

The importance of handling high-value biologicals: Physico-chemical instability and immunogenicity of monoclonal antibodies (Review)

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Received September 19, 2017; Accepted January 9, 2018

DOI: 10.3892/etm.2018.5821

Abstract. The present review specifies the various chemical and physical factors that can influence drug stability and immunogenicity, and the treatment outcomes of antibody biologicals. Although monoclonal antibodies (mAbs) are known to be more resistant to environmental changes compared with other proteins, the molecules themselves can be subjected to chemical and physical processes that promote their degradation and transformation into their specific amino-acid moieties. With increasing use of medicinal products that contain mAbs, and their self-administration by the patients, the issue of the correct manipulation of these drugs is of increasing importance. This review summarises the correct handling of mAb biologicals from the point of view of the pharmacist, clinical biochemist and patient, as is supported by relevant cases from the literature and our own data and experience. In particular, if there is a break in the cold chain, both healthcare professionals and patients need to be aware of the potential pharmacokinetics and pharmacodynamics alterations to these biologicals. Furthermore, any alterations in the protein structure can induce harmful immune reactions, including anaphylaxis and cytokine storms, or result in the production of neutralising or blocking Abs. Overall, considering also that treatment costs usually remain high, drug stability can have a tremendous effect on the clinical, humanistic and economic outcomes of such treatments.

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1. Introduction

Monoclonal antibodies (mAbs) have revolutionised the treatment of oncological and autoimmune diseases over the past 10 years. Moreover, they are also successfully used in the management of asthma, hypersensitivity reactions, osteoporosis, skeletal-related events in patients with bone metastases from solid tumours, neovascular (wet) age-related macular degeneration, hyperlipidaemia, and many others. According to data from the U.S. National Institutes of Health and other publications, various ongoing clinical studies look promising for indications like Alzheimer's disease, infections, and type-1 diabetes (1,2).

According to our local drug registration database (EU-Slovenia), there are currently 64 medical products that contain 40 active mAbs (data retrieved in November 2017). Among these, 39 are intended for intravenous administration, 22 for subcutaneous, and one each for intramuscular and intravitreal administration. For their pharmaceutical forms, 50 are solutions or concentrates for solution, 13 are in the form of powder for solutions, and one is a kit for radiopharmaceutical preparations for infusion. The data from the Agency for Medicines and Medical Devices of the Republic of Slovenia show a wide spectrum of the designs, forms and routes of administration of pharmaceutical drugs. In the earlier years of clinical use, these preparations were compounded by healthcare professionals in controlled environments, and administered in healthcare facilities. However, with the introduction of TNF- α inhibitors in pre-filled syringes in the last 10 years, medicinal products have left such controlled environments, and can now be stored and administered by the patients themselves (3). Therefore, in many cases the responsibility for the appropriate storage conditions has shifted from the healthcare professional to the patients.

The correct handling of biologicals at all times is extremely important, from their production to their being released to the

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Key words: biologicals, immunogenicity, monoclonal antibodies, medicinal product, treatment outcome

market. As protein molecules retain their biological activities and pharmacokinetics and pharmacodynamics profiles only when the higher-order protein structure is maintained, various factors that can lead to immune reactions should be avoided. Risks to protein stability can arise from not only a low percentage of 'foreignness' of the protein, but also from minimal product impurities and the formation of aggregates during handling. These can result in unwanted immunogenicity and anti-drug Ab responses in the patient, with subsequent effects on their treatment outcome. The desired clinical outcome can be lost through immune reactions, creating the potential need to switch to another drug with a different mechanism of action; e.g., a switch from a TNF- α inhibitor to an interleukin-6 inhibitor in patients with rheumatoid arthritis. Such loss of efficacy of a treatment and the subsequent lower interest in a medication can be attributed to human as well as clinical outcomes. Also, considering the high value of these medicinal products, there are economic consequences that should not be ignored regarding the waste from discarded medicines, potential unwanted side-effects, and the costs of new medicines and hospital staff and facilities.

2. Potential instabilities of medicinal products and pharmaceutical preparations containing monoclonal antibodies

Compared to a broad range of known proteins, the molecular structure of Abs provides them with one of the most stable and resistant forms against changes to their environment. However, due to their therapeutic use, even small deviations from the native structure of these proteins can severely affect one or more of the pharmaceutical standards of quality, efficacy and safety of the therapeutic product.

The protein structure of therapeutic mAbs is highly similar to the structure of Abs produced daily by lymphocyte B cells in response to any invasion of microorganisms in our body. The majority of the biotechnological pipelines provide Abs of the IgG1k type (~150 kDa), although other derivatives are also produced, such as certolizumab pegol (48 kDa pegylated Fab' fragment) and radio-nuclide Ab drugs. The most important parts of the IgG molecule that contribute to binding specificity with the antigen are the CDR loops-the complementarity determining regions-of the variable regions (Fig. 1). Small variations in the amino-acid side chains in CDR loops can result in diversity of the molecular surface, and the subsequent loss of recognition and specificity for binding to the antigen.

Chemical instability

Oxidation. Although not all proteins are uniformly susceptible to oxidative damage, oxidation remains one of the most important mechanisms of covalent protein modification. This can lead to altered or diminished biological functions because of fragmentation, dimerisation, aggregation or denaturation of the protein. The native conformation of proteins in a medicinal product is usually protected by the addition of stabilisers. Oxidation reactions are catalysed by free radicals, light, and trace amounts of metal ions (4-8). Many studies have confirmed that oxidation of cysteine or methionine in the Fc region can alter the effector functions of mAbs, and decrease

their binding to Fc receptors on immune cells (9,10). The amino-acids histidine, tyrosine and tryptophan are potential sites of oxidation, and at the same time, they are also highly concentrated in antigen-binding sites of Ab molecules (11). The effects of the oxidation of these amino-acids in the binding regions have not been clearly defined to date, although important alterations to Ab specificity and proinflammatory activities have been reported in in-vitro studies (12,13).

Deamidation. Deamidation is one of the most common modifications to mAb structure, which introduces a high level of charge heterogeneity into both their light and heavy chains. Removal of the functional amide group from asparagine residues is generally favoured, and to some extent also from glutamine. These reactions are highly selective for individual Ab (14), and they have a detrimental effect on potency if a negative charge is introduced into the antigen-binding region (15).

Hydrolysis. Hydrolysis is a chemical process that affects the primary structure of a protein, and it can result in loss of protein conformation and mAb function. An example from the literature shows that even when stored at 5°C, muromonab-CD3 (withdrawn in 2010) undergoes hydrolysis in the hinge region between the cysteine in the variable region and the proline in the Fc region (2). Today, such hydrolysis of mAbs would not be expected under the conditions that these medicinal products are exposed to during normal formulation and storage (5), due to the use of excipients.

Physical instability. During processing and handling of proteins, changes in their molecule structure can result in many structural variants of their conformation as they adapt to the changes in their environment. Non-physiological conditions, shear stress, agitation and stirring represent stress factors that are routinely encountered during synthesis, purification, shipping and preparation of such medicinal products. These structural changes alter the physical properties of mAbs and can introduce physical instability into the protein molecule. This can include adsorption onto surfaces (e.g., containers, syringes, needles), unfolding and formation of soluble aggregates, or formation of insoluble precipitates. This instability will result in loss of efficacy of the therapeutic protein, and also potential immunogenicity *in vivo*.

In a complex, structure-function related protein, as in the case of immunoglobulin, the different regions/domains denature independently: The Fab region is more sensitive to heat treatment, and the Fc region is more sensitive to lower pH (16). Aggregates can form due to changes to pH, ionic strength or surface tension, or to the presence of organic solvents, although these are often reversible. However, elevated temperature usually leads to irreversible aggregation and loss of protein function. Aggregates are organoleptically recognized as turbidity of a solution, although, there can also be a period of transparency at the very beginning of the nucleation process. Aggregation poses greater problems for the use of pharmaceutical forms for subcutaneous and intramuscular administration, where the protein concentrations in the solution can reach 125 mg/ml, while the concentrates used for solutions for infusion are usually lower, from 1 to 25 mg/ml. For self-administration, a low solution volume is preferred due to lower levels of site reactions and greater tolerability for the patient (14).

Elevated temperatures. The stability of mAbs in pharmaceutical preparations greatly depends on the temperatures they are exposed to. Longer exposure to less-elevated temperatures mainly accelerates their chemical instability. Studies have shown that a mAb exposed to 40°C for 6 months mainly showed deamidation and hydrolysis (17). When mAbs are exposed to temperatures near their unfolding temperature (defined as the temperature at which 50% of the protein molecules are unfolded), the prevailing instability mechanism is aggregation (4,5).

The stability of pharmaceutical preparations with mAbs also depends on the pharmaceutical form (and consequently, the use of excipients) and the properties of the protein itself. Ye (18) exposed abciximab (Reopro®) and trastuzumab (Herceptin®) to temperatures up to 70°C for 15 min, and to room temperature for up to 42 days. Under these conditions, 95% of abciximab was degraded or aggregated with the 70°C treatment, while it remained stable at room temperature; trastuzumab did not undergo any physical or chemical changes under either condition. During the development of equine venom Abs that are suitable for storage at room temperature in climate zone IV, Segura *et al* (19) managed to achieve stability at 37°C for 1 year using sorbitol and phenol as excipients.

Freeze-thaw cycles. The main mechanisms of protein instability during their freezing and thawing result from their aggregation. This can be due to their exposure to elevated concentrations of excipients in the non-water phase [that do not freeze; (20)] and to pH changes (21), to their adsorption onto ice-liquid interfaces or the walls of the vessels (5,22), as well as gas-liquid interfaces caused by accelerated cooling (23). The effects of freeze-thaw cycles are also cumulative, where faster cooling can denature proteins at rates up to 11-fold greater than seen for slower cooling (24).

3. Protein stabilisation in pharmaceutical forms

Diverse instability mechanisms are prevalent under conditions that cause changes to pH or temperature. To achieve greater stability and longer shelf-life of mAbs, these parameters need to be adjusted accordingly during the development phase. The U.S. Food and Drug administration criteria on stability of pharmaceutical forms state that no more than 10% of the active ingredient should deteriorate over 2 years (25).

Excipients. Various excipients are used in all mAb preparations to assure their appropriate pharmacokinetics properties and stability, to enable the formulation of the pharmaceutical product, and to enhance the tolerability of the patient. Medicinal products that contain mAbs are generally either powders for solutions or concentrates for solutions for parenteral administration (Table I). Here, excipients are used to maintain the pH (e.g., Tris, acetate, histidine, citrate buffers), to enhance the protein stability and prevent oxidation (e.g., sugars, polyols), to achieve an appropriate viscosity, or to bind metal ions and free radicals (e.g., chelators, antioxidants). Their addition can therefore stabilise solutions or lyophilisates (see section 3.2) over long periods of time (6).

Lyophilisation. Hydrophilic solutions provide a favourable milieu for physico-chemical changes to proteins. Aqueous

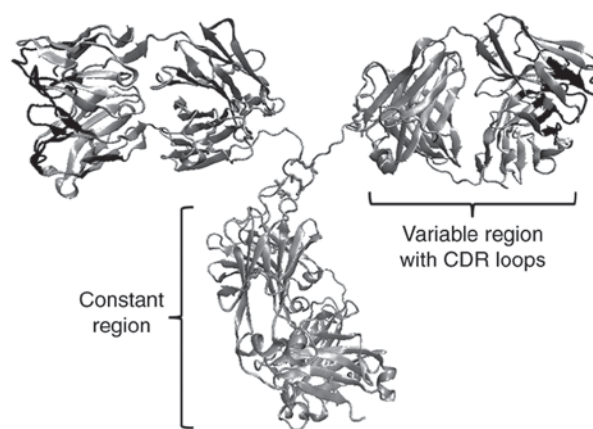


Figure 1. Three-dimensional molecular structure of an antibody (PDB_ID: 1IG).

media allow the transfer of the electrons needed for oxidation and deamidation reactions, and have an important role in protein aggregation. The exclusion of water from pharmaceutical forms can therefore provide important enhancement to the stability of proteins (14). The most commonly used method for this water exclusion is the three-phase process of freeze-drying, lyophilisation, and addition of cryoprotectants.

4. Unwanted immunogenicity of biologicals that contain monoclonal antibodies

In general, the immunogenicity of drugs refers to the formation of Abs against a certain drug (i.e., anti-drug Abs; ADAs). All such biologicals can potentially induce unwanted immune responses that can activate the mechanisms of innate and acquired immunity. It has been recognized that mAb drugs or their novel mAb derivatives (e.g., Fab fragments, scFv, nanobodies, fusion proteins) can induce both humoral and cellular immune responses. ADAs can alter the pharmacokinetics, pharmacodynamics and bioavailability of mAbs, thus affecting the safety and efficacy of these drugs.

There are two main mechanisms of immunogenicity: i) activation of classical immune reactions that are triggered by foreign proteins, which results in synthesis of ADAs and induction of memory cells, and leads to enhanced reactions upon rechallenge (26); and ii) breach of B-cell and T-cell immune tolerance. The clinical consequences of these can vary widely among individuals, and they are mostly unpredictable. The major safety concerns related to immunogenicity are induction of anaphylaxis, cytokine storms (the rapid release of proinflammatory cytokines), serum sickness disease, and delayed hypersensitivity. These can be accompanied by various clinical symptoms, including fever, rash, myalgia, haematuria, proteinuria and haemolytic anaemia, and can even induce autoimmune reactions.

Two types of ADA responses have been defined: i) Neutralising or blocking Abs (NABs) which block the effects of the exogenous drug or destroy the drug itself. These can neutralise the biological activity of the drug by either blocking the cell-surface molecule needed for its activity, or interfering with the binding of the drug to its receptor on the target cells;

Table I. Roles of excipients used in powder for concentrate for the solution for infusion with belimumab (Benlysta®).

Excipient	Role of the excipient
Citric acid monohydrate	Buffer component-weak acid (pH regulation)
Sodium citrate	Buffer component-strong base (pH regulation)
Sucrose	Cryoprotectant, bulking agent
Polysorbate 80	Surfactant

and ii) binding Abs, which (BAbs) bind to the drug but do not sterically hinder its biological actions (27).

However, many studies have confirmed the altered pharmacokinetics of a drug due to the formation of the immune complex between the drug and the binding Ab, and the resulting enhanced clearance (27-29). The levels of neutralising/blocking or binding Abs that are produced depend on the dose and the frequency and mode of injection or application of a drug (i.e., skin > intramuscular > intravenous > per os). There was a well-known case in 1998 where an increase in the incidence of pure red-cell aplasia (PRCA) was associated with the formation of anti-erythropoietin Abs after subcutaneous use of epoetin alpha (Eprex®) in patients under chronic dialysis (30). Also, in 2006, a phase I clinical study was being conducted for a humanised CD28 superagonist Ab, TGN1412, as a potential drug candidate for the treatment of B-cell lymphoma and rheumatoid arthritis. Within 8 h of the first infusion at a dose 500-fold more dilute than that shown to be safe in animal studies, all six of the human volunteers faced multiorgan failure. This was later recognized to have been the result of cytokine storm (31).

The earliest mAbs used as drugs originated from mice, and their degree of 'foreignness' was a pivotal force for development of immunogenicity. Indeed, overall some 90% of these treated patients produced human anti-mouse Abs (32), which greatly diminished the clinical objectives. The second generation of mAbs were chimeric mAbs, which were fusions between the murine epitope-specific variable region and the human constant region, and were produced by genetic engineering methods. These Abs were much more successful for therapeutic purposes. This technological advance from murine origin to humanised Abs greatly improved their *in vivo* tolerability, although 50% of treated patients still produced human anti-chimeric Abs (HACA) (32).

The more recent production of fully human Abs further decreased the unwanted immune response, although anti-idiotypic response remain, which might influence the outcome of immune responses. Anti-idiotypic Abs are raised against the antigen-binding site because the individual T-cell receptors and immunoglobulins are also immunogenic by virtue of the unique sequence within their variable regions. Therefore, even with human mAbs it is not reasonable to expect immunogenicity rates of zero, as this can be affected by numerous patient-related and product-related factors (Fig. 2). Aggregation, polymerization, denaturation or partial unfolding of native proteins, expose neoepitopes, cryptic

epitopes or repeated epitopes. Subsequent immune response to the aggregates involves previously mentioned mechanisms of the immunogenicity.

Protein aggregates in the size range of 0.1-10 µm are believed to be the most immunogenic (33). Recently, new animal testing methods have been developed that together with *in-silico* prediction models, can provide additional help to explain potential immunogenicity (34,35). For mAbs intended for *in-vivo* clinical use, the European Medicines Agency has issued regulatory guidance about screening and confirmatory assays, as well as risk assessment (EMA/CHMP/BMWP/86289/2010). Precise control of the manufacturing processes with concomitant systematic evaluation of immunogenicity would help to bridge this gap between product quality and clinical immunogenicity and understanding of the complex immune responses to mAbs. Due to the biological variability and broad spectrum of biological agents that contain mAbs, their different origins and different manufacturing processes, the 'one size-fits all' strategy is no longer acceptable. Therapies should be personalised and adapted to the individual patient, and supported by therapeutic drug monitoring. Together with the assured quality of mAb drugs, this will assure optimal treatment efficacy with minimal toxicity.

5. Handling of biologicals

The main critical phases that can affect the stability of mAb biologicals include the transportation and storing of these medicinal products, and the reconstitution of pharmaceutical preparations. Handling by the patient or a caregiver can also prove critical, as the medicinal products are no longer kept in a controlled environment.

Handling by pharmacy staff and healthcare professionals.

The transport to the healthcare provider is well established, with the maintenance of the cold-chain conditions, and this does not generally represent a risk to a medication. We therefore describe here critical preparation steps and procedures after the delivery of a biological to a healthcare facility. In 1976, the Breckenridge report stated that if possible, all parenteral pharmaceutical preparations administered to a patient should be compounded centrally in a hospital pharmacy, to ensure patient safety and maintain the sterility of the final product (36). The U.K. National Health Service guidelines HC(76)9 from 2008, the American Society of Clinical Oncology guidelines from 2012, and the South Australian Health Service guidelines from 2015 all added pharmaceutical preparations with mAbs used for oncological indications to their list of medications that should be compounded in the hospital pharmacy setting. In rare cases, where centralised preparation is neither possible nor feasible, such preparations can be carried out by a specially trained nurse (37).

Even though the establishing of a clean room is associated with high costs, this investment returns through rationalisation of the use of high-cost medicinal products that contain mAbs. This is achieved by using large-volume vials and being able to store the unused drug through the use of aseptic conditions. The dosing of mAbs is usually based on patient weight or body surface area. Due to limitations in the availability of such powders or solutions of mAbs, during on-ward preparation,

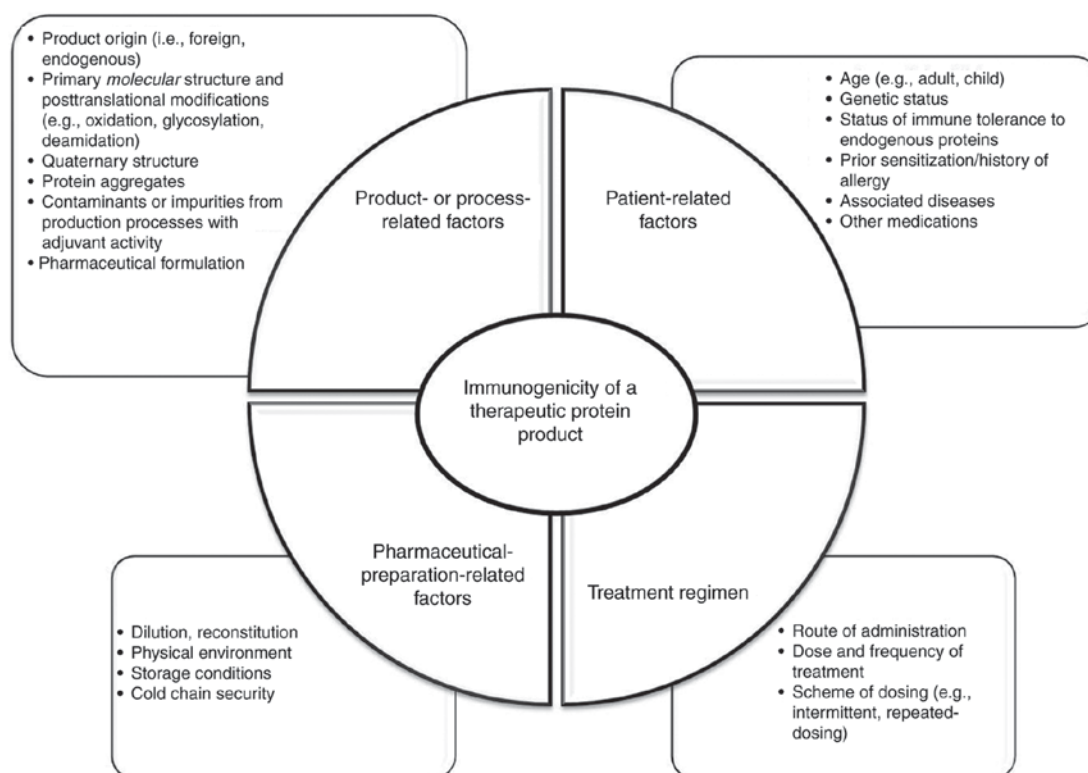


Figure 2. Product-related and patient-related factors that can influence the immunogenicity of medicinal products or pharmaceutical preparations that contain monoclonal antibodies.

the unused drug has to be discarded, according to the manufacturer instructions.

The preparation of intravenous pharmaceuticals should be carried out by a pharmacy technician under the supervision of a registered pharmacist, and more recently this can be guided by specific compounding computer software. Two methods are commonly in use. For volumetric preparation, every addition of a drug to the base solution has to be confirmed by a pharmacist. As this requires additional staff, more recently the gravimetric method is in greater use as the workflow is guided by a predefined protocol provided by the computer software (BD Cato™ medication workflow solutions compounding), and the validity of every step involving additions of the drug is confirmed by weighing. The use of bar codes, defined drug solution densities, and predefined tolerance levels, can exclude the human factor, and this ensures the traceability and safety of every pharmaceutical preparation, as well as providing better management of the unused drug. Nevertheless, both methods require skilled staff who have undergone training for working under aseptic conditions, as well as for handling of medicinal products containing mAbs.

Especially in cases where prescriptions are not verified in the pharmacy, it is crucial that medical doctors consider incompatibility issues regarding medicinal products with mAbs, such as the use of the correct dilution media and the concentration ranges needed. In some cases, it is recommended that the patients receive premedication prior to administration. Also, administration of medicinal products with mAbs can be accompanied by various adverse reactions. Again, it is crucial for physicians to recognize and manage these. Based on local guidelines, medical doctors

are usually obligated to report these adverse reactions. The European Medicines Agency pharmacovigilance legislation has also placed mAbs under so-called 'additional monitoring', and these medicines are labelled with an inverted black triangle to encourage healthcare professionals, and especially medical doctors, to report any suspected adverse effects (EMA/169546/2012).

Practical points for pharmacists and healthcare professionals. i) Elevated temperature. All currently commercially available pharmaceutical forms that contain mAbs have to be kept between 2 and 8°C until the compounding of the pharmaceutical preparation, or the administration of the drug itself (Agency of the Republic of Slovenia for Medicines and Medical Devices). It is highly unlikely that a product with mAbs would be exposed to temperatures that would threaten their instability during storage in a pharmacy, except in the case of refrigerator failure.

In rare cases, when such a product is accidentally exposed to elevated temperatures, the manufacturer should be consulted to obtain the necessary stability studies data, to determine whether the drug is safe or should be discarded.

ii) Freeze-thaw cycles. Although medical products with mAbs are not expected to be subjected to freeze-thaw cycles after the formulation of the final drug product, the possibility of accidental freezing cannot be fully ignored.

iii) Reconstitution of lyophilisates. The required solvent should be added slowly to a lyophilisate that contains mAbs, to minimise foaming and promote the formation of the native conformation of the protein. During lyophilisation, the protein molecules can form certain non-native conformations, due to interactions with the molecules that substitute the water during

Table II. Details of prescriptions filled for medicinal products containing mAbs for self-administration in Slovenia EU (estimated population, ~2 million) from 2004 to 2016.^a

Year	Number of prescriptions	Total value (€)
2004	278	150,697
2005	827	1,154,331
2006	1,308	2,370,870
2007	1,966	4,491,672
2008	2,495	7,432,900
2009	3,367	10,854,609
2010	4,185	13,284,280
2011	7,490	17,141,873
2012	14,133	21,542,757
2013	20,115	24,701,471
2014	27,330	26,892,197
2015	39,507	30,276,936
2016	50,233	31,996,181

^aData were extracted from the Registry of Medication Use of the Health Insurance Institute of Slovenia.

this process (14). Solvation can be accelerated with rotation of the vial around its axis. Newer medicines with lyophilised powders also provide the end-user with parameters for use with mechanical reconstitution devices (European Medicines Agency, Summary of product characteristics Benlysta). The reconstitution of pharmaceutical preparations from lyophilisates is technically challenging and time consuming.

iv) The effects of interfaces and adsorption. Manufacturers often state that the shaking of vials with concentrates for infusions and with the pharmaceutical preparations themselves should be avoided. Solutions of mAbs can be exposed to an air-liquid interface while shaking, and also during removal from the vial and addition into the dilution medium. The transfer from a syringe should be as slow as feasible, with the needle always submerged in the dilution medium to avoid unnecessary contact of the solution with the air. It has become common practice to use polymer spikes with separate channels for fluid transfers and air-pressure equalisation. These are usually equipped with 0.22 or 0.45- μ m hydrophobic air filters (38). Before removal of an Ab solution from the vial or after its reconstitution, the solution has to be visually checked for turbidity or the presence of floating non-transparent particles that indicate protein aggregation. Newer products that are prepared for longer periods of use (e.g., up to 96 h) can include a separate stabilising solution that is added to the infusion bag immediately before preparation of the pharmaceutical (European Medicines Agency, Summary of product characteristics Blincyto).

v) Protection from light. Until the compounding of the pharmaceutical preparation, the medicinal products containing mAbs are kept in secondary packaging to protect the solution from light. Should the duration of administration to the patient last several hours, the pharmaceutical preparation should be further protected from light. The same applies to any remaining solutions kept in preparation areas.

vi) Long-term stability of pharmaceutical preparations with mAbs. There are several situations when a solution that contains a mAbs cannot be administered immediately after its preparation. The manufacturers define the time limits in which prepared infusions or injections should be used if they were not prepared under aseptic conditions. Pharmaceutical preparations with mAbs do not contain preservatives. If preparations have been compounded under validated aseptic conditions, these periods can be longer. It appears that even for aseptic preparations, storage times for diluted solutions are based on microbiological integrity, rather than physico-chemical stability. For example, for diluted rituximab solutions, the manufacturer states that these are physically and chemically stable for up to 24 h at temperatures between 2 and 8°C, and then for an additional 12 h at room temperature (European Medicines Agency, Summary of product characteristics MabThera). On the other hand, Paul *et al* (39) reported that 1% rituximab solutions in 0.9% NaCl stored at 4°C for 6 months did not show any physical or chemical instability. The direct cytotoxic effects of rituximab were also fully retained. The availability of ready-to-use solutions with mAbs that are prepared in a controlled environment by the manufacturers themselves would cut the healthcare staff preparation costs and lessen their burden.

Handling by patients. The use of medicinal products containing mAbs for self-administration has steadily been rising worldwide. As more prescriptions for medications with mAbs are filled, the number of patients or caregivers that handle them also rises. All of these people should be properly informed about the concept of the cold chain, and they should know to follow the recommendations of the manufacturers and healthcare professionals.

The number of prescriptions dispensed and the total cost of medicines in Slovenia are given in Table II. The rise of prescriptions filled in 2012 and 2013 without a corresponding rise in costs can be attributed to the use of denosumab in the prevention of osteoporosis. The unusual increase in the number of prescriptions filled in 2015 can be attributed to changes in dispensing regulations, where most medicinal products that contain mAbs can now only be dispensed for 1 month at a time, instead of the previous 3 months limit (Health Insurance Institute of Slovenia).

The first time a patient receives a medicinal product that contains mAbs, they are usually given a package that is prepared by the manufacturer. This contains information about the safety of the drug and several practical aspects of its handling. The roles of all of the healthcare professionals involved in the care of a patient include the education of patients about the correct transport and storage of their drug. The risks include both the low and high temperatures that medicines can be exposed to.

The conditions when a medicinal product containing mAbs might be exposed to higher temperatures, and especially those above the protein unfolding temperature, are frequent in the warmer months of the year. The temperatures inside vehicles exposed to direct sunlight can reach 90°C in summer and 60°C in spring and autumn (40). Although these maximum temperatures were measured on sunny days, on cloudy summer days the temperatures are only 10°C lower (40). The colour of a

vehicle is not a major contributing factor. The patient has to be informed that the transport time from the pharmacy should be as short as possible, and that the medicine should not be left in a vehicle for long periods under any conditions. Even greater risk is posed by refrigerators used for storing medicines at the home of the patient. As it would be unrealistic to expect that patients have a medical-grade refrigerator for the sole purpose of storing their medicinal products, these are mostly kept in domestic refrigerators. Domestic refrigerators use heat exchange via the walls, where the chilling liquid runs through. The temperature near the walls where the heat exchangers are placed can reach -5°C (41), while for the doors or in the corners of the refrigerator, this can rise to 15°C (42). Domestic refrigerators also work in cycles that can pose additional risks to medicines, and especially those kept near the wall. As temperature can vary from below to above freezing point, a medicine might be subjected to freeze-thaw cycles. Patients are advised to keep medicines in the central part of the refrigerator, where the temperature is closest to the pre-set temperature (42), and to frequently monitor the temperature conditions using a thermometer.

6. Conclusions

Various factors have to be taken into consideration to assure the maximum safety of medicinal products containing mAbs. Current guidelines recommend compounding of pharmaceutical preparations with mAbs under the controlled aseptic conditions of the hospital pharmacy, with this performed by trained and experienced pharmacy staff. As well as chemical and physical reactions, a panel of patient characteristics and degrees of 'foreignness' of a protein are important for individual treatments. The main goal is to determine the bioavailability and to use data on unwanted immunogenicity to optimise the therapeutic dose for each individual while reducing possible immune reactions and preventing incorrect therapy choice. Due to the high economic cost of these biologicals and to safety concerns because of potential protein instability, health-care professionals (i.e., medical doctors, nurses, pharmacists) have to be properly and continuously educated in terms of the prescribing and preparation of biological medicinal products. Furthermore, their knowledge should also be transferred to the patient, to ensure the correct handling of these medicinal products at home. Where adverse reactions do occur, it is highly recommended that they are reported, according to local legislation. Only with strict and regular supervision all the way from production to final use will a medicinal products that contains mAbs provide the desired clinical, humanistic and economic effects designed for the therapy.

Acknowledgements

The authors acknowledge Dr. Chris Berrie for editing of the manuscript.

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