Editorial

CAR-T cell *in vivo* tracking method using PET scan with the reporter gene and new investigational tracer [^{18}F] FHBG

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Development and clinical effects of chimeric antigen receptor (CAR)-T cells

Recent development of cancer immunotherapy, and CAR-T cell therapy in particular, attracts considerable attention among researchers. In antitumor immunity, cytotoxic T lymphocytes (CTLs) specifically recognize tumor-associated antigens (TAAs) in an MHC-class I-restricted manner and attack tumor cells. CTL activation and its clonal expansion require co-stimulatory receptors such as CD28, 4-1BB and OX40, and CTL stimulatory cytokine including IL-2 and IFNγ in addition to antigen recognition. However, tumor cells escape from the immune surveillance via decreased expression of MHC class 1, increased expression of co-inhibitory molecule, inhibitory cytokine production and accumulation of inhibitory immune cells during *in vivo* growth. CAR gene-designated extracellular binding domain consists of a target-specific antibody or a targeting ligand fused with the intracellular activating CD3zeta chain and the co-stimulatory molecule gene (1). Unlike conventional activated lymphocyte therapy or natural adaptive immunity, genetically engineered CAR-T cells can attack tumor cells in non-MHC class 1-restricted manner without co-stimulatory molecule signals. Complete remission and durable clinical effect of CD19 CAR-T cell therapy for CD19 positive leukemia are reported in previous studies (2). Treatment strategies using CAR-T cells have also been developed for high-grade gliomas (HGGs). IL-13Rα2 is a cell surface receptor, over-expressed in more than a half of HGG patients (3). IL-13 zetakine contains point-mutated IL-13 sequence and it does not bind to IL-13Rα1 but specifically binds to IL-13Rα2 (4). Therapeutic effects of IL-13 zetakine-transfected T cells have been reported in *in vitro* and *in vivo* studies using several cell lines containing a tumor stem cell line and isolated HGG cells (5,6). A case study of HGG patients treated with IL-13 zetakine transfected T cells showed safety of the cell injection and efficacy during clinical monitoring (7). In this study, CTLs, genetically modified to express the herpes simplex virus 1 thymidine kinase (HSV1-tk) suicide gene, were injected in HGG patients, and HSV1-tk positive CTLs were detected by the PET scan with the uptake of a ^{18}F radiolabelled 9-[4-[^{18}F] fluoro-3-(hydroxymethyl)butyl]guanine ([^{18}F] FHBG) as a probe.

Determination of treatment effect—problems of prognosis estimation

Easy and exact method to predict therapeutic effect of these immunotherapies and patient prognosis has not been found to date. In general, detection of the delayed-type hypersensitivity (DTH) reaction using TAA is performed after immunotherapy. Also in our study, DTH-positive patients have been found to experience a good prognosis (8). A recent study shows the correlation between preoperative PD-L1 expression of circulating mononuclear cells and prognosis after immunotherapy, and that is expected as a

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prognostic marker for immunotherapy (9). However, they are not real-time tools for evaluating treatment effect during immunotherapy. MR imaging is a repeatable imaging tool for detection of the treatment effect. Response evaluation criteria in solid tumors (RECIST) based on MR imaging are most frequently used for evaluation of the local reaction of antitumor therapy including chemotherapy. However, the use of these criteria for immunotherapy has some issues such as underestimation of progression-free survival due to pseudoprogression caused by the cell influx into the tumor microenvironment and no correlation with long-term prognosis (10). Although immune related response criteria (irRC) were advocated to solve these issues (11), it takes 2–3 months for the first response assessment to determine the therapeutic effect, and patients lose treatment opportunity if true progression occurs during this period. For these reasons, development of a method of immune cells tracking and early therapeutic effect prediction has been urged. This non-invasive *in vivo* imaging of dynamics of immune cells in the human body has become available in clinical practice with the development of several new molecular imaging methods. It will be an important method to improve development and optimization of immunotherapy.

CTL Tracking using [^{1}8F] FHBG

[^{18}F] FHBG is a PET tracer with an ^{18}F-labelled side chain of penciclovir used for detecting HSV1-tk or HSV-sr39tk mutant protein (12). [^{18}F] FHBG is not degraded in mammalian TK and does not emit a radio signal. In preclinical models using rats and rabbits, [^{18}F] FHBG showed to be safe after administration in doses 100 times higher than for humans, and it was approved by FDA as an investigational new drug (13). Yaghoubi *et al.* also studied human pharmacokinetics and dosimetry of [^{18}F] FHBG with ten healthy volunteers and showed no toxicity (14). The safety and feasibility of radiolabeled [^{18}F] FHBG PET is studied in several clinical fields including recurrent glioma (7,15).

Keu *et al.* reported the *in vivo* kinetics of CAR-T cells detected by PET scan using [^{18}F] FHBG (16). Genetically engineered IL-13 zetakine CAR-T cells were transfected with hygromycin phosphotransferases/HSV-tk fused in-frame gene and glucocorticoid resistance locus. Those CAR-T cells were positively selected using hygromycin B and indirect labeling with [^{18}F] FHBG. In addition, glucocorticoid receptor resistance locus allowed using that CAR-T cells in HGG patients during steroid therapy. Those sequences were transfected into CTL by electroporation using plasmid DNA. In this Keu's study, 7 patients including the first patient who administrated [^{18}F] FHBG with relapsed/recurrent HGG were enrolled. These patients received autologous or allogeneic CAR-T cells, and *in vivo* monitoring was performed by the [^{18}F] FHBG-PET scan. Safety and stability of CAR-T cells and [^{18}F] FHBG were examined. Rickham reservoir/catheter was installed to reach the tumor resection site after surgery or biopsy and CAR-T cells were injected directly into the tumor site. Patients No. 1–2 administered autologous CAR-T cells and the patients No. 3–7 administered allogeneic CAR-T cells (GRm13Z40-2 CTL line) derived from healthy donors. Patients No. 3–7 simultaneously administered recombinant human IL-2. More than 99% of the produced CAR-T cells were CD8 positive T cells, and [^{18}F] FHBG uptake of CAR-T cells was significantly higher than that of the parental cells. After the [^{18}F] FHBG uptake, CAR-T cells retained proliferation ability for 2 days.

One patient had rash and redness in the lower part of the face after administration of FHBG, but the symptoms improved after administration of diphenhydramine. There were no serious adverse events other than in this patient. After administration of [^{18}F] FHBG, the [^{18}F] FHBG accumulated in the tumor area and tumor resection site were observed. One patient whose tumor was attached to the dura matter with rich tumor blood vessels showed 3–4 times higher accumulation of [^{18}F] FHBG compared to the baseline. Since [^{18}F] FHBG cannot pass through the BBB, [^{18}F] FHBG accumulated in areas rich in tumor blood vessels without the BBB. PET scan data calculation methods need to be validated, however, an increase in signal was observed after the CAR-T cells administration in all patients except Patient 1, who could not receive pre-CAR-T cell injection PET scan. Furthermore, in patients with multiple tumors an increase in [^{18}F] FHBG signal was observed in areas where CTLs were not injected, suggesting transition of CAR-T cells between tumors. These data proved that PET scan with [^{18}F] FHBG can visualize HSV1-tk expressing CAR-T cells accumulated in the tumor site after injection. Data on safety of [^{18}F] FHBG needs to be collected in the future. In this study, the compound targeted recurrent gliomas, and most patients died within 12 months after administration of CAR-T cells. Further research will be focused on methods to evaluate the therapeutic effect of CAR-T cell therapy.

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Footnote

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