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**DIFFERENT DOSE REGIMENS OF ANTI-CD 20
MONOCLONAL ANTIBODY (RITUXIMAB) IN PATIENTS
WITH SEVERE AND RECALCITRANT AUTOIMMUNE
BULLOUS DISEASE PEMPHIGUS**

**Case series of the University Dermatologic Clinic of Chieti-Ortona
Systematic Review of the literature and Meta-analysis**

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INTRODUCTION

Pemphigus represents a spectrum of potentially life-threatening autoimmune-mediated skin blistering conditions caused by antibody production against desmoglein 1 and 3 (anti-DSG 1 and 3) in keratinocytes. Greater than 50% of pemphigus patients experience relapse, which complicates long-term medical management, including risks associated with re-treatment and complications such as infection and dehydration. Mechanisms of relapse include the persistence of auto-reactive B-cell populations post-treatment and CD20-B-cell populations that reactivate after B-cell depletion. Risk factors for relapse include high body surface area (BSA) of pemphigus involvement, high body mass index, high severity according to the Pemphigus Disease Area Index (PDAI) at onset, treatment delay, and high anti-DSG1 and DSG3 titers post-treatment.

Anti-CD20 monoclonal antibody rituximab is an approved treatment for patients with pemphigus vulgaris, who have severe course and prognosis, such as recalcitrant pemphigus or resistant to high dose corticosteroids and immunosuppressive agents.

Updated approaches to the management of pemphigus vulgaris support rituximab as a first-line adjuvant treatment to induce remission early in the course of disease; however, its feasibility in the clinical setting is often reduced by a series of limitations, including high cost of this biological drug, risk of severe adverse reactions, and uncertainty regarding the optimum dosing and schedule of administration. The standard approved rituximab dosages, which are derived from lymphoma protocols, have been recognized to exceed the effective dose required for inducing B cell depletion, since the B cell burden in pemphigus vulgaris is much lower than in lymphoproliferative disorders. To overcome these limitations, recent research has investigated alternative regimens of rituximab, using lower doses of the drug. The current literature suggests that lower-dose regimens of rituximab are not only tolerable and cost-effective but may also be associated with a positive response in pemphigus vulgaris, comparable to that achieved with higher doses especially in early disease.

For all these mentioned reasons, I decided to focus my PhD research on the study of different low-dose regimens of Rituximab in patients affected by Pemphigus, to better understand the therapeutic scenario. So I reported our experience in the use of ultra-low dose Rituximab protocol, showing case series of patients with Pemphigus referred to our Dermatologic Clinic of the University Hospital of Chieti-Ortona. I also investigated through a systematic review of the literature and meta-analysis the presence of any differences, in terms of efficacy and safety outcomes, among different dose regimens of Rituximab in patients affected by Pemphigus.

SECTION I

AUTOIMMUNE BULLOUS DISEASES: PEMPHIGUS

I.1 Definition and Pathophysiology

The autoimmune bullous diseases are a large group of dermatologic diseases characterized by bullae and/or erosion on the skin and mucosae and pathogenic auto-antibodies directed on target antigens whose function is either cell-to-cell adhesion within the epidermis or adhesion of stratified squamous epithelium to dermis or mesenchyme. These target antigens are components of desmosomes (*Fig 1*) or the functional unit of the basement membrane zone known as the adhesion complex. Depending on the location of the molecular target and the type of detachment we distinguish two large families of autoimmune bullous diseases: pemphigus, in case of intraepidermic cleft and pemphigoid family, with a sub-epidermic detachment.

We will talk about the first group of pathologies. A comprehension of the ultrastructural anatomy and composition of the areas under discussion greatly facilitates an understanding of the diseases that produce chronic blistering of the skin and mucous membranes (1).

Intercellular adhesion in the epidermis

Adhesion between keratinocytes is mediated predominantly by cell adhesion molecules of the cadherin family, localized in two specialized intercellular adhesion junctions: desmosomes and intermediate or adherens junctions. Desmosomes are small, electron-dense structures that link the intermediate filament within cells to the plasma membrane and to adjacent cells. The possible arrangement of desmosomal components is illustrated in *Fig.1*. The major adhesion components of desmosomes are the desmosomal cadherins, desmogleins and desmocollins. The cadherins are transmembrane glycoproteins each having an extracellular domain, a transmembrane domain and a cytoplasmic domain, which links to the cytoskeleton. Adhesion is probably regulated by complex

interactions between the cytoplasmic domains of cadherins and cytoplasmic proteins such as catenins or plakoglobin.

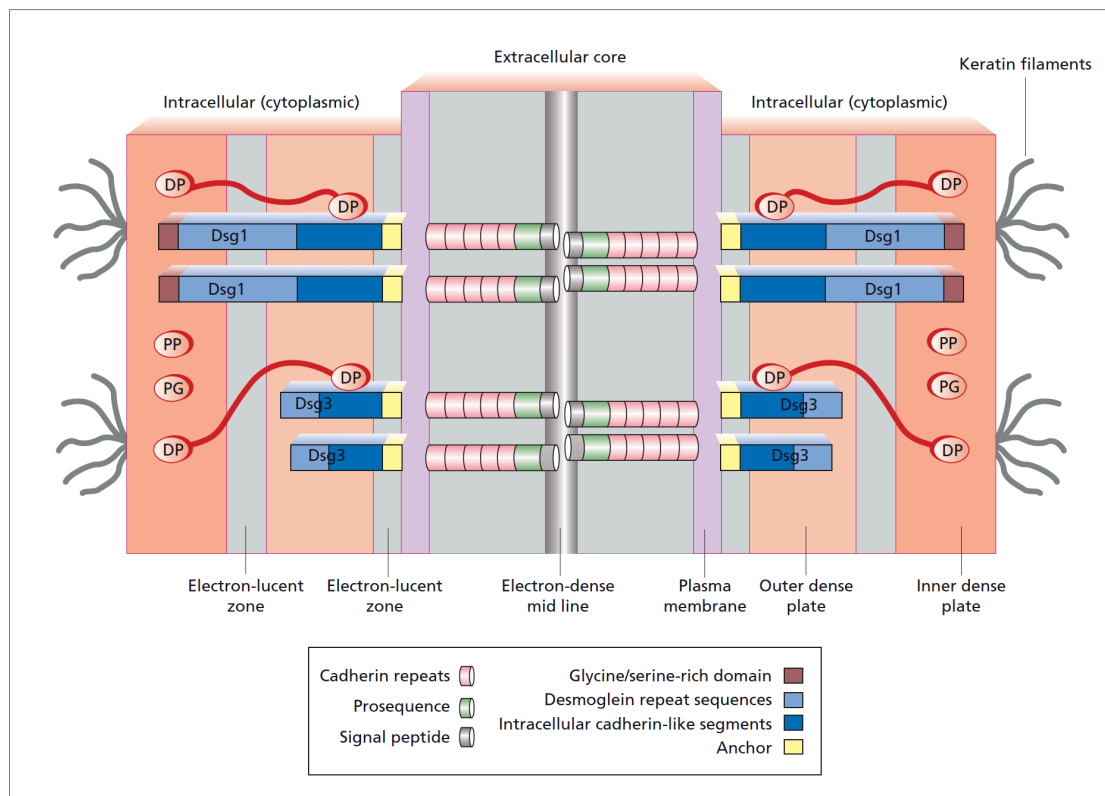


Fig. 1 Representation of the molecular components of the desmosome. DP, desmoplakin; Dsg1, desmoglein 1; Dsg3, desmoglein 3; N, amino terminal; PG, plakoglobin; PP, plakophilin (Rook's Textbook of Dermatology 8th Ed).

Type of autoantibody and genetics in Pemphigus

In patients with PV, most types of antibodies are oriented against desmosomal cadherins, Dsg1 and Dsg3, but other autoantibodies have been identified targeting other metabolic and structural proteins, such as Dsc1 and Dsc3 desmocollins, mitochondrial antigens, hSPCA1, thyroid peroxidase, muscarinic and nicotinic acetylcholine receptors, plakoglobin, E-cadherin and plakophilin 3. The pathogenic role of these non-Dsg autoantibodies is mentioned by some studies, which suggest that

they synergistically complement the classic effects of anti-Dsg autoantibodies in the complex process of pemphigus pathogenesis. The two antigens targeted by autoantibodies in PV are the 130-kDa glycoprotein Dsg3 and 160-kDa glycoprotein Dsg1. Dsg1 is mainly expressed on the surface of the epidermis, while Dsg3 accumulates predominantly in the mucous membranes and deeper epidermal layers. Patients with mucosal-dominant-type PV have only anti-Dsg3 antibodies, and those with mucocutaneous-type PV have both anti-Dsg3 and anti-Dsg1 antibodies (2).

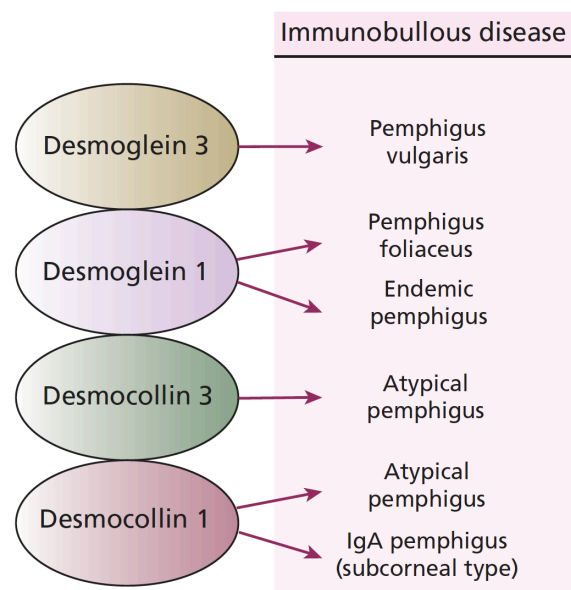


Fig. 2 Different antigens involved in different type of Pemphigus

There is a genetic predisposition for developing PV; certain major histocompatibility complex (MHC) class II molecules, such as DR4 (DRB1*0402) and DRw6 (DQB1*0503) occurring more frequently among those affected. Although these alleles are rare in the European population, they are more common in certain ethnic groups (e.g. Jewish population) and in countries of the eastern Mediterranean and the Middle East (Turkey, Iran, Iraq) (3)

I.2 Clinical and classification

The intraepidermic autoimmune bullous diseases are classified largely by their clinical presentation, histopathology and immunopathology.

Pemphigus vulgaris (PV) is the most common type of intraepidermic autoimmune bullous diseases, is a rare disease and its incidence has been estimated between 1 and 16 new cases per million per year. It usually begins in adulthood and there are no differences between men and women. There are no racial differences although a genetic predisposition is suggested by the association with some HLA class II molecules. Pemphigus can be associated with other autoimmune diseases (e.g. myasthenia gravis). Clinically, start in the majority of cases from the oral mucosa with painfull erosions and aftoid lesions, often misdiagnosed. It can involve frequently also larynx and pharynx. Suddenly, flaccid bullae with citrine content, aphlegmatic, appear on the skin, which break easily, resulting in painful erosions. Swiping the fingertip around a bulla causes a characteristic detachment (Nicholsky's sign).

Pemphigus Vegetans (PVEG) is a subclasses of PV, characterized by oozing, macerated, vegetating lesions, associated with pustules, located mainly at the level of large skin folds.

Pemphigus Foliaceus (PF) is characterized by a few very fragile vesicular lesions and above all by squamous-crusts and erosions on an erythematous basis, which often start from the head and trunk and spread throughout the entire skin area. There is burning and itching. The oral cavity and mucous membranes are not affected.

Pemphigus Erythematousus (PE) is similar to PF with greater involvement of the face: it differs from this due to the presence of antinuclear antibodies which are also highlighted with IFD.

Endemic Pemphigus Foliaceus (PFE), widespread in some areas of the Amazon and Tunisia, presents extensive skin lesions similar to those of PF. In the Amazon population, it occurs in genetically predisposed individuals when they come into contact with a particular type of

entomophagous fly: a protein from the saliva of this insect has cross-reactivity with DGL 1 and is the initial cause of the production of the antibodies that cause the skin disease.

Pemphigus Herpetiformis (PH) presents with vesicles and even evanescent pustules, grouped together to form often annular figures; severe itching is generally present. It almost always has a benign trend, but many times it transforms into the foliaceous or vulgar form.

Paraneoplastic Pemphigus (PNP) is a very serious form. It generally begins with very painful ulcerative and erosive lesions in the oral cavity and pharynx, which are followed by erosive and crusted manifestations of the labial, perioral, perinasal and periocular regions; these are associated with blisters with the characteristics of pemphigus and/or pemphigoid, lichen planus-type papular manifestations, polymorphic erythema-type aspects or even Graft Versus Host Disease-type aspects. There is often involvement of the digestive tract and lung with a characteristic and often fatal bronchiolitis obliterans. May be associated with lymphoma, thymoma, chronic lymphocytic leukemia, Waldstrom's macroglobulinemia, sarcoma, and Castleman's disease

Drug-Induced Pemphigus (DIP) presents the clinical aspect of PF, but is triggered in genetically predisposed subjects by the intake of certain drugs which predominantly have thiol groups (penicillamine, captopril) or phenolic groups (rifampicin, levodopa) and other products in their molecule, such as NSAIDs and calcium channel blockers. Despite the suspension of the trigger drug, in some cases the disease persists.

IgA pemphigus (PIgA) is very rare and is characterized by flaccid pustular lesions with typical hypopyon, which group together to form annular or circinate figures in the "subcorneal pustulosis" type or sunflower-shaped figures in the "intraepidermal neutrophilic" type. The oral mucosa is rarely affected. May be associated with monoclonal gammopathy and other lymphoproliferative diseases. It differs immunologically from other forms of pemphigus, because the pathogenic antibodies are IgA and their target is almost always desmocollins rather than desmogleins (2).

Disease	Direct IMF	Isotype	Target antigens	Antigens (kDa)	Epitopes	Location	Immunogenetics
							MHC class I and II
Pemphigus vulgaris/ pemphigus vegetans	Intercellular	IgG (few IgM, IgA)	Desmoglein 3, sometimes desmoglein 1 desmocollins	130	Amino-terminal of extracellular domain	Desmosome	DRB1*0402 DRB1*1401
Pemphigus foliaceus	Intercellular	IgG	Desmoglein 1, sometimes desmocollins	160	Amino-terminal of extracellular domain	Desmosome	HLA-DRB1*14
Endemic pemphigus foliaceus	Intercellular	IgG	Desmoglein 1, sometimes desmocollins	160	Amino-terminal of extracellular domain	Desmosome	Several susceptibility alleles, all with same amino acid sequence in <i>DRB-1</i> gene DRB1*0102 DRB1*0404, *1402 or *1406
Paraneoplastic pemphigus	Intercellular and subepidermal	IgG	Plakins (desmoplakin, envoplakin BP230, periplakin)		Various	Desmosomes, BMZ; stratified, simple and transitional epithelia	Unknown

BMZ, basement-membrane zone; HLA, human leukocyte antigen; IMF, immunofluorescence; MHC, major histocompatibility complex.

Tab 1. Immunopathologic and immunogenetics characteristics of Intraepidermal autoimmune bullous diseases

Disease	Patients	Cutaneous distribution	Mucosal involvement	Lesions	Disease associations	Treatment	Prognosis
Pemphigus vulgaris	Middle age	Scalp, face, flexures, may be generalized	Always oropharynx, conjunctiva, genital	Flaccid blisters, erosions, flexural vegetations	Autoimmune disease, thymoma	Steroids, immunosuppressives, dapsone	Variable, may remit
Pemphigus vegetans	Middle age	Flexural	Oral	Vesicles, pustules, erosions, vegetating plaques		Steroids, immunosuppressives, dapsone	Variable, may remit
Pemphigus foliaceus	Middle age	Scalp, face, chest, upper back, rarely generalized 'seborrheic'	None	Scaly papules, crusted erosions, erythroderma		Steroids (topical, intralesional, systemic), immunosuppressives	Benign but chronic
Endemic pemphigus foliaceus	Children, young adults	Head, neck, generalized	Uncommon	Flaccid blisters, erosions, verrucous lesions, erythroderma		Steroids, immunosuppressives, antimalarials	Chronic mortality <10%
Intercellular IgA dermatosis	Adults, children	Axillae, groins, face, scalp, proximal limbs	Uncommon	Flaccid pustules annular or circinate configuration	IgA monoclonal gammopathy	Dapsone	Chronic indolent
Paraneoplastic pemphigus	Adults, children	Upper body, palmoplantar	Severe mucositis	Polymorphous, bullae, erosions, 'target lesions'	Lymphoproliferative disease, Castleman's, other malignancies	Tumour resection, steroids, immunosuppression	Very poor

Tab 2. Clinical characteristics of Intraepidermal autoimmune bullous diseases

Severity and Comorbidity

Pemphigus Disease Area Index (PDAI), Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), and Pemphigus Vulgaris Activity Score (PVAS) are the most comprehensively validated measures.

PDAI

PDAI has the greatest reliability and is least affected by variability in rater's pemphigus expertise. All three pemphigus measures have acceptable convergent validity with Physician Global Assessment, but PVAS seems to have the greatest value. PVAS is the fastest and simplest score to complete. Responsiveness studies may be needed for comprehensive validation. There are inconsistencies in severity cutoffs. Recently, PDAI, as the internationally accepted pemphigus disease measure, has been increasingly used in different studies around the world. (4)

Pemphigus disease area index (PDAI)

Skin	Activity	Damage
Anatomical location	Erosion/Blister or new erythema	Post-inflammatory hyperpigmentation or erythema from resolving lesion
	0 absent 1 1-3 lesions, up to one >2 cm in any diameter, none > 6 cm 2 2-3 lesions, at least two > 2 cm diameter, none > 6 cm 3 >3 lesions, none > 6 cm diameter 5 >3 lesions, and/or at least one >6 cm 10 >3 lesions, and/or at least one lesion >16 cm diameter or entire area	Number lesions if ≤ 3 0 absent 1 present
Ears		
Nose		
Rest of the face		
Neck		
Chest		
Abdomen		
Back, buttocks		
Arms		
Hands		
Legs		
Feet		
Genitals		
Total skin	/120	/12

Scalp

Scalp	Erosion/Blister or new erythema	Damage
	0 absent 1 in one quadrant 2 two quadrants 3 three quadrants 4 affects whole skull 10 at least one lesion > 6 cm	Number lesions if ≤ 3 0 absent 1 present
Total Scalp (0-10)	/10	/1

Mucous membrane

Anatomical Location	Erosion/Blister	Damage
	0 absent 1 1 lesion 2 2-3 lesions 5 >3 lesions or 2 lesions >2 cm 10 entire area	Number lesions if ≤ 3
Eyes		
Nose		
Buccal mucosa		
Hard palate		
Soft palate		
Upper gingiva		
Lower gingiva		
Tongue		
Floor of mouth		
Labial bucosa		
Posterior pharynx		
Anogenital		
Total Mucosa	/120	

Total Activity Score:

Total Damage Score:

Fig. 3 PDAI (Pemphigus Disease Area Index) Scoring System

Aside from extension and severity involvement of skin and mucosae, the severity of the disease is based on its progressive course which is accompanied by an increased body catabolism with loss of body fluids and proteins and secondary bacterial and viral infections which may lead to sepsis and heart failure. So a tempestive and efficacy treatment is mandatory to avoid complications of this sometimes life-threatening condition.

I.3 Diagnostic assessment

The clinical, immunopathological and immunogenetic features of these diseases are shown in Tables 1 and 2. The most important techniques for the investigation of patients with immunobullous disease are histopathology, and direct and indirect immunofluorescence. Research techniques such as immunoblotting and immunoelectron microscopy may refine the diagnosis in the individual patient but do not replace the clinical findings [1]. Serological detection of serum autoantibodies against intercellular substance (ICS) by indirect immunofluorescence microscopy (IIF) and/or enzyme-linked immunosorbent assay (ELISA).

Histopathology

Preferentially, a 4 mm- punch excision should be taken of a fresh (<24 h) small vesicle or 1/3 of the peripheral portion of a blister and 2/3 perilesional skin (placed in 4% formalin solution) for routine histopathological analysis: intraepidermal suprabasal acantholysis in PV and PNP, or acantholysis at the granular layer in PF is usually seen.

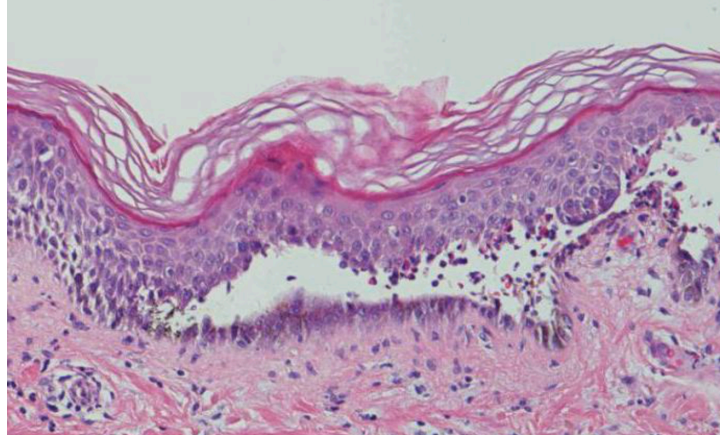


Fig. 4 Histopathologic features of Pemphigus

Direct Immuno Fluorescence (DIF)

Biopsy of perilesional skin or mucosa (up to 1 cm from a fresh lesion), put into a cryotube for transportation in a cylinder of liquid nitrogen, or in saline (delivery <36 h) or Michel's fixative for DIF analysis:

- DIF: IgG and/or C3 deposits at the ICS of epithelial keratinocytes;
- The ICS staining for in vivo IgG deposits is normally linear/granular in DIF, and not smooth such as in IIF;
- IgA deposits with an ICS pattern in addition to IgG may be present in a minority of cases. When only IgA is found, the diagnosis of IgA pemphigus is established;
- ICS deposits can sometimes be associated with linear deposits of IgG or C3 along the dermal-epidermal junction, suggestive of PNP/PAMS or pemphigus erythematosus, or the coexistence of pemphigus and pemphigoid.

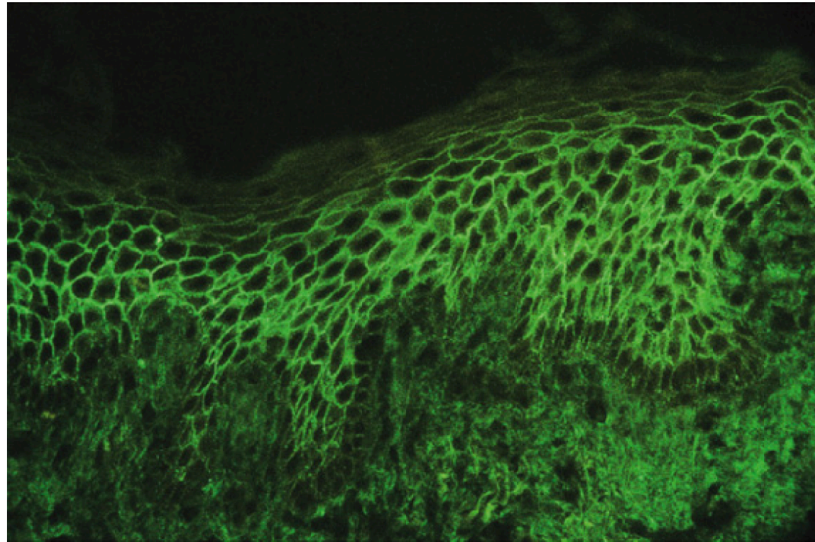


Fig. 5 Direct Immuno Fluorescence of Pemphigus, showing deposition of intercellular IgG in the epidermis.

Immune serological tests

In addition to DIF, IIF and additional techniques with defined native or recombinant proteins are commonly used to detect serum autoantibodies in patients with pemphigus.

Indirected Immuno Fluorescence (IIF)

IIF test on monkey esophagus or human skin to search for autoantibodies against proteins of epithelial keratinocytes; the ICS pattern by IIF on substrates is smooth and reticular, which is also referred to as “chicken wire”, “honeycomb”, or “fishnet-like” pattern.

ELISA

- Detection of antidesmoglein 1 (Dsg1) (PF/cutaneous PV) and/or antidesmoglein 3 (Dsg3) IgG autoantibodies (mucosal PV) by ELISA (commercial kits are available);
- the detection of IgG autoantibodies by ELISA is positive in more than 90% of cases;
- in general, the ELISA index correlates with the extent and/or activity of disease (see remark above and prognostic value for relapse, helping to guide treatment). Large prospective cohort studies are however missing in this context to provide reliable data about predictive value;
- in case of atypical presentation or the suspicion of an unrelated autoimmune bullous disorder,

additional immunopathological tests may be performed, such as II F on rat bladder and immunoblot/immunoprecipitation (5)

I.4 Therapeutic options

The main objectives of the PV treatment are to control and healing the bullous skin and/or mucous lesions, minimizing, as much as possible, serious side effects of treatment.

TABLE II.—*Therapeutic management in pemphigus.*

First line	Second line /adjuvant	Third line
Prednisone 0.5-1.5 mg/kg/day Anti CD-20* 1000 mg twice (2 weeks apart) or 375 mg/m ² (1 week apart 4 times)	Azathioprine 1-3 mg/Kg/day Mycophenolate mofetil 2 g/day Mycophenolic acid 1440 mg/day	Intavenous immunoglobulins 2 gr/kg/month Immunoadsorption Cyclophosphamide 500 mg bolus or 2 mg/Kg/day Dapsone** 100 mg/day Methotrexate + folate 10-20 mg/week
*Third line treatment in guidelines but recently included by a panel of experts JAAD 2018 as first line treatment. Only in Severe and refractory cases (G.U. 7/12/12 n. 286 [law 648]); **in IgA pemphigus consider Dapsone as first choice with corticosteroids.		

Tab 3. *Therapeutic management in pemphigus from EDF and EADV guidelines*

First line treatments

Systemic Corticosteroid

Systemic Corticosteroid therapy, usually prednisolone at 0.5 mg to 1.5 mg/kg/day. If initial control of PV is not reached within 2 weeks, a higher prednisolone dose (up to 2 mg/kg) is optional; Control of PF generally requires lower doses than PV. Systemic corticosteroids are generally combined with an immunosuppressive adjuvant immediately at the start of therapy, particularly in cases of increased risk of corticosteroid therapy, complications due to expected prolonged use (>4 months) or dose dependency above minimal therapy (>10 mg/day).

Immunosuppressive adjuvants

Based on the current evidence, adjuvants have only a steroid-sparing effect, and may lead to steroid-free remission.

First line adjuvants

Azathioprine (1-3 mg/kg/day). Start first week 50 mg /day to detect idiosyncratic reactions (and in case stop immediately), and then raise to desired dose. Check TPMT activity before treatment;

Mycophenolate mofetil (2g/day) or mycophenolic acid (1440 mg/day). Consider to raise daily dose by 1 capsule per week for better gastrointestinal tolerance

Second line adjuvants

Anti-CD20 monoclonal antibody, such as rituximab 2 x 1 g IV (2 weeks apart) or 4x375 mg/m² (each 1 week apart);

Intravenous immunoglobulins (IVIg, 2g/kg/month);

Immunoadsorption (2 cycles on 4 consecutive days, 4 weeks apart);

Cyclophosphamide (500 mg as IV. bolus or given orally at 2 mg/kg/day);

Methotrexate (10-20 mg/week);

Dapsone 100 mg/day or up to ≤ 1.5 mg/kg/day

Additional supportive treatment

Intralesional injections of corticosteroids (triamcinolone acetonide) may be beneficial for isolated lesions of oral mucosa, lips, and skin.

Topical treatment with potent corticosteroids (clobetasol propionate) or calcineurin inhibitors applied directly to the lesions, and oral topical corticosteroids (such as triamcinolone acetonide gel) directly to oropharyngeal erosions for use in combination with systemic therapy, may be beneficial.

The use of baths containing antiseptics such as chlorhexidine could also be useful.

If there are erosive lesions, they may be covered by using different low adhesive wound dressings or local emollients, and compresses. Analgesics (paracetamol, metamizol, and opioids) may be necessary. Gels containing local anesthetics may be used for application at the mucosal surfaces (5).

Anti-CD 20: Rituximab

Rituximab, a chimeric monoclonal antibody, specifically targets the CD20 antigen, predominantly expressed on the surface of pre-B and mature B lymphocytes, enabling its selective lysis of these cells. This targeting mechanism is facilitated through two primary pathways: complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) . In CDC, rituximab binds to specific amino acid regions on CD20, triggering complement system activation and subsequent lysis of CD20+ cells. Similarly, ADCC involves rituximab-induced lysis of CD20+ cells through complement system activation and the engagement of natural killer (NK) cells. Additionally, rituximab exhibits indirect effects by inducing structural changes, apoptosis, and sensitization of cancer cells to chemotherapy, further contributing to its efficacy in treating various B cell malignancies. (Fig. 5)

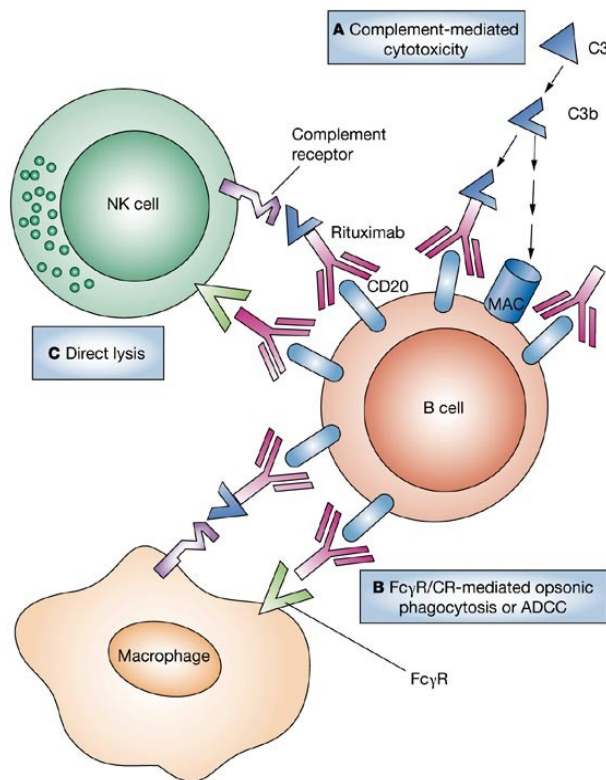


Fig. 5 Immunological mechanisms of action of Rituximab on B cell depletion

Notably, rituximab's therapeutic benefits extend to conditions such as non-Hodgkin's lymphoma, chronic lymphocytic leukemia (CLL), and pemphigus vulgaris. Rituximab, an anti-CD20 monoclonal antibody, has gained FDA approval for treating various conditions, encompassing non-Hodgkin lymphoma, CLL, granulomatosis with polyangiitis, and microscopic polyangiitis. Furthermore, the FDA has endorsed rituximab for managing pemphigus vulgaris, suggesting it as a primary treatment for severe cases. Rituximab can be administered via intravenous (IV) or subcutaneous (SC) routes. Recent studies indicate comparable clinical effectiveness between SC and IV rituximab, with the SC route offering advantages such as faster drug delivery, reduced potential for dosage errors, and minimized drug wastage [14]. Consequently, rituximab is viable for administration via both IV and S.

Clinical Outcomes for evaluation of disease activity and treatment monitoring

The consensus statement on definitions of disease, end points and therapeutic response for pemphigus are defined as follows:

- ***Time to disease control (TDC)*** was defined as the time interval between the baseline and the time at which new lesions cease to form.
- ***Complete remission off therapy (CROT)***, was defined as the absence of new or established lesions while the patient was off all systemic therapy for at least 2 months.
- ***Complete remission with minimal therapy (CRMT)*** was defined as the same above while the patient was receiving minimal therapy.
- ***Partial remission off therapy (PROT)*** was defined as the presence of transient new lesions that heal within 1 week while the patient was off systemic therapy for at least 2 months.
- ***Partial remission with minimal therapy (PRMT)*** was defined as the same state as that of PROT while the patient was receiving minimal therapy.
- ***Minimal therapy*** was defined as prednisone at 10 mg per day or half the dose required to control disease activity.
- ***Relapse*** was defined as the appearance of three or more lesions over 1 month that do not heal spontaneously within 1 week.
- ***Refractory disease*** was defined as recurrent blister formation despite immunosuppressive therapy with corticosteroids (>10 mg daily prednisolone equivalent) and azathioprine 2-2.5 mg/kg daily or equivalent immunosuppression.

SECTION II

DIFFERENT DOSE REGIMENS OF ANTI-CD 20 MONOCLONAL ANTIBODY (RITUXIMAB) IN PATIENTS WITH SEVERE AND RECALCITRANT PEMFIGUS: A SYSTEMATIC REVIEW OF THE LITERATURE AND META-ANALYSIS

Introduction

Treatment regimens that utilize RTX regimens for patients with PV increase the chance of the patient achieving complete remission (CR) to almost three times higher than the corticosteroids-alone regimen (Citation11,Citation12). A lymphoma protocol (375 mg/m² each week × 4 times) in 2002 was the first to use RTX to treat refractory PV (Citation13). In addition to this protocol, the rheumatoid arthritis (RA) protocol (1000 mg at 2 week intervals × 2 times) is also recommended by the international expert consensus as the first-line treatment for PV (Citation14). When administered at a dosage of less than 1 mg/m², RTX can deplete circulating B-cells in healthy individuals (Citation15,Citation16). Additionally, because of factors such as the expense of the full-dosage medication and the risk of infection caused by depletion of B-cells and infusion reactions, the clinical utility of the full dose of the drug is often limited. Various studies have investigated different RTX dosages for the treatment of PV, so we propose a systematic review of the literature to better understand the efficacy and safety data among different dosages.

II. 1 Background and Aims

The objectives were to evaluate the efficacy and safety of different dose regimen of anti-CD 20 monoclonal antibody (Rituximab) in patients with Pemphigus Vulgaris, an autoimmune bullous disease. Our aims were also to explore the presence of any differences on the efficacy response to Rituximab between the two subclasses of pemphigus, vulgaris and foliaceus.

II. 2 Material and methods

II.2.1 Inclusion and exclusion criteria

Type of studies

We included observational studies, cohort studies, case series and phase III randomized clinical trials (RCTs) and relevant literature reviews that were published within the last 25 years with more or equal than 5 participants. Phase I and II trials were not eligible because participants, outcomes, dosages, and schema of administration of interventions are too different from phase III trials. Cross-over trials were eligible if they had almost 4 weeks of wash-out prior re-randomization. We excluded data from conference abstract.

Type of participants

We considered trials that included adults (over or equal 18 years of age) with moderate to severe pemphigus (vulgaris or foliaceus, PDAI >12), with cutaneous, mucosal or mucocutaneous involvement, and/or with an history of recalcitrant pemphigus, non responder to high dosage of systemic corticosteroids (1 mg/kg/die or 0.2-1g bolus) or immunosuppressants (Azathioprine, Mofetile Micophenolate, Methotrexate, Cyclosporin, etc). All patients included have had a pemphigus diagnosis according to both clinical and hystolgic criteria, including Direct Immuno-Fluorescence (DIF). We excluded studies regarding paraneoplastic pemphigus.

Type of interventions

We considered studies that assessed anti-CD 20 monoclonal antibody (Rituximab or L01XC02) at the Hematologic doses (375 mg/mq of BSA) also called “Low-Dose” or “Lymphoma Protocol” and at inferior dosage (200 mg), also called “Ultra-Low-Dose” or “Very Low-Dose”, irrespective of the duration treatment. We excluded reports using the Rheumatologic Dosage of Rituximab or conventional protocol (1000 mg twice 2 weeks apart). We excluded studies with concomitant adjuvant immunosuppressive therapy during the administration of Rituximab, other than systemic corticosteroids (such as Azathioprine, Micophenolate, Methotrexate, etc.)

Type of outcomes

We considered these primary and secondary effectiveness outcomes:

Primary Effectiveness Outcomes

- a) Complete Remission (Off Therapy – CROT- and with Minimal Therapy –CRMT)
- b) Time to reach Complete Remission (TTCR)

Secondary Effectiveness Outcomes

- a) Relapses (%)
- b) Time To Relapse (TTR)

For more details on effectiveness outcomes see the Glossary table.

We considered these primary and secondary safety outcomes:

Primary Safety Outcomes

- a) SAE (Serious Adverse Event)*

Secondary Safety Outcomes

- a) Progressive Multifocal Leukoencephalopathy (LMP)

*International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, which includes death, life-threatening events, initial or prolonged hospitalisation, and adverse events requiring intervention to prevent permanent impairment or damage.

II.2.2 Search methods for studies identification

Electronic sources

We searched studies from the three following databases from 01 January 2000 up to 16 February 2025: MEDLINE Ovid (from 1946), OVID Embase Classic and Embase 1947, Cochrane Central Register of Controlled Trials (CENTRAL; 2016, Issue 11). We also applied the filters only Human study and English language. To view the search term for all three platforms go to the Appendix.

Trial Registers

We searched the following trials registers up to 16 February 2025 with the following search terms: pemphigus AND one by one each drug name listed in Types of interventions: US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov); EU Clinical Trials Register (www.clinicaltrialsregister.eu). We excluded unpublished reports.

II.2.3 Data collection

Selection of studies

Two physicians independently (FG and PDS) proceeded to abstracts screening, according to inclusion and exclusion criteria. These researchers independently examined full-text articles to determine eligibility and, when necessary, discussed disagreements to reach consensus. We list excluded studies and document the primary reason for exclusion.

Data extraction

Three review authors (FG, PDS, PA) each extracted the data from published reports independently using a standardised form. We pilot-tested this form (Data Extraction Form) on a set of included trials. We extracted the data from the US National Institutes of Health Ongoing Trials Register ClinicalTrials.gov (www.clinicaltrials.gov) and finally from the published reports.

II.2.4 Statistical analysis

We used a standard inverse-variance random-effects meta-analysis to combine the trials, with the estimate of heterogeneity being taken from the Mantel-Haenszel model (STATA command “metan”, option “random”).

Assessment of heterogeneity

We quantified heterogeneity between trials using the I^2 statistic (6), which describes the percentage of variation across trials that is attributable to heterogeneity rather than to chance. I^2 values of 25% may be interpreted as low, 50% as moderate, and 75% as high between trial heterogeneity, although its interpretation depends on the size and number of trials included (7).

Sub-group analysis

For each of the outcomes where at least 5 studies contributed, we performed stratified analyses by type of intervention doses (500 mg, 375mg/mq, 200 mg). We derived p values for interactions from univariable, random-effects meta-regression models to evaluate if treatment effects were affected by type of intervention dose (8). We considered a two-sided p value <0.05 as statistically significant. We then performed subgroup analyses by computing the effect within each subgroup and then comparing the summary effects across subgroup.

Meta-regression

We also performed simple meta-regression analyses from primary outcomes CR and TTCR by pre-specified variables, including sex, age, mucosal involvement, severity (PDAI), duration of disease, concomitant Corticosteroid.

We performed all analyses and drew graphs using the Review Manager (RevMan version 5.3, The Nordic Cochrane Centre, The Cochrane Collaboration, 2008, Copenhagen, Denmark), OpenMetaAnalyst (Brown University School of Public Health Providence, RI, USA), and STATA (1996–2018 StataCorp LLC, College Station, Texas, USA) software packages.

Missing data

We extracted, when possible, both the number of randomised and analysed participants in each study arm. We contacted trial authors or sponsors by email to request missing outcome data when these were not available in study reports. For the main analysis, we assumed that any participant with missing outcome data did not experience clearance (NRI, non-responders imputation), whatever the group. When in studies reports missing values were imputed by both multiple imputation and non-responder imputation, we chose the non-responder imputation data.

Prisma Flow diagram

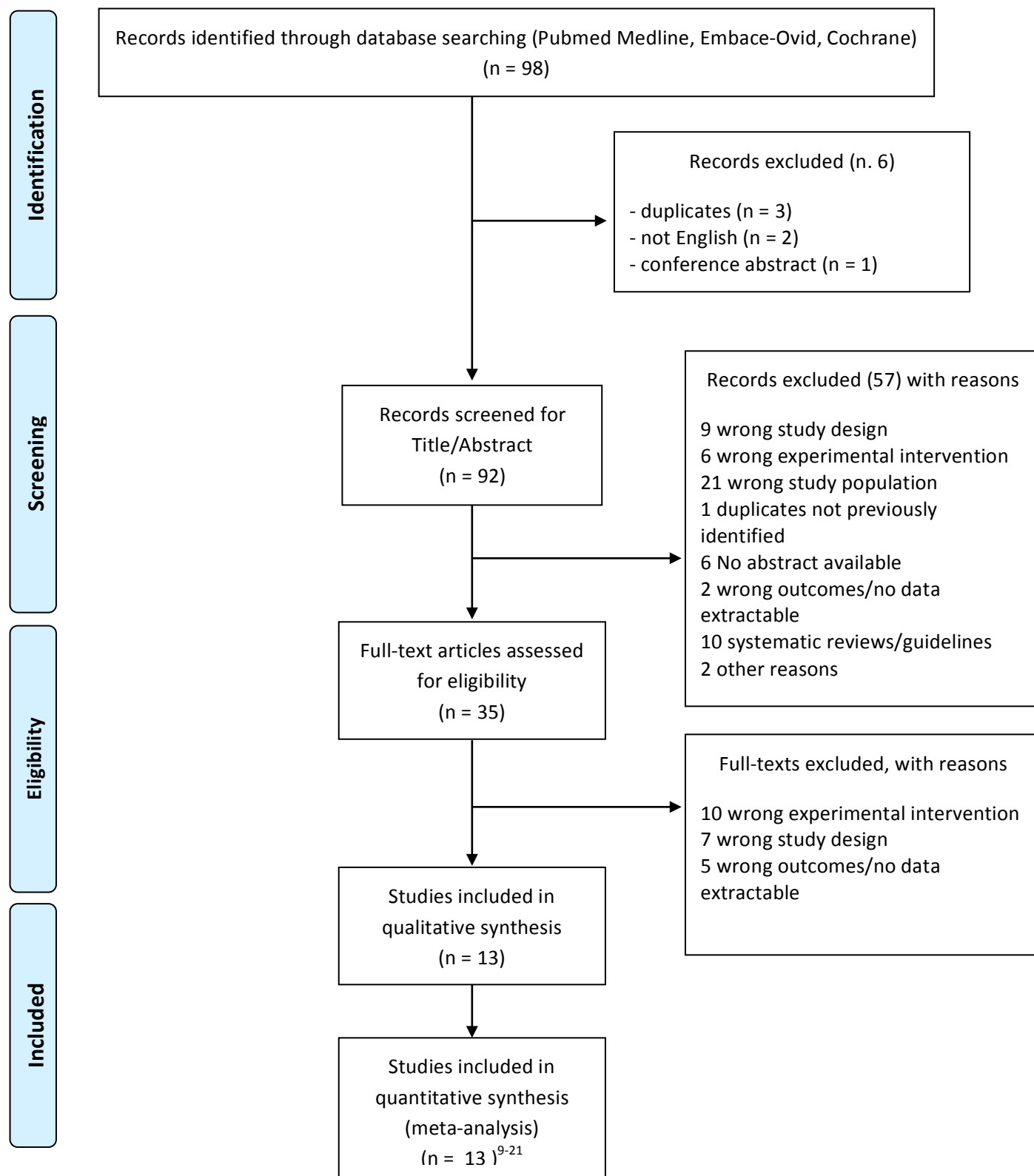


Figure 6 Flow-diagram of studies selection

II.3 Results

II.3.1 Search results

We identified through database searching 98 records: 6 have been excluded because duplicates or conference abstract or not English; 92 records have been screened for titles and abstracts; then we excluded 57 records and we assessed 35 full text articles for eligibility. Of these, 22 full text articles were excluded and 13 records were included for qualitative synthesis. We extract data from a quantitative analysis of 13 studies (9) (10) (11) (12) (13) (14) (15) (16) (17) (18) (19) (20) (21). For further descriptions see the study flow-diagram (*Fig. 6*)

II.3.2 Included studies

All 13 reports included in qualitative and quantitative analysis are reported in table 4. One was RCT, one randomized not controlled study, four Cohort studies, one prospective non randomized study, three observational retrospective study, three case series one of which was multicentric. The mean sample size was 11,6 (range: 5 to 27).

Population baseline characteristics (tab)

We included 13 reports with a total of 245 patients. We excluded from qualitative and quantitative analysis patients from studies who assumed immunosuppressants other than corticosteroids at the time of Rituximab initiation therapy. We also excluded patients with unclear history of concomitant Immunosuppressor therapy at the time of Rituximab initiation, not understandable from the articles (see below in *Intervention Characteristics*). In detail, we considered:

- 6 out of 11 patients from the Kanwar et al. study (9);

- 11 out of 50 patients from Gupta et al. study (11);
- 8 out of 19 patients from Gregoriou et al. study;
- 7 out of 23 patients from Cho et al. study (18);
- 5 out of 6 patients from Marzano et al. Study (19);
- 13 out of 35 patients from Zhang et al. Study (12);
- all patients from other studies.

So we included in the final quantitative analysis 151 patients, 128 with PV and 23 with PF.

We summarised the characteristics of the participants in the table below (*Table 4*). The overall mean age of participants was 45,2 years (SD 6,9); there were 77 women (51%) and 74 men (49%). 65,86% had a mucosal involvement. The overall mean duration of disease was 39,3 months (21,8 SD). The mean PDAI score at baseline, when reported, was 52,85.

Reports	Type of study	Patients (n)	Male (n)	Age M	PV (n)	PF (n)	Mucosal involvement (%)	PDAI	Duration (m)	CS	RTX 200	RTX 375/mq	RTX 500	Posology
Kanwar et al	RCT	6 (11)	4	38,2	4	2	.	.	14,4	100			x	1x2/14d
Singh et al	RT	11	3	42,6	10	1		46,6	21,6	100			X	1x2/14d
Gupta et al	Cohort study	11 (50)	4	37,8	10	1	.	.		27,3			X	1x2/14d
Zhang et al	Cohort study	13 (35)	6	52	11	2	.	78	13,5	100			X	1x2/14d
Zhou et al	Cohort study	19	6	44,2	19	0	94,7	22,5	22,5	100			X	single infusion
Russo et al	Prospective nonrandomize	8	5	54,3	8	0	.	64,3	52,5	100	X			single infusion
Fortuna et al	Retrospective	10	3	44,8	10		100	.	.	0		X		1 weekly x 4 w
Gregoriou et al	Cohort study	8 (19)	4	48	7	1			63,9	100		X		1 weekly x 4 w
Joly et al	Multicenter series	21	14	53,7	14	7	33		50,1	100		X		1 weekly x 4 w
Cho et al	Observ Retrospect	7 (23)	4	50,4	3	4			60,6	100		X		1 weekly x 2 w
Marzano et al	Case series	5 (6)	3	43,6	2	3	40	.	62,4	80		X		1 weekly x 4 w
Kim JH et al	Retrospective	27	14	48	25	2	81,5		13,5			X		1 weekly x 2,7 w
Antonucci et al	Case series	5	4	30,5	5	0	40		57,6			X		1 weekly x 4 w

Table 4 _ Population baseline characteristics of included studies

Intervention characteristics

We included study testing low-dose Rituximab or very-low-dose Rituximab. We excluded from the analysis patients undergoing Rituximab at conventional dosage or RA protocol (1000 mg). We also excluded patients who received immunosuppressor other than corticosteroids at the time of Rituximab infusion (*see population baseline characteristics above*). So we included in the final quantitative analysis 151 patients: 60 received Rituximab at the dose of 500 mg (1 or 2 infusions 14 days apart from each other), 83 received Rituximab at the hematologic dose of 375 mg/mq weekly (Lymphoma Protocol) for 2 or 3 or 4 weeks consecutively and 8 patients received a single infusion of Rituximab 200 mg.

II.3.3 Efficacy

Reports	Patients (n)	RTX 200	RTX 375/mq	RTX 500	Posology	CR n	CR %	CROT %	CRMT %	PR	TTCR (w)	TTDC (w)	Relapse (%)	TTR (m)	FU (m)	SAE
Kanwar et al	6 (11)			x	1x2/14d	6	100				16	5	83.3	9,2	12	0
Singh et al	11			X	1x2/14d	11	100				26					0
Gupta et al	11 (50)			X	1x2/14d	10	90	72,7	27,3	1	.	.	0		12	0
Zhang et al	13 (35)			X	1x2/14d	13	100		13		23,1	1,5	7,7	.		
Zhou et al	19			X	single infusion	10	52,63		100		34,5				12	0
Russo et al	8	X			single infusion	5	62,5	1	4	3		7,5	12,5		13,1	1 sepsi 1pneum
Fortuna et al	10		X		1 weekly x 4 w	7	70	40	30	3	19,8		20	7,4	14,3	0
Gregoriou et al	8 (19)		X		1 weekly x 4 w	8	100	100	0	0	19,5	10,5	0		18	0
Joly et al	21		X		1 weekly x 4 w	20	95	40	60				42,86	18,9	34	1sepsi 1Pneum
Cho et al	7 (23)		X		1 weekly x 2 w	4	57,1	25	75	3	15,5	3,7	28,6	9,5	17,1	0
Marzano et al	5 (6)		X		1 weekly x 4 w	4	80	25	75	1					16,4	0
Kim JH et al	27		X		1 weekly x 2,7 w	16	59			5	41,5	4	29,6		14,7	0
Antonucci et al	5		X		1 weekly x 4 w	5	100	40	60		4,5	7	40	13	12	0

Table 5_ Efficacy and safety endpoints of included studies

II.3.3.1 Efficacy Primary Outcomes

a) COMPLETE REMISSION

We analyzed all studies testing low-dose and ultra low-dose Rituximab. Pooled analysis showed an estimate rate of 84% to achieve complete remission (CR) (figure 7), in a mean of 15,9 months of Follow-up period, with 49% of patients experimented Complete Remission Off Therapy (CROT).

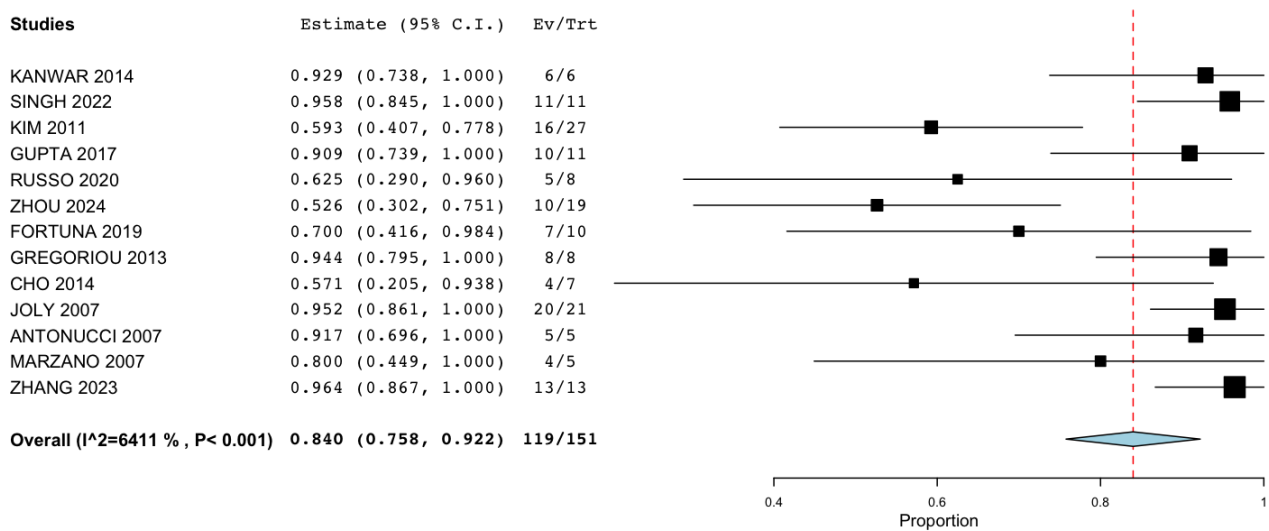


Fig. 7 Forest plot of achievement of CR

Further, although considering small sample sizes, there was no difference between Rituximab different dose regimens to achieve CR (Fig 8)

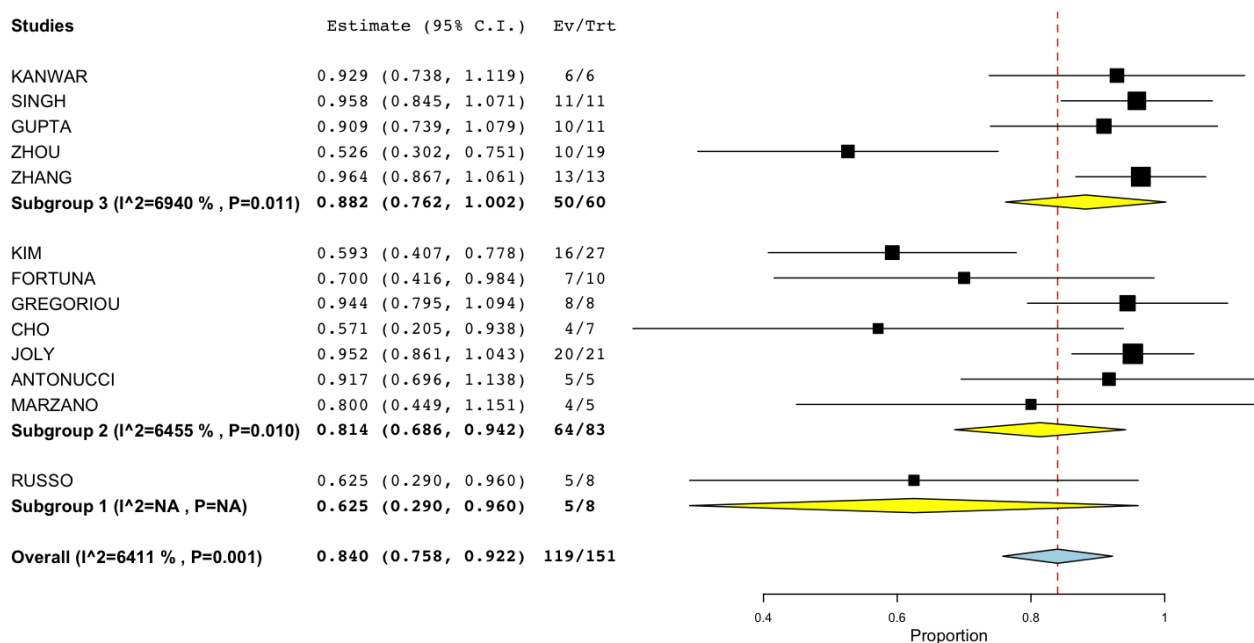


Fig. 8 Forest plot of achievement of CR with Rituximab different dose regimens (Subgroup 1: Rituximab 200 mg; Subgroup 2: Rituximab 375 mg/mq; Subgroup 3: Rituximab 500 mg)

Meta-regression

We documented a significant heterogeneity among studies about the primary efficacy end-point CR ($I^2=64.1\%$, $p<0.001$). Hence, we performed meta-regressions to explore sources of clinical and statistical heterogeneities among studies. Univariable meta-regression analysis revealed a significant inverse association between the estimate ratio of achieving CR and the percentage of mucosal involvement ($p<0.001$), showing that in studies including patients with higher mucosal involvement the probability of achieving CR with Rituximab low-dose was lower (Figure 9).

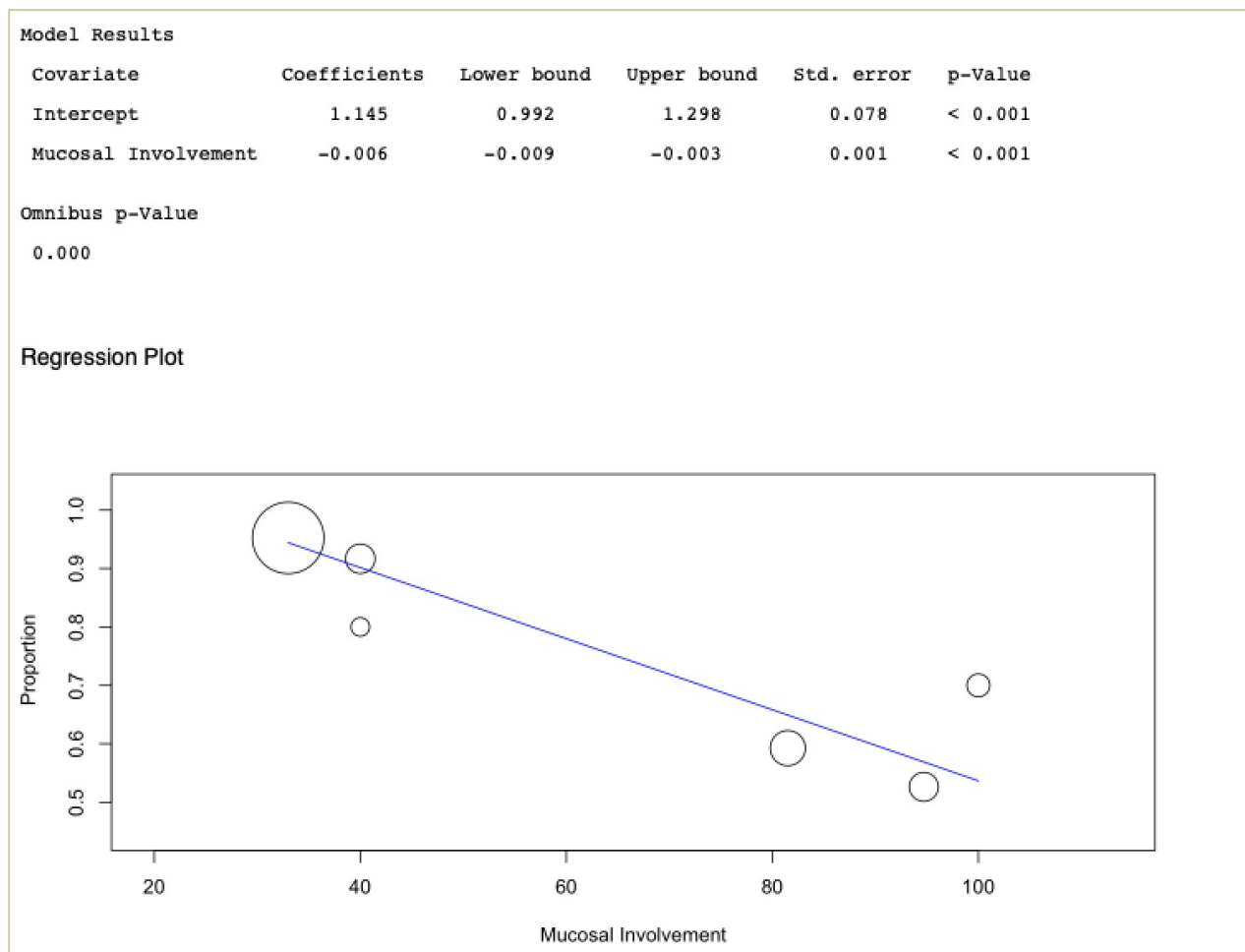


Figure 9_ Meta-regression bubble plot correlating CR and mucosal involvement

No association with CR was found at metaregression analysis with other covariates such as: male sex, age, type of pemphigus (vulgaris vs foliaceus) (fig 10), PDAI and use of corticosteroids.

Covariate	Coefficients	Lower bound	Upper bound	Std. error	p-Value
Intercept	0.957	0.532	1.383	0.217	< 0.001
PV%	-0.001	-0.006	0.004	0.003	0.578

Omnibus p-Value

0.578

Regression Plot

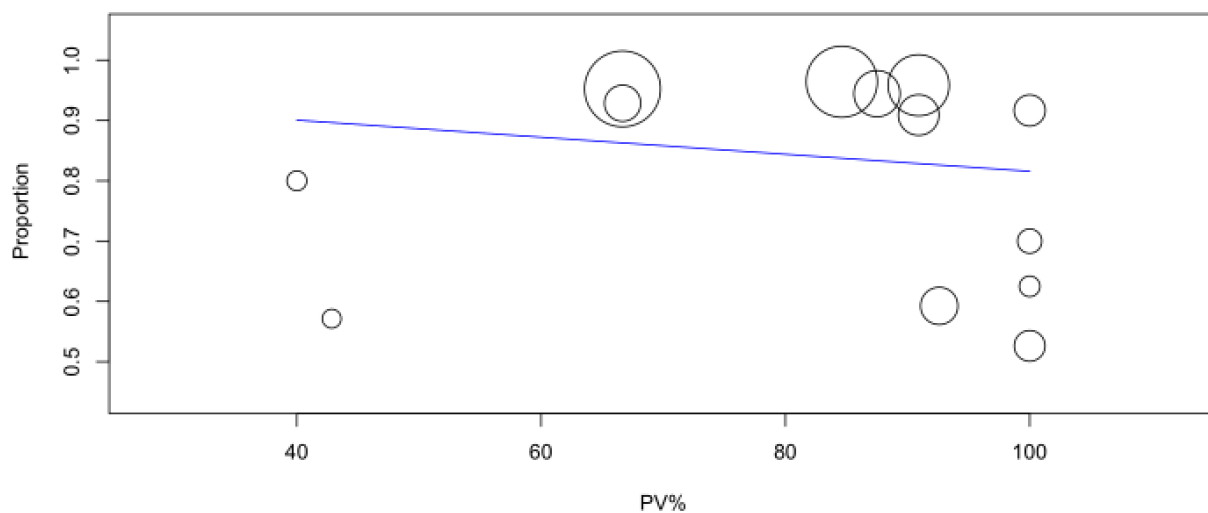


Figure 10_ Meta-regression bubble plot correlating CR and PV %

b) TIME TO REACH COMPLETE REMISSION (TTCR)

We assessed TTCR pooling data from 9 studies reporting these data. Mean pooled TTCR was 22.2 weeks (*Figure 11*).

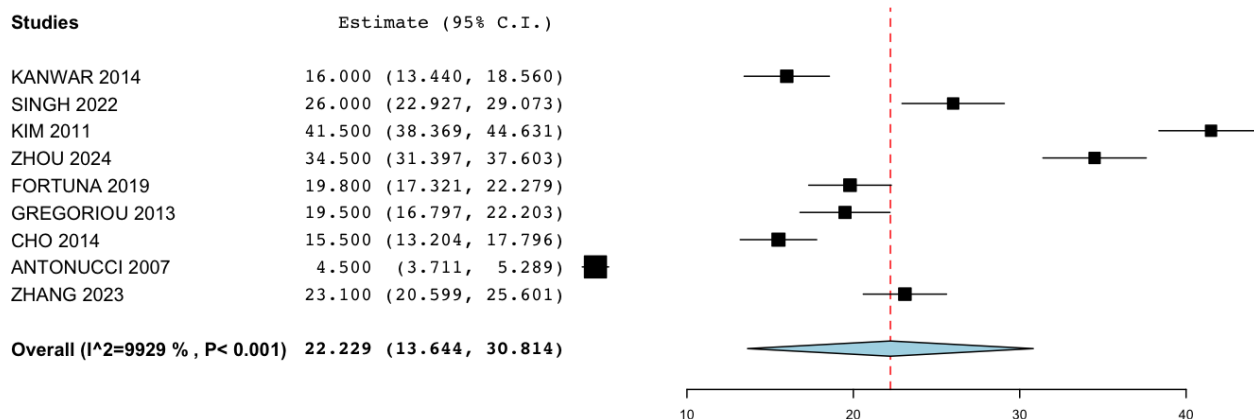


Figure 11_ Forest plot for TTCR.

No significant differences were observed between different Rituximab dose regimens (Figure 12).

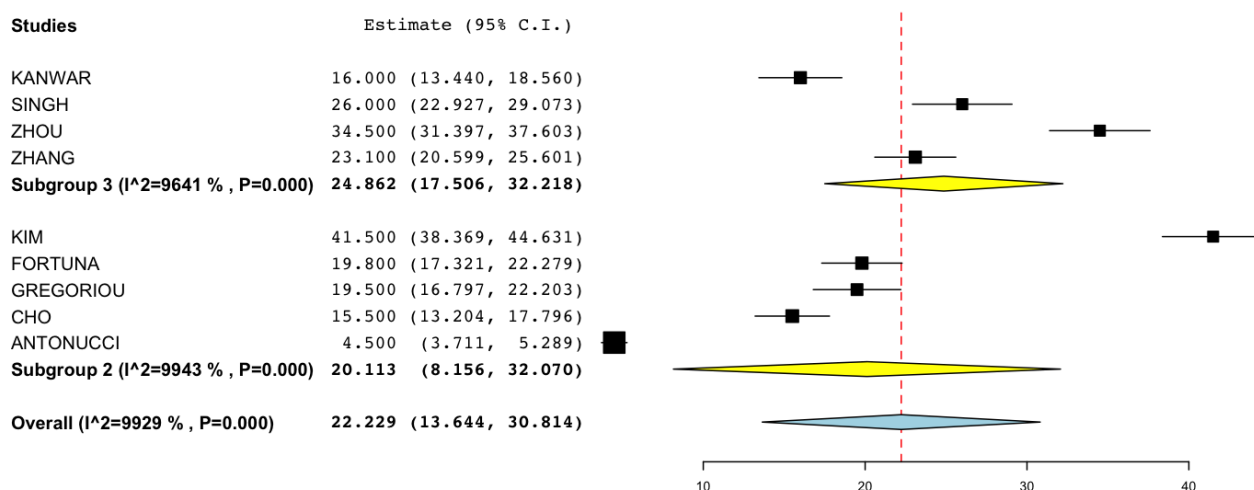


Figure 12_ Forest plot of TTCR between different rituximab doses regimens regimens (Subgroup 1: Rituximab 200 mg; Subgroup 2: Rituximab 375 mg/mq; Subgroup 3: Rituximab 500 mg).

Meta-regression

We documented a significant heterogeneity among studies about the primary efficacy end-point CR ($I^2=99.2\%$, $p<0.001$). Hence, we performed meta-regressions to explore sources of clinical and statistical heterogeneities among studies. Univariable meta-regression analysis didn't reveal association between the pooled mean TTCR with other covariates such as: male sex, age, type of pemphigus (vulgaris vs foliaceus), PDAI and use of corticosteroids.

II.3.3.2 Efficacy Secondary Outcomes

A) RELAPSE (%)

We analyzed 10 studies reporting rates of relapse after low-dose Rituximab. Pooled analysis showed an estimate rate of 24.4% of relapse (figure 13).

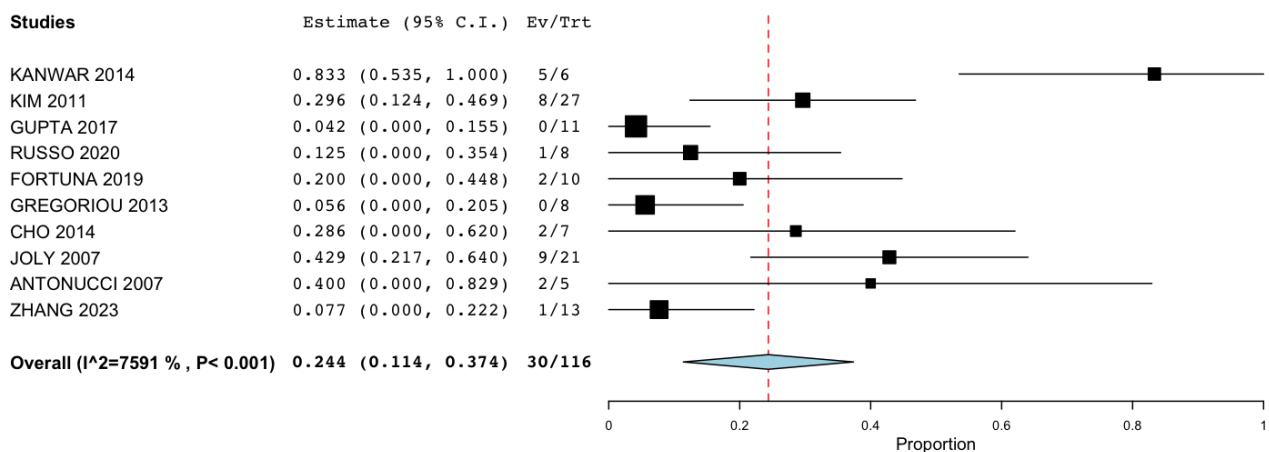


Fig. 13 Forest plot of pooled estimate ratio of relapse

Further, although considering small sample sizes, there was no difference between Rituximab different dose regimens and the risk of relapse (Fig 14)

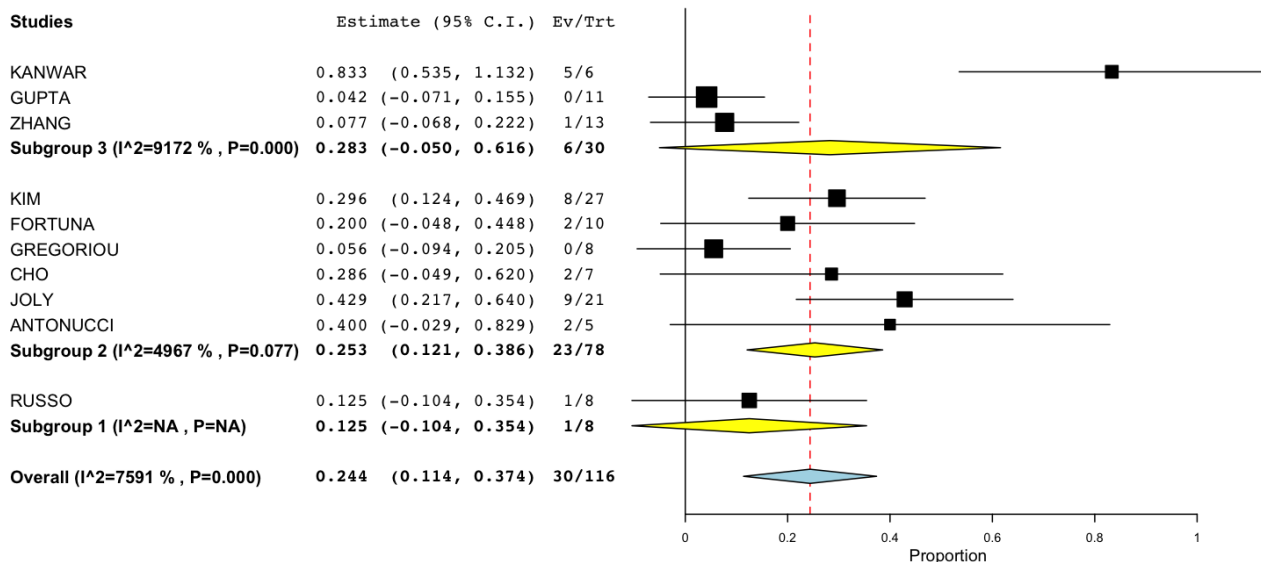


Fig. 14 Forest plot of pooled estimate ratios of relapse with Rituximab different dose regimens (Subgroup 1: Rituximab 200 mg; Subgroup 2: Rituximab 375 mg/mq; Subgroup 3: Rituximab 500 mg)

Meta-regression

We documented a significant heterogeneity among studies about the primary efficacy end-point CR ($I^2=75.9\%$, $p<0.001$). Hence, we performed meta-regressions to explore sources of clinical and statistical heterogeneities among studies. Univariable meta-regression analysis revealed a trend towards an inverse association between the estimate ratio of relapse and the percentage of pemphigo vulgaris ($p=0.077$), showing that in studies including more patients with PV as compared to foliaceus pemphiguss the probability of relapse with low-dose Rituximab is lower (Figure 15).

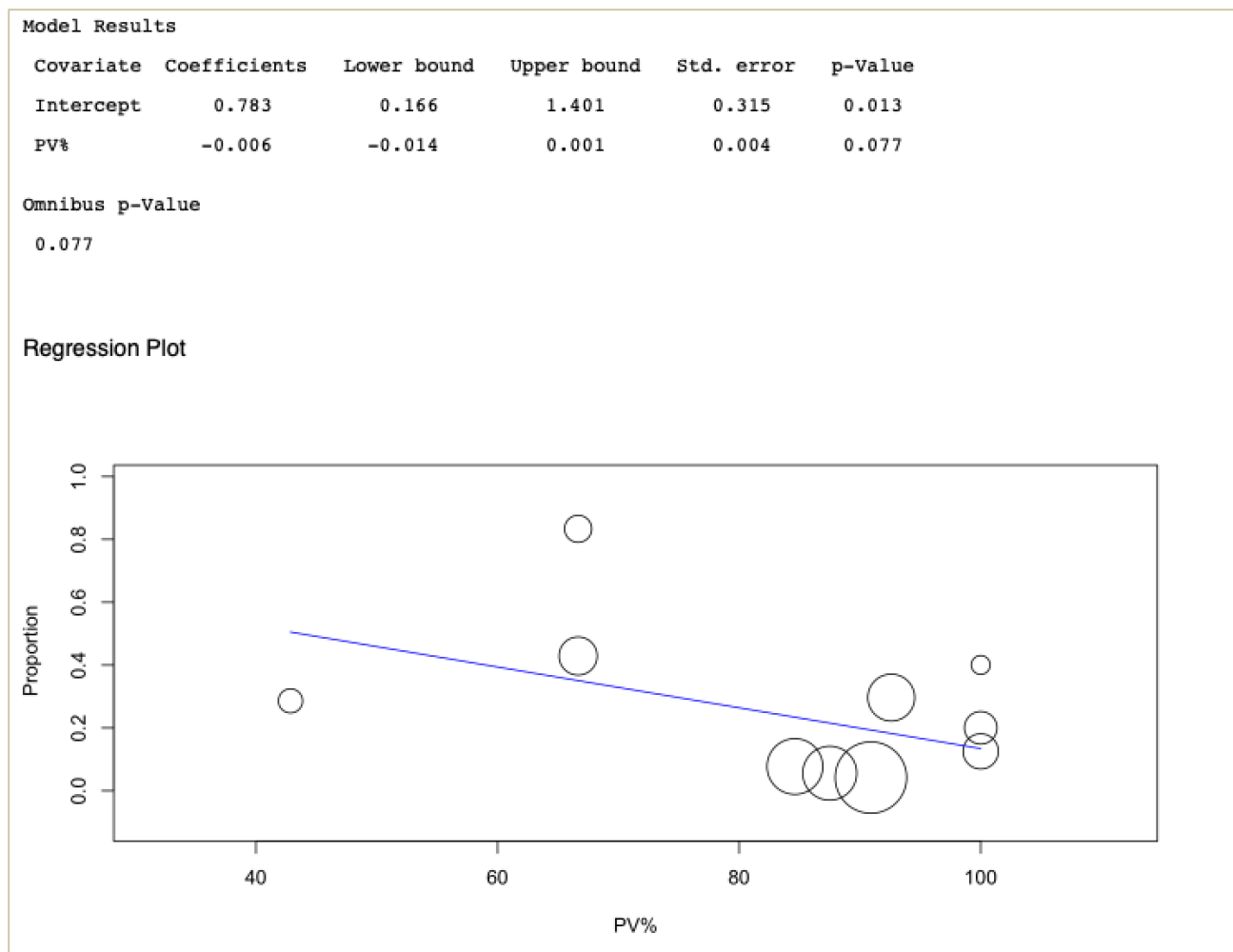


Figure 15_ Meta-regression bubble plot correlating Relapse % and PV

No association with percentage of Relapse was found at metaregression analysis with other covariates such as: male sex, age, mucosal involvement, length of disease, PDAI and use of corticosteroids.

B) TIME TO RELAPSE (TTR)

Only 5 studies reported TTR. Mean pooled TTR was 11.5 months (*Figure 16*).

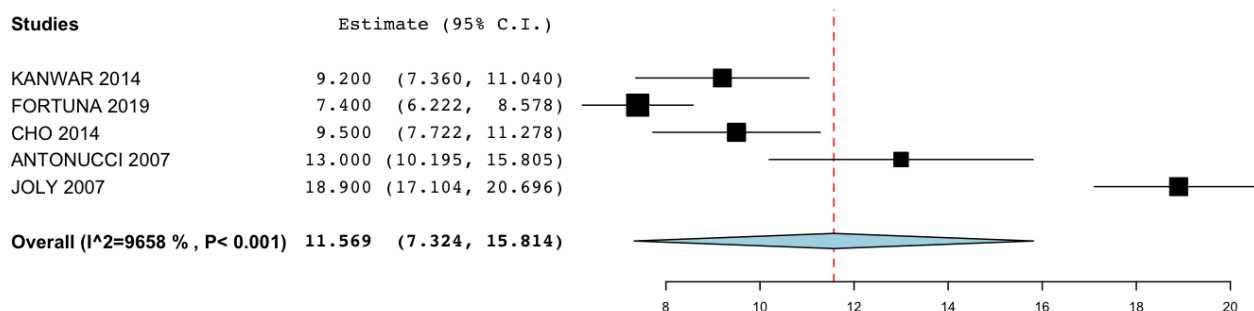


Figure 16_ Forest plot for TTR.

We documented a significant heterogeneity among studies ($I^2=96.5\%$, $p<0.001$), but due to the low number of studies included for this endpoint, no meta-regression or subgroup analysis has been performed.

II.3.4 Safety

No SAE was reported from the majority of studies. Only 2 authors out of 13 (Russo et al (14). and Joly et al (17)) described 2 SAE per each respectively, with a total number of 4 patients with SAE on a total number of 151 patients (2,6%), 2 sepsi, 1 of which occurred 12 months later rituximab infusion and was fatal (Joly et al), and two Pulmonitis.

Other AEs mentioned were infusion-related events predominantly, due to missed / not correct pre-medication before Rituximab infusion or high speed of infusion; they were usually reversible and temporary AEs and consisting in headache, nausea, chills, vomiting.

II.4 Discussion

This Systematic review and Meta-analysis confirmed the excellent response of patients affected by Pemphigus at low dose regimens of Rituximab other than conventional one.

In particular, in this SR we found that patients with Pemphigus undergoing low and ultra-low dose RTX, reached a Complete Remission rate (CR) in 87% of cases, about an half of them without systemic Therapy (49% CROT) with a very speed of action, as demonstrated by the mean of 5,6 weeks to reach Disease Control (TTDC) and the mean of 22,2 weeks necessary to reach Complete Remission (TTCR).

We did not see any differences among the three different dose regimens of 200 mg, 375mg/mq and 500 mg respect to the primary and secondary efficacy outcomes explored.

Many interesting data have emerged from Metaregression analysis.

Studies including a major percentage of patients having a mucosal involvement seems to have a worse outcomes in terms of CR% responses, instead of studies with a lower prevalence of mucosal involvement. Therefore, the mucosal involvement in patients with PV e PF could represent a negative predictive factor of response to treatment in terms of CR.

Moreover, our metaregression showed that reports with a major prevalence of patients with PV respect PF, have a lower probability to have relapses. So the type of pemphigus could influence the response to the treatment and the course of the illness, with PV type assuming a positive predictive value of response and better clinical outcome after low-dose RTX therapy.

The use of concomitant systemic corticosteroid during low-dose RTX therapy seems not to influence primary and secondary efficacy and safety outcomes in studies analysed, as well as the age, gender, severity and duration of disease.

Besides, in this SR we underlined the safety of the low and ultra-low doses RTX therapy for Pemphigus, as only 4 patients on 151 expressed SAE.

SECTION III

DIFFERENT DOSE REGIMENS OF ANTI-CD 20 MONOCLONAL ANTIBODY (RITUXIMAB) IN PATIENTS WITH SEVERE AND RECALCITRANT AUTOIMMUNE BULLOUS DISEASES: CASE SERIES OF THE UNIVERSITY DERMATOLOGIC CLINIC OF CHIETI-ORTONA

III.1 Introduction

Pemphigus includes a group of rare autoimmune bullous diseases characterized by flaccid blisters and erosions of the mucous membranes and skin. This sometimes life-threatening condition, is characterized by the presence of IgG autoantibodies against the desmosomal adhesion proteins, desmoglein 3 and/or desmoglein 1, on epidermal keratinocytes, that are responsible of intraepithelial blister formation.

The severity of the disease is based on its progressive course which is accompanied by an increased body catabolism with loss of body fluids and proteins and secondary bacterial and viral infections which may lead to sepsis and heart failure.

Rituximab is a chimeric monoclonal antibody approved for treatment of adults with severe and refractory pemphigus vulgaris. Recent studies have focused on assessments of efficacy and safety of low-dose rituximab (<2 gram in each cycle).

We reported our experiences based on a case series of seven patients treated with Rituximab at rheumatologic dose regimen (1000) and ultra-low (200) dose regimen.

III.2 Materials and Methods

Seven patients with extensive pemphigus were selected (Table 1), who either had recalcitrant pemphigus, were steroid dependent, had relapsed after pulse therapy, had contraindications to conventional treatment or wanted to avoid conventional treatment and its side effects. Two doses of conventional rituximab (1000 mg) or ultra low-dose rituximab (200 mg) were given 2 weeks apart and patients were regularly followed up every 2 weeks for the first month and then every three months. Complete blood counts, liver function tests, renal function tests, flow cytometry assessments of B-cell subtypes, skin biopsy, direct immunofluorescence and desmoglein levels were checked before and after rituximab administration. Pre-rituximab chest X-ray, electrocardiograph, ophthalmic evaluation and computed bone mineral density were also obtained. Clinical response was defined by the criteria outlined in the consensus statement on definitions of endpoints and therapeutic response for pemphigus (22). Complete remission off therapy (CROT) was defined as complete epithelialization and absence of new or established lesions while the patient is off all systemic therapy for at least 2 months while complete remission on minimal therapy (CRMT) was defined as the absence of new lesions while the patient is receiving minimal doses of systemic therapy. Partial remission off therapy (PROT) was defined as the presence of transient new lesions that healed within 1 week without treatment and while off all systemic therapy. Minimal therapy was defined as prednisone up to 10 mg/day or azathioprine up to 1.25 mg/kg/day. Relapse was defined as the appearance of at least three new lesions in 1 month that did not heal spontaneously within 1 week or the extension of established lesions in a patient who had previously achieved disease control.

III.3 Results

We recruited seven patients with an average age of 63.1 years old, three were males and four females (Table 6). All of them undergoing and failed previous systemic corticosteroid therapy (Metilprednisolone 1 mg/kg/die or multiple boluses of 1 gr) and/or systemic immunosuppressant (Azathioprine or Mycophenolate). Two male patients were treated with two doses of Rituximab at rheumatologic regimen (1000 mg) two weeks away. One male patient and three female patients were treated with two ultra-low doses of Rituximab (200 mg) two weeks away.

PATIENTS	AGE	SEX	DESEASE	PREVIOUS THERAPY	DOSE T0	DOSE T1 (2w)
1	82	M	Pemfigo	CS	1000	1000
2	32	M	Pemfigo	CS, AZT	1000	1000
3	74	M	Pemfigo	CS	200	200
	66	F	Pemfigo	CS	200	200
5	55	F	Pemfigo	CS, MMF, AZT	200	200
6	64	F	Pemfigo	CS, Dapsone, MMF, evIG	200	200
7	69	F	Pemfigo	CS, RTX	200	200

Table 6. Population demographic characteristics of our case series

At 3 months, the two patients treated with Rituximab at rheumatologic regimen (1000 mg) showed respectively a complete remission off therapy (CROT) and a complete remission on minimal therapy (CRMT). At 3 month, the five patients treated with ultra-low doses of Rituximab (200 mg)

achieved respectively: three patients a complete remission off therapy (CROT), one patient a complete remission on minimal therapy (CRMT) and one patient a partial remission off therapy (PROT) (Table 2; Fig 17).

Two patients showed adverse events, in detail, one patient treated with Rituximab 1000 mg showed defect of vision like visual blurring and one patient treated with Rituximab 200 mg developed dizziness (Table 2).

Flow cytometry assessments of B-cell subtypes displayed a lasting maintenance of the zeroing of linf B cd19 + and cd19 / 45 + at three months and at 6 months in all patient except one male patient treated with 200 mg (Table 6; Fig 18).

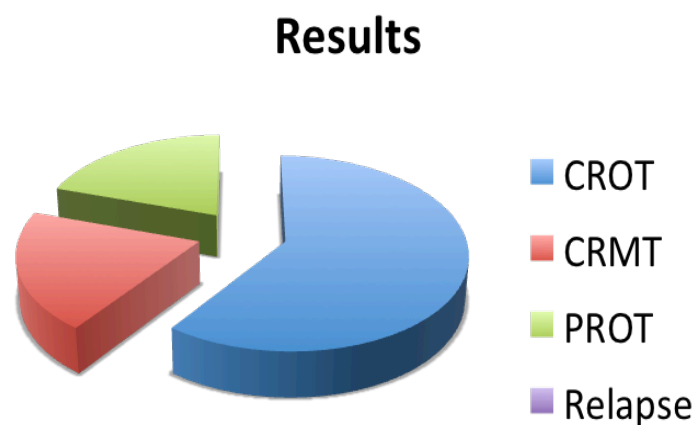


Figure 17. Results at week 12, in patients undergoing two doses of low-dose Rituximab (200 mg). CROT complete remission off therapy; CRMT: complete remission on minimal therapy; PROT: partial remission off therapy.

PATIENTS	AGE	SEX	DOSE T0	RESULTS (12w)	CD19+ CD19+/CD45+ +% (4 w)	CD19+ CD19+/CD45+ % T2 (12w)	CD19+ CD19+/CD45+ % T3 (24w)	AEs
1	82	M	1000	CROT	0/0	0/0	0/0	-
2	32	M	1000	CRMT	0/0	0/0	0/0	EP
3	74	M	200	CROT	2/0	2/0	1/0	headache
4	66	F	200	PROT	0/0	0/0	191/10	dizziness
5	55	F	200	CROT	0/0	0/0	0/0	-
6	64	F	200	CROT	0/0	1/0	1/0	-
7	69	F	200	CRMT	0/0	1/0	1/0	-

Table 6. Results of efficacy and safety in patients undergoing Rituximab at conventional and ultra low-dose regimen

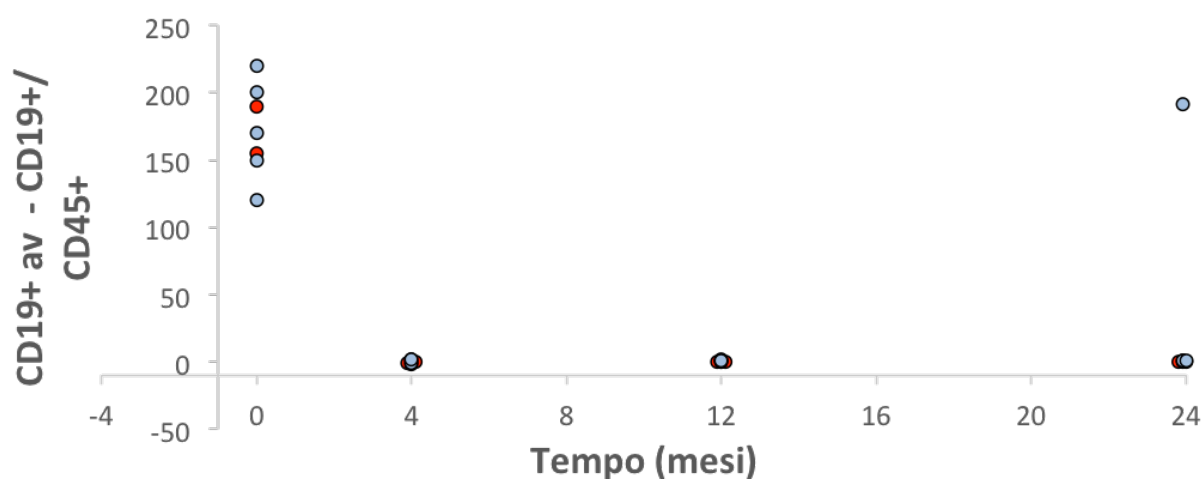


Figure 18. Maintenance of zeroing cd19+ and cd19+/45+ B lymphocytes since first week, maintained after 3 and 6 months from the first infusion.

III.4 Discussion

Rituximab has been approved as a treatment for moderate-to-severe Pemphigus Vulgaris (PV). By depleting B cells, rituximab decreases circulating anti-desmoglein autoantibodies. However, the optimal dosage has not been standardized. Since the B-cell burden in autoimmune blistering skin diseases is much lower than that in lymphoproliferative disorders, lower dosage regimens, as low as a single infusion of 200 mg has been reported to be effective for PV (14). Schoergenhofer et al. (23) showed that CD20+ cells were depleted by 68%, 74%, and 97% at 1 h after the infusion of rituximab at the doses of 0.1, 0.3, and 1 mg/m², respectively, and the CD20+ cell counts gradually returned to baseline in 1–9 months. Therefore, the authors extrapolated that 100 mg Rituximab may be sufficient to fully suppress CD20+ cells for 3 months.

In our series low-dose Rituximab regimen was associated with a good and prolonged clinical response with zeroing of cd19+ and cd19+/45+ B lymphocytes since first week, maintained after 3 and 6 months from the first infusion (Graphic 2). No serious side adverse events was reported, and only one male developed dizziness.

Our data show that this strategy can be effective even in patients with extensive and recalcitrant pemphigus, with a lower probability of side-effects, thus potentially reducing the negative consequences deriving from long-term use of corticosteroids and other immunosuppressive drugs.

Instead higher Rituximab doses “at rheumatologic regimen”, may be necessary in very severe cases with high levels of anti-desmoglein 3 antibodies, which are considered predictive of therapy failure. Such preliminary hypothesis need to be confirmed on larger and possibly randomized prospective clinical trials.

Our results suggest that ultra low-dose rituximab can be a well-tolerated and effective adjuvant therapy in recalcitrant and/or severe pemphigus which may also act as a steroid-sparing strategy, avoiding long-term side effects of chronic corticosteroids intake.

CONCLUSIONS

Rituximab is a monoclonal antibody approved for treatment of adults with severe and refractory pemphigus vulgaris. Concerns about side effects and high costs of conventional doses have risen the hypothesis that low-dose rituximab regimen may be cost-effective with a better safety profile.

Targeted B-cell localization is associated with better clinical outcomes, including less frequent relapses. Relapse rates across lymphoma dosing (375 mg/m²) versus rheumatoid arthritis dosing (1 g dosing weekly) was inconsistent. Moreover, differences in patient and disease characteristics that are highlighted in the literature strongly suggest that therapy should be tailored individually on a case-by-case basis.

Here we report our experience of seven patients with extensive/recalcitrant pemphigus, who either were steroid dependent, had contraindications or refused conventional treatment. Two patients received conventional rituximab (1000 mg 2 weeks apart) while five received ultra low-dose rituximab (200 mg 2 weeks apart). At 3 months, the two patients treated with high Rituximab regimen showed respectively a complete remission off therapy (CROT) and a complete remission on minimal therapy (CRMT), while among the five patients treated with ultra low-doses, three achieved CROT, one achieved CRMT and one a partial remission off therapy (PROT).

All patients treated with ultra low-dose rituximab achieved complete depletion of cd19+ and cd19/45+ B lymphocytes after three months and all patients except one male maintained the zeroing after 6 months. No serious side effect was documented with low dose regimen except for a case of dizziness. Our data suggest that ultra low-dose rituximab can be effective even in patients with extensive/recalcitrant pemphigus, with a lower probability of side-effects respect to higher dose regimen and may act as a steroid sparing strategy.

We also highlighted from the systematic review of the literature and meta-analysis that the mucosal involvement in patients with PV e PF could represent a negative predictive factor of response to treatment in terms of CR. Moreover, the type of pemphigus could influence the response to the

treatment and the course of the illness, with PV type assuming a positive predictive value of response with lower rate of relapse in % and better clinical outcome after low-dose RTX therapy respect of PF type. No differences was seen among different regimens of low and ultra-low dose RTX treatment.

Further evidence from rigorous clinical trials will be required to optimize lower-dose regimens of RTX and establish their position within the treatment scenario of pemphigus vulgaris.

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APPENDIX

1. Glossary

PDAI	The PASI combines the extent of body surface involvement in 4 anatomical regions (head, trunk, arms, and legs). For each region the percent area of skin involved was estimated from 0 (0%) to 6 (90%-100%) and severity was estimated by clinical signs of erythema, induration and scaling with a scores range from 0 (no involvement) to 4 (severe involvement). Each area is scored by itself and the scores were then combined for the final PASI. Final PASI calculated as: sum of severity parameters for each region * area score * weighing factor (head [0.1], upper limbs [0.2], trunk [0.3], lower limbs [0.4]). Overall scores range from 0 (no Ps) to 72 (the most severe disease). Patients achieving PASI90 were defined as having an improvement of $\geq 90\%$ in the PASI score compared to baseline
CROT	CROT was defined as the absence of new or established lesions while the patient was off all systemic therapy for at least 2 months
CRMT	CRMT was defined as the same above while the patient was receiving minimal therapy.
PROT	PROT was defined as the presence of transient new lesions that heal within 1 week while the patient was off systemic therapy for at least 2 months.
PRMT	PRMT was defined as the same state as that of PROT while the patient was receiving minimal therapy
LLCR	Longlasting complete response (LLCR) was defined as being lesion-free period without the need for glucocorticoids or adjuvant immunosuppressives for a continuous period of 6 months.
TDC	Time to disease control (TDC) was defined as the time interval between the baseline and the time at which new lesions cease to form
TCR	Time to complete remission (TCR) was defined as the time interval between the baseline and the time at wich patient achieve Complete Remission (CR)
MINIMAL THERAPY	Minimal therapy was defined as prednisone at 10 mg per day or half the dose required to control disease activity.
RELAPSE	Relapse was defined as the appearance of three or more lesions over 1 month that do not heal spontaneously within 1 week.
DLQI 0/1	The DLQI consists of 10 standardised questions concerning the last week health-related quality of life of patients with psoriasis. Patients score every question based on the level of strain from “not at all” (0 points) to “very much” (3 points). The total score ranges from 0 to 30, with scores of 10 or above indicating a severe impairment of quality of life. On an international level, the DLQI is one of the most important instruments when measuring quality of life of psoriasis patients. DLQI 0/1 mean no impact on quality of life.

2. MEDLINE search strategy

("pemphigus"[MeSH Terms] OR "pemphigus"[All Fields]) AND ("rituximab"[MeSH Terms] OR "rituximab"[All Fields] OR "rituximab s"[All Fields] OR "anti CD 20"[All Fields] OR "anti CD20"[All Fields] OR "Rituxan"[All Fields] OR "Mabthera"[All Fields]) AND ("500"[All Fields] OR "375"[All Fields] OR "200"[All Fields] OR "low dose"[All Fields] OR "Low dosage"[All Fields] OR "low dose"[All Fields] OR "very low dose"[All Fields] OR "very low dosage"[All Fields] OR "very low dose"[All Fields] OR "very low dose"[All Fields] OR "ultra-low dose"[All Fields] OR "ultra-low dosage"[All Fields] OR "ultra-low dose"[All Fields] OR "ultra-low dosage"[All Fields] OR "ultralow-dose"[All Fields] OR "ultralow dosage"[All Fields] OR "ultralow-dose"[All Fields] OR "reduced dose"[All Fields] OR "reduced dosage"[All Fields] OR "half-dose"[All Fields] OR "half dosage"[All Fields] OR "half-dose"[All Fields] OR "intermediate dose"[All Fields] OR "intermediate dosage"[All Fields] OR "modified dose"[All Fields] OR "modified dosage"[All Fields] OR "dose comparison"[All Fields] OR "dosage comparison"[All Fields])) AND (2000/1/1:2025/2/3[pdat])

Database(s): Ovid MEDLINE(R) Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R) Daily and Ovid MEDLINE(R) 1946 to Present
Search Strategy run on Feb 03, 2025 through OVID.