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Development and validation of immunoassays for monitoring of guselkumab and anti-guselkumab antibodies in patients with moderate-to-severe psoriasis

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Development and validation of immunoassays for monitoring of guselkumab and anti-guselkumab antibodies in patients with moderate-to-severe psoriasis

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Highlights

- 20 specific anti-guselkumab monoclonal antibodies were generated and characterized
- An accurate and precise ELISA to quantify guselkumab was developed
- The guselkumab ELISA was validated in moderate-to-severe psoriasis patients
- Two different ELISAs to detect anti-guselkumab antibodies were developed

Abstract

Therapeutic drug monitoring, which is the measurement of drug concentrations in the blood, is a useful tool to guide clinical decision-making and treatment adjustments, on the condition that drug concentrations are correlated with treatment response. For guselkumab, an anti-IL-23 monoclonal antibody for the treatment of moderate-to-severe psoriasis, such a concentration-response relationship could not yet be determined as no commercial assays for the quantification of this drug or antibodies against this drug are available. Therefore, the aim of this study was to develop and validate immunoassays for the quantification of guselkumab and anti-guselkumab antibodies according to the guidelines of the European Medicines Agency (EMA). A diverse panel of 20 highly specific anti-guselkumab monoclonal antibodies (MA-GUS) was generated of which eight revealed a neutralizing capacity of ≥65%. At least seven different antibody clusters were identified based on their epitope binning profile. Using MA-GUS9F6 as the capture antibody and MA-GUS12G12 as the detection antibody, an ELISA was developed with a dose-response curve ranging from 0.08 to 5 ng/mL. The assay was specific, selectivity and could accurately and precisely quantify guselkumab concentrations in spiked healthy control serum and serum from guselkumab-treated psoriasis patients with a cut-off for quantification of 0.014 μg/mL. The presence of IL-23 in physiological concentrations or of non-neutralizing antibodies did not impact the quantification of guselkumab, while the presence of neutralizing antibodies did. Using MA-GUS12A9 as a calibrator, two anti-guselkumab antibody assays were developed to detect anti-guselkumab antibodies, which differ in the threshold for detection and quantification and the tolerance to the presence of guselkumab. Together, these validated immunoassays are essential to establish a concentration-response relationship and will allow the future implementation of therapeutic drug monitoring in moderate-to-severe psoriasis patients receiving guselkumab treatment.

1. Introduction

Guselkumab (Tremfya®) is a fully human IgG1λ monoclonal antibody that targets the p19 subunit of interleukin (IL)-23, thereby preventing the activation of T helper 17 cells and inhibiting the production of the pro-inflammatory cytokines IL-17 and IL-22 [1]. The pivotal phase III trials (VOYAGE 1 and VOYAGE 2) have demonstrated the efficacy of this biological in psoriasis patients resulting in the approval of guselkumab for the treatment of adult patients with moderate-to-severe plaque psoriasis [2-4].

Despite its proven therapeutic efficacy, the treatment response to guselkumab can vary considerably between patients. Some patients respond favorably to therapy while others do not show any improvement or show an initial response but lose this response over time. In certain patients, this non-response could be explained by a drug concentration that is too low, or by the presence of anti-drug antibodies [5].

Preventing loss of response to biologicals is a challenge for clinicians treating patients with psoriasis but strategies to aid in clinical decision-making are emerging. An important strategy is therapeutic drug monitoring (TDM), the measurement of drug concentrations which can serve as a tool to optimize biological treatment by identifying under- or overexposed patients and evaluating potential reasons for treatment failure [6]. Two fundamental requirements to perform TDM are the availability of an assay to quantify drug concentrations and the presence of a concentration-response relationship [7].

Several research groups have demonstrated the relationship between the serum concentration of adalimumab, an anti-tumor necrosis factor antibody, and clinical response in psoriasis patients [8, 9]. More recently, a similar relationship was observed for the anti-IL-12/23 antibody ustekinumab and the anti-IL17A antibody secukinumab [10-12]. For guselkumab, no information on the presence of a concentration-response relationship or the value of TDM could be found in literature as there is currently no commercial assay available for the quantification of this drug.

Therefore, the aim of this study was to develop and validate immunoassays for the quantification of guselkumab and anti-guselkumab antibody concentrations in serum of moderate-to-severe psoriasis patients, using in-house generated monoclonal antibodies. The assay validation was performed according to the guidelines of the European Medicines Agency (EMA) for bioanalytical method validation of ligand-binding assays [13].

2. Material and methods

2.1 Materials
Bovine serum albumin (BSA), Tween-80, citric acid, sulfuric acid (H_2SO_4), Ethylene Diamine Tetraacetic Acid (EDTA), TRIS base and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich. Sodium chloride, sodium carbonate and disodium hydrogen phosphate dihydrate ($Na_2HPO_4 - 2H_2O$) were purchased from VWR International. Potassium dihydrogen phosphate (KH_2PO_4) and Sulfo-NHS-LC-Biotin were purchased from Thermo Fisher Scientific Inc. Potassium chloride (KCl) and o-phenylenediamine (OPD) were purchased from Acros Organics and streptavidin poly-HRP from Sanquin Reagents. Acetic acid was purchased from Chem-Lab NV. Polystyrene 96-well EIA/ RIA plates (flat bottom) were purchased from Corning and the CELLine bioreactor flasks from Merck.
Guselkumab (Tremfya®) was purchased from Johnson and Johnson, secukinumab (Cosentyx®) from Novartis, whereas the human IgG-mixture (Multigam®) was purchased from C.A.F-D.C.F. IL-23 was obtained from PeproTech. Human serum from healthy donors further referred to as "healthy control serum", was purchased from Valley Biomedical.

The following buffers were used: PBS (140 mM NaCl, 8 mM Na_2HPO_4-$2H_2O$, 2.7 mM KCl and 1.5 mM KH_2PO_4), PTAE (PBS with 0.1% BSA; 0.002% Tween-80; 5 mM EDTA), citrate buffer (0.1 M sodium citrate, 0.2 M disodium phosphate), block buffer (1% BSA in PBS) and wash buffer (PBS with 0.002% Tween-80).

2.2 Patients

Serum samples of ten moderate-to-severe psoriasis patients treated with guselkumab were collected by the Department of Dermatology of Ghent University Hospital (Ghent, Belgium) in the framework of the BIOLOPTIM-GUS study (ClinicalTrials.gov Identifier: NCT04080648). The local ethics committee approved the study (B670201836638) and all patients provided written informed consent. Patients received the standard treatment regimen of 100 mg subcutaneously at weeks 0 and 4 followed by 100 mg every 8 weeks. Blood was collected in serum-separating tubes and serum was obtained by centrifugation of the tubes for 15 min at 1700 g at room temperature. The serum samples were divided into aliquots and stored at -20°C until analysis. Serum samples were prospectively collected before the start of treatment and at weeks 1, 2, 3, 4, 12 and 20. The Psoriasis Area and Severity Index (PASI) was used to assess disease activity which takes into account the severity and the body area that is affected.

2.3 Generation and characterization of a unique panel of monoclonal antibodies against GUS (MA-GUS)

2.3.1 Monoclonal antibody generation and purification

Monoclonal antibodies against guselkumab (MA-GUS) were generated in SJL/J mice according to the hybridoma technology of Galfré and Milstein with approval from the ethical committee (172/2017 KU Leuven, Leuven, Belgium) [14]. MA-GUS were purified on a ProSep-vA Ultra column (Merk Millipore) as described by Van Stappen *et al* [15].

2.3.2 Selection of 20 hybridomas to create a diverse panel of MA-GUS

2.3.2.1 Evaluation of the cross-reactivity of MA-GUS

Using a sandwich-type enzyme-linked immunosorbent assay (ELISA), the cross-reactivity towards secukinumab (an anti-IL17A antibody) and Multigam® (a human immunoglobulin G mixture) was determined. Briefly, guselkumab, secukinumab, and Multigam® were coated overnight at 4°C in a 96-well plate at a concentration of 4 µg/mL in PBS. After blocking for 2 h at room temperature (RT), hybridoma medium containing MA-GUS was applied in a 1:10 and 1:100 dilution and incubated overnight at 4°C. An HRP-conjugated rabbit anti-mouse antibody (RAM/mIg/PO; Nordic-MUbio, 1:10000 diluted) was used to detect bound MA-GUS. Subsequently, the color reaction was initiated using OPD (0.4 g/L) and H_2O_2 (0.003%) in citrate buffer. The reaction was stopped with H_2SO_4 (4 M) and the absorbance was measured at 492 nm. MA-GUS revealing a cross-reactivity less than 20% towards secukinumab and Multigam® were considered as specific antibodies and selected for further characterization.

2.3.2.2 Evaluation of the bridging capacity of MA-GUS

The bridging capacity of each MA-GUS was evaluated by incubating the respective hybridoma medium (1:40 diluted) for 2 h at 21°C on a guselkumab-coated plate. Guselkumab was labeled with Sulfo-NHS-LC-Biotin according to the manufacturer's instructions and applied on the plate in a 1:400 dilution for 1 h at 21°C to detect bound guselkumab. Thereafter, HRP-streptavidin was added for 30 min at 21°C and shaken at 300 rpm. The bridging capacity of each MA-GUS was based on the respective optical density (OD) relative to the OD of the MA-GUS with the highest OD. MA-GUS of which the OD was ≤5%, 6-20%, 21-50% and >50% of the MA-GUS with the highest OD was categorized as "no bridging

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capacity", "low bridging capacity", "intermediate bridging capacity" and "high bridging capacity", respectively.

2.3.3 Evaluation of the neutralizing capacity of MA-GUS

Using an IL-23-based competition assay, the neutralizing capacity of the generated MA-GUS was determined. IL-23 was coated at 0.5 µg/mL on a 96-well plate and incubated overnight at 4°C. Guselkumab (4 µg/mL) was pre-incubated with MA-GUS (8 µg/mL, 1:2 ratio) for 15 min at 37°C. After blocking for 2 h at RT and washing the plate, the GUS:MA-GUS mix was applied on the plate followed by a 2 h incubation period at 37°C. Residual binding of guselkumab to IL-23 was measured by the addition of HRP-conjugated polyclonal goat anti-human IgG Fc (GAHu/IgG(Fc)/PO; Nordic-MUbio, 1:4000 diluted) and incubation for 2 h at RT. MA-GUS were considered to be neutralizing when the binding of guselkumab was reduced with at least 65%.

2.3.4 Epitope binning of MA-GUS

A competitive ELISA was carried out to establish the binning profile of each MA-GUS. Specifically, 4 µg/mL of guselkumab was coated on a 96 well-plate for 72 h at 4°C. After blocking for 2 h at RT, 10 µg/mL MA-GUS ("cold-antibody") was applied on the plate and incubated overnight at 4°C. Then, plates were washed and a biotin-labeled conjugate of each MA-GUS was applied at a final concentration of at least 25 ng/mL followed by incubation for 2 h at RT. After washing, binding of biotin-labeled MA-GUS was determined using streptavidin poly-HRP. The OD obtained for each biotin-labeled MA-GUS in the absence and in the presence of the "cold antibody" was compared. MA-GUS of which the $\frac{OD+cold\ antibody}{OD-cold\ antibody}$ gave a percentage lower than 20%, were considered as competitive with the cold antibody. Principal component analysis (PCA) and clustering were performed using the online web tool ClustVis (https://biit.cs.ut.ee/clustvis/) to group the 20 MA-GUS according to similar binding sites towards guselkumab.

2.4 Development and validation of the guselkumab ELISA

2.4.1 Development of the guselkumab ELISA

MA-GUS was coated at 4 µg/mL overnight at 4°C followed by blocking with PBS containing 1% BSA. Samples were diluted 1:100 to 1:800 in PTAE buffer for concentrations <0.5 µg/mL, 1:400 to 1:3200 for concentrations between 0.5 µg/mL and 5 µg/mL and 1:1600 to 1:12800 for concentrations >5 µg/mL. Thereafter, the samples were applied on the plate and shaken at 300 rpm for 2 h at 21°C. Biotinylated MA-GUS was added and incubated for 1 h at 21°C to detect bound guselkumab. After washing, HRP-streptavidin was applied and incubated for 30 min at 21°C. Subsequently, the plate was developed using OPD (0.4 g/L) and H_2O_2 (0.003%) in citrate buffer and the reaction was stopped with H_2SO_4 (4 M). The absorbance was measured at 492 nm and the guselkumab dose-response curve was analyzed by non-linear regression using Graphpad Prism 8.2.1 (Graph Software, San Diego, CA, USA).

2.4.2 Specificity

The specificity of the guselkumab assay was determined by spiking secukinumab (10; 20 and 30 µg/mL) and guselkumab (0.10 and 5.00 µg/mL) to guselkumab-naive serum samples of psoriasis patients. Thereafter, the guselkumab concentration was determined. The assay is defined as specific when the recovery of guselkumab is 75-125% [13].

Additionally, five serum samples of secukinumab-treated psoriasis patients were measured with the guselkumab assay.

2.4.3 Selectivity

2.4.3.1 Impact of the matrix on guselkumab determination

The impact of unrelated compounds in the matrix, i.e. serum of psoriasis patients, on the determination of guselkumab was evaluated by spiking guselkumab (0.10 and 5.00 µg/mL) to 10 guselkumab-naive psoriasis serum samples. Thereafter, the guselkumab concentration was determined. The assay is defined as selective when the recovery of guselkumab is 80-120% in at least 80% of the evaluated samples [13].

2.4.3.2 Impact of IL-23 on guselkumab determination

To evaluate the potential interference of IL-23 on the determination of guselkumab, varying concentrations of IL-23 (0.1-2.5 µg/mL, molecular weight = 53.5 kDa) were spiked to 1 µg/mL guselkumab (molecular weight = 146.61 kDa) in PTAE buffer. The IL-23-GUS mixture was pre-incubated for 15 min at 37°C on a shaker (300 rpm) and applied in a 1:400 dilution on a MA-GUS coated plate for the detection of guselkumab. Subsequently, the residual binding of guselkumab was determined.

2.4.3.3 Impact of anti-guselkumab antibodies on guselkumab determination

The impact of anti-guselkumab antibodies on the determination of guselkumab was assessed by the addition of a two-fold excess (2 µg/mL) of MA-GUS to guselkumab (1 µg/mL) in PTAE buffer. For this experiment, 5 MA-GUS with varying neutralizing capacities were selected. After incubation for 15 min at 37°C and 300 rpm, the mixture was diluted 1:400 and applied on the plate. Subsequently, the residual binding of guselkumab was determined.

2.4.4 Determination of the assay cut-off values for detection and quantification, accuracy, imprecision and dilutional linearity

The assay lower limit of detection (LLOD), the lower limit of quantification (LLOQ), and cut-off values for detection and quantification were determined using a panel of 15 guselkumab-naive serum samples of psoriasis patients. The LLOD and LLOQ were calculated based on the mean OD of 15 guselkumab-naive serum samples plus 3 times and 10 times the standard deviation, respectively. The cut-off values for detection and quantification were set based on the calculated LLOD and LLOQ multiplied by the assay dilution factor.

The intra- and inter-assay accuracy and precision were determined by preparing 5 quality control samples (0.10; 0.25; 0.50; 2.00 and 5.00 µg/mL guselkumab) in healthy control serum and measuring four sets within one run for the intra-assay evaluation and six times one set, each on a different day, for the inter-assay evaluation. Acceptable criteria for accuracy and imprecision were defined as a recovery of 80-120% (75-125% for LLOQ) and a coefficient of variation (CV) of ≤20% (≤25% for LLOQ). Furthermore, the total error (i.e. the sum of the absolute value of the % relative error and % CV) should not exceed 30% (40% at LLOQ)[13].

To evaluate dilutional linearity, two quality control samples (2.00 µg/mL and 5.00 µg/mL guselkumab) were prepared in healthy control serum and applied on the plate in a dilution of 1:400 to 1:3200 and 1:1600 to 1:12800, respectively. Acceptable criteria for accuracy and imprecision were defined as a recovery of 80-120% and a CV of ≤20%. Moreover, the precision between the measured values in a dilution series should not exceed 30% [13].

2.4.5 Validation of the guselkumab ELISA in a cohort of moderate-to-severe psoriasis patients

Serum samples of 10 moderate-to-severe psoriasis patients under standard guselkumab treatment were measured with the guselkumab ELISA. The accuracy of incurred samples was evaluated by reanalysis of the samples in separate runs at different days with a total of four repeats. The concentration obtained for the initial analysis and the concentration obtained by reanalysis should be within 30% of their mean for at least 67% of the repeats. Moreover, the precision between the measured values in a dilution series should not exceed 30% [13].

2.5 Development and validation of the anti-guselkumab antibody ELISAs

2.5.1 Development of the anti-guselkumab antibody bridging ELISA

Guselkumab was coated at 0.5 µg/mL overnight at 4°C followed by blocking with PBS containing 1% BSA for 2 h at RT. Samples were diluted 1:20 to 1:160 in PTAE buffer, applied on the plate and incubated for 2 h at 21°C on a plate shaker (300 rpm). After washing, biotinylated guselkumab (diluted 1:4000) was added and shaken for 1 h at 21°C for the detection of bound MA-GUS. Afterward, HRP-streptavidin was applied and incubated for 30 min at 21°C. Plates were washed and developed using OPD (0.4 g/L) and H_2O_2 (0.003%) in citrate buffer and the reaction was stopped with H_2SO_4 (4 M). The absorbance was measured at 492 nm and the guselkumab dose-response curve was analyzed by non-linear regression using Graphpad Prism 8.2.1 (Graph Software, San Diego, CA, USA).

2.5.2 Development of the anti-guselkumab antibody ACE ELISA

An affinity capture elution (ACE) assay was developed that allows the detection of anti-guselkumab antibodies in the presence of drug. In this assay, guselkumab/anti-guselkumab antibody complexes were dissociated by acidification with acetic acid (pH 3, 300 mM) for 10-15 min at RT. The acidified samples were immediately applied on a plate coated with 5 µg/mL guselkumab that contained 25 µl of TRIS buffer (pH 9.5, 1 M) to neutralize the samples. After incubation at RT for 1 h, plates were washed and a second acidification step was performed to dissociate the anti-guselkumab antibodies from the coated drug. Next, the supernatant was transferred into a new plate containing TRIS buffer (pH 9.5, 1 M) and incubated for 1 h at RT to allow the anti-guselkumab antibodies present in the supernatant to bind to the plate. Taken all acidification and neutralization steps into account, the final dilution of the sample is 1:17.78. After blocking and washing the plate, anti-guselkumab antibodies bound to the plate were detected by the addition of biotinylated guselkumab for 1 h at RT. Finally, HRP-streptavidin was applied and incubated for 30 min at 21°C. Plates were washed and developed using OPD (0.4 g/L) and H_2O_2 (0.003%) in citrate buffer and the reaction was stopped with H_2SO_4 (4 M). The absorbance was measured at 492 nm and the

guselkumab dose-response curve was analyzed by non-linear regression using Graphpad Prism 8.2.1 (Graph Software, San Diego, CA, USA).

2.5.3 Specificity

The specificity of the anti-guselkumab antibody bridging and ACE assay was determined by spiking secukinumab (10; 20 and 30 µg/mL) and MA-GUS12A9 (50 and 200 ng/mL for the bridging assay and 2250 and 9000 ng/mL for the ACE assay) to guselkumab-naive serum samples of psoriasis patients. Thereafter, the anti-guselkumab antibody concentration was determined. The assay is defined as specific when the recovery of anti-guselkumab antibodies is 75-125% [13].

2.5.4 Selectivity

The impact of unrelated compounds in the matrix, i.e. serum of psoriasis patients, on the determination of anti-guselkumab antibodies in the bridging and ACE assay was evaluated by spiking MA-GUS12A9 (50 ng/mL for the bridging assay and 2250 ng/mL for the ACE assay) to 10 guselkumab-naive psoriasis serum samples. Thereafter, the anti-guselkumab antibody concentration was determined. The assay is defined as selective when the recovery of anti-guselkumab antibodies is 80-120% in at least 80% of the evaluated samples [13].

2.5.5 Determination of the assay cut-off values for detection and quantification, accuracy and imprecision

The assay LLOD, LLOQ and cut-off values for detection and quantification for the anti-guselkumab antibody bridging and ACE assay were determined using a panel of 25 guselkumab-naive serum samples of psoriasis patients as described in paragraph 2.4.4.

The intra- and inter-assay accuracy and imprecision were determined by preparing 3 quality control samples (50; 100 and 200 ng/mL MA-GUS12A9 for the bridging assay and 2250; 4500 and 9000 ng/mL MA-GUS12A9 for the ACE assay) in healthy control serum and measuring six sets within one run for the intra-assay evaluation and four times one set, each on a different day, for the inter-assay evaluation. Acceptable criteria for accuracy and imprecision were defined as a recovery of 80-120% (75-125% for LLOQ) and a coefficient of variation (CV) of ≤20% (≤25% for LLOQ). Furthermore, the total error (i.e. the sum of the absolute value of the % relative error and % CV) should not exceed 30% (40% at LLOQ) [13].

2.5.6 Evaluation of the drug tolerance

The interference of guselkumab with the determination of anti-guselkumab antibodies, i.e. the drug tolerance, was assessed by incubating increasing concentrations of guselkumab (1-8 µg/mL) to 4 µg/mL MA-GUS12A9, the antibody selected as calibrator. After 15 min incubation of the GUS:MA-GUS complex at 37°C, residual binding of MA-GUS12A9 was measured in both the bridging assay and the ACE assay. The percentage recovery of MA-GUS12A9 in the presence of guselkumab was expressed relative to the percentage recovery of MA-GUS12A9 in the absence of guselkumab, which was set at 100%.

3. Results

3.1 Characterization of a unique panel of monoclonal antibodies against guselkumab (MA-GUS)

3.1.1 Selection of 20 hybridomas to create a diverse panel of MA-GUS

A total of 144 hybridomas producing monoclonal antibodies that bind to guselkumab were generated. The cross-reactivity was evaluated by counter-screening for binding to secukinumab (an anti-IL17A antibody) and Multigam®(a human IgG mixture) resulting in a total of 73 hybridomas binding exclusively to guselkumab (Figure 1). To facilitate further antibody selection for assay development, the growth rate of the hybridoma and the ability of the antibody to cross-link coated guselkumab with biotin-labeled guselkumab, i.e. the bridging capacity, was evaluated. Of the 49 viable hybridomas, 28 had no bridging capacity, 8 had a low bridging capacity, 7 an intermediate bridging capacity and 6 a high bridging capacity. Of each group, several hybridomas were selected in order to create a diverse panel of 20 different guselkumab-specific antibodies that can be used for further assay development.

3.1.2 Neutralizing capacity of guselkumab-specific MA-GUS

The neutralizing capacity of the 20 MA-GUS was determined using an IL-23-based competition assay. Eight MA-GUS reduced the binding signal of guselkumab to IL-23 with ≥ 65% when using at 2-fold excess of antibody over guselkumab and were considered neutralizing. One antibody had no neutralizing capacity (<10%), while the 11 remaining antibodies had an intermediate neutralizing capacity (ranging from 37 to 62%).

3.1.3 Epitope binning of guselkumab specific MA-GUS

The results of the epitope binning experiment were visualized using principal component analysis (PCA). The score plot shows the first two principal components (PC), PC1 on the X-axis and PC2 on the Y-axis, explaining 76.3% and 9.7% of the total variance, respectively (Fig. 2). The 20 MA-GUS were clustered in seven different groups, each group containing antibodies that are directed towards a similar guselkumab binding site.

3.2 Development and validation of the guselkumab ELISA

3.2.1 Development of the guselkumab ELISA

The 20 selected MA-GUS were pairwise tested for their suitability as capture and detection antibody in the development of an ELISA to quantify the concentration of guselkumab. Based on the affinity, the absence of serum interference and the interdilution, MA-GUS9F6 was selected as the capture antibody and MA-GUS12G12 as the detection antibody. MA-GUS9F6 and MA-GUS12G12 had a neutralizing capacity of 72% and 58%, respectively and bind to different epitopes of guselkumab (clusters 1 and 6). Using this combination, a non-linear guselkumab calibration dose-response curve, that ranged from 0.08 to 5 ng/mL was obtained (Suppl. Fig. 1).

3.2.2 Specificity

The specificity of the guselkumab assay was determined by spiking secukinumab, a human anti-IL-17A monoclonal antibody frequently used in the treatment of psoriasis, and

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guselkumab to drug-naive serum samples of psoriasis patients. In these samples, guselkumab could accurately be measured (recovery of 95-111%), demonstrating the specificity of the assay. Moreover, in five serum samples of secukinumab-treated guselkumab-naive psoriasis patients (with secukinumab concentrations ranging from 13.1-49.0 µg/mL) guselkumab was undetectable.

3.2.3 Selectivity

The selectivity of the guselkumab assay was determined by evaluating the impact of the matrix (serum of psoriasis patients), the target (IL-23) and anti-guselkumab antibodies on the measurement of guselkumab.

3.2.3.1 Impact of the matrix on guselkumab determination

Guselkumab was spiked to guselkumab-naive serum samples of psoriasis patients to evaluate the impact of unrelated compounds in the matrix on guselkumab determination. The matrix did not affect the quantification of guselkumab concentrations as the accuracy of 95% of the spiked samples was within the acceptable range (recoveries ranging from 81-117%).

3.2.3.2 Impact of IL-23 on guselkumab determination

Varying concentrations of IL-23 were spiked to 1 µg/mL guselkumab to determine the impact of IL-23 on guselkumab determination. The assay could quantify guselkumab (recovery of 80%) in the presence of up to 1 µg/mL IL-23 (= 2.74:1 molar ratio of IL-23 versus guselkumab, Fig. 3A). Once exceeding this IL-23 concentration, guselkumab could still be detected but not accurately quantified (recovery below 80%). Nevertheless, even in the presence of 2.5 µg/mL IL-23 (= 6.85:1 molar ratio of IL-23 versus guselkumab), the detection of guselkumab was not completely blocked.

3.2.3.3 Impact of anti-guselkumab antibodies on guselkumab determination

The influence of a two-fold excess of five MA-GUS with varying neutralizing capacities on the determination of 1 µg/mL guselkumab was evaluated. In the control samples with only guselkumab or with the addition of a non-relevant anti-ixekizumab antibody (MA-IXE66F11), the concentration of guselkumab was accurately detected (Fig. 3B). The addition of the non-neutralizing antibody (MA-GUS14D11, neutralizing capacity = 5%) did not significantly impair the quantification of guselkumab (recovery of 84%). In contrast, the addition of intermediate neutralizing antibodies (MA-GUS6G1, neutralizing capacity = 40% and MA-GUS18A4 neutralizing capacity = 38%) revealed less than 25% recovery of guselkumab, while the addition of neutralizing antibodies (MA-GUS12A9 and MA-GUS13A10, neutralizing capacity more than 65%) completely hampered guselkumab detection.

3.2.4 Assay cut-off values for detection and quantification, accuracy and imprecision

The cut-off values for detection and quantification were determined based on the OD of 15 guselkumab-naive serum samples of psoriasis patients. The mean OD was 0.061 with a standard deviation of 0.012, resulting in a LLOD of 0.073 ng/mL and LLOQ of 0.137 ng/mL. Taking into account the dilution factor of 1:100, the cut-off value for guselkumab detection and quantification was 0.007 µg/mL and 0.014 µg/mL, respectively.

The assay complied with the requirements for intra-assay accuracy and precision with recoveries ranging from 81% to 101% and CV of ≤12% and inter-assay accuracy and

precision with recoveries ranging from 89% to 107% and CV of ≤13% for all quality control samples tested. Furthermore, the total error did not exceed 17% and 25%, for the inter-assay and intra-assay evaluation, respectively.

3.2.5 Minimum required dilution and dilutional linearity

The minimum required dilution was determined to be 1:100. For samples with a guselkumab concentration between 0.5 µg/mL and 5 µg/mL, a dilution of 1:400 to 1:3200 was applied and concentrations >5 µg/mL were diluted 1:1600 to 1:12800. Quality control samples of 2 and 5 µg/mL guselkumab could accurately (recovery of 89% and 94%, respectively) and precisely (CV of 3% and 4%, respectively) be quantified showing that concentrations exceeding the range of quantification can be reliably measured by the guselkumab assay after proper dilution. Moreover, the precision between the measured values in a dilution series of each quality control sample was acceptable (CV <30%).

3.2.6 Validation of the guselkumab ELISA in a cohort of moderate-to-severe psoriasis patients

The ability of the newly developed assay to measure guselkumab concentrations in patient's serum was determined by the application of the guselkumab ELISA on serum samples of 10 moderate-to-severe psoriasis patients under standard guselkumab treatment (Figure 4). The guselkumab concentrations that were measured ranged from 0.46 to 10.36 µg/mL. Guselkumab could be detected during induction at weeks (w) 1, 2, 3 and 4 after the start of guselkumab treatment (w1, 2, 3: intermediate time points and w4: trough, i.e. the time point right before the next administration) and at weeks 12 and 20 (trough).

To evaluate accuracy of incurred samples, all patient samples were measured four times. For each sample, at least 67% of the repeats lied within 30% of its mean. Moreover, the precision between the measured values in a dilution series of all patient samples was acceptable (CV <30%) demonstrating parallelism between the calibration standard curve and serially diluted patient samples and excluding a possible matrix effect.

3.3 Development and validation of the anti-guselkumab antibody ELISAs

3.3.1 Development of the anti-guselkumab antibody assays

Two assays to measure anti-guselkumab antibodies were developed: an anti-guselkumab antibody bridging assay and an anti-guselkumab antibody ACE assay.

The suitability of different MA-GUS as calibrator antibody in the anti-guselkumab antibody bridging ELISA was evaluated. Based on the ability to cross-link plate-coated guselkumab with biotinylated guselkumab and the obtained dose-response curve, MA-GUS12A9 was selected as calibrator antibody for anti-guselkumab antibody quantification. Using GUS12A9 as a calibrator, a non-linear dose-response curve was obtained in the range of 0.16-10 ng/mL (Suppl. Fig. 2, panel A).

Additionally, an anti-guselkumab antibody ACE assay was developed that allows the detection of anti-guselkumab antibodies in the presence of drug. The same calibrator as in the bridging assay, GUS12A9, was used and yielded a non-linear dose-response curve ranging from 0.98 to 1000 ng/mL (Suppl. Fig. 2, panel B).

3.3.2 Specificity

The specificity of the anti-guselkumab antibody assays was determined by spiking secukinumab, a human anti-IL-17A monoclonal antibody frequently used in the treatment of psoriasis, and MA-GUS12A9 to drug-naive serum samples of psoriasis patients. In both the bridging and ACE assay, anti-guselkumab antibodies could accurately be measured, as the recovery of MA-GUS12A9 was 110-124% and 82-118%, respectively, demonstrating the specificity of the assays.

3.3.3 Selectivity

MA-GUS12A9 was spiked to guselkumab-naive serum samples of psoriasis patients to evaluate the impact of unrelated compounds in the matrix on anti-guselkumab antibody determination. In the bridging assay, the matrix in a dilution of 1/20 did not affect the quantification of anti-guselkumab antibodies as the accuracy of 90% of the spiked samples was within the acceptable range with recoveries ranging from 90-120%. In the ACE assay, the recovery of anti-guselkumab antibodies in spiked psoriasis serum was within 88-107% in 80% of the samples.

3.3.4 Assay cut-off values for detection and quantification, accuracy and imprecision

The cut-off values for detection and quantification of anti-guselkumab antibodies in the bridging and ACE assay were determined based on the OD of 25 guselkumab-naive serum samples of psoriasis patients. In the bridging assay, the mean OD was 0.06 with a standard deviation of 0.01, resulting in a LLOD of 0.186 ng/mL and LLOQ of 0.348 ng/mL. Taking into account the dilution factor of 1:20, the cut-off value for guselkumab detection and quantification was 3.7 ng/mL and 7.0 ng/mL MA-GUS12A9 equivalents, respectively. In the ACE assay, the mean OD was 0.075 with a standard deviation of 0.012, resulting in a LLOD of 25.56 ng/mL and LLOQ of 50.38 ng/mL. Taking into account the dilution factor of 1:17.78, the cut-off value for guselkumab detection and quantification were set at 454 ng/mL and 896 ng/mL MA-GUS12A9 equivalents, respectively.

The bridging assay complied with the requirements for intra-assay accuracy and precision with recoveries ranging from 81% to 119% (and CV of ≤12%) and inter-assay accuracy and precision with recoveries ranging from 101% to 103% (and CV of ≤17%) for all quality control samples tested. A similar conclusion can be drawn for the ACE assay as the intra-assay accuracy and precision was shown to be 83% to 118% (and CV of ≤19%) and the inter-assay accuracy and precision was 91% to 113% (and CV of ≤14%) for all quality control samples tested.

3.3.5 Evaluation of the drug tolerance

To evaluate the impact of guselkumab on the detection of anti-guselkumab antibodies, i.e. the drug tolerance, increasing concentrations of guselkumab were spiked to 4 µg/mL MA-GUS12A9 resulting in GUS:MA-GUS ratios ranging from 0.25:1 to 2:1. The addition of guselkumab reduced the detection of MA-GUS12A9 in a concentration-dependent manner (Fig. 5). The presence of 1 µg/mL guselkumab (= ratio 0.25:1) already decreased the recovery of MA-GUS12A9 to below 80% in both the bridging assay and the ACE assay. In the anti-guselkumab antibody bridging assay, MA-GUS12A9 became undetectable in the presence of 3 µg/mL guselkumab (= ratio 0.75:1), while an average recovery of 29% was

observed in the ACE assay with the same guselkumab concentration. Interestingly, in contrast to the bridging assay, MA-GUS12A9 remains detectable in the presence of up to 8 µg/mL guselkumab in the ACE assay, although in low concentrations.

4. Discussion

TDM of biologicals is an emerging tool to optimize treatment as drug concentrations have been linked to clinical outcomes in various chronic inflammatory diseases [9-11, 16-19]. Multiple studies indicate that monitoring drug concentrations together with a clinical evaluation, will not only maximize treatment outcomes but will also improve the cost-effectiveness of biological therapies [20, 21]. To date, TDM of guselkumab in patients with moderate-to-severe psoriasis cannot be performed in daily clinical practice because no commercial assays to measure guselkumab or anti-guselkumab antibody concentrations are available. In this study, we aimed to develop immunoassays for the quantification of guselkumab and anti-guselkumab antibody concentrations according to the guidelines of EMA.

A diverse panel of 20 highly specific anti-guselkumab monoclonal antibodies was generated and characterized for their neutralizing capacity and epitope binning profile. This allowed to carefully select the best antibody combination for the development of an assay to measure guselkumab. Using MA-GUS9F6 as the capture antibody and MA-GUS12G12 as the detection antibody, an ELISA was developed which accurately and precisely quantified guselkumab concentrations with a cut-off for quantification of 0.014 µg/mL. The mean (±SD) steady-state trough serum guselkumab concentrations in two phase III studies were reported to be 1.15±0.73µg/mL and 1.23±0.84 µg/mL [5]. As TDM is most often performed at trough, our in-house developed guselkumab assay can quantify guselkumab in a clinically relevant concentration range with a good sensitivity.

The guselkumab assay was shown to be selective as no matrix effect of serum of psoriasis patients was observed. Moreover, the presence of the drug's target, IL-23, did not influence the quantification of guselkumab until a concentration of 0.25 µg/mL, indicating that the presence of IL-23 at physiological concentrations (pg/mL) will not have an impact on the quantification of guselkumab [22]. In contrast, the presence of neutralizing anti-guselkumab monoclonal antibodies, but not non-neutralizing ones, severely hampered the detection of guselkumab. This can be explained by the fact that the coating antibody MA-GUS9F6 is also a neutralizing antibody and other neutralizing antibodies consequently compete for a similar binding site. Hampering of drug detection by neutralizing anti-drug antibodies has also been observed with other ELISAs that quantify biologicals [15, 23].

With the aim to perform a clinical validation, serum samples of 10 moderate-to-severe psoriasis patients collected at several time points during standard guselkumab treatment were applied on the guselkumab assay. The high sensitivity of the assay allowed to measure guselkumab concentrations during the induction and maintenance phase, both at intermediate time points (weeks 1, 2 and 3 after the start of treatment) and at trough (weeks 4, 12 and 20), indicating that this assay can be used to perform TDM in guselkumab-treated psoriasis patients. Moreover, the guselkumab concentrations observed at week 20 were comparable to the data reported in clinical trials [5]. Currently, clinical studies evaluating the efficacy and safety of guselkumab in the treatment of inflammatory bowel disease and psoriatic arthritis are ongoing [24-26]. If guselkumab is approved for these indications, the developed assays will also be very useful to evaluate the value of monitoring guselkumab and anti-guselkumab antibodies in these other chronic inflammatory diseases.

Anti-drug antibodies can have a significant impact on treatment response. Multiple studies have shown that anti-infliximab and anti-adalimumab antibodies are associated with lower serum drug concentrations and loss of response in psoriasis patients [27-29]. The clinical relevance of anti-guselkumab antibodies is not well established because of the lack of appropriate assays. Therefore, two assays were developed to detect anti-guselkumab antibodies, i.e. a bridging assay and an ACE assay. The specificity and selectivity of both anti-guselkumab antibody assays was demonstrated.

Both anti-guselkumab antibody assays use the same calibrator, MA-GUS12A9, to detect anti-guselkumab antibodies but differ in the threshold for anti-guselkumab antibody detection and quantification and the tolerance to the presence of guselkumab. In the bridging assay, the cut-off value for detection and quantification was determined to be 3.7 ng/mL and 7 ng/mL respectively. In contrast, the cut-off values of the ACE assay are 454 ng/mL and 896 ng/mL for detection and quantification, respectively. These are considerably higher compared to the bridging assay, which can be explained by the fact that due to the different acidification steps, the sample is automatically diluted to a certain extent. On the other hand, the acid dissociation steps allow the detection of anti-drug antibodies that were previously in complex with the drug. As a result, the ACE assay can still detect antibodies in the presence of up to 8 µg/mL guselkumab, whereas the bridging assay cannot detect anti-guselkumab antibodies from a guselkumab concentration of 3 µg/mL onwards. These assays will allow to further elucidate the clinical relevance of anti-guselkumab-antibodies, either free or in complex with the drug.

Together, the guselkumab and anti-guselkumab antibody assays will allow to evaluate the presence of a concentration-response relationship and establish a therapeutic window in patients receiving guselkumab treatment and may support the use of TDM in guselkumab-treated patients.

5. Conclusion

In conclusion, 20 highly specific anti-guselkumab monoclonal antibodies were generated, characterized and pairwise tested for the development of an ELISA for the quantification of guselkumab concentrations in serum of patients with moderate-to-severe psoriasis. Additionally, an anti-guselkumab antibody bridging and ACE assay were developed for the detection of anti-guselkumab antibodies, either free or in complex with guselkumab. All three assays were validated according to the EMA guidelines. These validated immunoassays are essential to establish a concentration-response relationship and will allow the future implementation of TDM in moderate-to-severe psoriasis patients receiving guselkumab.

Author statement

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Declaration of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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Figure captions

Figure 1: Flowchart of the selection of 20 guselkumab-specific MA-GUS

Figure 2: Principal component analysis and clustering of the 20 MA-GUS

Figure 3: Effect of IL-23 (panel A) and different antibodies (panel B) on the determination of guselkumab (1 µg/mL) in the ELISA using MA-GUS9F6 as the capture antibody and MA-GUS12G12 as the detection antibody (mean ± SD). Both graphs are representative of 2 independent experiments with 2 replicates each.

Figure 4: The average guselkumab serum concentration (mean ± SD) of 10 moderate-to-severe psoriasis patients at different time points after the start of guselkumab treatment. The patients received 100 mg guselkumab subcutaneously at weeks 0, 4, 12 and 20 according to the standard dosing regimen. The graph shows the results of 2 independent experiments with 2 replicates each.

Figure 5: The drug tolerance of the anti-guselkumab antibody bridging assay (black dots) and anti-guselkumab antibody ACE assay (white dots) was evaluated by incubating increasing concentrations of guselkumab with 4 µg/mL MA-GUS12A9. The percentage recovery of MA-GUS12A9 in the presence of guselkumab was expressed relative to the percentage recovery of MA-GUS12A9 in the absence of guselkumab, which was set at 100%. The graph shows the results of 2 independent experiments with 2 replicates each.
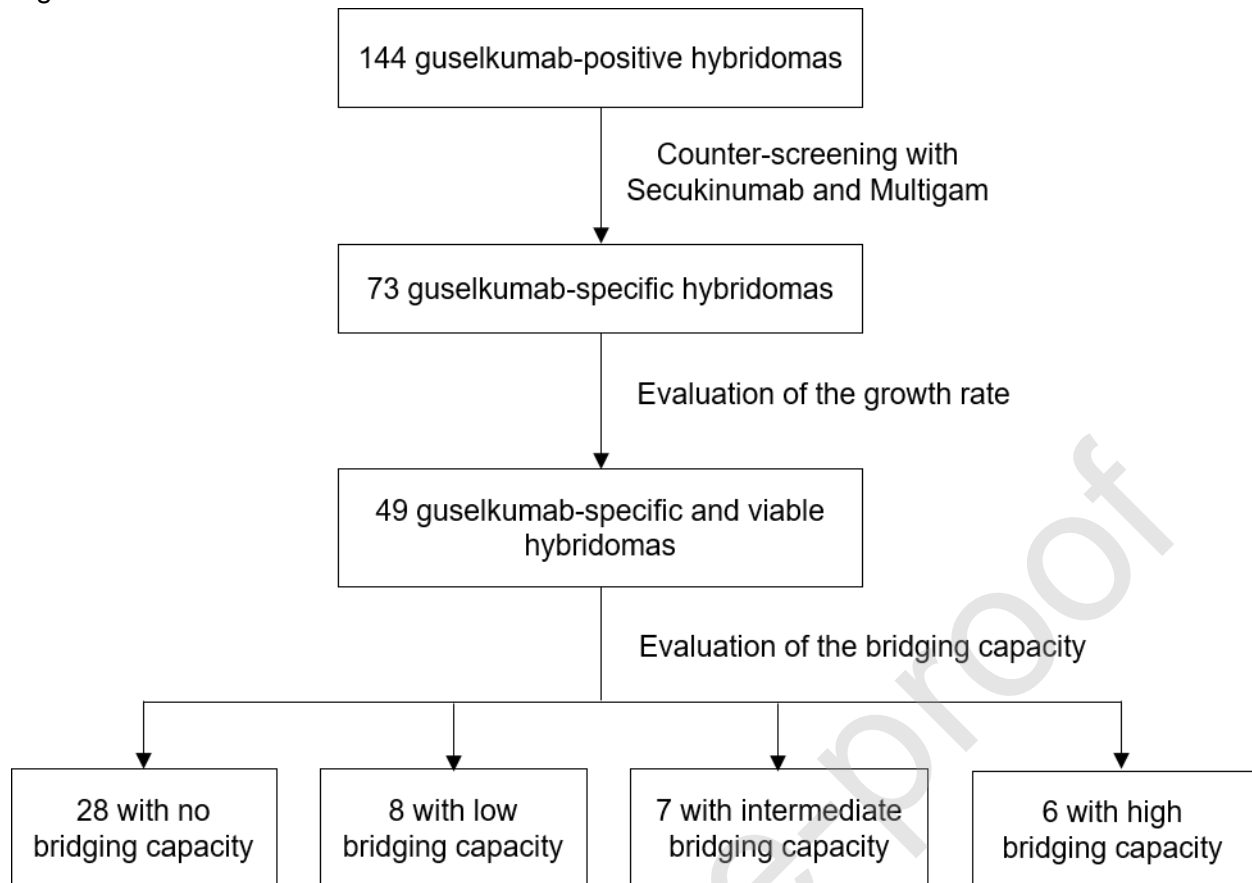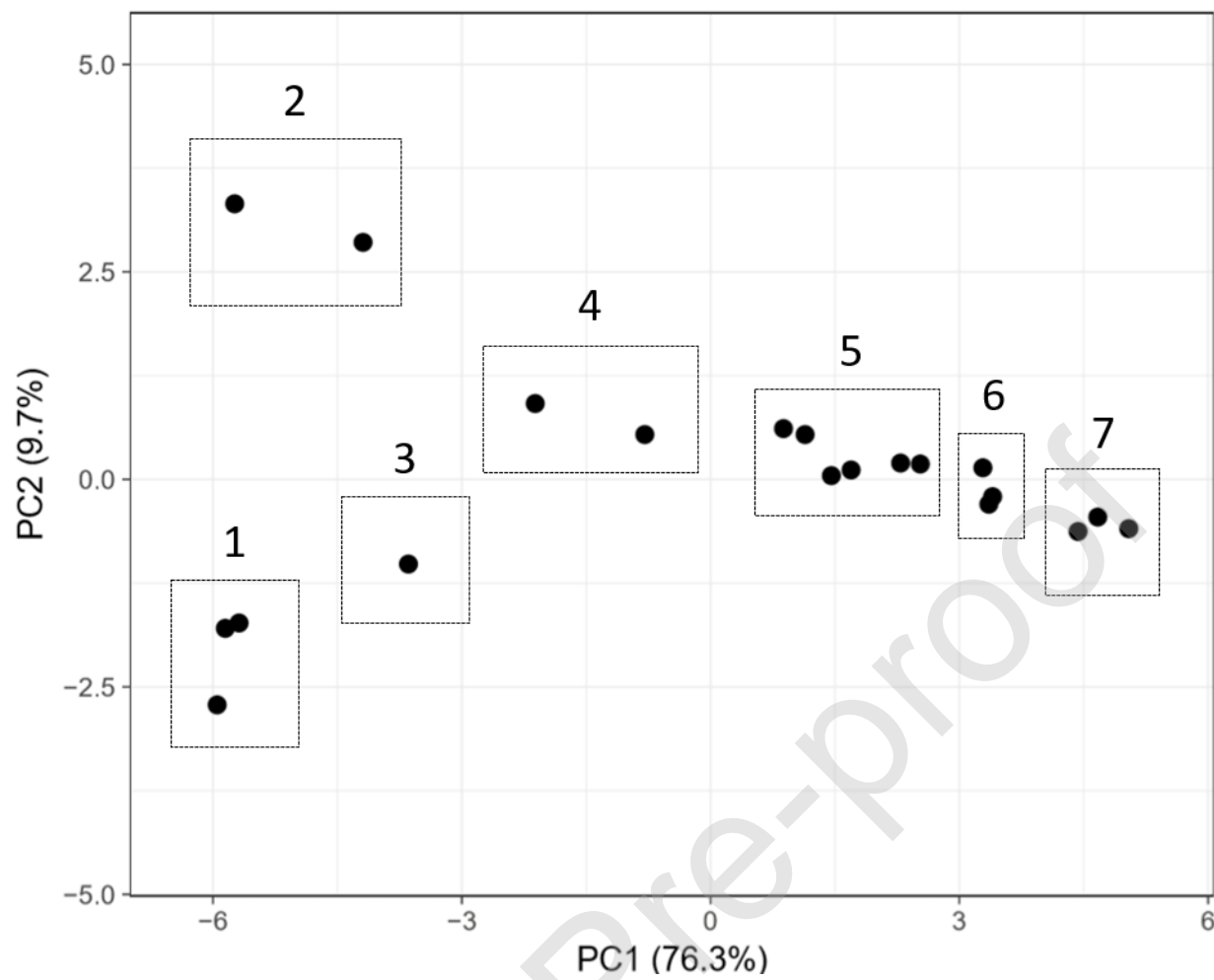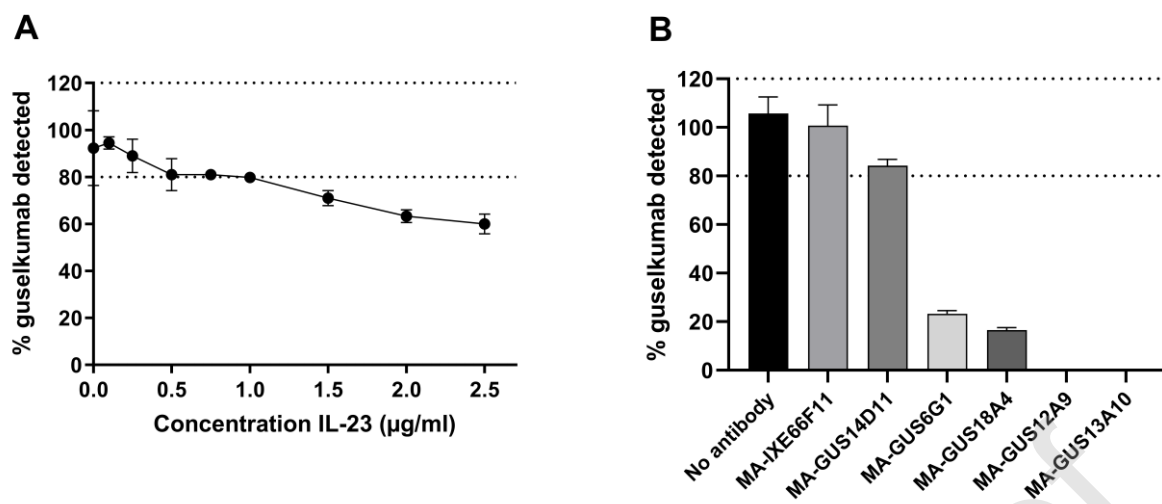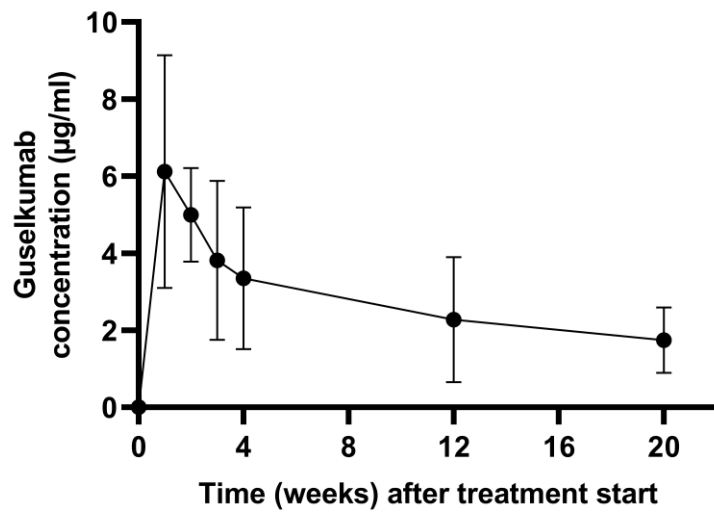
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Fig 1


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                    ┌─────────────────────────────────────┐
                    │ 144 guselkumab-positive hybridomas  │
                    └─────────────────────────────────────┘
                                     │
                                     │  Counter-screening with
                                     │  Secukinumab and Multigam
                                     ▼
                    ┌─────────────────────────────────────┐
                    │ 73 guselkumab-specific hybridomas   │
                    └─────────────────────────────────────┘
                                     │
                                     │  Evaluation of the growth rate
                                     ▼
                    ┌─────────────────────────────────────┐
                    │ 49 guselkumab-specific and viable   │
                    │              hybridomas             │
                    └─────────────────────────────────────┘
                                     │
                                     │  Evaluation of the bridging capacity
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28 with no bridging capacity	8 with low bridging capacity	7 with intermediate bridging capacity	6 with high bridging capacity

Fig 2

Fig 3

A

B

Fig 4

Fig 5