New Immunosuppression Drugs in kidney transplantation

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INTRODUCTION

- Finding new innovative drugs for kidney transplantation is not easy but looking for unmet needs it is possible to find new interesting drugs and opportunities to use in kidney transplantation.

Principal unmet needs are

1. Treatment and prevention of delayed graft function
2. Improve the long-term outcomes
3. Desensitization
4. Treatment of acute antibody-mediated rejection.
5. Transplant from non-heart beating donor
6. Transplant in ABO incompatibles pairs.

Drugs for unmet therapeutic needs

These drugs may be categorized as follows

(1) Therapy for ischemia-reperfusion injury (IRI) that results in delayed graft function (DGF).

(2) Therapy to preserve optimal kidney function over the long-term.

(3) Therapy for desensitization and antibody-mediated rejection (ABMR).

THERAPY FOR DGF

- DGF refers to acute kidney injury (AKI) occurring in the first week of transplantation that cannot be ascribed to acute rejection.
    - DGF is associated with
- Increased immune activation
- Complement activation
- Release of damage associated molecular patterns, such as hypomethylated DNA, hyaluronic acid, heparin sulfate, fibrinogen and heat shock proteins.
- Consequently, NFκB is activated and induces inflammatory cytokines such as IL-1, IL-6, TNF-alpha and interferon beta.
- Due to this complex mechanism, although several drugs to treat DGF have been tried, many of them failed to prove their effectiveness.
- Indeed, DGF has also been called the graveyard of drugs for transplantation.

THERAPY FOR DGF

- **Anti-apoptotic strategies**

- Apoptosis plays an important role in shaping DGF. Indeed, the pro-apoptotic gene p53 is activated by hypoxia and induces cell cycle arrest and apoptosis.

- QPI-1002 also known as 15 NP, is a short interfering RNA that inhibits the expression of gene p53. The results of a phase I/II clinical trial in kidney transplant recipients demonstrated beneficial effects on IRI/DGF in humans.

- Additionally, two studies reported good results in mice.

THERAPY FOR DGF

- **Pegylated carboxyhemoglobin Carbon monoxide (CO)** is involved in regulating endothelial cell survival and proliferation. It also plays roles in protecting against DGF through IRI, vessel relaxation and inhibition of proinflammatory responses.
- CO is a very powerful anti-apoptotic substance and has anti-inflammatory effects.
- In animal studies, CO is extremely effective in both cold and warm ischemia.
- The use of pegylated carboxyhemoglobin is currently the object of a phase 2/3 study to analyze the efficacy and safety for reducing the DGF rate in patients receiving a kidney transplant.
- In a recent study by Thuillier et al , 3 oxygen transporters, HBOC-201, BbV and M101, were tested in organ preservation.

THERAPY FOR DGF

- **Relaxin** (RLX) has an anti-inflammatory effect by Reducing the
- 1. expression of ICAM-1
- 2. Inducing the expression of Notch1 in macrophages (DEACRESE APOPTOSIS)
- 3. Reducing neutrophil adhesion through increased synthesis of nitric oxide.
- 4.vasodilatation through increased NO production
- 5. inhibition of endothelin 1 production.
- Two studies documented improved renal function, histologic improvement in damaged tissue after DGF, and a reduced number of apoptotic cells.

THERAPY FOR DGF

- **Hepatocyte growth factor**

- ANG-3777, formerly BB3, is a hepatocyte growth factor (HGF) mimetic that binds to its transmembrane tyrosine kinase receptor, cMET.

- In preclinical studies, ANG-3777 was renoprotective in a variety of animal models of AKI, exerting anti-inflammatory and regenerative effects and preventing tubular cell apoptosis, epithelial to mesenchymal transition and fibrosis.

- In a randomized, placebo-controlled phase 2 trial on oliguric patients after kidney transplantation, patients treated with ANG-3777 had a larger increase in urine output, a greater reduction in CRP and neutrophil gelatinase associated lipocalin (NGAL) and a higher eGFR.

- More recently, Vincenti et al started the Graft Improvement Following Transplant (GIFT) trial, which is a phase 3 trial on the HGF mimetic (ANG-3777) in kidney transplant recipients with DGF.
- In addition, the authors stress that a significant factor is that ANG-3777 may also be effective when administered after AKI-related DGF

THERAPY FOR DGF

- **Complement inhibition**

- Complement activation causes and precedes DGF. The most studied among the complement inhibitor drugs to minimize DGF has been Miroccept (APT 070), which inhibits C3/C5 convertases and C1 esterase inhibitors.
- Miroccept, still in a phase 1 trial, is a potent membrane-localizing complement inhibitor and may be administered ex vivo to the donor kidney prior to transplantation.
- However, a recent dose finding study in animals documented that a high dose of Miroccept might be needed to achieve adequate complement inhibition. More promising results have been obtained with C1 esterase inhibition.
- This drug may also be administered as a donor pretreatment strategy in high-risk recipients (NCT02435732), but the trial results are still unknown.
- Better results have been obtained by administering C1 esterase inhibitors to recipients of kidneys from high-risk donors or in the case of donation after circulatory death (DCD).
- A recent study from Huang et al, studied the three-year outcomes of patients treated with C1 esterase inhibitors to avoid DGF in a randomized controlled study.
- The study found that the treatment was associated with a lower incidence of graft failure

THERAPY TO PRESERVE RENAL FUNCTION

- These drugs may be divided into the following categories:
- (1) Therapy to avoid nephrotoxicity, usually by elimination of calcineurin inhibitors (CNIs).

- (2) Therapy to control inflammation and fibrosis (principally when inflammation overlaps fibrosis).

- (3) Therapy to prevent donor-specific antibodies (DSAs) and treat chronic ABMR (cABMR).

Therapy to avoid nephrotoxicity induced by CNIs

- Until recently and even today, the two main strategies for a CNI-free regimen have been as follows:
- 1. MTOR- inhibitor based immunosuppression
- 2. Belatacept based immunosuppression.

- Several studies have documented the efficacy of Everolimus therapy in conjunction with low-dose CNIs.
- The study by Pascual et al “the Advancing renal TRANSplant eFficacy and safety Outcomes with eveRoliMus based regimen (TRANSFORM)” was a randomized open label, two-arm study with 2037 de novo kidney transplant recipients recruited in 186 centers worldwide.
- Everolimus efficacy was demonstrated, but the administration of low-dose tacrolimus (TAC) was needed. The complete withdrawal of CNIs is difficult to achieve and is only appropriate for low-risk patients and donors and for living donors, and in the absence of DSAs.
- The use of belatacept or other agents blocking the costimulatory pathways is the other method to avoid CNIs.

Therapy to avoid nephrotoxicity induced by CNIs

- The blockade of CD28/cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) on T effector lymphocytes and CD80/CD86 on antigen presenting cells (APCs) was the first pathway to be targeted in the trials BENEFIT and BENEFIT-EXT .
- Independent of well-preserved kidney function, the use of Belatacept in a subset of patients was associated with an increased number of severe rejections and an increased number of opportunistic infections , including cytomegalovirus.
- In addition a correlation between the incidence of post-lymphoproliferative disease and Epstein-Barr virus seronegative patients in the belatacept group was found.
- These drawbacks are related to the fact that belatacept, which binds to CD80 and CD86 on APCs, blocks not only the T effectors that represent the positive signal but also the regulatory T (Tregs) that constitute the inhibitory signal (Figure 1).
- In 2015, a report showed that the blockade of CD28 on (effector T cells) without inhibition of Treg cells prolonged survival in a nonhuman primate kidney transplant model. In this way, effector cells can be inhibited without inhibiting Tregs because selective CD28 blockade allows inhibitory signals via CTLA-4 and programmed cell death ligand-1 to remain intact while blocking T cell activation by CD28 (Figure 2).
- Selective targeting of the CD28 antigen on T cells might be a more effective immunosuppressive therapy than belatacept, since this blockade leaves the inhibitory signal of CTLA-4 intact and may preserve Treg functions.

Therapy[1] to avoid nephrotoxicity induced by CNIs

belatacept, which binds to CD80 and CD86 on APCs, blocks not only the T effectors that represent the positive signal but also the regulatory T (Tregs) that constitute the inhibitory signal

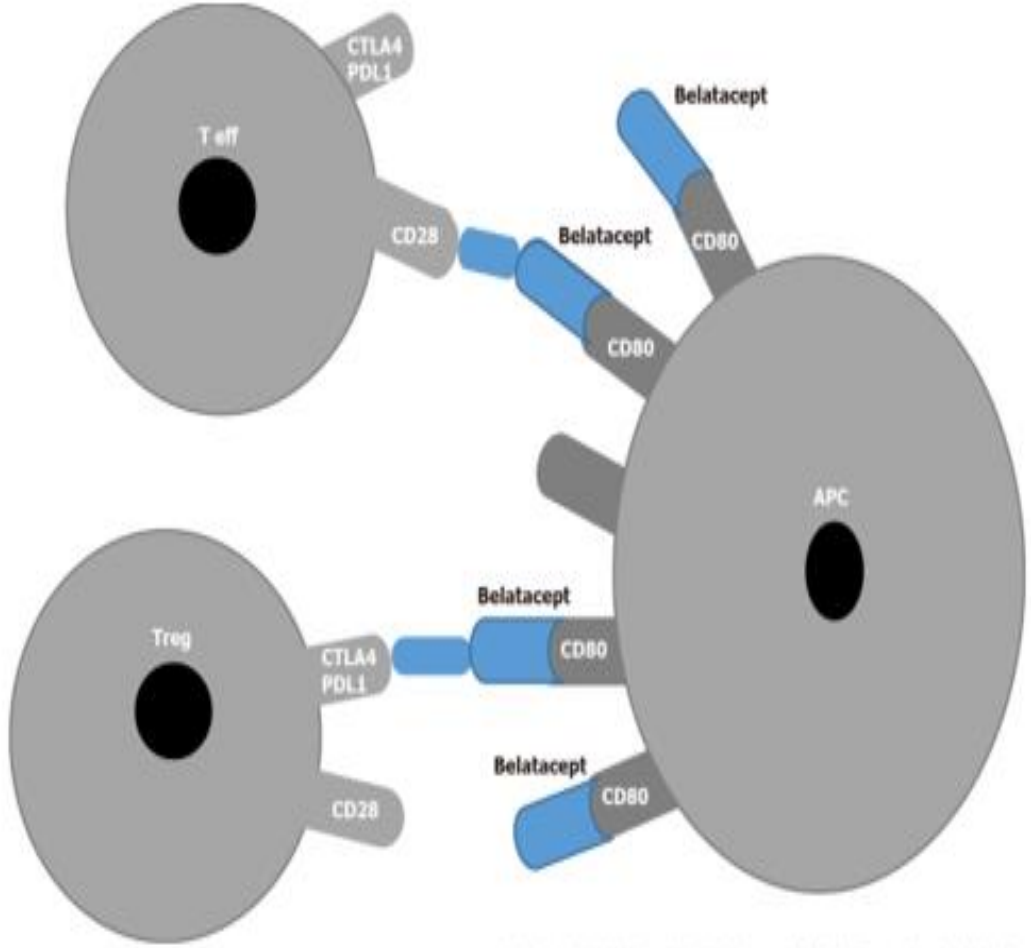

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Figure 1 Block of co-stimulation with Belatacept. APC: Antigen presenting cell; T eff: T effector; T reg: Regulatory T cells; PDL1: Programmed cell death receptor ligand 1; CTLA4: Cytotoxic T-lymphocyte-associated antigen 4.

(2)

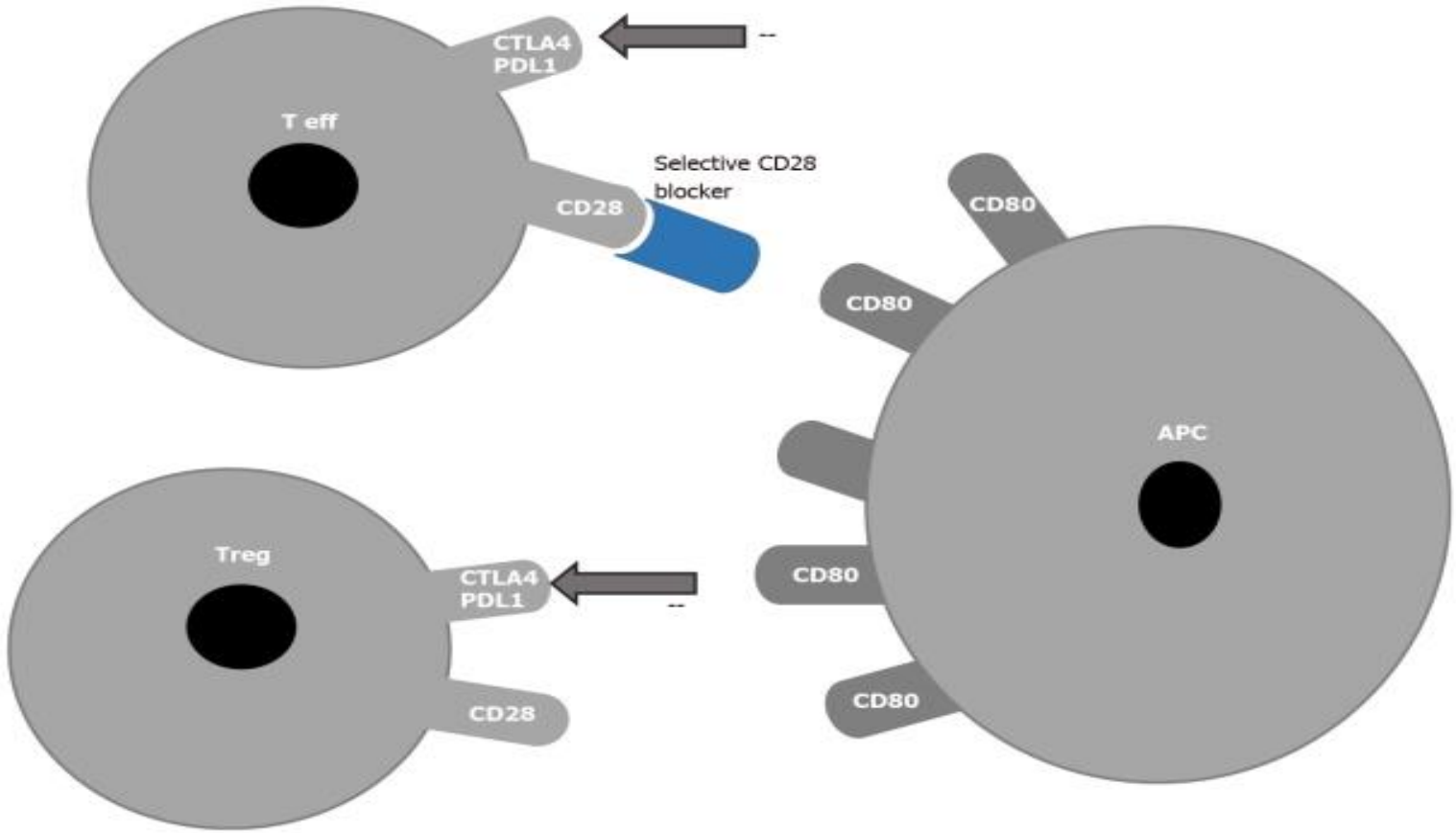

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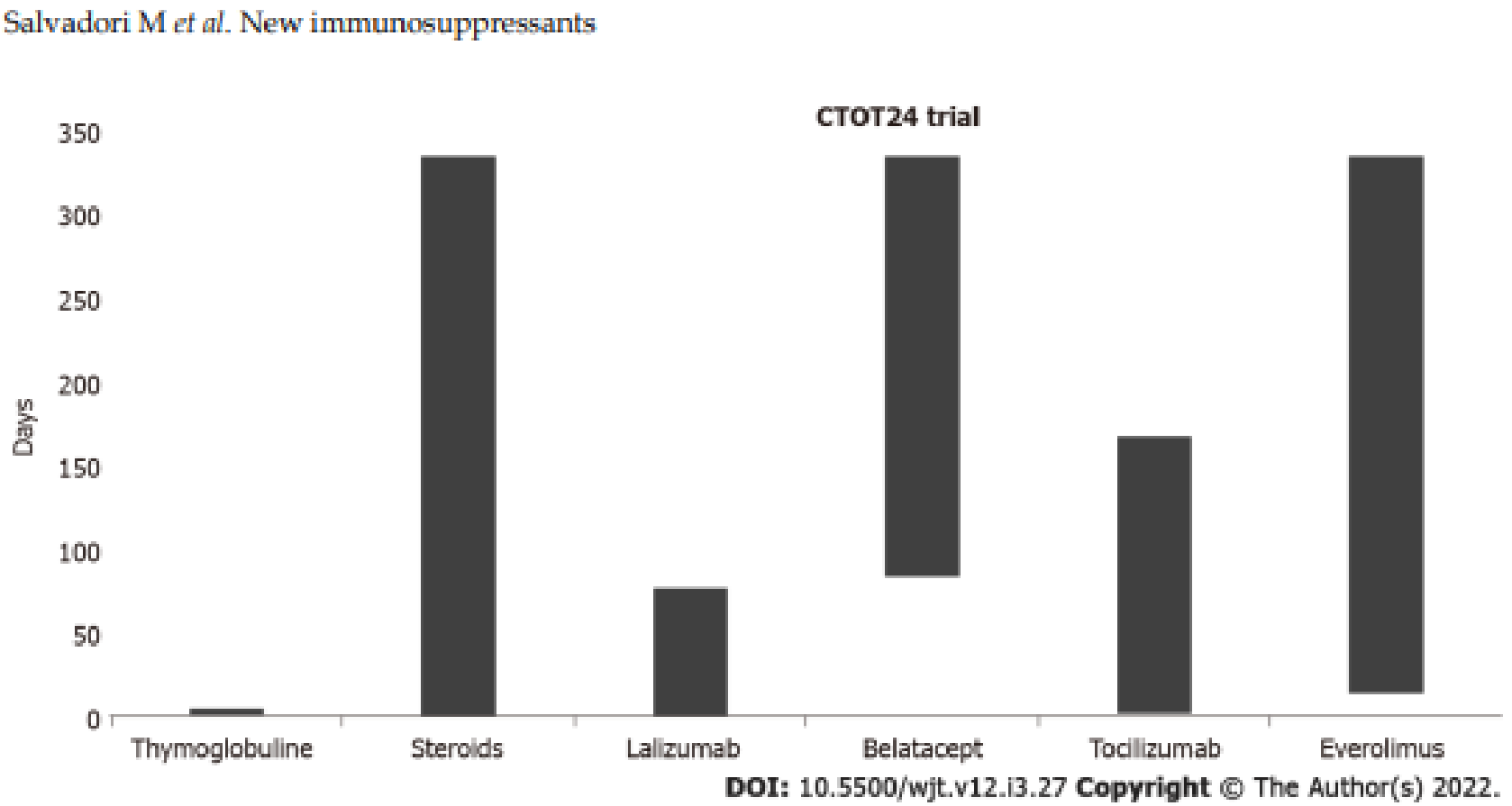
Figure 2 Block of co-stimulation with anti CD28. APC: Antigen presenting cell; T eff: T effector; T reg: Regulatory T cells; PDL1: Programmed cell death receptor ligand 1; CTLA4: Cytotoxic T-lymphocyte-associated antigen 4.

Therapy to avoid nephrotoxicity induced by CNIs

- Currently, two monovalent antibodies, FR104 and lulizumab-pegol are under development for clinical application. These antibodies have antagonistic activity against CD28 (T- CELL eff) alone. To date, an RCT has been conducted at the University of California to modulate Tregs with combinatorial treatment with CD28 and IL-6 receptor antagonists (Figure 3).
- The addition of an IL-6 receptor antagonist (tocilizumab) aims to further stimulate Treg cells and exert an anti-inflammatory effect.
- In the CTOT24 trial, after induction with thymoglobulin, steroids are administered from the beginning, lulizumab is started at the beginning and then continued weekly through day 77, belatacept is started on day 84 and administered every 4 wk, tocilizumab is started at the beginning and continued every 2 wk through day 168, and everolimus is started on day 14 and administered twice daily.
- A different way to block costimulation is to block the interaction between CD40 and CD40 L. A first attempt was made to block the CD 40 receptor, but the studies were interrupted because of a number of thromboembolic complications. This was because CD40 L is also expressed on platelets, which causes thromboembolic complications.

An RCT has been conducted at the University of California to modulate Tregs with combinatorial treatment with CD28 and IL-6 receptor antagonists (3)



Therapy to avoid nephrotoxicity induced by CNIs

- In 2014, Okimura et al reported that ASKP 1240, a fully human antibody targeting human CD40 (APC), had a potent immunosuppressive effect that did not interfere with platelets.
- Bleselumab, a fully human anti-CD40 monoclonal antibody, was documented by Vincenti et al. The results were confirmed by a phase 2, randomized, open label, noninferiority study by Harland et al.
- Novartis claimed to have developed another anti-CD40 monoclonal antibody (CFZ-533, Iscalimab).
- Until recently, it was believed that the main cause of kidney injury over time after transplantation was primarily due to CNI nephrotoxicity but the first study questioning this opinion was the DeKAF study by Gaston et al .

Therapy to avoid nephrotoxicity induced by CNIs

- The decline in kidney function was not only due to CNI nephrotoxicity but also due to the recipient of DSAs and the consequent activation of the humoral response.
- Indeed, long-term graft survival was lower in patients with DSAs in the serum and C4d+ on graft.
- A separate *study* documented that both de novo and pre-existing DSAs caused ABMR and reduced graft survival.
- A more recent study by Stegall et al found that 82% of patients whose grafts survived 10 years were affected by inflammatory lesions not related to CNI toxicity or to immunological mechanisms.
- Preserving renal function requires other therapies in addition to safely reducing or withdrawing CNIs.

Therapy to control inflammation and fibrosis not related to immunological causes

- Hyperuricemia
- Glucose intolerance
- Hypertension
- Dyslipidemia
- Infection, may induce an inflammatory state in kidney transplant patients.
- In addition, chronic hypoxia mediated by IL-1 and IL-6, angiotensin II and transforming growth factor beta may result in the accumulation of extracellular matrix, which can lead to interstitial fibrosis.
- It seems that IL-6 leads to allograft injury by acute inflammation, adaptive cellular/humoral responses, innate immunity and fibrosis.
- IL-6 is a mainstay in inducing inflammation and allograft injury. Several drugs have been proposed to control the graft inflammatory state, including low-dose aspirin, statins, renin-angiotensin inhibitors, and xanthine-oxidase inhibitors, but no prospective trial with these drugs has been conducted in kidney transplantation. The only drug object of an RCT is the IL-6R inhibitor.

Therapy to control inflammation and fibrosis not related to immunological causes

- Currently, available agents for IL-6 signaling inhibition include monoclonal antibodies against IL-6 or IL-6R and Janus kinase inhibitors.

- The most often studied is tocilizumab, an IL-6R blocker. In a study conducted by Chandran et al, IL-6 blockade with tocilizumab increased Tregs and reduced T effector cytokines in renal graft inflammation. Tocilizumab-treated patients showed an improved tubulointerstitial .

Therapy to control chronic humoral rejection

- Until recently, attempts to treat cABMR had been limited to a combination of plasmapheresis and intravenous immunoglobulins (IVIGs) and rituximab (RTX).
- Recently, proteasome inhibitors such as bortezomib and carfilzomib have also been studied, but these drugs were not as effective as anticipated.
- In addition, complement inhibitors such as C1 inhibitors (C1-INH) and eculizumab, failed to control cABMR probably because antibodies may injure the endothelium in a complement-independent pathway.
- Better results have been obtained with the use of IL-6R or IL-6 inhibitors.

Therapy to control chronic humoral rejection

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- In a previous study, Shin et al, documented the efficacy of tocilizumab in blocking monocyte activation in an in vitro model, to inhibit the inflammatory cascade induced by alloantibodies.
- In a more recent study, Shin et al documented a beneficial effect of tocilizumab on cABMR owing to a reduction in antibody production by B cells.

Therapy to control chronic humoral rejection

- Similarly, Choi et al documented a reduction in DSAs and cABMR and stabilization of renal function in patients with cABMR, DSAs and transplant glomerulopathy treated with tocilizumab.

- A phase 4 RCT in patients with cABMR was recently designed. Clazaki zumab is a humanized monoclonal antibody directed against IL-6.
- In a study by Dobere et al , clazakizumab reduced DSAs and demonstrated beneficial effects on cABMR and renal function.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- To better understand the mechanism of action of these drugs, Figure 4 represents how DSAs are formed and where the immunosuppressant drugs may act.
- Naïve CD4+ T cells recognize the antigen presented by APCs. Activated CD4+ cells process antigens, which are presented to naïve B cells.
- Costimulatory molecules mediate the presentation through CD80/86 and CD28.
- B cell maturation and development into B-memory cells and plasma cells (PCs) is regulated by cytokines (principally IL-6 and IL-21), B cell activating factor (BAFF) and a proliferation-inducing ligand that interact with B cell maturation antigen.
- PCs produce antibodies that bind to donor-specific human leukocyte antigen (HLA) molecules, activate complement and initiate injury leading to ABMR.
- Agents capable of interfering with this complex system are numerous and act at different levels.

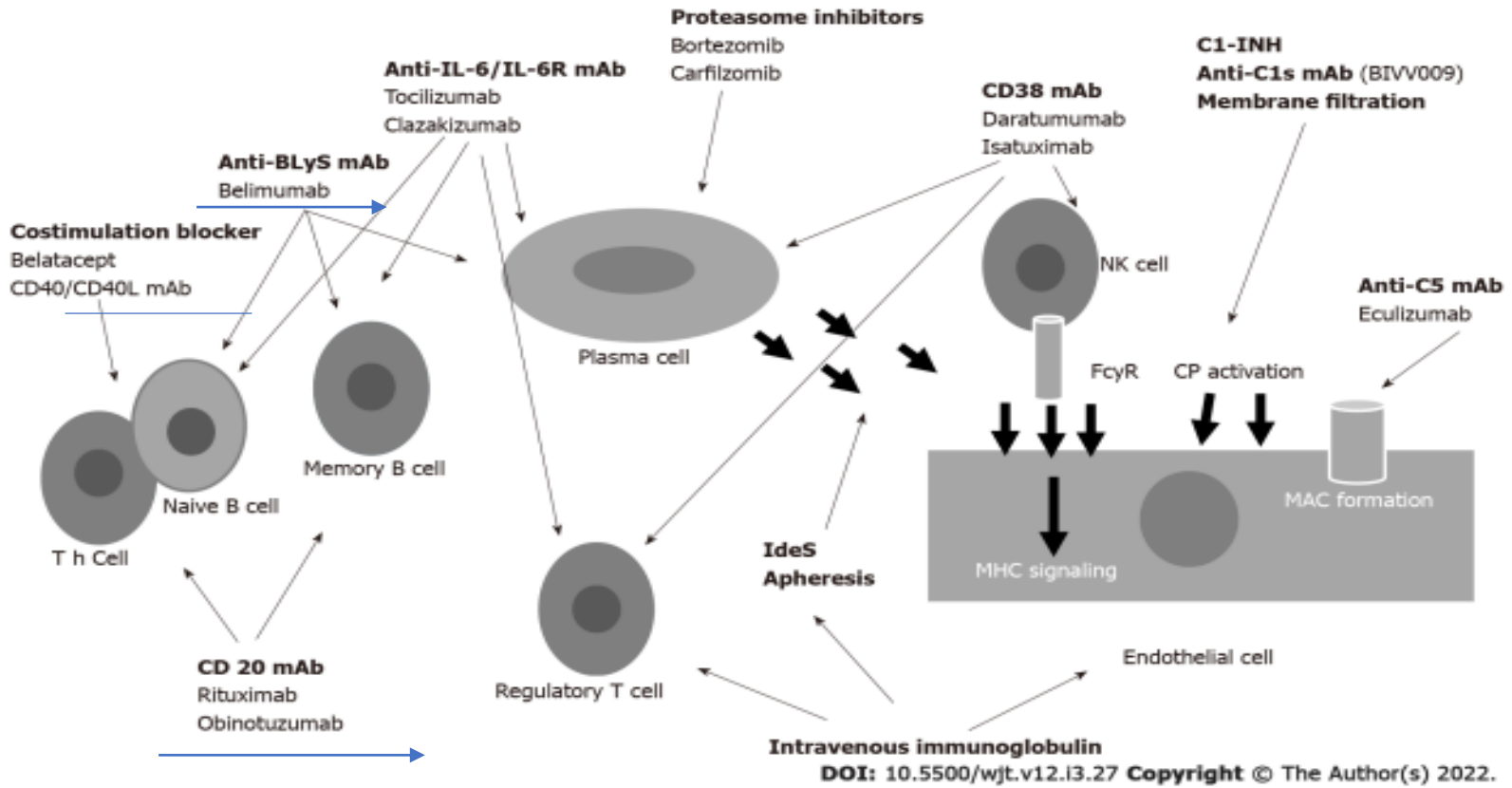

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Figure 4 Drugs acting at different levels to control the antibody formation. BLyS: B Lymphocyte stimulating factor; mAb: Monoclonal antibody; C1-INH: C1 inhibitors; NK: Natural killer; Cp: Complement; FcyR: FcyReceptor; MAC: Membrane attacking complex; MHC: Major histocompatibility complex; IL: Interleukin.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- Several studies and reviews have described the drugs used in desensitization and in the treatment of ABMR.
- Novel agents will be discussed :
- Obintuzumab is a type 2 anti-CD20 antibody that induces more robust B cell depletion than RTX.
- To date, the drug has been evaluated in a phase 1b study to induce desensitization.
- Belimumab belongs to the anti BAFF family. The drug is effective in treating systemic lupus erythematosus but **less effective** in treating ABMR due to possible infective complications.
- Proteasome inhibitors such as bortezomib and carfilzomib act on PCs, but are not as effective as anticipated. Carfilzomib has been studied in desensitization in a nonhuman primate model.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- Drugs acting directly on PCs target CD38. Several studies or case reports have documented the efficacy of daratuzumab in the treatment of ABMR.
- Isatuximab is effective on PCs and other immune cells, such as Tregs and Bregs. This fact may limit its applicability in the treatment of ABMR .
- Inebilizumab is a humanized anti-CD19 monoclonal antibody approved for neuromyelitis optica . An RCT with inebilizumab for pretransplant desensitization was suspended due to the coronavirus disease pandemic.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- In ABMR, the activation of the complement cascade is triggered by ligation of the C1 complex to HLA antigens that are bound by DSAs. Several drugs are capable of blocking complement activation (Figure 5).
- The C1 complex is activated upon antibody binding. The humanized monoclonal antibody BIVV009 (sutinlimab) targets its enzymatic subcomponent C1 s and this therapy blocks C4 and C2 cleavage and the formation of C3 convertase.
- A phase 1 study with this drug was concluded, and Eskandary et al[80] studied 10 kidney transplant recipients with ABMR. Repeated biopsies documented a reduction in C4d deposition even if DSA levels and microvascular inflammation were unchanged.

principal Drugs affecting Complement

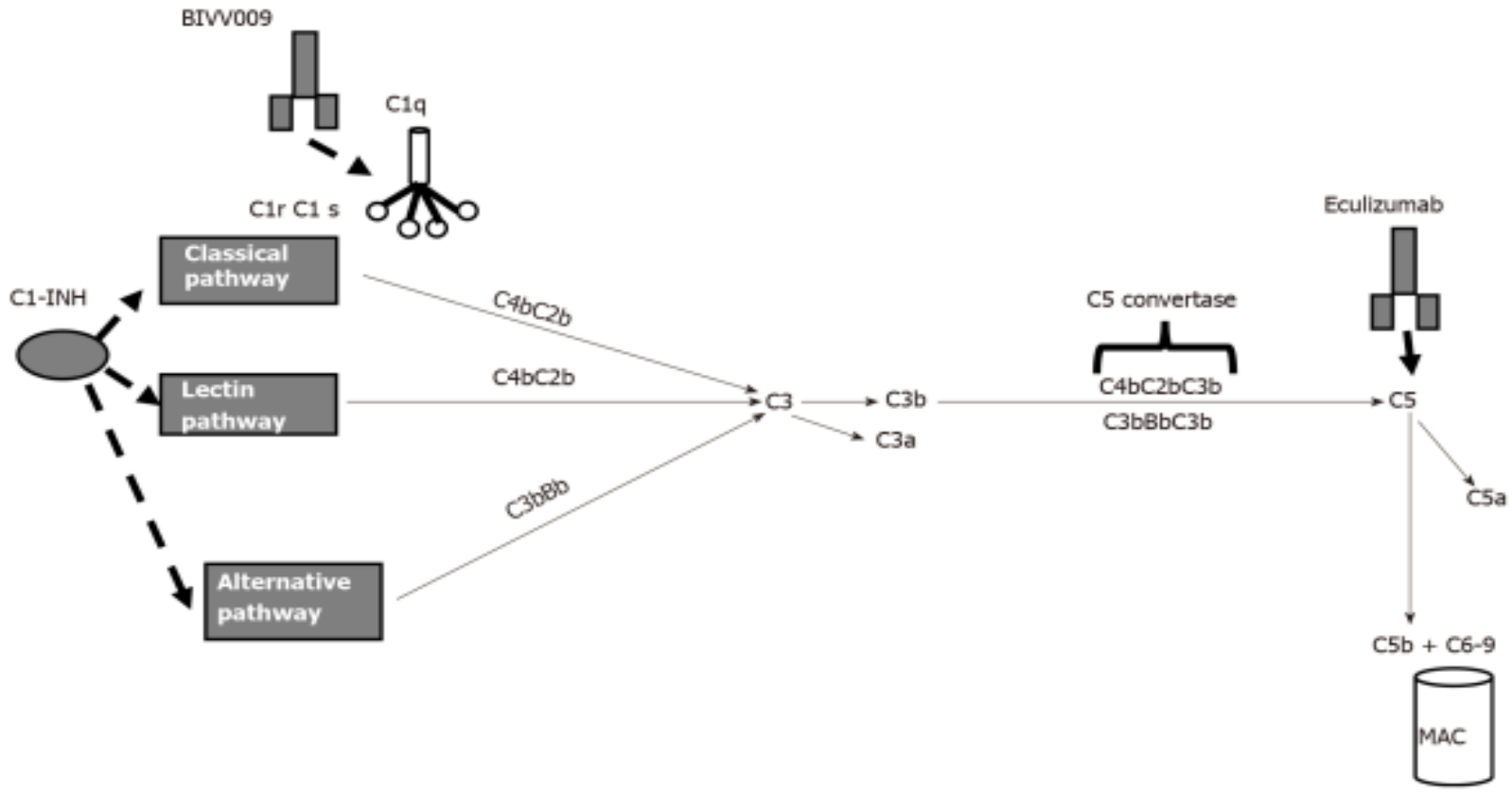

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Figure 5 Principal drugs affecting complement. C1-INH: C1 inhibitor; MAC: Membrane attacking complex.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- C1-INH regulates several pathways that contribute to complement activation and cause ABMR.
- In 2015, in a phase I/II placebo-controlled trial, Vo et al[106] reported the efficacy of C1-INH in the prevention of ABMR in HLA-sensitized patients. Later, Montgomery et al in a randomized controlled pilot study, documented the efficacy of C1-INH in controlling ABMR.
- More recently, two more studies are ongoing to document the efficacy of human plasma C1 esterase inhibition as an addition to the standard of care for the treatment of ABMR

THERAPY
FOR DESENSITIZATION AND ACUTE ABMR

- The humanized monoclonal antibody eculizumab binds to C5 with high affinity and prevents C5 convertase-mediated cleavage to C5a and C5b. In the past, several studies documented the efficacy of eculizumab in treating ABMR.
- Recently, other studies documented the efficacy of eculizumab in treating and preventing ABMR.

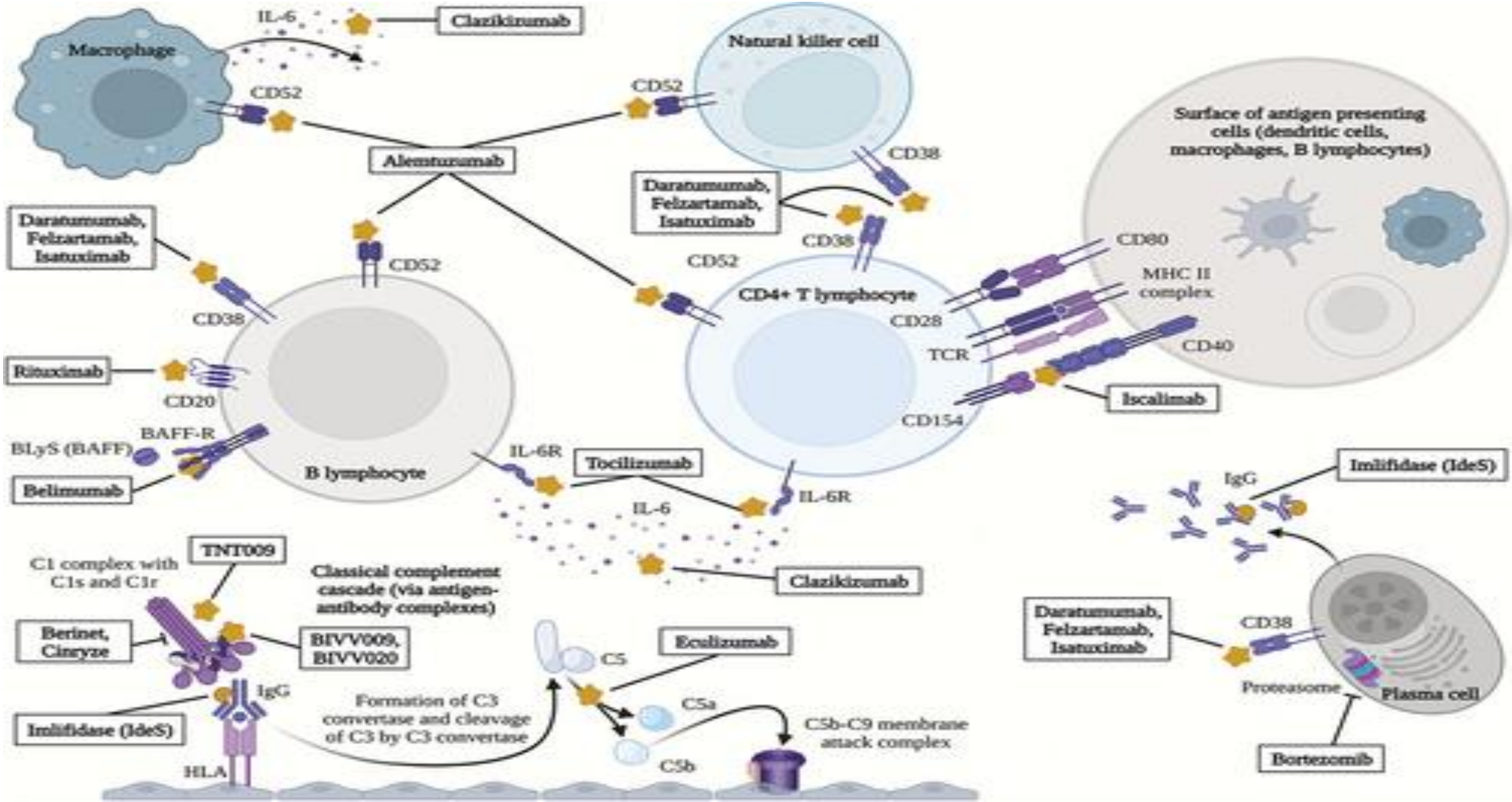
- Antibody removal is another therapeutic technique that may be applied primarily to desensitize patients with preformed DSAs before transplantation. Until recently, antibody removal and/or inhibition have been performed by plasmapheresis and IVIGs.
- Recently, it was documented that imlifidase (IdeS), a recombinant cysteine protease derived from Streptococcus pyogenes, rapidly cleaves IgG in the lower hinge region to a Fab fragment and a dimeric Fc fragment.

THERAPY FOR DESENSITIZATION AND ACUTE ABMR

- In addition to eliminating HLA antibodies, Ge et al[116] demonstrated that IdeS is a potent inhibitor of antibody-dependent cell cytotoxicity. A drawback of IdeS treatment is antibody recurrence after the interruption of the treatment. Incorporation of plasmapheresis and RTX to this treatment may overcome this drawback.

THERAPY FOR DESENSITIZATION AND ACUTE AB MR

- An international phase 2 trial was conducted in five transplant centers[117] for desensitization of cross-match-positive, highly sensitized kidney transplant recipients. Antibody rebound occurred 3-14 d after lipopolysaccharide administration, but graft survival at six months was 88.9%. The study conclusion was that IdeS converted positive cross matches to negative cross matches and achieved the transplantation of high-sensitized patients with optimal results at 6 mo.
- In a more recent study, Kjellman et al[118] documented that lipifidase treatment administered to 39 cross-match-positive patients accomplished a 3-year graft survival of 93% with an ABMR incidence of 38% in the first month post-transplantation.


Macrophage
IL-6
Clazikizumab
CD52
Alemtuzumab
Natural killer cell
CD52
CD38
Daratumumab, Felzartamab, Isatuximab
CD38
CD52
Surface of antigen presenting cells (dendritic cells, macrophages, B lymphocytes)
CD80
MHC II complex
CD28
TCR
CD40
CD154
Iscalimab
CD4+ T lymphocyte
Daratumumab, Felzartamab, Isatuximab
CD38
CD52
Rituximab
CD20
BLyS (BAFF)
BAFF-R
Belimumab
B lymphocyte
IL-6R
Tocilizumab
IL-6
IL-6R
Clazikizumab
TNT009
C1 complex with C1s and C1r
Classical complement cascade (via antigen-antibody complexes)
Berinet, Cinryze
BIVV009, BIVV020
C5
Eculizumab
IgG
Imlifidase (IdeS)
Formation of C3 convertase and cleavage of C3 by C3 convertase
C5a
C5b
C5b-C9 membrane attack complex
HLA
IgG
Imlifidase (IdeS)
Daratumumab, Felzartamab, Isatuximab
CD38
Proteasome
Plasma cell
Bortezomib

Thanks

CONCLUSION

- Lack of interest by industries and optimal outcomes reached by the drugs used to date has resulted in little progress in finding new drugs.
- However, examining unmet needs in the field of kidney transplantation may help us to find new drugs.
- Needs not optimally covered by current drugs are control of DGF, improvement of the long-term immunosuppression with graft outcomes reduced by chronic damage and the control of desensitization and ABMR.
- The control of these needs is of outmost importance, considering the expanding numbers of new kinds of kidney transplantation as transplantation from older donors and from NHBDs and transplantation from antibody-incompatible donors.
- In the first kind, controlling or reducing DGF is essential; in the latter kind, the reduction of antibodies against HLA is essential.

Cellular-depleting therapies

Alemtuzumab

- Alemtuzumab is a monoclonal antibody directed against the CD52 membrane protein.
- Its mechanism of action is to cause depletion of circulating CD52 positive cells by
- Complement-dependent cytotoxicity
- Antibody-dependent cellular cytotoxicity
- Induction of apoptosis.
- In solid organ transplantation (SOT), Alemtuzumab has been evaluated as an alternative to rATG, because of its apparent efficacy, easier mode of administration and superior tolerability
- . In kidney transplantation, in addition to induction therapy, alemtuzumab has been used to treat severe or glucocorticoid-resistant acute rejection. Although alemtuzumab is not a novel therapy in kidney transplantation, recent publications and new insights regarding its pharmacokinetic and pharmacodynamic properties are reason to cover it in this review.

Alemtuzumab

- Compared with rATG, a slower lymphocyte reconstitution was observed after alemtuzumab. Only 55.7% of patients had a T cell count >200 × 10^6/L one year after treatment .

- This prolonged lymphocyte depletion may in part explain the increased risk of infection, secondary auto-immunity and malignancy that have been associated with alemtuzumab .
- Currently, alemtuzumab is most often prescribed as a fixed-dose of 30 mg. Plasma alemtuzumab concentrations have, however, shown substantial interpatient variability.
- An individualized dose might lead to faster lymphocyte recovery and less adverse events.
- In kidney transplantation, weight-based dosing led to faster lymphocyte repopulation, less infection, and comparable rejection rates . Furthermore, a lower dose (20 mg) was found to be effective .
- These findings indicate that individualized alemtuzumab dosing may improve the balance between efficacy and toxicity. The use of a pharmacokinetic model, such as was recently developed for children who underwent stem cell transplantation , may allow for such individualized alemtuzumab dosing.

Rituximab

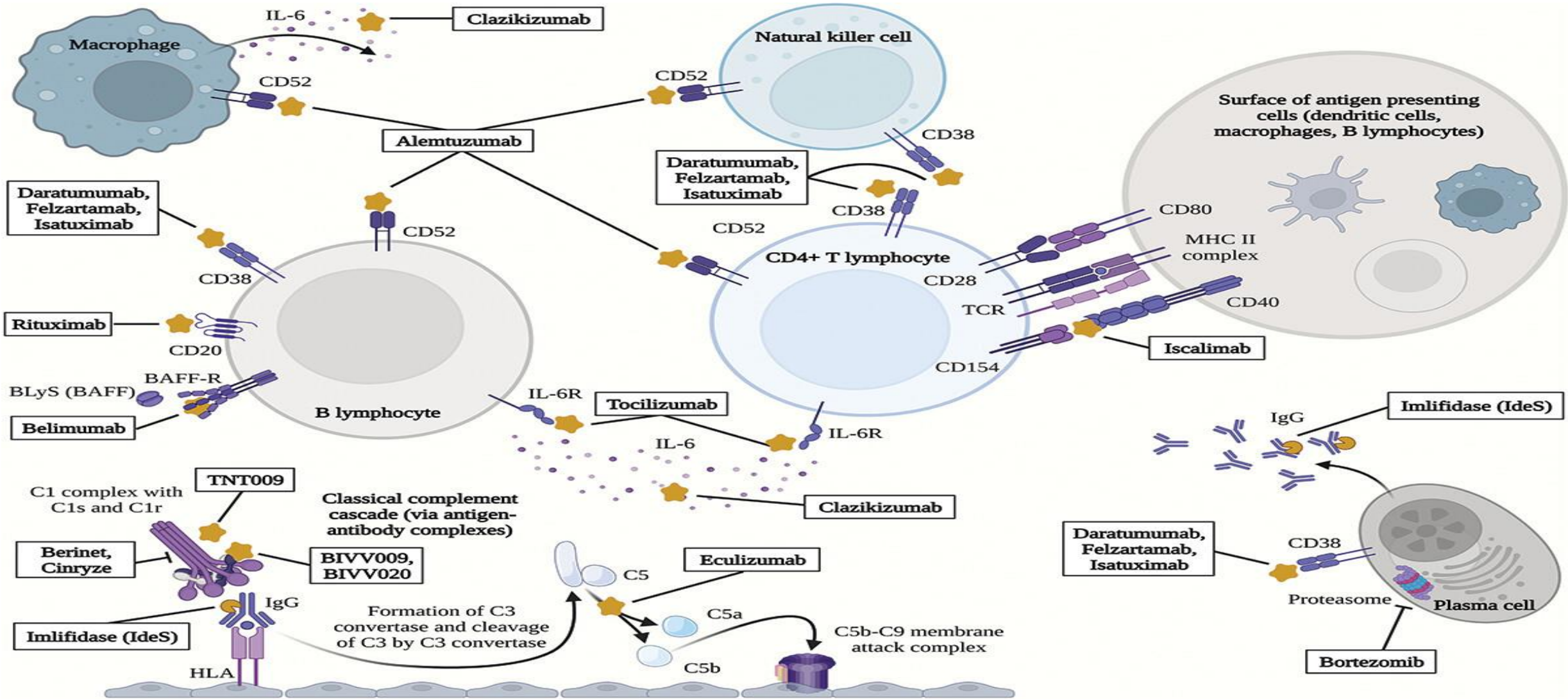
- Rituximab is a monoclonal antibody directed at CD20, which is expressed on B lymphocytes and its precursor cells, but not on plasma cells. Its mechanism of action is to deplete B lymphocytes by various mechanisms .
- Rituximab is registered for the treatment of hematologic malignancies, rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis. It has also been used off-label in a variety of other diseases characterized by pathogenic auto-antibody formation.
- In transplant rejection, B lymphocytes play a versatile role. They can differentiate into (donor-specific) antibody-secreting plasma cells and influence the T lymphocyte response by acting as antigen presenting cells (APC) and through the production of cytokines .
- Because of the central role of B lymphocytes and donor-specific anti-HLA antibodies (DSA) in ABMR, rituximab has been investigated extensively for this indication.
- In human kidney transplantation, rituximab was effective in reducing blood group antibodies in blood group-incompatible transplantation and in reducing the concentration of DSA in highly immunized recipients .
- However, rituximab was not effective in preventing TCMR when prescribed as an induction agent

- Sautenet *et al.* conducted a RCT for active ABMR in which n = 38 patients were randomized to receive methylprednisolone, plasma exchange and high-dose IVIG or methylprednisolone, plasma exchange, high-dose IVIG plus rituximab. They found a comparable one-year graft survival between the two groups . There was no significant difference in DSA concentrations . Associated side effects of rituximab were infection-related: opportunistic infections occurred more frequently after rituximab (six *versus* one) [Citation26].
- A recently published, follow-up study of this cohort study reported equal long-term outcomes between the two therapies [Citation27].
- The modest sample size and high cross-over (8 out of 19 control patients received rituximab) should, however, be taken into account when these results are interpreted.

Bortezomib

- Bortezomib is a proteasome inhibitor that is registered for the treatment of multiple myeloma [Citation28]. Its mechanism of action is to inhibit the degradation of intracellular proteins, such as misfolded immunoglobulins, pro-apoptotic kinases, and protein inhibitors of cell survival pathways, which in the end causes apoptosis (Figure 1) [Citation28]. Malignant and normal plasma cells are hypersensitive to bortezomib, possibly because of their extremely high protein synthesis [Citation29]. In vitro, bortezomib caused human plasma cell apoptosis and prevented DSA production [Citation30]. Therefore, bortezomib was suggested as a treatment option for ABMR.

Schematic representation of investigational drugs for the treatment of kidney transplant rejection and their targets. This figure presents a B- and T lymphocyte, macrophage, natural killer cell, plasma cell, and a cluster of antigen-presenting cells (dendritic cells, macrophages, B lymphocytes) with their surface receptors. Abbreviations: IL, interleukin; IL-6 R, interleukin-6 receptor; BAFF-R, B cell-activating factor-receptor; BLyS, B lymphocyte stimulator; BAFF, B cell-activating factor; IdeS, immunoglobulin-G-degrading enzyme of the human pathogen *Streptococcus pyogenes*; Ig, immunoglobulin; HLA, human leukocyte antigens; TCR, T cell receptor; MHC, major histocompatibility complex



of investigational drugs for the treatment of kidney transplant rejection.

Mechanism of action	Therapeutic effect	Advantages	Disadvantages	Reference
es				
B/T lymphocyte and NK cell depletion	In retrospective analysis, allograft survival comparable to rATG	Applicable in ABMR, TCMR and mixed rejection	Long-lasting lymphocyte depletion with risk of infection, malignancy, auto-immunity	[8]
B lymphocyte depletion	No clear evidence for beneficial effect in ABMR	Specifically targets B lymphocytes	Higher risk of infection, plasma cells unaffected	[26]
Inhibits degradation intracellular protein	No conclusive evidence for beneficial effect in ABMR	Specifically targets plasma cells	High rate of gastro-intestinal and hematological toxicity	[32]
Plasma cell, B/T lymphocyte and NK cell depletion	Anecdotal evidence only, regarding use in ABMR	Targets plasma cells and lymphocytes	Possibly increased rejection rate due to loss of regulatory cells	[36–38]
Blocks binding of BLyS to B cell receptor, preventing B lymphocyte survival and differentiation	No clear evidence for beneficial effect in ABMR. B lymphocytes possibly have greater capacity to produce IL-10 compared with IL-6 post-treatment	Specifically targets B lymphocytes	Ineffective in lowering DSAs	[58]
Binds CD40, preventing activation of the CD40-CD154 costimulatory pathway	*In vitro* and non-human primate studies only. Clinical trial results awaited	B lymphocyte sparing, no platelet activation (in comparison to anti-CD154 antibodies)	Short-term effect, necessitating continued dosing.	[84]
rapy				
Blocks IL-6 R, thereby preventing effector functions of IL-6	No conclusive evidence for beneficial effect in chronic ABMR	Specifically targets IL-6, modulating immune responses without cellular depletion, potential co-treatment	Risk of neutropenia	[92]
Binds IL-6, thereby preventing effector functions of IL-6	Stabilization of kidney function in small numbers of patients with chronic ABMR	Specifically targets IL-6, modulating immune response without cellular depletion, potential co-treatment	Risk of gastro-intestinal adverse effects, most notably diverticulitis.	[95,96]
Cleavage of IgG-molecules and antigen-bound IgG	Ongoing trial in ABMR (Clinicaltrials.gov identifier NCT03897205), registered as desensitization drug	Specifically targets IgG molecules resulting in rapid removal of circulating DSAs, so high potential in ABMR	Short-term effect (week-months) with possibility of rebound effect of DSAs	[108]
Binding and inactivating C1 esterase	No conclusive evidence for beneficial effect acute and late, active ABMR	Specifically targets complement, modulating immune responses without cellular depletion	Gastro-intestinal toxicity	[124]
Inhibits cleavage of C5 in active components	No conclusive evidence for beneficial effect in ABMR	Specifically targets complement, modulating immune responses without cellular depletion	Increased meningococcal infections and hepatotoxicity	[133]
Binds and blocks activated C1 protein	No conclusive evidence in ABMR, only phase I trials	Specifically targets complement, modulating immune responses without cellular depletion	Safety unclear. Safety data only available from small patient numbers	[134]

Table 1. Summary of investigational drugs for the treatment of kidney transplant rejection.

Type of immunosuppression	Mechanism of action	Therapeutic effect	Advantages	Disadvantages	Reference
Cellular-depleting therapies					
Alemtuzumab	B/T lymphocyte and NK cell depletion	In retrospective analysis, allograft survival comparable to rATG	Applicable in ABMR, TCMR and mixed rejection	Long-lasting lymphocyte depletion with risk of infection, malignancy, auto-immunity	[8]
Rituximab	B lymphocyte depletion	No clear evidence for beneficial effect in ABMR	Specifically targets B lymphocytes	Higher risk of infection, plasma cells unaffected	[26]
Bortezomib	Inhibits degradation intracellular protein	No conclusive evidence for beneficial effect in ABMR	Specifically targets plasma cells	High rate of gastro-intestinal and hematological toxicity	[32]
Daratumumab	Plasma cell, B/T lymphocyte and NK cell depletion	Anecdotal evidence only, regarding use in ABMR	Targets plasma cells and lymphocytes	Possibly increased rejection rate due to loss of regulatory cells	[36–38]
Non-depleting antibodies					
Belimumab	Blocks binding of BLyS to B cell receptor, preventing B lymphocyte survival and differentiation	No clear evidence for beneficial effect in ABMR. B lymphocytes possibly have greater capacity to produce IL-10 compared with IL-6 post-treatment	Specifically targets B lymphocytes	Ineffective in lowering DSAs	[58]
Iscalimab	Binds CD40, preventing activation of the CD40-CD154 costimulatory pathway	*In vitro* and non-human primate studies only. Clinical trial results awaited	B lymphocyte sparing, no platelet activation (in comparison to anti-CD154 antibodies)	Short-term effect, necessitating continued dosing.	[84]
Interleukin-6 directed therapy					
Tocilizumab	Blocks IL-6 R, thereby preventing effector functions of IL-6	No conclusive evidence for beneficial effect in chronic ABMR	Specifically targets IL-6, modulating immune responses without cellular depletion, potential co-treatment	Risk of neutropenia	[92]
Clazakizumab	Binds IL-6, thereby preventing effector functions of IL-6	Stabilization of kidney function in small numbers of patients with chronic ABMR	Specifically targets IL-6, modulating immune response without cellular depletion, potential co-treatment	Risk of gastro-intestinal adverse effects, most notably diverticulitis.	[95,96]
Antibody targeted therapy					
Imlifidase	Cleavage of IgG-molecules and antigen-bound IgG	Ongoing trial in ABMR (Clinicaltrials.gov identifier NCT03897205), registered as desensitization drug	Specifically targets IgG molecules resulting in rapid removal of circulating DSAs, so high potential in ABMR	Short-term effect (week-months) with possibility of rebound effect of DSAs	[108]
Complement inhibition					
C1 esterase inhibitors	Binding and inactivating C1 esterase	No conclusive evidence for beneficial effect acute and late, active ABMR	Specifically targets complement, modulating immune responses without cellular depletion	Gastro-intestinal toxicity	[124]
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Anti-C1s antibodies	Binds and blocks activated C1 protein	No conclusive evidence in ABMR, only phase I trials	Specifically targets complement, modulating immune responses without cellular depletion	Safety unclear. Safety data only available from small patient numbers	[134]

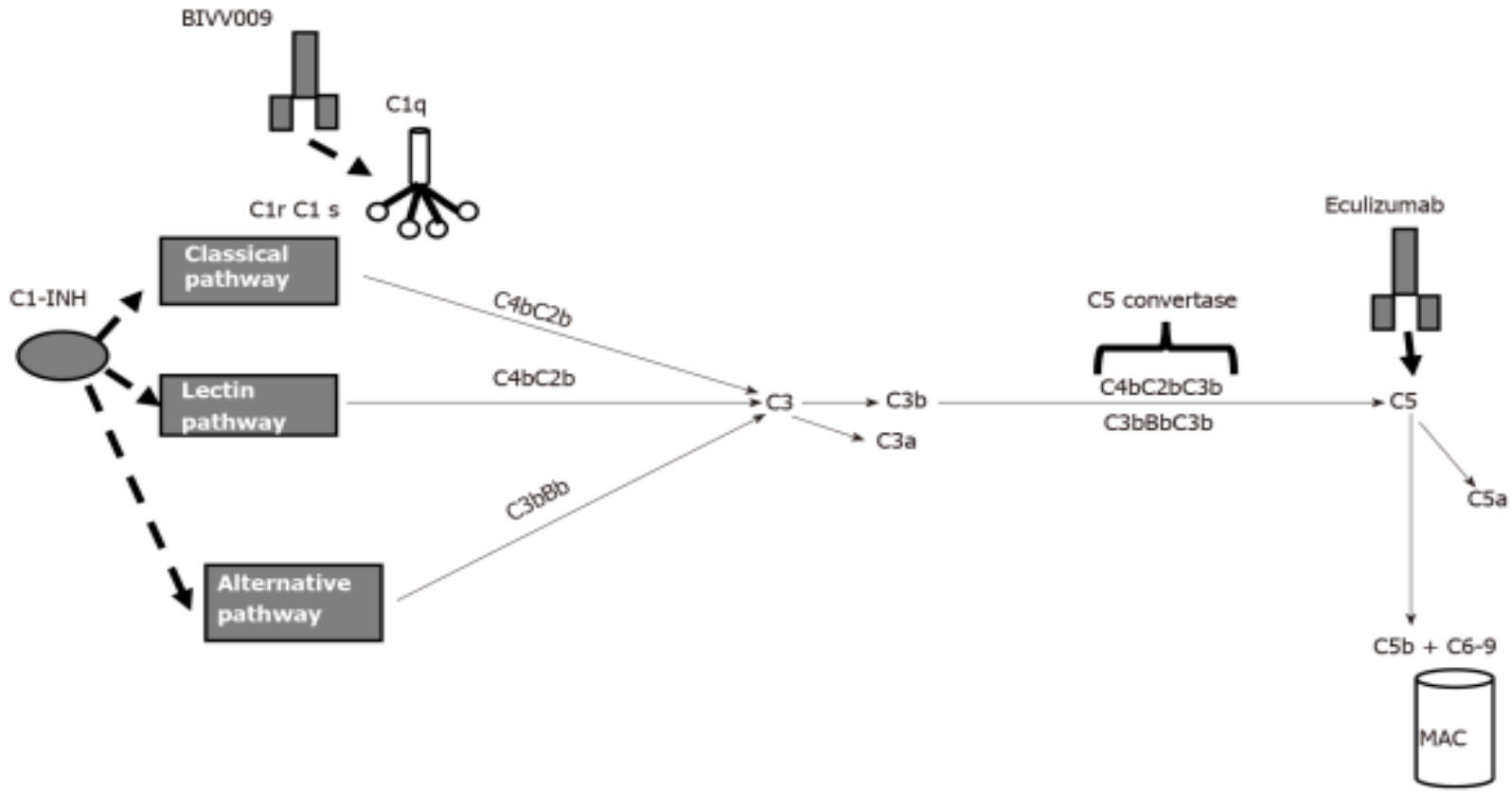

Figure 5 Principal drugs affecting complement. C1-INH: C1 inhibitor; MAC: Membrane attacking complex.

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