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# GMP-Compliant Production of a Fluorescent Antibody for *in vivo* Molecular Endoscopy in a Phase I/IIa Clinical Study in Inflammatory Bowel Disease Patients

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## Abstract

Therapeutic response to anti-TNF antibody treatment in inflammatory bowel diseases is strongly influenced by transmembrane tumor necrosis factor (mTNF) expression in the intestinal mucosa. Fluorescent anti-TNF antibodies applied for in vivo molecular endoscopy have shown to be able to quantify mucosal mTNF expression in real-time and predict response to subsequent anti-TNF treatment in individual patients with Crohn's disease. We herein describe a straightforward approach to generate a fluorescein-labeled anti-TNF antibody (FITC-adalimumab) as a valuable molecular tool for in vivo imaging via molecular endoscopy to depict mTNF expressing intestinal cells. The GMP-compliant production of FITC-adalimumab served as a key prerequisite in order to gain a more precise insight in the therapeutic options and hence improved pharmacotherapy for patients with IBD. A total of 30 GMP-compliant batches of FITC-adalimumab concentrate were produced and applied as investigational medicinal product (IMP) in a phase IIa clinical study to predict response to adalimumab in ulcerative colitis patients. The methodology described can also be widely applied to generate variable fluorescent probes in other immune-mediated inflammatory diseases to address clinical problems associated with antibody therapies and to facilitate personalized medicine.

**Keywords:** fluorescent antibody; adalimumab; GMP-compliant production; inflammatory bowel diseases; in vivo; molecular endoscopy

**List of Abbreviations:**

AMG: Arzneimittelgesetz; CHO: chinese hamster ovary; CLE: confocal laser endomicroscopy; FACS: fluorescence activated cell sorting; FITC: Fluoresceinisothiocyanate; GMP: good manufacturing practice; HPLC: High performance liquid chromatography; IBD: inflammatory bowel disease; IMP: investigational medicinal product; mTNF: transmembrane tumor necrosis factor; PBS: phosphate-buffered solution; TNF: tumor necrosis factor; TRIS: Tris(hydroxymethyl)aminomethan

## Introduction

Patients with inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis, suffer from relapsing inflammations of the intestinal mucosa. Both IBD subtypes are progressive conditions that can lead to gut damage and disability, having a major impact on an individual's quality of life and their ability to work. Clinical symptoms include rectal bleeding, chronic diarrhea, abdominal pain and fatigue. Ongoing inflammatory activity is also causative for occurrence of strictures, fistula, abscesses and heightened incidence of colitis-associated neoplasia, often requiring surgical intervention [1, 2]. There is therefore the heightened need for optimized anti-inflammatory therapy in affected patients. Growing insights into underlying immunopathogenic mechanisms have led to the advent of targeted therapies, which selectively inhibit crucial mediators of the inflammatory process.

Genetic and environmental factors have a major role in the complex and multifactorial pathogenesis of ulcerative colitis. A combination of these risk factors seems to initiate alterations in epithelial barrier function, thereby allowing the heightened translocation of luminal bacterial antigens into the bowel wall [3]. Subsequently, aberrant, and uncontrolled activation of the mucosal immune system, marked by excessive cytokine responses cause acute mucosal inflammation. Pro-inflammatory cytokines, produced by mucosal immune cells have emerged as promising targets for IBD therapy, as they play a crucial role in controlling intestinal inflammation and the associated clinical symptoms of IBD [4, 5]. Here, numerous studies have suggested that the proinflammatory cytokine tumor necrosis factor alpha (TNF-α) is one of the major pathogenic cytokines involved in the pathogenesis of IBD [6]. Increased numbers of TNF producing mucosal cells could be found in IBD patients with active inflammation [7, 8] and produced amounts of TNF correlated with severity of intestinal inflammation [9]. The relevance of TNF is also reflected by the fact, that TNF-neutralizing antibodies such as infliximab, certolizumab and adalimumab have been approved for induction and maintenance therapy in IBD [10]. However, approximately one third of treated patients exhibit insufficient response to anti-TNF inhibitor therapy (primary non-response). Available data indicate that primary and 30–50% of initial responders are prone to lose response to ongoing anti-TNF treatment (secondary non-response) [11, 12]. The optimized clinical use of these agents in IBD remains unclear, as suitable predictive biomarkers for response are lacking. This results in the persistence of clinical symptoms due to lack of efficacy under anti-TNF therapy but at the same time, patients are exposed to potential treatment-related side effects. These include allergic reactions, skin disorders and heightened risk for opportunistic infections, thus potentially worsening the patient's overall condition [13, 14]. Moreover, ineffective use of anti-TNF antibodies is also associated with substantial costs for the health care systems. It is therefore crucial to consider in advance, whether an anti-TNF therapy is beneficial for a patient in order to improve patient's outcomes, enhance safety and cost-effectiveness and therefore utilization of this treatment.

However, therapeutic response is highly challenging to predict. The failure of anti-TNF therapy can either be due to well described pharmacokinetic mechanisms, such as inadequate serum or tissue concentrations due to immunogenicity [15], or pharmacodynamic mechanisms that are poorly characterized [16]. However, there are currently no highly sensitive and specific biomarkers available which could be employed in routine diagnostic for an estimation of therapeutic efficacy in advance [17]. There is therefore a major unmet clinical need for predictive markers of response in order to allow personalized medicine in individual patients with higher response rates to available targeted therapies. Induction of intestinal T cell apoptosis has been suggested as a central molecular mechanism of action of anti-TNF agents in IBD [18]. Here, mucosal expression of mTNF has

been linked to the mode of action of anti-TNF antibody therapy in IBD. Evidence suggests that anti-TNF agents bind to immune cells expressing membrane-bound TNF (mTNF), thereby inducing apoptosis in intestinal T cells which results in the amelioration of mucosal inflammation. This concept proposes an indirect mucosal T cell apoptosis induction by neutralizing the mTNF binding side for TNFR2 by anti TNF antibody treatment, resulting in apoptosis of pathogenic TNFR2 expressing T cells [19]. Therefore, a molecular probe visualizing mTNF expression levels in the intestinal tissue might represent a valuable tool to predict therapeutic response in regard to anti-TNF therapy in IBD patients. Based on these findings the anti-TNF agent adalimumab was labeled with the fluorescein derivate fluorescein isothiocyanate (FITC) under GMP criteria. The fluorescent-labeled anti-TNF antibody was then sprayed on the surface of the most inflamed area of the mucosa in 25 Crohn's disease patients with moderate-to-severe disease activity during ongoing endoscopy and mTNF expressing mucosal cells were visualized in real-time via confocal laser endomicroscopy (CLE). Confocal laser endomicroscopy allows to capture high-resolution images not only of morphologic changes in the mucosa but also of single molecules of interest *in vivo* [20]. Topical antibody administration of the fluorescent anti-TNF agent led to specific signal detection of intestinal mTNF+ immune cells in vivo during CLE. It could be shown that patients with higher mucosal TNF expression had a much higher probability of responding to subsequent anti-TNF therapy than patients with lower mTNF expression levels. Crohn's disease patients with high numbers of mTNF+ cells showed statistically significantly ($p= 0.0002$) higher clinical response rates (92%, 11 out of 12 patients) to subsequent anti- TNF therapy with adalimumab at week 12, compared with patients with low numbers of tissue mTNF+ cells (15%, 2 out of 13 patients). Furthermore, the clinical response was sustained over a follow- up period of 1 year and was associated with mucosal healing [21]. Findings of this study suggest that in vivo molecular endoscopic imaging with fluorescent antibodies directed against specific molecules could be used to predict responsiveness to subsequent biological therapy. As fluorescent probes for *in vivo* use in humans fall under the German Drug Law, they have to be manufactured in compliance with GMP guidelines. We developed a protocol for GMP-compliant manufacturing of a FITC-adalimumab, which was successfully applied as an IMP in a first-in-human phase IIa clinical study to predict response to therapy with the anti-TNF antibody adalimumab in patients with active ulcerative colitis.

## Materials and Methods

### Production of FITC-adalimumab concentrate

Fluorescein-labeled adalimumab concentrate was prepared in accordance to GMP regulations in the production premises of the Pharmacy Department according to Paragraph 13 of the German Pharmaceuticals Act (§13 AMG). The materials and equipment summarized in Table 1 were applied. A stability study at 2-8 °C was performed and FITC-adalimumab concentrate was found to be in specification for all time points (t0, t28d, t56d), hence resulting in a shelf life of 56 days.

| Material | Supplier | Use |
|---|---|---|
| 1 ml tuberculin syringes Injekt-F Solo | B. Braun Melsungen AG, Melsungen, Germany | Primary packaging |
| Boric acid, Ph. Eur. | Caesar & Loretz GmbH, Hilden, Germany | Buffer preparation |
| Combi-stopper | B. Braun Melsungen AG, Melsungen, Germany | Primary packaging |
| Eppendorf cups type 3810X, 1.5 ml | VWR International GmbH, Darmstadt, Germany | Reaction vessel |
| Glass bottle (amber, 100 ml), Ph. Eur. | Iphas Pharma-Verpackung GmbH, Würselen, Germany (article no. 07050) | Buffer storage |
| Humira® 40mg/0.4ml | AbbVie Deutschland GmbH & Co. KG, Ludwigshafen, Germany | Starting material |
| light-protection bag, PE, green | MacoPharma, Langen, Germany | Secondary packaging |
| NaOH 1 N, Ph. Eur. | Merck Chemicals GmbH, Darmstadt, Germany | Buffer preparation |

| | | |
|---|---|---|
| Pierce® FITC Antibody Labeling Kit | Thermo Fisher Scientific, Rockford, IL, USA (article no. 53027) | Labeling reagent + purification of product |
| Screw cap, Ph. Eur. | Zscheile&Klinger GmbH, Hamburg, Germany | Buffer storage |
| Water for injection, Ph. Eur. | in-house distillation plant, Pharmacy Department of Erlangen University Hospital, Germany | Buffer preparation |
| Sterican canula 18G | B. Braun Melsungen AG, Melsungen, Germany | Product collection |
| Pipette tips 100µl | Biotix, San Diego, CA, USA | Dosing |
| Pipette tips 1250µl | Biotix, San Diego, CA, USA | Dosing |
| **Equipment** | **Supplier** | **Use** |
| pH Meter: SevenEasy | Mettler Toledo, Columbus, OH, USA | pH adjustment (buffer preparation) |
| Centrifuge 5424 | Eppendorf AG, Hamburg, Germany | Quantitative collection of reaction mixture |
| Water bath Memmert Typ WB7 | Memmert GmbH + Co. KG, Schwabach, Germany | Incubation |
| Eppendorf pipette 10-100µl | Eppendorf AG, Hamburg, Germany | Dosing |
| Eppendorf pipette 100-1000µl | Eppendorf AG, Hamburg, Germany | Dosing |

**Table 1:** Materials and equipment used for production of FITC-adalimumab.

## Preparation of 50 mM Borate Buffer, pH 8.5

0.31 g of boric acid Ph. Eur. was dissolved in 100 ml water for injection. pH was adjusted to 8.5 ± 0.1 using 1 N sodium hydroxide solution and a SevenEasy pH Meter. The buffer was stored in an amber glass bottle, sealed with a cap at 2-8 °C and used within 7 days.

## Coupling and Purification of Fluorescein Labeled Antibody

FITC-adalimumab was prepared using the approved drug Humira® 40mg/0.4ml and the Pierce® FITC antibody labeling kit. Coupling was performed according to manufacturer's instruction. In brief, adalimumab was diluted in 50 mM borate buffer to achieve a concentration of 50 µg/µl. 50 µg of fluorescein isothiocyanate were dissolved in 475 µl of 50 mM borate buffer, mixed with 25 µl of adalimumab solution and incubated at 25 °C ± 2 °C for 60 min, protected from light. The labeled protein was purified using the purification resin and spin columns contained in the kit. The product was stored at 2-8 °C and protected from light in 1 ml tuberculin syringes Injekt-F Solo, closed with a combi-stopper.

**Characterization of FITC-Adalimumab**

FITC-adalimumab was characterized applying the methods and specifications listed in Table 2. Analytical methods are described in the following subsections.

## Physicochemical Analysis of FITC-Adalimumab

## UV-Vis Spectroscopy

A method validation was performed in terms of precision, specificity, robustness and a system suitability test. Measurements were performed using phosphate-buffered solution (PBS) as diluent. PBS was prepared by dissolving 4.0 g sodium chloride (Caesar & Loretz GmbH, Hilden, Germany), 0.1 g potassium chloride (Merck KgaA, Darmstadt, Germany), 0.72 g di-sodium hydrogen phosphate (Merck KgaA, Darmstadt, Germany) and 0.12 g potassium dihydrogen phosphate (Merck KgaA, Darmstadt, Germany) in 495.14 g water for injection and was used within 3 months. After blank correction with PBS, UV absorption of the sample was measured at 495 nm and 280 nm. The sample was obtained by diluting 12 µl of FITC-adalimumab solution in 60 µl PBS in a quartz sub-micro cell cuvette. To ensure uniform mixing, it was pipetted up and down 10 times while moving the pipette tip in the solution, followed by right-left motion of the pipette tip for 15 times and repetition of the whole process for 2 more times. Three individual measurements were performed.

Protein concentration and coupling degree were determined using the following equations[†]:

$$Protein\ concentration(M) = \frac{A_{280} - (A_{max} \times CF)}{\varepsilon_{protein}} \times dilution\ factor$$

$$CF = corrective\ factor = \frac{A_{280}}{A_{max}} = 0.3$$

$$dilution\ factor = 6$$

$$\varepsilon_{Protein} = molar\ extinction\ coefficient\ of\ IgG \approx 210.000 M^{-1} cm^{-1}$$

$$Protein\ concentration[\mu g/\mu l] = \frac{Protein\ concentration(M) \times 1,48}{10}$$

$$Coupling\ degree = \frac{A_{max}}{\varepsilon_{FITC} \times Protein\ concentration(M)} \times dilution\ factor$$

$$\varepsilon_{FITC} = molar\ extinction\ coefficient\ of\ FITC \approx 70.000 M^{-1} cm^{-1}$$

## Size Exclusion Chromatography

Size exclusion chromatography was performed using the device specified in Table 2 and the parameters described in Table 3. Validation of the method included the following aspects: ideal dilution of sample, content of free FITC, identity, precision, specificity, linearity, robustness and a system suitability test.

[†] See also: Manual for Pierce® FITC antibody labeling Kit, Thermo Fisher Scientific, 2014

FITC-adalimumab concentrate was injected 3 times undiluted and 3 times diluted to 0.05 mg/ml in 50 mM borate buffer (prepared as described in above). A system suitability test was carried out by injecting undiluted Humira® 6 times with the passing criteria being that the relative standard deviation of peak area in both detectors must not be more than 1%. Content of free FITC was determined after analysis of peak areas of FITC (undiluted sample of FITC-adalimumab concentrate) and FITC-adalimumab (diluted sample of 0.05 mg/ml), respectively, detected in the fluorescence detector ($\lambda_{ex}$ = 495 nm, $\lambda_{em}$ = 518 nm) and calculated using the following equation:

$$Content\ of\ free\ FITC[\%] = \frac{area(FITC) \times 0.05mg/ml}{area(FITC-adalimumab) \times 2.5mg/ml} \times 100$$

| Parameter | Method/Device | Specification |
|---|---|---|
| appearance of solution | visually by the unaided eye | clear, yellow to green, free from visible particles |
| coupling degree | UV-Vis Spectrometer: Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA. Software: Insight 2 Software, Thermo Fisher Scientific, Waltham, MA, USA. Quartz sub-micro cell 50 µl cuvette: Thermo Electron, Madison, WI, USA (article no. 268-809900) | 1.5 – 3.0 |
| protein concentration | UV-Vis Spectrometer: Evolution 201, Thermo Fisher Scientific, Waltham, MA, USA. Software: Insight 2 Software, Thermo Fisher Scientific, Waltham, MA, USA. Quartz sub-micro cell 50 µl cuvette: Thermo Electron, Madison, WI, USA (article no. 268-809900) | 1.0 – 2.5 µg/µl |
| content of free FITC | Size exclusion chromatography: Ultimate 3000 Dionex HPLC system, Thermo Fisher Scientific. Software: Thermo Scientific Chromeleon 7.2 SR4. Column: Phenomenex Yarra, 3 µ, 300 x 7.8 mm with precolumn Phenomex HPLC Security Guard | <1% |
| identity | Size exclusion chromatography: Ultimate 3000 Dionex HPLC system, Thermo Fisher Scientific. Software: Thermo Scientific Chromeleon 7.2 SR4. Column: Phenomenex Yarra, 3 µ, 300 x 7.8 mm with precolumn Phenomex HPLC Security Guard | retention time of FITC-adalimumab complies to retention time of Humira (acceptance criterium ± 1%) |
| biological affinity | Flow cytometry: BD FACSVerse, BD Biosciences Heidelberg, Germany. Software: BD FACSuite Version 1.0.6 | 2.5 – 7.5% TNF positive CHO-cells |

**Table 2:** Specifications for physical and chemical analysis

| | |
|---|---|
| Flow agent | TRIS buffer *, isocratic |
| Flow rate | 0.5 ml/min |
| Injection volume | 5 µl |
| Column | Phenomenex: Yarra, 3 µ; 300 x 7.8 mm with Phenomenex HPLC SecurityGuard pre-column |
| Column temperature | 22 °C |
| Detection | Diode Array Detector: 280 nmFluorescence Detector: $\lambda_{ex}$ = 495 nm, $\lambda_{em}$ = 518 nm |
| Run time | 35 min |

*TRIS buffer was freshly prepared before SEC analysis: A TRIS buffer concentrate (10x) was diluted 1:10 with aqua destillata and filtrated using a membrane filter (0.45 µm, Sartorius AG, Goettingen, Germany, article number 18406-50-N). The 10x concentrate was prepared by dissolving 30.29 g 2-amino-2(hydroxymethyl)-1,3-propanediol (Merck KgaA, Darmstadt, Germany) and 58.44 g sodium chloride (Caesar and Lorentz GmbH, Hilden, Germany) in 411.27 g water. pH was adjusted to 7.0 using 1N hydrochloric acid (Merck KgaA, Darmstadt, Germany) and the concentrate was used within 6 months.

**Table 3:** Parameters used for size exclusion chromatography

## Affinity Analysis of FITC-Adalimumab

Biological affinity of FITC-adalimumab was determined by fluorescence activated cell sorting (FACS), using materials and equipment summarized in Table 4 and Table 5. Validation of the method covered ideal dilution of the sample, specificity, precision/repeatability, robustness, precision/intermediate precision and a system suitability test.

## Preparation of FITC-IgG1 Concentrate

Human IgG1 Isotype control (Enzo Life Sciences GmbH, Loerrach, Germany) was labeled with FITC using the Pierce® antibody labeling kit (Thermo Fisher Scientific, Rockford, IL, USA, article no. 53027). Coupling was performed according to manufacturer's instruction. In brief IgG1 was diluted in 50 mM borate buffer to achieve a concentration of 2 µg/µl. 50 µl of the resulting solution were used to dissolve 50 µg of fluorescein isothiocyanate and the solution was incubated at 25 °C ± 2 °C for 60 min, protected from light. The labeled protein was purified using the purification resin and spin columns contained in the kit. FITC-IgG1 concentrate was stored at -80 °C in aliquots à 10 µl and used within 1 year.

## FACS Buffer Preparation

FACS buffer was prepared by mixing 0.5 ml fetal calf serum and 99.5 ml Dulbeccos PBS. The buffer was stored at 2-8 °C and used within 28 days.

## FACS Measurement

CHO cell aliquots (approx. 1.5 x $10^6$ cells per aliquot) of wild type CHO cells and mTNF CHO cells, respectively, were thawed at 37 °C and washed using FACS buffer (3x 50 ml, 10 min centrifugation at 300 G, with an additional filtration step using Falcon® Cell Strainer 40 µm prior to the second resp. third centrifugation step). Cells were suspended in 200 µl FACS buffer and equally distributed in 3 tubes (100 µl each), resulting in a cell number of approx. 0.5 x $10^6$ cells per tube. To each tube, 1 µl of FC Receptor Block was added and the mixture was then put on ice for 10 minutes. Staining of cells was performed by addition of 2.5 µg FITC-adalimumab to the first tube and addition of 2.5 µg FITC-IgG1 to the second tube, while applying neither FITC-adalimumab nor

FITC-IgG1 to the third tube to serve as a negative control. Subsequently, each tube was incubated at ambient temperature and protected from light for 15 min. Finally, cells were washed with FACS buffer (2 ml, centrifugation at 300 G, 10 min) and resuspended in 500 µl FACS buffer. Samples were stored protected from light until analysis with BD FACSVerse. Analysis was performed using approximately 50.000 cells per tube.

| Material | Supplier | Use |
|---|---|---|
| BD FACSFlow™ | BD Bioscience, Franklin Lakes, NJ, USA | Flow agent |
| CHO cells, wild type | Leibniz Institute DSMZ GmbH, Braunschweig, Germany | negative control |
| CHO cells with stable TNF α expression | BSRC Alexander Fleming, Athens, Greece | affinity determination |
| Dulbeccos PBS | Life Technologies, Carlsbad, CA, USA | FACS buffer preparation |
| Falcon® Cell Strainer 40 µm | Fa. Corning, Corning, NY, USA | Sample preparation |
| Falcon® tubes 50 ml, sterile | Greiner Bio-One, Kremsmünster, Austria | Sample preparation |
| FC Receptor Block | eBioScience GmbH, Frankfurt, Germany | Sample preparation |
| Fetal Calf Serum | Sigma-Aldrich, St. Louis, MO, USA | FACS buffer preparation |
| Round bottom polystyrene tube | Fa. Corning, Corning, NY, USA | Sample preparation |
| Serologic pipette tips | Greiner Bio-One, Kremsmünster, Austria | Sample preparation |

**Table 4:** Materials used for affinity analysis of FITC-adalimumab

| Equipment | Supplier | Use |
|---|---|---|
| Water bath | Greiner Bio-One, Kremsmünster, Austria | Thawing of cell aliquots |
| Pipetting aid | Eppendorf AG, Hamburg, Germany | Sample preparation |
| Eppendorf Research Plus Pipette 100µl | Eppendorf AG, Hamburg, Germany | Sample preparation |
| Eppendorf Research Plus Pipette 1000µl | Corning, NY, USA | Sample preparation |
| BD FACSVerse | BD Biosciences Heidelberg, Germany | Measuring device |

**Table 5:** Equipment used for affinity analysis of FITC-adalimumab

## Results

With a manufacturing authorization in accordance with §13 AMG we successfully produced FITC-adalimumab concentrate as a novel investigational medicinal product (IMP) in compliance to GMP. Manufacture according to the provisions of Good Manufacturing Practice is a key factor for the conduction of clinical studies involving novel IMPs. FITC-adalimumab concentrate was approved by the national competent authority (Paul-Ehrlich-Institut, Federal Institute for Vaccines and Biomedicines, No. 3179/01, EudraCT-No. 2014-001594-14) to be applied in a phase I/IIa clinical study. The study was also approved by the Ethics Committee, Medical Faculty Friedrich-Alexander-University Erlangen-Nuremberg, application number ”362 17 AZ”. A total of 30 batches of FITC-adalimumab concentrate were manufactured and successively used in the ongoing study. After dilution of the concentrate to a final concentration of 25 µg/ml, FITC-adalimumab was sprayed on the colon mucosa with heaviest signs of inflammation during endoscopy in ulcerative colitis patients with moderate-to-severe disease activity. The application of this fluorescent probe for *in vivo* imaging generated valuable insights on therapeutic options in ulcerative colitis.

## Discussion

Therapeutic response in inflammatory bowel diseases, e.g. Crohn's disease and ulcerative colitis, is strongly influenced by the expression of transmembrane tumor necrosis factor in the gut [16]. We herein describe a straightforward approach to generate a valuable molecular tool for *in vivo* molecular endoscopy imaging. This was achieved using FITC as an easily accessible reactive fluorescein derivative and the approved anti-inflammatory drug adalimumab. Due to its favorable toxicity profile, fluorescein is a widely used fluorophore for human *in vivo* studies [20, 22, 23].

The GMP-compliant production of FITC-adalimumab concentrate served as a key prerequisite in order to gain a more precise insight in the therapeutic options and hence improval of pharmacotherapy for patients with inflammatory bowel diseases. Application of the high standards required for GMP-compliant IMP-production in combination with approval of the study project by the competent authorities results in a high level of product safety. The methodology described can also be widely applied to generate variable fluorescent probes in order to address other questions associated with antibody therapies.

## Conclusion

Herein we describe a method for a GMP-compliant production of a fluorescent antibody which was used in a diagnostic tool. This straightforward method can be applied to easily access other fluorescent antibodies or peptide-based drugs and is therefore an excellent tool to elucidate further diagnostic questions.

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## Author Contributions

Conceptualization, R.A. and M.N.; methodology, R.H-B. and T.B.; validation, T.B., R.H.-B., H.S. and J.S.; formal analysis, T.B., J.S., R.H-B. and H.S.; investigation, I.K., T.B., M.B. and H.S.; resources, R.A., F.D. and M.N.; data curation, R.A.; writing—original draft preparation, I.K.; writing—review and editing, T.B.; visualization, R.A.; supervision, F.D. and M.N.; project administration F.D. and M.N..; funding acquisition, R.A., F.D. and M.N. All authors have read and agreed to the published version of the manuscript.

## Potential Conflicts of Interest

R.A. has served as a speaker, or consultant, or received research grants from AbbVie, Amgen, Arena Pharmaceuticals, Biogen, Boehringer Ingelheim, Bristol-Myers Squibb, Cellgene, Celltrion Healthcare, DrFalk Pharma, Galapagos, Gilead, InDex Pharmaceuticals, Janssen-Cilag, Lilly, MSD Sharp & Dohme, Novartis, Pandion Therapeutics, Pfizer, Roche Pharma, Samsung Bioepsis, Takeda Pharma, Viatris.

F.D. has received consulting fees from E. Lilly, Pfizer, Boehringer Ingelheim, Sandoz and honoraria for lectures from Sanofi, B.Braun Melsungen and E. Lilly. FD was president of the Federal Association of German Hospital Pharmacists (2018-2020).

## References

1. Kobayashi T, Siegmund B, Le Berre C, Wie SC, Ferrante M, et al. (2020) Ulcerative colitis. Nat Rev Dis Primers 6: 74.

2. Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L (2017) Crohn's disease. Lancet 389: 1741-55.

3. Atreya R, Neurath MF (2015) IBD pathogenesis in 2014: Molecular pathways controlling barrier function in IBD. Nat Rev Gastroenterol Hepatol 12: 67-8.

4. Atreya R, Neurath MF (2008) New therapeutic strategies for treatment of inflammatory bowel disease. Mucosal Immunol 1: 175-82.

5. Billmeier U, Dieterich W, Neurath MF, Atreya R (2016) Molecular mechanism of action of anti-tumor necrosis factor antibodies in inflammatory bowel diseases. World J Gastroenterol 22: 9300-13.

6. Gareb B, Otten AT, Frijlink HW, Dijkstra G, Kosterink JGW (2020) Review: Local Tumor Necrosis Factor-α Inhibition in Inflammatory Bowel Disease. Pharmaceutics 12: 539.

7. MacDonald TT, Hutchings P, Choy MY, Murch S, Cooke A (1990) Tumour necrosis factor-alpha and interferon-gamma production measured at the single cell level in normal and inflamed human intestine. Clin Exp Immunol 81: 301-5.

8. Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB et al. (1994) Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 106: 1455-66.

9. Reinecker HC, Steffen M, Witthoeft T, Pflueger I, Schreiber S et al. (1993) Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1 beta by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clin Exp Immunol 94: 174-81.

10. Rehman M, Cancarevic I, Iskander B, Lalani S, Malik BH (2020) Biologics Targeting in the Treatment of Inflammatory Bowel Disease: A Conundrum. Cureus 12: e10621.

11. Atreya R, Neurath MF, Siegmund B (2020) Personalizing Treatment in IBD: Hype or Reality in 2020? Can We Predict Response to Anti-TNF? Front Med (Lausanne) 7: 517.

12. Hanauer SB, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M et al. (2006) Human Anti–Tumor Necrosis Factor Monoclonal Antibody (Adalimumab) in Crohn's Disease: the CLASSIC-I Trial. Gastroenterology 130: 323-33.

13. Segaert S, Hermans C (2017) Clinical Signs, Pathophysiology and Management of Cutaneous Side Effects of Anti-Tumor Necrosis Factor Agents. Am J Clin Dermatol 18: 771-87.

14. Shivaji UN, Sharratt CL, Thomas T, Smith SCL, Iacucci M et al. (2019) Review article: managing the adverse events caused by anti-TNF therapy in inflammatory bowel disease. Aliment Pharmacol Ther 49: 664-80.

15. Allez M, Karmiris K, Louis E, Van Assche G, Ben-Horin S et al. (2010) Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: definitions, frequency and pharmacological aspects. J Crohn's Colitis 4: 355-66.

16. Digby-Bell JL, Atreya R, Monteleone G, Powell N (2020) Interrogating host immunity to predict treatment response in

inflammatory bowel disease. Nat Rev Gastroenterol Hepatol 17: 9-20.

17. Atreya R, Neurath MF (2018) Mechanisms of molecular resistance and predictors of response to biological therapy in inflammatory bowel disease. Lancet Gastroenterol Hepatol 3: 790-802.

18. Atreya R, Neurath MF (2008) New therapeutic strategies for treatment of inflammatory bowel disease. Mucosal Immunol 1: 175-82.

19. Atreya R, Zimmer M, Bartsch B, Waldner MJ, Atreya I et al. (2011) Antibodies against tumor necrosis factor (TNF) induce T-cell apoptosis in patients with inflammatory bowel diseases via TNF receptor 2 and intestinal CD14⊠ macrophages. Gastroenterology 141: 2026-38.

20. Bojarski C, Waldner M, Rath T, Schürmann S, Neurath MF et al. (2021) Innovative Diagnostic Endoscopy in Inflammatory Bowel Diseases: From High-Definition to Molecular Endoscopy. Front. Med 8: 655404.

21. Atreya R, Neumann H, Neufert C, Waldner MJ, Billmeier U et al. (2014) In vivo imaging using fluorescent antibodies to tumor necrosis factor predicts therapeutic response in Crohn's disease. Nat Med 20: 313-8.

22. Krishna SG, Modi RM, Kamboj AK, Swanson BJ, Hart PA et al. (2017) In vivo and ex vivo confocal endomicroscopy of pancreatic cystic lesions: A prospective study. World J Gastroenterol 23: 3338-48.

23. Coron E, Mosnier JF, Ahluwalia A, Le Rhun M, Galmiche JP et al. (2012) Colonic mucosal biopsies obtained during confocal endomicroscopy are pre-stained with fluorescein in vivo and are suitable for histologic evaluation. Endoscopy 44: 148-53.