzed by flow cytometry (FACScalibur, Becton Dickinson).

**Animal experiments.** The three types of mice including female FVB/neu mice, BALB/c mice and female Balb/C athymic nude mice of 7 to 10-weeks-age, were obtained from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences. All mice were housed under specific pathogen-free conditions. Experiments were carried out according to the National Institute of Healthy Guide for Care and Use of Laboratory Animals, and were approved by the Institutional Animal Care and Use Committee at the Shanghai Institute of Materia Medica, Chinese Academy of Sciences.

**Pharmacokinetic analysis.** BALB/c mice (48 animals per group) were injected with 25 or 100 µg of the purified HFI sample in a volume of 0.2 ml in the tail vein using a slow push, respectively. At various time points, small blood samples (6 animals per time
point) were taken by retro-orbital bleeding and collected in tubes coated with heparin to prevent clotting. After centrifugation, the plasma was assayed by ELISA with goat anti-human IgG and goat anti-human IL-2 antibody. The results were normalized to the initial concentration in the serum of each mouse taken immediately after injection.

In vitro antitumor activity. When the signals of HER2 were blocked on the surface of the HER2 overexpression cells, the growth of cells was inhibited or the cells entered apoptosis. The three types of cells including SKBR3 cells (HER2+), SKOV3 cells (HER2++) and MCF7 cells (HER2+) were provided by Professor N. Guo (Institute of Basic Medical Sciences, China), and were chosen to evaluate HFI antitumor activity in vitro (33). Firstly, the cells were plated on 96-well cell plates, then, serial dilution of the samples were added to the plates and incubated at 37°C in a humidified atmosphere of 5% CO2. Apoptosis of the cells were assayed for different time points by MTT cell proliferation assay methods.

In vivo antitumor activity. i) Six to seven-week-old female Balb/C athymic nude mice were subcutaneously injected 1x107 Calu-3 cells (human non-small cell lung cancer) into the right flank. The mice were treated with PBS (iv: 0.2 ml per mouse) (control) or HFI (iv: 1.0, 0.5 and 0.25 mg/kg mice weight, respectively). Seven or eight mice were used in each group. ii) The antitumor activity of HFI was evaluated in FVB/neu transgenic mouse model, which expressed the ErbB2/neu proto-oncogene and closely recapitulated the ontogeny and progression of human breast cancer. When tumors reached a size of more than 300 mm3, the mice were grouped (n=5) and PBS or HFI (1.0 mg/kg mice weight) or Herceptin (3.0 mg/kg mice weight) in a volume of 0.2 ml, was injected i.v. into the lateral tail vein. Dosage regimen: the first week for five continuous days, then once every other day. The mice weight were monitored daily and tumor volume was measured with a caliper once per two days, using the formula volume = length x width²/2.

Splenocyte culture. Splenocytes were harvested on day 20 after HFI administration and were cultured in triplicate (5x10⁷/well) in 96-well culture plates (Corning) for 24 h with 0.5 µg/ml of concanavalin A (Con A) or 1 µg/ml of lipopolysaccharide (LPS). The cells were pulsed with 0.5 µCi/well of [3H]thymidine for 8 h and harvested onto glass fiber filters. The incorporated radioactivity was then counted using a Beta Scintillation Counter (MicroBeta Trilux, PerkinElmer Life Sciences, Boston, MA).

ELISA detection of cytokines. Splenocytes were cultured in 24-wells culture plates (Corning) for 24 and 48 h with 0.5 µg/ml of Con A or 1 µg/ml of LPS. Culture supernatants were harvested and submitted to assays for the determination of cytokine concentrations. Cytokines in culture supernatant was assayed using mouse IFN-γ ELISA kit (BD Pharmingen), according to the manufacturer's instructions.

Cytotoxicity assays. Murine YAC-1 cells were used as targets in the NK assay. Target tumor cells were labeled with 250 kBq [3H]thymidine by incubating overnight in a culture dish (Corning) containing 10 ml of culture medium. Targeted cells (2x10⁶) were cultured in triplicate with different numbers of effector cells (effector: Target ratio = 100:1, 50:1 or 12.5:1) for 4, 8 and 12 h in 96-wells flat-bottomed plates. Cells were harvested onto glass fiber filters. The incorporated radioactivity was then counted using a Beta Scintillation Counter (MicroBeta Trilux, PerkinElmer Life Sciences, Boston, MA). Cytotoxic activity was calculated and expressed as the mean of percent cytotoxicity. Percent cytotoxicity = (cpm in effector-free wells - cpm in effector positive wells)/(cpm in effector-free wells) x 100.

Flow cytometric analysis. A single-cell suspension of spleen cells harvested on day 20 after HFI administration were blocked with anti-mCD16/CD32 (2.4G2, purified inhouse), stained with fluorescein isothiocyanate (FITC)-conjugated, phycoerythrin (PE)-conjugated, (PerCPeCy5.5)-conjugated, (APC)-conjugated, and analyzed using a FACScalibur system with Cell Quest software (Becton Dickinson). (FITC), (PE), (PerCPeCy5.5) or (APC)-conjugated mAb against CD3 (145-2c11), CD4 (Gk1.5), CD8 (Ly-2) (53-6.7), CD1 (1d3), TCR-γδ (GL3) were purchased from BD Pharmingen. PerCPeCy5.5-conjugated anti-mouse/rat FoxP3 (FJK-16s), PerCPeCy5.5-conjugated rat IgG2a and the FoxP3 staining buffer set were purchased from ebioscience.

Statistics. The statistical significance of the time course data was determined by two-way analysis of variance testing and Student's t-test was used to compare two treatment groups. Statistical significance was set at p<0.05.

Results

HFI protein expression and its characterization. The stable HFI expression was first assayed in the differential course of cell passage by using a pair of goat anti-human IgG antibodies in a 'sandwich ELISA', SDS-PAGE and IL-2 dependent T cell proliferation. The fusion proteins of HFI were stably expressed in different cell lines, such as the primary cell line (PCL), the master cell line (MCL), the working cell line (WCL) and the production of end-stage cell line (ECL) in 1,000 ml tissue culture roller bottles with a culture volume of 200-300 ml and at (2-4)x10⁶ cells/ml density at 30°C for 7 days. The culture supernatants were harvested following 1,500 rpm for 5 min to 8,000 rpm for 30 min. The molecular weight of HFI was found in a bivalent form of 140 kDa (Fig. 1A) and a monovalent form of 70 kDa (Fig. 1B) on 8% SDS-PAGE stained with Coomassie Brilliant Blue. The HFI expression of the four kinds of cells cultured for 7 days was examined at 100-150 mg/l by ELISA (Fig. 1C). The IL-2 bioactivity of HFI was assessed at (2-2.5) x10⁶ IU/mg by the standard IL-2 dependent T cell proliferation assay and ELISA calibration concentration (data not shown). Fig. 1 shows that the HFI gene copy number and HFI expression were relatively stable in the differential course of cell passage.

Since the expression of HFI in the cell lines was stable, the purification and stability of HFI was crucial to the ability of this fusion protein. The supernatant of HFI reaction liquid was sampled at 30°C and assayed for one month by the Cell Bioreactor under a continuous flow mode. In the HFI purification process, insoluble cell debris and other irrelevant substances were first removed from the HFI supernatant by 0.2 or 0.45 µm microfiltration membrane bag (Millipore), then...
The microfiltration sample was concentrated with the 30-50 kD nitrocellulose membrane bag (Millipore) to a proper volume. This concentration step was critical for HFI purification, as it could not only remove a large number of cellular components, but also deplete certain host proteins and small molecules in culture medium. In order to protect the affinity chromatography media from erosion, the ultra-filtration sample was purified by DEAE ion exchange in 0.2-0.4 M NaCl, 40 mM phosphate-citrate buffer and pH 7.2 was maintained, this process was mainly to remove endotoxin, nucleic acids and other substances. Purity of antibody protein reached 97% or higher after affinity chromatography purification. Therefore, the HFI was purified over Mabselect Sure (GE Healthcare) affinity chromatography, eluting with acidic conditions.
purified solution of the HFI was obtained by Superdex 200 (GE Healthcare) chromatography media to remove HFI monomer and polymer (Fig. 2). After the protein purification process, the HFI yield was measured by a microplate spectrophotometer at 490 nm, and the final HFI sample was more than 40% yield, the HFI purity was examined by reducing and non-reducing SDS-PAGE (Fig. 2A), the quantity of HFI monomers and polymers were less than 5% of HFI dimers (Fig. 2A; lanes 7 and 8), more than 98% purity of the HFI sample was determined by HPLC (Fig. 2B), fluorescent PCR, and host protein ELISA. Endotoxin was monitored by LAL agglutination. The HFI bioactivity was consistent with IL-2 activity, measured by a standard IL-2-dependent T cell proliferation assay (Fig. 2C). Human CD3+ and CD16+ cells were detected from the peripheral blood of mice at several doses of the HFI treated groups (33). The binding characteristics of the HFI was identified, and then three HER2 expressing cells, SKBR3 (HER2+++), SKOV3 (HER2++) and MCF7 cells (HER2±) were used to test HFI binding activity in different tumor cell lines. In the same molar concentration, HFI, Herceptin (Trastuzumab Injection, Roche Pharma Ltd.) and HF (antibody part of HFI, self-made sample) showed the same binding ability (Fig. 3). The HFI binding capacity was not affected by IL-2 fusion with HFI as compared to HF (Fig. 3A-D). This result demonstrated that the HFI binding ErbB2 protein characteristics depended on the expression of the ErbB2 protein (Fig. 3A-D). HFI showed a similar characteristic of binding HER2 with the Herceptin (Fig. 3A-D).

HFI binds specifically to HER2. The HFI built targeting was based on the binding properties of the ant-ErbB2 antibody and interleukin-2 bioactivity. The biological activity of IL-2 of the HFI was based on the ability of the HFI to support IL-2 dependent T cell proliferation (Fig. 2C). Human CD3+ and CD16+ cells were detected from the peripheral blood of mice at several doses of the HFI treated groups (33). The binding characteristics of the HFI was identified, and then three HER2 expressing cells, SKBR3 (HER2+++), SKOV3 (HER2++) and MCF7 cells (HER2±) were used to test HFI binding activity in different tumor cell lines. In the same molar concentration, HFI, Herceptin (Trastuzumab Injection, Roche Pharma Ltd.) and HF (antibody part of HFI, self-made sample) showed the same binding ability (Fig. 3). The HFI binding capacity was not affected by IL-2 fusion with HFI as compared to HF (Fig. 3A-D). This result demonstrated that the HFI binding ErbB2 protein characteristics depended on the expression of the ErbB2 protein (Fig. 3A-D). HFI showed a similar characteristic of binding HER2 with the Herceptin (Fig. 3A-D).

Figure 3. The specific binding activity of the HFI was analyzed by FACS. In the same molar concentration of hIgG, human IgG1; HFI, the purified HFI sample; Herceptin, trastuzumab injection; HF, antibody of HFI. (A) Results of MCF7 cell line (HER2±). (B) Results of SKOV3 cell line (HER2++). (C) Results of SKBR3 cell line (HER2+++). (D) The binding rate compared with the three proteins. The rates of HFI loading to HER2 proteins were 40%-45% (SKOV3 cells, HER2++) and 85%-95% (SKBR3 cells, HER2+++), respectively. There was no significant difference in the binding capacity of HFI, when compared with HFI, HF and Herceptin.
Pharmacokinetic properties. The pharmacokinetic properties of immunocytokines were reported to be dependent on their uptake by the FcR-bearing cells and the intracellular proteolysis (28). Half-life of drugs was related to their molecular size. The structure of HFI was determined by the in vivo stability, and properties of IL-2 and anti-ErbB2 protein. The half-life of HFI was detected in the mouse serum at different time points by ELISA. The results showed that the concentration of HFI decreased quickly during the first 4 h, and showed a relatively stable plateau following 64 h (Fig. 4). At 100 µg HFI maintained relatively higher levels than 25 µg HFI, demonstrating that the higher levels were maintained with higher concentrations (Fig. 4). The results indicated that the HFI structural design was conducive to recruitment around the cells, and also extended the half-life of IL-2.

In vitro antitumor activity. The HFI has a combination of activities, i.e., binding properties of HER2 receptor and interleukin-2 bioactivity. The ability of the HFI binding HER2 receptor depends on the HER2 overexpression on the cell surface. In this study, whether the HFI inhibited the cell proliferation when the HFI was bound to HER2 receptor was evaluated. The three cell types, breast cancer (SKBR-3, HER2+++), breast cancer (MCF-7, HER2-) and ovarian cancer cells (SKOV-3, HER2-) were incubated with the different concentrations of HFI for 72 h. The results showed that the HFI did not inhibit cells proliferation, and proved that the HFI did not have chemical toxicity. In vitro, the role of HFI was to inhibit tumor cell growth by activating the effector cells.

The in vivo antitumor activity was investigated using a Calu-3 xenograft model. Calu-3 is known to be a HER2-overexpressing tumor. A total of 1x10⁷ Calu-3 cells suspended in 0.15 ml PBS were injected subcutaneously into the right flank of the mice. Treatment was started when the tumor volume reached 200-350 mm³ after tumor cell implantation. (A) Tumor volume was measured by a vernier caliper and calculated by the formula (long measurement x short measurement²/2). HFI inhibition rate reached 32.2% (0.25 mg/kg), 51.4% (0.5 mg/kg), and 61.8% (1.0 mg/kg), respectively. (B) The average weight of mice was observed at different time points. The mouse weights had no significant differences, P>0.05; n=7; error bars represent SEM.
of FVB/neu in the transgenic mouse model. Moreover, after treatment for 15 days, the tumor volume which was initially <400 mm$^3$, was undetectable.

**HFI promoted mitogen-induced splenocyte proliferation and natural killer (NK) cell-mediated cytotoxicity.** The *ex vivo* immunoenhancement of HFI was evaluated on splenocyte proliferation induced by Con A and LPS. The results showed that the splenocytes of FVB/neu tumor bearing mice treated with 1 mg/kg HFI for 3 weeks had significant proliferation activity compared to those treated with PBS induced by LPS (Fig. 7A) or Con A (Fig. 7B). The production of IFN-$\gamma$ was induced by FVB/neu in mice treated with HFI (Fig. 7C) or Herceptin (Fig. 7D). HFI also increased the NK cell-mediated cytotoxicity (Fig. 7E-G).

**Effect of HFI treatment on regulatory T cells and T cell percentage.** The therapeutic mechanisms of HFI were investigated *ex vivo* to determine whether its effects were attributable to influenced FoxP3$^+$ Treg cells, and T cells. Intracellular staining of transcription factors showed that HFI treatment significantly reduced the expression of FoxP3 (Fig. 8A and C). HFI also increased the T cell population (Fig. 8B and D).

**Discussion**

Proteins are marginally stable and readily denatured by various stresses encountered in solution or in the frozen or dried states. Various additives are known to minimize the damage and to enhance the stability of proteins (34). The recombinant anti-ErbB2 scFv-Fc-IL-2 fusion gene was transfected into CHO dhfr- cells and selected for the dhfr-phenotype (31,32,35). The CHO cells stably expressing the fusion proteins (HFI) were cultured in serum-free medium, so that HFI stability was guaranteed when the protein purification process was downstream. HFI purified liquid was stable at 4˚C for two months (Fig. 2), indicating that HFI can be produced in big batches. Stability data of freeze-dried HFI products were obtained after one year's experiment, including quality analysis and activity detection, there was no stability data of the HFI available for more than three years, yet the current process and the data showed that the stability was not a problem for HFI, in drug development.

IL-2 is an important pleiotrophic cytokine and exhibits a wide variety of biological activities, including the stimulation of antitumor effector cells (18,36,37). IL-2 treatment can augment the activation of pre-existing antigen-specific T cells, enhance their recognition and destruction of neoplastic tissue, and activate NK cells (33,38,39). However,
systemic cytokine therapy frequently caused severe problems with toxicity, and made it impossible to achieve an effective dose at the tumor sites. The recombinant human IL-2 gene is fused with the anti-ErbB2 scFv-Fc gene at the C-terminal (31,32,35). The biological activities of both antibody and IL-2 were maintained. The specific local activation of IL-2 at tumor sites provided more effective tumor destruction (33,40,41). HFI not only extended IL-2 half-life in vivo (Fig. 4), but also reduced the retention time of IL-2 in the blood because of HFI targeted activity (Fig. 3), and thus reduced the systemic toxicity of IL-2.

Patients with ErbB2-overexpressing tumors have shown a significantly lower overall survival rate and shorter relapse time than those with ErbB2-negative tumors (33,42,43). In therapy, ErbB2 overexpression is a significant negative prognostic indicator for a variety of therapies (33,44). In the pre-clinical trials using murine models, antibody-IL-2 fusion proteins have shown to be very effective antitumor agents (11,45-48). HFI was effective in killing SKBR3 and SKOV3 cells, which expressed ErbB2 at higher levels, than MCF7 cells (33,49), suggesting that the killing effect mediated by HFI was dependent on the expression level of ErbB2 molecules on the target cells. To further determine the efficacy of HFI in vivo, the xenograft models of human non-small cell lung cancer (Calu-3) cells (HER2+++ in nude mice were treated with different concentrations of HFI (Fig. 5). The 1.0 mg/kg dose of HFI showed that the HFI had more than 60% of antitumor activity in vivo (Fig. 4), but also reduced the retention time of IL-2 in the blood because of HFI targeted activity (Fig. 3), and thus reduced the systemic toxicity of IL-2.

In conclusion, HFI purified solution was stable, and could be prepared in mass for drug development. Fusion protein of the HFI retained ErbB2 specificity and IL-2 biological activity. Pharmacokinetics of HFI was not only associated with its molecular weight, but also positively correlated with HFI concentration. In vivo, HFI had significant activity in inhibiting HER2-overexpressing tumor growth. HFI demonstrated potency of initiating a cytotoxic activity on unstimulated PBMC against human breast and ovarian cancer cells at low effector-to-target ratios in vitro experiments. In this study, it was observed that HFI promoted apoptosis had no impact on HER2 expression, indicating HFI had potential toxicity. There are many issues to be resolved during the process of HFI drug development, including the long-term stability, mechanism of action, toxicological effects as well as the immunogenic effects.
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References


