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Validated detection of human anti-chimeric immune responses in serum of neuroblastoma patients treated with ch14.18/CHO

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ABSTRACT

Human/mouse chimeric monoclonal antibody (mAb) ch14.18/CHO is directed against disialoganglioside GD_2. Activity and efficacy of this mAb are currently determined in ongoing clinical Phase II and -III studies in high-risk neuroblastoma (NB). Based on the chimeric nature of this mAb, some patients may develop a human anti-chimeric immune response (Mirick et al., 2004) which impacts on pharmacokinetics and may induce anti-anti-idiotype (Id) mAb with a potential survival benefit. Therefore, a validated method of quantitative detection of human anti-chimeric antibodies (HACA) in serum samples of NB patients treated with ch14.18/CHO is an important tool for monitoring of clinical trials.

Here, we report a validated sandwich enzyme-linked immunosorbent assay (ELISA) according to the one arm binding principle using ch14.18/CHO as a capture mAb and biotinylated ch14.18/CHO mAb for detection. Ganglidiomab, a monoclonal anti-Id Ab to ch14.18/CHO (Lode et al., 2013), was used as a standard for assay validation and HACA quantification. Systematic evaluation of the established ELISA procedure revealed an optimal serum sample dilution factor of 1:160. Assay validation was accomplished with a set of tailored quality controls (QC) containing distinct concentrations of ganglidiomab (3 and 15 μg/ml). The coefficients of variation (CV) for all within-assay and inter-assay measurements using QCs were under 20% and the limit of detection (LOD) was 1.1 μg/ml. Three patients (P1, P2, P3) treated with a 10 day continuous infusion of 100 mg/m^2 of ch14.18/CHO were selected for analysis with this assay. Selection was based on ch14.18/CHO drug level on day 8 in cycle 2 of >10 μg/ml (expected) (P1) and of <2 μg/ml (unexpected) (P2 and P3). Both patients with unexpected low ch14.18/CHO levels revealed a strong signal in the HACA ELISA.

Interestingly, ch14.18/CHO-mediated complement-dependent cytotoxicity (CDC) could not be detected in P2 in contrast to P3 suggesting anti-NB activity even in the presence of HACA. We showed that neither eight freeze–thaw cycles nor storage at room temperature for up to 168 h affected HACA stability in serum. In summary, we describe a validated ELISA method suitable for the assessment of HACA in NB patients treated with ch14.18/CHO.

Abbreviations: mAb, monoclonal antibody; NB, neuroblastoma; anti-Id, anti-idiotype; HACA, human anti-chimeric antibody; ELISA, enzyme-linked immunosorbent assay; CV, coefficient of variation; LOD, limit of detection; QC, quality controls; CDC, complement-dependent cytotoxicity; HAMA, human anti-murine antibody; CDR, complementarity-determining regions; HAHA, human anti-humanized antibody; DMEM, Dulbecco's modified Eagle's medium; TMB, 3,3′,5,5′-tetramethylbenzidine; RT, room temperature; SD, standard deviation; LTI, long term infusion; AM, acetoxymethyl ester; HRP, horseradish peroxidase; IL-2, interleukin-2; CHO, Chinese hamster ovary; BSA, bovine serum albumin; OD, optical density.

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1. Introduction

Neuroblastoma (NB) patients treated with mAb directed against the tumor-associated antigen disialoganglioside GD_2 had an improved outcome (Parsons et al., 2013). However, administration of anti-GD_2 mAb of murine origin (3F8 and 14G2a) induced development of high levels of human anti-murine Ab (HAMA) resulting in a rapid clearance of the anti-GD_2 mAb from the patient circulation. To reduce murine origin and associated immunogenicity of anti-GD_2 mAb in humans, chimeric mAbs were generated by genetic engineering of the murine variable regions into the backbone of human IgG constant regions. Clinical benefit of immunotherapies based on application of chimeric human/mouse mAb ch14.18 was reported to be accompanied by lack of HAMA indicating efficacy of Ab chimerization (Parsons et al., 2013). However, administration of ch14.18 resulted in development of HACA directed against the remaining murine component of the chimeric mAb. To overcome this problem, humanized hu14.18 mAbs were generated containing only the complementarity-determining regions (CDR) of the murine variable domains, grafted into the intact human IgG1 molecule. Since these humanized versions of this antibody (hu14.18) are currently in the early phase of clinical trials, development of human anti-humanized Ab (HAHA) directed against murine components of hu14.18 as well as its effects on the therapy outcome remain to be evaluated.

Based on the fact that development of HACA affects immunotherapy with a chimeric mAb, a validated assay for detection of HACA Ab is an important tool.

In Europe, three clinical trials with different ch14.18/CHO treatment schedules are ongoing to investigate the influence of a combined immunotherapy of ch14.18/CHO, IL-2 and 13-cis-retinoid acid on the outcome of patients with high-risk NB in the absence or presence of haploidentical blood stem cell transplantation. The European Phase III clinical trial (HR-NBL 1.5/ESIOP, Eudra CT: 2006-001489-17) and the trial in the context of haploidentical stem cell transplantation (Eudra CT: 2009-015936-14) are based on a bolus infusion of 20 mg/m^2/day ch14.18/CHO over 8 h on five subsequent days. To reduce side effects including neuropathic pain, a Phase I/II clinical trial was initiated based on the same cumulative dose of ch14.18/CHO (100 mg/m^2/cycle) infused over a longer time period (ten days) (Eudra CT: 2009-018077-3).

We developed an ELISA method following the one arm binding principle (Fig. 1), to quantify HACA to ch14.18/CHO in serum samples of patients in these clinical trials. One arm of HACA Abs binds immobilized ch14.18/CHO allowing the second arm to bind a biotin-conjugated ch14.18/CHO for detection. The previously described anti-Id mAb ganglidiomab (Lode et al., 2013) specifically binds ch14.18/CHO and was therefore used as a standard and QC for HACA quantification and assay validation.

We first determined an optimal dilution factor for standards, patient samples and QC. Next, we demonstrated consistency and validity of this method, determined limit of detection (LOD) and coefficients of variation (CV) and defined an acceptable limit of deviation of QCs at 20% from their nominal values for each experimental run. This ELISA method was further used for detection of HACA in serum samples of selected patients treated in a European clinical trial with ch14.18/CHO. To analyze a potential therapy benefit based on HACA-dependent induction of anti-anti-Id mAb, we determined complement-dependent cytotoxicity (CDC) against NB in serum of patients who developed HACA. Finally, using this ELISA assay, we demonstrated stability of HACA in serum samples after eight freeze–thaw cycles and storage at room temperature (RT) for up to 168 h.

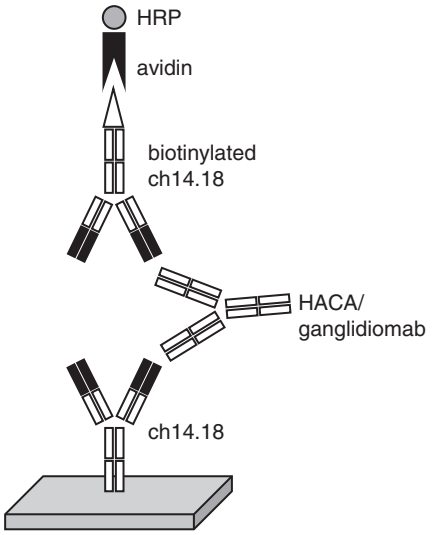

Fig. 1. Schematic illustration of the one arm binding HACA-ELISA method. To detect HACA in patient serum samples, solid bound ch14.18/CHO was used as a capture mAb. Free arms of bound HACA antibodies were detected with biotinylated ch14.18/CHO in combination with an avidin-HRP conjugate and 3,3′,5,5′-tetramethylbenzidine (see also the Materials and methods section). Ganglidiomab, a monoclonal anti-Id of ch14.18/CHO (Lode et al., 2013), was used as a standard.

In conclusion, the present study supports the utility of this validated ELISA method for systematic clinical evaluation of patients treated with ch14.18/CHO.

2. Materials and methods

2.1. Cell culture

Human NB cell line LA-N-1 was cultured in RPMI supplemented with 4.5 g/l glucose, 2 mM glutamine, 10% FCS, 100 U/ml penicillin and 0.1 mg/ml streptomycin (PAA, Pasching, Austria). Ganglidiomab-producing hybridoma cells were cultured in serum-free Dulbecco's modified Eagle's medium with stable glutamine (DMEM, 4.5 g/l glucose; PAA, Pasching, Austria) supplemented with 1 × non-essential amino acids (PAA, Pasching, Austria) and 50 μM β-mercaptoethanol (Sigma Aldrich, Steinheim, Germany).

2.2. Isolation of ganglidiomab from hybridoma supernatants

Hybridoma cells were cultured in serum-free medium for three weeks followed by isolation of ganglidiomab as previously described (Lode et al., 2013). Briefly, prior to isolation, the concentration of ganglidiomab in hybridoma

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