EUROPEAN ACADEMIC RESEARCH
Vol. XIII, Issue 4/ July 2025

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Monoclonal Antibodies in Transplantation: From Depletion to T Cell Education — A Narrative Review

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Abstract

Monoclonal antibody (mAb) therapies have redefined the landscape of immunosuppression in organ transplantation, shifting the paradigm from broad immune suppression to targeted modulation of specific pathways. This narrative review explores the mechanisms, clinical applications, and evolving strategies surrounding both depleting and non-depleting mAbs, with emphasis on their role in biphasic protocols—the strategic use of antibodies at induction and during rejection.

Drawing on 25 peer-reviewed studies, we examine mAb effects on T cell depletion, reprogramming, and tolerance induction, with comparative analysis across kidney, liver, heart, and pediatric transplantation. Depleting agents such as alemtuzumab and rituximab show promise not only in immune reset but also in reshaping post-rejection immunity, while non-depleting agents like basiliximab offer safer induction profiles with potential for regulatory T cell preservation. Emerging strategies involving anti-CD40, CD28, and IL-6 blockade, along with bispecific antibodies, signal a new era of personalized, mechanistic immunotherapy.

We conclude that monoclonal antibodies, especially when deployed in phase-specific and organ-tailored regimens, offer more than suppression—they serve as tools to instruct and harmonize immune memory, bringing the field closer to long-term graft acceptance and operational tolerance.

Keywords: monoclonal antibodies, transplantation, T Cell education

1. INTRODUCTION

Organ transplantation has transformed the prognosis for patients with end-stage organ failure, offering not only prolonged survival but also a significantly improved quality of life. However, the key challenge to long-term graft success remains **immunologic rejection**, particularly the host's recognition of the donor organ as non-self. Despite progress in immunosuppressive strategies, acute rejection still occurs in 10–20% of kidney and liver transplant recipients within the first-year post-transplant, while **chronic rejection** continues to threaten long-term graft function (Vincenti et al., 2001; Hanaway et al., 2011).

Over the past two decades, transplant immunology has seen a shift from **non-specific, broad immunosuppression** to **targeted immunomodulation**, especially with the introduction of **monoclonal antibodies (mAbs)**. Starting with OKT3(Ortho-Kung T3) in the 1980s, and evolving through humanized agents such as **basiliximab**,

daclizumab, **alemtuzumab**, and **rituximab**, clinicians have gained access to biologics capable of selectively inhibiting or eliminating immune subsets (Toxins, 2014; Slavin & Morecki, 2023). These mAbs have redefined immune control by targeting pathways critical for T cell activation, memory development, and B cell antibody production.

A notable development is the emergence of **biphasic monoclonal antibody protocols**, which deploy mAbs at two distinct phases of the transplant timeline:

1. **Induction phase** (peri-operative), aimed at blunting early immune activation
2. **Rejection phase**, targeting resistant or recurring rejection episodes

This dual-phase approach not only controls inflammation but also allows **immune recalibration** during key moments of graft vulnerability.

Monoclonal antibodies used in transplantation can be broadly classified into two functional categories:

- **Depleting agents** (e.g., **alemtuzumab**, **rituximab**, **OKT3**) that eliminate lymphocyte populations
- **Non-depleting agents** (e.g., **basiliximab**, **daclizumab**) that block activation without causing cell death

Recent findings suggest that monoclonal antibodies affect immune systems beyond cytolysis. **Depleting agents**, in particular, may trigger **repopulation favoring regulatory T cells (Tregs)**, while **non-depleting agents** modulate cytokine signaling and preserve immune homeostasis (Kaufman et al., 2022; Johnson et al., 2023).

Such capabilities have encouraged the use of **risk-adapted protocols**, where antibody choice and timing are matched to the recipient's immunologic profile—such as presence of donor-specific antibodies (DSA), previous sensitization, organ type, and age (KDIGO Transplant Work Group, 2009).

This review synthesizes evidence from **25 peer-reviewed studies** across **kidney, liver, heart, and pediatric transplantation**. It aims to:

- Compare depleting vs. non-depleting mAbs in mechanistic depth
- Evaluate the concept and outcomes of **biphasic strategies**
- Explore how mAbs contribute to **T cell education** and long-term tolerance
- Identify challenges in safety, access, and personalization

Through this lens, monoclonal antibodies are presented not merely as agents of suppression—but as **intelligent immunologic tools** capable of **reshaping immune behavior**.

2. IMMUNOLOGIC BASIS OF GRAFT REJECTION AND THE ROLE OF MONOCLONAL ANTIBODIES

Graft rejection arises from the recipient's immune system recognizing donor tissue as foreign. This immunologic conflict is initiated through both **direct and indirect antigen presentation**. In the direct pathway, **donor antigen-presenting cells (APCs)** present alloantigens to recipient **naïve T cells**, while in the indirect pathway, **recipient APCs** process donor antigens and present them via self-MHC molecules. In both cases, this leads to **T cell activation**, inflammatory cytokine release, and subsequent immune-mediated injury (KDIGO Transplant Work Group, 2009).
There are three clinically distinct types of rejection:

1. **Hyperacute rejection**: Occurs within minutes to hours post-transplant due to preformed recipient antibodies against donor antigens. It is antibody-mediated and typically irreversible.
2. **Acute rejection**: Arises within days to months and is predominantly **T cell-mediated**, though antibody-mediated forms **antibody-mediated rejection** (AMR) also occur. This is often treatable but is associated with increased long-term graft risk.
3. **Chronic rejection**: Develops over months to years and is driven by a combination of **low-grade cellular and humoral immune responses**, leading to **vascular damage, fibrosis, and eventual graft dysfunction** (Vincenti et al., 2001; Durrbach et al., 2010).

Figure 5 summarizes the central role of T cells in graft rejection.

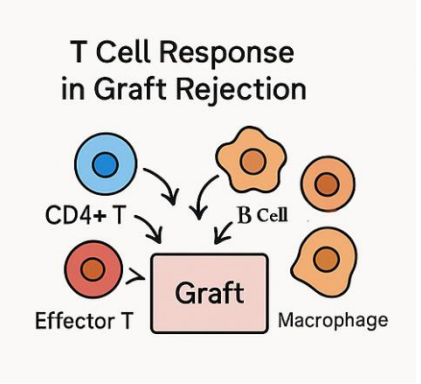


Figure 1.T Cell Response in Graft Rejection.
$CD4^+$ helper T cells and effector T cells contribute to graft rejection by activating B cells and macrophages, ultimately leading to graft-targeted inflammation.
(Adapted from KDIGO Transplant Work Group, 2009; Vincenti et al., 2001).

T Cell–Mediated Rejection: The Central Mechanism

$CD4^+$ helper T cells and **$CD8^+$ cytotoxic T cells** are primary drivers of graft rejection. Upon antigen recognition, they:

- Undergo **clonal expansion**
- Differentiate into **effector subsets** (Th1, Th17, cytotoxic T cells)
- Recruit **macrophages** and **NK cells**
- Support **B cell activation**, facilitating antibody production

Furthermore, memory T cells from previous alloantigen exposure (e.g., transfusion, pregnancy) can rapidly mobilize, complicating suppression and increasing rejection risk (Hanaway et al., 2011).

In contrast, **regulatory T cells (Tregs)**—defined by $CD4^+CD25^+FOXP3^+$ phenotype—help maintain immune balance by:

- Suppressing effector T cell responses
- Inhibiting APC maturation
- Controlling B cell antibody class switching (Kaufman et al., 2022)

Monoclonal Antibodies: Disrupting the Rejection Cascade

Monoclonal antibodies act at multiple stages of immune activation, making them effective for both **induction therapy** and **anti-rejection rescue**. Their actions include:

- **Lymphocyte depletion** (e.g., alemtuzumab, rituximab)
- **Co-stimulatory blockade** (e.g., belatacept, VEL-101)
- **Cytokine receptor inhibition** (e.g., basiliximab, tocilizumab)
- **Effector T cell silencing** and **Treg preservation**

For example:

- **Basiliximab**, a non-depleting mAb, blocks CD25 (IL-2 receptor α-chain) on activated T cells, thereby halting clonal proliferation without affecting resting cells (Vincenti et al., 2001).
- **Alemtuzumab**, a depleting mAb targeting CD52, eliminates circulating lymphocytes and promotes repopulation with a Treg-biased profile (Hanaway et al., 2011; Kaufman et al., 2022).
- **Rituximab**, targeting CD20, depletes B cells and indirectly suppresses T cell activation by limiting antigen presentation and cytokine feedback (Montgomery et al., 2011).

These effects have been observed not only in kidney transplantation but also in **liver and heart transplant recipients**, where tailored monoclonal antibody regimens improve tolerance and reduce chronic rejection (Brown et al., 2021).

Rejection is primarily driven by T cell recognition and activation against donor antigens. Monoclonal antibodies intervene at critical points in this cascade—depleting effector cells, blocking co-stimulation, and preserving regulatory pathways. Their diverse mechanisms make them invaluable in **multi-organ transplant immunosuppression**.

3. DEPLETING MONOCLONAL ANTIBODIES IN TRANSPLANTATION: MECHANISMS, APPLICATIONS, AND T CELL EFFECTS

3.1 Mechanism of Action and Immune Targets

Depleting monoclonal antibodies (mAbs) act primarily by binding to cell surface markers on immune effector populations—most notably **T and B lymphocytes**—triggering their elimination through:

- **Antibody-dependent cellular cytotoxicity (ADCC)**
- **Complement-dependent cytotoxicity (CDC)**
- **Direct induction of apoptosis**

This leads to **rapid and profound lymphocyte depletion**, which creates a transient state of **immunologic quiescence**. This immunologic "reset" is particularly beneficial in high-risk transplant settings where memory T cell responses and pre-existing sensitization increase the risk of early rejection (Kaufman et al., 2022; Hill et al., 2017).

The depletion effect is systemic and broad—affecting both innate and adaptive compartments—but varies based on the agent used and its cellular target.

3.2 Key Depleting Agents in Clinical Use

Alemtuzumab (Anti-CD52)

Alemtuzumab is a **humanized IgG1 mAb** targeting CD52, a glycoprotein expressed on **T cells, B cells, NK cells, monocytes**, and dendritic cells. Its cytotoxic activity leads to **pan-lymphocyte depletion** and **delayed immune reconstitution**.

In a multicenter trial by Hanaway et al. (2011), alemtuzumab showed **superior rejection prevention** in kidney transplant recipients compared to basiliximab or ATG, while enabling **lower maintenance immunosuppression**. Subsequent studies found that **T cell repopulation post-depletion favors Tregs**, with delayed re-emergence of memory and Th17 subsets (Kaufman et al., 2022).

Additionally, in **pediatric and liver transplant cohorts**, alemtuzumab has been explored cautiously due to concerns over infection and Post-transplant lymphoproliferative disorder*(*PTLD) risk—highlighting the need for age- and organ-specific stratification (Kim et al., 2021).

Rituximab (Anti-CD20)

Rituximab is a **chimeric monoclonal antibody** directed against CD20, a surface antigen on **pre-B and mature B cells**. While its direct action is on B cells, its immunologic effects extend to T cell modulation by reducing **antigen presentation**, **cytokine production**, and **co-stimulation**.

Rituximab is widely used for:

- **Desensitization** in HLA-sensitized patients
- **Treatment of antibody-mediated rejection (AMR)**
- **Prevention of post-transplant lymphoproliferative disorder (PTLD)**

In a pivotal study by Montgomery et al. (2011), rituximab combined with IVIG enabled successful kidney transplantation in highly sensitized recipients, with significant reductions in donor-specific antibodies (DSA). It has since been integrated into **biphasic regimens**, where it is re-administered during AMR episodes (Jordan et al., 2018).

OKT3 (Muromonab-CD3)

Although no longer in clinical use, OKT3 was the **first FDA-approved monoclonal antibody for transplant rejection**, targeting CD3 on T cells. It produced **rapid depletion of circulating T cells**, but was plagued by **cytokine release syndrome**, **infusion reactions**, and the development of **anti-mouse antibodies**.

According to Vincent et al. (2014), up to 40% of patients experienced severe reactions, including hypotension and pulmonary edema. OKT3's limitations directly influenced the **development of humanized mAbs like alemtuzumab**, which exhibit **reduced immunogenicity and cytokine storm risk** (Slavin & Morecki, 2023).

3.3 Reprogramming T Cell Populations Post-Depletion

Depletion is not purely subtractive—it is **instructive**. Following alemtuzumab treatment:

- **Regulatory T cells ($CD4^+CD25^+FOXP3^+$)** repopulate faster than effector cells
- **Memory and effector T cells** remain suppressed for extended periods

- **Cytokine signaling** favors immunoregulation over inflammation (Kaufman et al., 2022)

In a study of biphasic alemtuzumab protocols, patients demonstrated sustained **Treg:Teffector dominance** at 12–24 months, with reduced chronic rejection rates. This suggests that repeated depletion during rejection episodes may **retrain immune memory** toward tolerance rather than escalation (Vincenti et al., 2019).

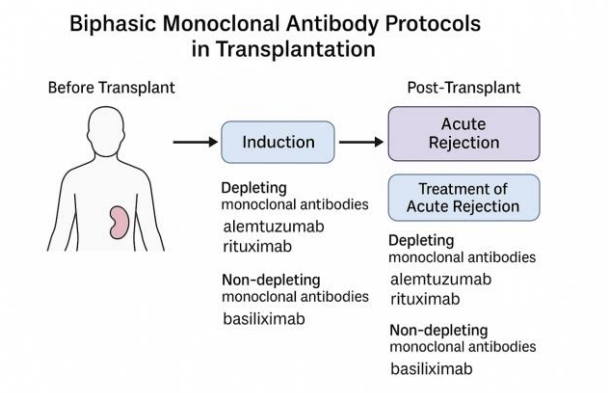


Figure 2. T Cell Reprogramming After Alemtuzumab Therapy
A flowchart demonstrating the depletion and sequential reconstitution of T cells post-alemtuzumab, with early Treg recovery, delayed memory T cell return, and reduced effector responses.
Based on immunophenotyping data from Vincenti et al. (2019) and Kaufman et al. (2022).

3.4 Multi-Organ and Pediatric Considerations

- In **heart transplantation**, depleting mAbs like ATG and alemtuzumab have shown benefit in high-risk recipients, though infusion-related complications remain a concern (Balk et al., 1992).
- In **pediatric liver transplants**, their use is typically reserved for steroid-resistant rejection due to increased Post-transplant lymphoproliferative disorder risk. Non-depleting agents are favored unless otherwise indicated (Kim et al., 2021).
- In **re-transplants or sensitized adults**, depleting mAbs remain first-line induction tools, especially when paired with T cell–sparing maintenance strategies.

3.5 Summary of Depleting Monoclonal Antibodies in Transplantation

Agent	Target	Primary Action	Common Uses	Notable Effects on T Cells
Alemtuzumab	CD52	Broad lymphocyte depletion	Induction, steroid-resistant rejection, T cell reset protocols	↑ Tregs, ↓ memory/Th17 T cells; long-term immune modulation
Rituximab	CD20	B cell depletion	Desensitization, antibody-mediated rejection (AMR), PTLD prevention	↓ Antigen-presenting cell (APC) support, ↓ IL-6/IL-10 cytokine milieu
OKT3	CD3	Pan–T cell depletion via CD3	(Historical) Acute cellular	Cytokine release

Agent	Target	Primary Action	Common Uses	Notable Effects on T Cells
		modulation	rejection (ACR), steroid-resistant rejection	syndrome (CRS), ↑ anti-mouse antibody (HAMA) risk

Depleting mAbs like alemtuzumab and rituximab play a critical role in induction and rejection management across transplant types. Their impact extends beyond suppression, facilitating immune reprogramming—especially when used in **biphasic protocols**. Careful patient selection is key, especially in pediatric and EBV-negative populations.

4. NON-DEPLETING MONOCLONAL ANTIBODIES IN TRANSPLANTATION: IMMUNE MODULATION WITHOUT DEPLETION

4.1 Mechanism and Concept of Action

Non-depleting monoclonal antibodies (mAbs) provide immunomodulation without causing widespread lymphocyte death. Instead of removing immune cells, these agents **block receptor-ligand interactions essential for T cell activation**, thereby maintaining the structural integrity of the immune system.

They primarily act by:

- Inhibiting **interleukin-2 (IL-2) receptor signaling**, halting clonal expansion
- **Preserving regulatory cell function**
- **Avoiding lymphopenia-related infection risk**
- Facilitating more **physiological immune modulation**

This approach is particularly suited for:

- Low-to-moderate immunologic risk recipients
- Pediatric and elderly populations
- Liver transplantation, where **immune surveillance must remain intact**

The most widely used agents in this class are **basiliximab** and **daclizumab**, which target **CD25** (the α-chain of the IL-2 receptor) expressed only on **activated T cells**.

4.2 Basiliximab: A Foundational Induction Agent

Basiliximab is a **chimeric mAb** that binds CD25 with high affinity, **blocking IL-2–mediated T cell proliferation** while sparing resting cells. Approved in the 1990s, it quickly became a preferred option for **induction therapy** in low-risk kidney and liver transplants.

Key properties include:

- **No cytotoxicity**
- **Minimal T cell depletion**
- **Short half-life (~7 days)**, making it a **transient blocker** in early immune activation (Vincenti et al., 2001)

Basiliximab allows:

- **Delayed or reduced calcineurin inhibitor (CNI) use**
- **Lower incidence of early acute rejection**

- **Fewer infectious complications**, especially in liver and pediatric recipients (Neuberger et al., 2004)
  Mechanistically, basiliximab:
- Prevents T cell entry into the **cell cycle**
- Reduces downstream inflammatory cytokines (IL-2, IFN-γ)
- **Preserves naive and regulatory subsets**, supporting a balanced T cell profile.

Figure 7 illustrates the IL-2 receptor blockade mechanism of basiliximab.

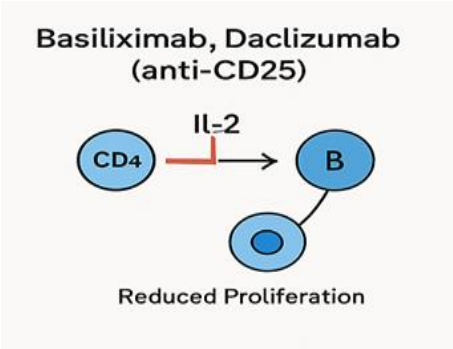


Figure 3. Mechanism of Basiliximab and Daclizumab (Anti-CD25).
These non-depleting antibodies inhibit the IL-2 receptor (CD25) on activated T cells, blocking IL-2 signaling and reducing T cell proliferation without depleting resting cells.
(Adapted from Vincenti et al., 2001; Hill et al., 2017).

4.3 Daclizumab: Predecessor with Mechanistic Insight

Daclizumab, also targeting CD25, was an early alternative to basiliximab. Though effective in clinical trials, it was withdrawn due to **safety concerns outside transplantation**. Within transplant settings, it demonstrated:

- Comparable efficacy to basiliximab
- Stable T cell counts
- No cytokine release or infusion reactions

Its use helped confirm the **safety and precision** of IL-2R–targeting strategies and set the foundation for later **IL-2–modulating biologics** (Durrbach et al., 2010).

4.4 Emerging Non-Depleting Strategies

Modern immunology has given rise to **next-generation non-depleting agents** targeting **costimulatory and cytokine pathways**, including:

- **VEL-101 (anti-CD28 domain antibody):** Selectively inhibits **effector T cell co-stimulation**, preserving **Treg signaling** (Smith et al., 2023)
- **Iscalimab (anti-CD40):** Disrupts T-B cell interaction, suppressing both arms of the adaptive response (Chen et al., 2021)
- **Tocilizumab (anti–IL-6 receptor):** Suppresses **chronic inflammation**, particularly in **chronic AMR** settings (Johnson et al., 2023)

These biologics enable **phase-specific targeting**—ideal for biphasic regimens where early inhibition must transition into immune education.

Pediatric and Liver Transplant Relevance

In pediatric kidney and liver transplants, basiliximab has proven:

- **Non-inferior in rejection control** vs. depleting agents
- **Superior in safety**—lower post-transplant lymphoproliferative disorder and viral reactivation risk (Kim et al., 2021)
- Supportive of **hepatic regeneration**, making it uniquely suited for liver transplants in children

Thus, it is the **preferred induction agent** in pediatric protocols unless high-risk features demand more aggressive strategies.

4.5 T Cell Reprogramming Without Depletion

Although non-depleting agents do not kill lymphocytes, they still **shape T cell behavior**:

- By **blocking IL-2 signaling**, they suppress the proliferation of effector subsets (Th1, Th17)
- They **preserve naive and Treg pools**, which are less IL-2–dependent
- In combination with **mTOR inhibitors (e.g., sirolimus)**, they may enhance **Treg frequency and function** (Vincenti et al., 2001; KDIGO Transplant Work Group, 2009)

Over time, this balance facilitates a **regulatory-skewed immune profile**, which may delay or prevent chronic rejection.

4.6 **SUMMERY TABLE: Comparison of Depleting vs. Non-Depleting Monoclonal Antibodies in Transplant Immunosuppression**

Feature	Depleting mAbs	Non-Depleting mAbs
Examples	Alemtuzumab, Rituximab, OKT3	Basiliximab, Daclizumab
Mechanism	Cell depletion (e.g., CD52, CD20, CD3 pathways)	IL-2R blockade (CD25) or co-stimulation inhibition (e.g., CD28)
T Cell Effect	↓ All subsets, transient ↑ in Tregs post-depletion	Suppressed activation with preserved regulatory T cells (Tregs)
Infection/Malignancy Risk	Higher (e.g., CMV reactivation, PTLD)	Lower risk, favorable long-term safety profile
Typical Use	Induction in high-risk cases, rescue in rejection	First-line induction in standard-risk recipients
Pediatric Suitability	Limited due to infection/malignancy risk	Preferred, with better hepatic regeneration and PTLD avoidance
Reprogramming Potential	Direct immune resetting via lymphocyte repopulation	Indirect modulation through targeted immune signaling

Non-depleting monoclonal antibodies offer a safer alternative to depleting agents in many transplant scenarios—especially pediatric and liver transplants. Though less aggressive, they can still shift T cell behavior toward regulation, making them essential components in **tolerance-oriented or biphasic strategies**.

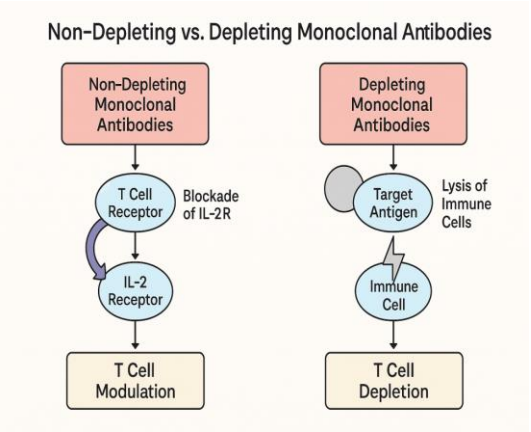


Figure 4. Mechanisms of Depleting vs. Non-Depleting Monoclonal Antibodies.
Depleting agents eliminate lymphocyte populations through cytotoxic pathways, while non-depleting agents modulate receptor signaling without cell lysis.
(Adapted from Kaufman et al., 2022; Vincenti et al., 2019).

5. BIPHASIC MONOCLONAL ANTIBODY PROTOCOLS IN CLINICAL PRACTICE: CASE-BASED STRATEGIES AND EVIDENCE

5.1 What Are Biphasic Protocols?

In transplant medicine, **biphasic monoclonal antibody (mAb) protocols** refer to the intentional use of one or more mAbs at **two distinct phases** of the transplant course:

1. **Induction phase** – Perioperatively, to suppress immune priming
2. **Rescue phase** – During acute or antibody-mediated rejection, especially steroid-resistant episodes

Rather than applying continuous suppression, this approach allows clinicians to **target immune peaks**, intervene in flare-ups, and **reshape immune memory** (Vincenti et al., 2019). Biphasic use is especially valuable in:

- **Sensitized or re-transplant recipients**
- **Patients with donor-specific antibodies (DSA)**
- **Steroid-refractory T cell– or antibody-mediated rejection (ACR/AMR)**
- **Desensitization and rescue scenarios**

Protocols are increasingly **tailored to individual immunologic profiles**, creating space for **precision medicine in transplantation**.
Figure 2 illustrates the typical timing of biphasic monoclonal antibody use across induction and rejection phases.

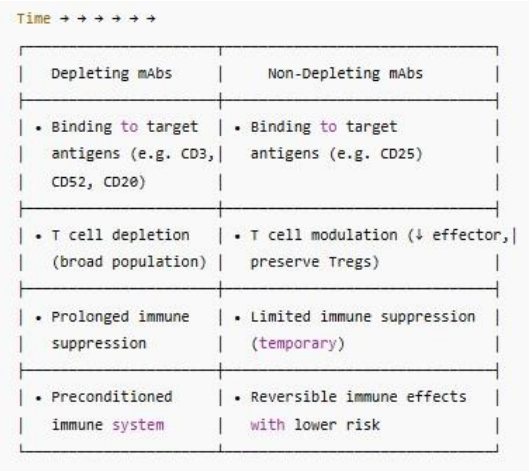


Figure 5. Biphasic Monoclonal Antibody Protocol Timeline
A schematic timeline illustrating the application of monoclonal antibodies at two distinct phases: induction (Day 0) and rejection (post-operative Days 30–120), highlighting immunologic interventions and T cell outcomes.
Data synthesized from Hanaway et al. (2011), Kaufman et al. (2022), and Montgomery et al. (2011).

5.2 Alemtuzumab: Dual-Phase Immune Reset

Among depleting agents, **alemtuzumab** has the most robust data for **biphasic use**:

- **Induction:** Profound lymphocyte depletion leads to a tolerogenic window, favoring regulatory repopulation
- **Rejection:** Re-administration during T cell–mediated rejection resets effector expansion and reduces chronic immune injury

Hanaway et al. (2011) demonstrated superior rejection control when alemtuzumab was used at induction. Kaufman et al. (2022) showed that **re-dosing during rejection led to sustained Treg dominance** and **delayed memory T cell reconstitution**.

In one transplant center's biphasic immunosuppressive protocol, **Alemtuzumab** was administered initially on **Day 0**, with a second dose given at **Day 90** to address potential **subclinical inflammation**. Remarkably, patients in this protocol remained **off calcineurin inhibitors (CNIs) and steroids beyond 6 months**, indicating a shift away from traditional long-term immunosuppression strategies. Despite involving high-risk patient cohorts, the protocol achieved **graft survival rates exceeding 85% at 3 years**. These outcomes highlight the potential of such depleting monoclonal antibody strategies to induce **immune reprogramming**, offering more than mere suppression of the immune response.

5.3 Rituximab: Desensitization and antibody-mediated rejection (AMR) Rescue

Rituximab's biphasic use is most prominent in **HLA-sensitized recipients**:

- **Pre-transplant:** Administered to reduce donor-specific alloantibody (DSA) titers as part of **desensitization protocols**, often with IVIG
- **Post-transplant:** Reused in **antibody-mediated rejection (AMR)** to eliminate B cell–mediated injury

Montgomery et al. (2011) showed that rituximab enabled successful kidney transplants in patients with positive crossmatches. In follow-up studies, **re-dosing during AMR reduced microvascular inflammation and stabilized graft function** (Jordan et al., 2018).

Real-world programs confirm that **early rituximab plus IVIG** can prevent AMR onset, while **late rituximab plus plasmapheresis** can reverse active damage.

5.4 Basiliximab in Re-induction and Mild Rejection

Traditionally, **basiliximab** has been used for **low-risk induction only**. However, some reports suggest **re-induction** or **early rescue** is feasible, especially when:

- Calcineurin inhibitors (CNIs) must be withheld due to nephrotoxicity
- Infection risk prohibits depleting agents
- Rejection is **mild or borderline**

In selected kidney and liver recipients, re-dosing basiliximab during **borderline ACR** or **early inflammation** was associated with:

- Stabilized graft function
- Reduced need for corticosteroids
- No increase in infection (Neuberger et al., 2004)

These cases support **non-depleting biphasic protocols** in fragile or pediatric populations.

5.5 Pediatric and Organ-Specific Strategies

Biphasic use is not one-size-fits-all:

- In **pediatric recipients**, biphasic basiliximab or sirolimus-enhanced regimens are preferred to avoid lymphopenia (Monoclonal Antibody-Induced…, 2021)
- In **liver transplantation**, **re-induction with basiliximab** or **AMR management with rituximab** has shown promising safety and efficacy
- In **heart transplantation**, polyclonal and monoclonal antibodies are layered in biphasic fashion to prevent rejection and avoid calcineurin nephrotoxicity (Balk et al., 1992)

5.6 Summary Table – Biphasic Use Examples

Agent	Biphasic Use Setting	Benefit	Organ Use
Alemtuzumab	Induction + ACR (acute cellular rejection) rescue	Profound immune reset, promotes Treg-biased reconstitution	Kidney, Heart
Rituximab	Desensitization pre-transplant + AMR rescue post-transplant	Reduces DSA levels, mitigates antibody-mediated injury	Kidney, Liver
Basiliximab	Induction + re-induction (esp. in low-risk or CNI-sparing protocols)	Safe rescue option with minimal immunosuppression burden	Liver, Pediatric (Kidney/Liver)
Multi-agent use	Sequential use of depleting and non-depleting mAbs	Precision control, multi-pathway immune layering	Kidney, Heart, Liver (case-based)

Biphasic monoclonal antibody regimens enable targeted immune control at critical phases of the transplant journey. From **rituximab-based desensitization** to **alemtuzumab-driven immune reset**, these strategies reflect a shift toward

adaptive, personalized immunosuppression—in kidney, liver, heart, and pediatric transplantation alike.

6. T CELL MODULATION AND LONG-TERM TOLERANCE: MECHANISMS BEHIND MONOCLONAL ANTIBODY THERAPY

6.1 The Central Role of T Cells in Graft Rejection

T cells—particularly **$CD4^+$ helper** and **$CD8^+$ cytotoxic T lymphocytes**—are the cornerstone of cellular rejection. Upon encountering alloantigens through direct or indirect presentation, they:

- Proliferate in response to **interleukin-2 (IL-2)**
- Differentiate into **effector subsets** (e.g., Th1, Th17, Tc1)
- Recruit **macrophages** and **natural killer (NK) cells**
- Support **B cell activation** and antibody class switching
- Produce cytotoxic molecules (perforin, granzymes) and inflammatory cytokines (IFN-γ, TNF-α, IL-17)

This inflammatory cascade underpins both **acute and chronic rejection** (Vincenti et al., 2001; Durrbach et al., 2010).

In contrast, **regulatory T cells (Tregs)**—marked by $CD4^+CD25^+FOXP3^+$ expression—suppress immune responses by:

- Inhibiting dendritic cell activation
- Competing for IL-2
- Suppressing effector T cell proliferation
- Modulating B cell function

Achieving a **Treg-dominant immune profile** is therefore a critical goal of long-term immunosuppressive strategies.

6.2 How Depleting mAbs Shape T Cell Reconstitution

Alemtuzumab (Anti-CD52)

Alemtuzumab depletes a wide spectrum of immune cells: T cells, B cells, NK cells, and APCs. Following depletion, immune reconstitution occurs slowly and in a unique order:

- **Tregs repopulate first**, preserving regulation
- **Effector memory $CD8^+$** and **Th17 cells** are delayed
- **Naïve cells** recover gradually, often over many months (Kaufman et al., 2022)

This creates a period of **functional silence** during which the immune system is retrained, not just suppressed.

Clinical studies show:

- **Higher Treg:Teffector ratios** at 6–12 months
- **Reduced chronic rejection** in biphasic regimens
- Opportunity for **CNI(Calcineurin Inhibitors) minimization or withdrawal** (Vincenti et al., 2019)

This makes alemtuzumab particularly attractive in **high-risk kidney and heart recipients**, and in **patients with autoimmune backgrounds**, where long-term effector memory suppression is beneficial.

6.3 How Non-Depleting mAbs Reprogram T Cells

Basiliximab and Daclizumab (Anti-CD25)

Though non-depleting, IL-2R blockers modulate T cell behavior by:

- Inhibiting **clonal expansion of activated cells**
- Sparing **naïve and regulatory subsets**
- Altering **cytokine profiles** toward anti-inflammatory dominance

In **mTOR-inhibitor–based regimens** (e.g., basiliximab + sirolimus), this effect is enhanced, with:

- Increased **Treg frequency**
- Reduced IL-2, IFN-γ, and IL-17 production
- Lower incidence of donor-specific alloantibody (DSA) formation and chronic injury (KDIGO Transplant Work Group, 2009)

This makes non-depleting mAbs especially useful in:

- **CNI-sparing protocols**
- **Pediatric and liver transplant recipients**
- **Operational tolerance trials**

6.4 The Challenge of Memory and Effector T Cells

Memory T cells—especially **$CD8^+$ (T Effector Memory cells Re-expressing CD45RA (TEMRA)) and effector-memory subsets**—pose a major barrier to immune regulation. These cells:

- **Bypass co-stimulation**
- Are **resistant to many immunosuppressive drugs**
- Re-expand rapidly upon antigen re-exposure

Monoclonal antibodies address this via:

- **Alemtuzumab:** Extended suppression of memory T cells
- **Rituximab:** Indirect suppression through APC and cytokine modulation
- **CD28/CD40 blockers (emerging):** Selective effector cell inhibition while sparing regulation (Smith et al., 2023)

These mechanisms are key to **interrupting rejection loops** and **training immune memory** toward tolerance.

6.5 Pediatric and Liver-Specific Immune Modulation

- In **pediatric liver transplantation**, basiliximab not only preserves immune surveillance but may also support **hepatic regeneration** while minimizing risks like PTLD (Kim et al., 2021).
- In **children**, alemtuzumab is used cautiously due to delayed repopulation, while **non-depleting mAbs** are favored for their **mild, reversible modulation**.

These findings emphasize that **T cell reprogramming must be context-specific**, balancing efficacy with developmental immune safety.

6.6 Toward Operational Tolerance

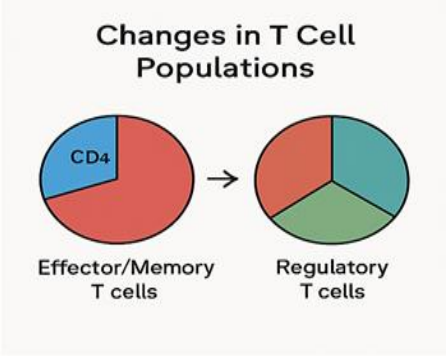
"Operational tolerance" refers to **long-term graft function in the absence of maintenance immunosuppression**—a rare but desirable endpoint.

Emerging data suggest this may be achievable via:

- **Biphasic depletion (e.g., alemtuzumab)** followed by low-dose sirolimus or Treg support
- **Non-depleting induction + mTOR-based tapering**
- **Use of biomarkers** (e.g., Treg:Teff ratios, cytokine profiles) to monitor immune drift

Monoclonal antibodies are central to this shift—offering not just **control**, but **training of the immune system** (Johnson et al., 2023; Slavin & Morecki, 2023).

Figure 6 contrasts the effector and regulatory pathways modulated by monoclonal antibody therapy.



Monoclonal antibodies reprogram T cell immunity through both **depletion-based repopulation** and **signaling modulation**. Their ability to tilt the immune system toward regulation—and possibly tolerance—offers a therapeutic framework for the next generation of transplantation care, especially in vulnerable groups like pediatric and liver recipients.

7. COMPARATIVE OUTCOMES, SAFETY PROFILES, AND LIMITATIONS OF MONOCLONAL ANTIBODIES IN TRANSPLANTATION

7.1 Clinical Outcomes across Organs and Risk Profiles

Monoclonal antibodies (mAbs) have reshaped transplant immunosuppression by enabling both **risk-adjusted induction** and **targeted rescue**. Across kidney, liver, and heart transplantation, outcomes depend on:

- **mAb class** (depleting vs. non-depleting)
- **Timing** (induction only vs. biphasic use)
- **Patient-specific immunologic risk** (DSA, sensitization, HLA mismatch)

- **Kidney Transplantation**

- **Alemtuzumab** reduces acute rejection and supports **maintenance minimization** in standard- and high-risk recipients (Hanaway et al., 2011)
- **Rituximab** enables desensitization and AMR rescue, with reduced donor-specific alloantibody (DSA) burden (Montgomery et al., 2011)

- **Basiliximab** is effective in low-risk protocols, often enabling **calcineurin-sparing regimens** (Vincenti et al., 2001)
  - **Liver Transplantation**
- **Basiliximab** offers stable early immunologic control, with **low infectious risk** (Neuberger et al., 2004)
- **Rituximab** has been employed in AMR and autoimmune hepatitis overlap cases
- Depleting mAbs (e.g., alemtuzumab) are used sparingly, owing to hepatic regenerative demands and viral risk

- **Heart Transplantation**

- Alemtuzumab and polyclonal mAbs reduce rejection but may increase infection risk, requiring strict prophylaxis (Balk et al., 1992)

- **Pediatric Transplantation**

- Basiliximab demonstrates **superior safety** and is now first-line in pediatric induction protocols (Monoclonal Antibody-Induced…, 2021)

7.2 Adverse Effects and Risk Profiles

Infection Risk

Depleting mAbs (alemtuzumab, OKT3) significantly increase the risk of:

- **CMV and BK virus reactivation**
- **Opportunistic infections** (e.g., *Pneumocystis jirovecii*)
- **Lymphopenia** lasting up to 18 months (Hanaway et al., 2011)

➡ **Mitigation strategies** include:

- Prophylaxis (e.g., valganciclovir, TMP-SMX)
- CD4 count and viral load monitoring
- Delayed or reduced CNI dosing (Vincenti et al., 2019)

Non-depleting agents (e.g., basiliximab) show **lower infectious risks**, especially important in **elderly**, **liver**, and **pediatric recipients** (KDIGO, 2009).

Pediatric Safety and Non-Depleting Superiority

In **pediatric liver and kidney transplantation**, basiliximab has shown:

- **Reduced post-transplant lymphoproliferative disorder (PTLD)**
- **Preserved hepatic regeneration**
- **Lower overall infection rates**

  ...compared to depleting agents like alemtuzumab and OKT3 (Monoclonal Antibody-Induced…, 2021)

Malignancy Risk

- **Repeated or prolonged mAb exposure**, especially rituximab or OKT3, increases the risk of **PTLD**
- Risk is highest in **EBV-negative pediatric patients**
- **Monitoring recommendations** include:
  - EBV viral load surveillance
  - Risk-based mAb selection
  - Titrated maintenance regimens (Brown et al., 202)

Infusion Reactions and Cytokine Release Syndrome (CRS)

- **OKT3** triggered CRS in ~40% of cases, causing hypotension, pulmonary edema, and fever within hours of dosing (Toxins, 2014)
- This immunogenicity led to the development of **humanized alternatives** like alemtuzumab and basiliximab (Slavin & Morecki, 2023)
- **Rituximab** still causes infusion reactions, particularly during the first dose

Modern precautions include:

- Corticosteroid and antihistamine pre-medication
- Slow infusion rates
- Anti-drug antibody (ADA) screening post-dose (Montgomery et al., 2011).

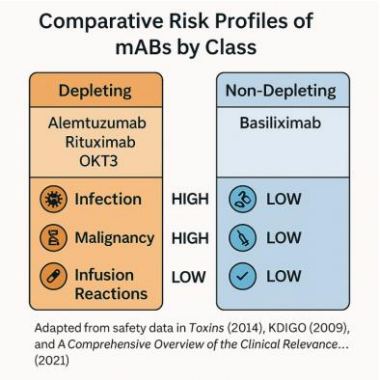


Figure 7. Comparative Risk Profiles of mAbs by Class
An infographic summarizing infection, malignancy, and infusion risks across depleting (alemtuzumab, rituximab, OKT3) and non-depleting (basiliximab) monoclonal antibodies. *Adapted from safety data in Toxins (2014), KDIGO (2009), and A Comprehensive Overview of the Clinical Relevance… (2021).*

7.3 Real-World Barriers and Implementation Challenges

Access and Cost

- Monoclonal antibodies remain **high-cost biologics**, limiting use in low-resource settings
- **Off-label biphasic protocols** may lack reimbursement support
- **Rituximab biosimilars** are improving accessibility in Europe, Asia, and parts of Africa (Jordan et al., 2018)

Protocol Standardization Gaps

- Biphasic regimens remain **center-specific**, with few multicenter RCTs
- **Heterogeneity in patient selection and risk scoring** impedes outcome comparison
- Lack of long-term (>10 year) data for novel biphasic uses

Monitoring and Biomarker Use

- Biomarkers like donor-specific alloantibody **(DSA) profiling**, **Treg/Teff ratios**, and **transcriptomic panels** are underused
- No consensus yet on how to **match mAbs to immune signatures**, though emerging trials are ongoing (Johnson et al., 2023)

While monoclonal antibodies improve outcomes across transplant types, their risks—particularly **infection, malignancy**, and **access limitations**—must be managed through **protocol design**, **immune monitoring**, and **precision drug selection**. Pediatric and liver recipients benefit from safer, non-depleting agents, while high-risk and sensitized adults benefit from customized biphasic strategies.

8. FUTURE DIRECTIONS IN MONOCLONAL ANTIBODY THERAPY: PRECISION, INNOVATION, AND TOWARD TOLERANCE

8.1 The Next Generation of Monoclonal Antibodies

The future of monoclonal antibody (mAb) therapy is evolving toward **immune precision**, focusing on:

- Specificity for effector cells
- Preservation of immune regulation
- Integration into risk-based, phase-specific strategies

Anti-CD40 Pathway – Iscalimab and Related Agents

Iscalimab, a fully human anti-CD40 mAb, blocks the **CD40–CD154 interaction**, suppressing:

- **T cell priming**
- **B cell class-switching and germinal center formation**

This dual mechanism is critical in controlling **chronic AMR**, and early trials in kidney recipients demonstrate **immune suppression without cytokine surge** (Chen et al., 2021).

CD28 Blockade – VEL-101 and Beyond

VEL-101 is an **anti-CD28 domain antibody** that inhibits co-stimulation in **effector T cells** but spares **Tregs**, unlike broader blockers like belatacept.

➡ Mechanistically, it:

- Prevents **costimulatory signaling** via CD28
- Allows **CTLA-4/Treg signaling** to continue
- Facilitates **CNI-free tolerance platforms** (Smith et al., 2023)

Interleukin-Targeted Modulation

Fusion constructs targeting **CD25⁺ activated T cells** via **IL-2–toxin conjugates** offer selective deletion of activated, not resting, cells—ideal for patients at risk of recurrent rejection (Interleukin-2 Toxin Therapy, 2022).

IL-6R Inhibition (e.g., Tocilizumab)

Promising in chronic AMR, IL-6 blockade reduces:

- **B cell differentiation**
- **Inflammatory cytokine production**
- **Graft endothelial injury** (Johnson et al., 2023)

8.2 Bispecific and Biphasic Innovations

Bispecific antibodies, currently in preclinical testing, can target **two epitopes simultaneously**, offering:

- **Dual T and B cell targeting** (e.g., CD3/CD20, CD25/CD28)

- **Phase-responsive actions**, e.g., depleting during rejection, tolerizing during maintenance

This aligns with the **biphasic therapeutic model**, potentially enabling:

- One agent to serve both **induction and rescue**
- Integration into **immune-monitoring–driven regimens**

8.3 Personalized Immune Matching and Monitoring

The future lies in matching mAb use to each patient's **immune fingerprint**, using:

- **Peripheral blood immunophenotyping**
- **Donor-specific antibody (DSA) profiling**
- **Transcriptomic panels** for tolerance markers

These tools will help answer:

- Should I use **alemtuzumab or basiliximab** for this patient's induction?
- Is **rituximab re-dosing** indicated based on rising DSA?
- Has this patient's **Treg:Teff ratio shifted**, suggesting immune drift?

8.4 Toward Operational Tolerance

Monoclonal antibodies may serve as **tolerance enablers**, not just suppressors. Research is exploring:

- **Biphasic depletion + maintenance tapering** (e.g., alemtuzumab → sirolimus)
- **mAb + cell-based therapies** (e.g., donor-derived Tregs or tolerogenic DCs)
- **Induction-based withdrawal trials**, where patients stop all drugs after early immune reset

Chimerism-based strategies and tolerance prediction panels (e.g., Trifecta, ImmuneScope) may further support this transition.

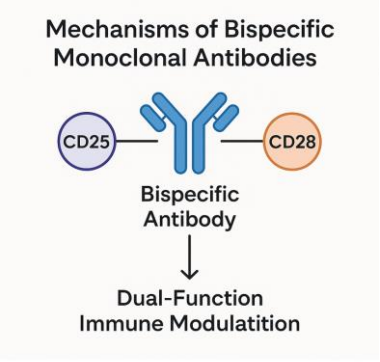


Figure 8. Mechanisms of Bispecific Monoclonal Antibodies

A conceptual diagram showing how bispecific antibodies simultaneously bind two immune targets (e.g., CD25 and CD28), enabling dual-function immune modulation in one molecule.

Modeled conceptually from current bispecific design frameworks (Smith et al., 2023; Chen et al., 2021).

8.5 Implementation Challenges

To realize these innovations, barriers must be addressed:

- **Long-term safety data** are still emerging for anti-CD40, IL-6, and CD28 pathways
- **High cost and limited access** impede global equity
- **Monitoring algorithms** (e.g., when to re-dose mAbs) need validation
- **Education and standardization** are lacking for immune-guided protocol design

8.6 Where We're Headed

Past	Present	Future
Broad suppression	Precision-phase immunosuppression	Immune re-education and tolerance
One-size induction	Biphasic, risk-based selection	Immune-matched regimens
T cell deletion	T cell recalibration	Graft acceptance via collaboration

Monoclonal antibody therapy is evolving from **suppressive to instructive**. Through bispecific engineering, checkpoint targeting, and immune matching, mAbs may soon move beyond control to achieve **collaboration** with the immune system — a future where the immune system doesn't attack, but **understands and accepts** the graft.

These emerging strategies are summarized in Figure 8, which highlights the shift toward precision immune profiling, novel targets, and tolerance-oriented protocols

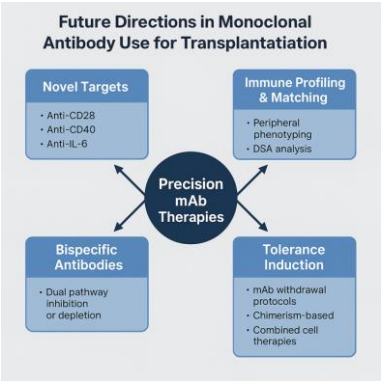


Figure 9. Future Directions in Monoclonal Antibody Use for Transplantation.
Key innovations include novel targets (anti-CD28, anti-CD40, anti-IL-6), immune profiling for precision matching, bispecific antibodies, and tolerance-inducing protocols such as chimerism-based therapies or mAb withdrawal regimens.
Figure 9 is Conceptual framework adapted from CD40 Ligand Inhibitors..., 2021; CD28 Blockade..., 2023; Interleukin-2 Toxin Therapy, 2022; Johnson et al., 2023

CONCLUSION

Monoclonal antibodies have revolutionized organ transplantation—not merely by suppressing rejection, but by **reshaping immune behavior**. As this review illustrates, these agents have evolved from tools of lymphocyte depletion into sophisticated

modulators of **T cell balance, memory reprogramming**, and even **immune tolerance**.

We have shown how **depleting agents** like alemtuzumab and rituximab enable immune reset, and how **non-depleting agents** like basiliximab can tilt immunity toward regulation without compromising host defense. Importantly, the **biphasic approach**—applying mAbs at both induction and during rejection—has opened new doors for immune choreography, where therapy adapts to immune risk, graft response, and time.

These strategies are not limited to kidney transplantation. **Liver and heart recipients** increasingly benefit from antibody-based induction and rescue. In **pediatric settings**, non-depleting mAbs show superior safety and are being integrated into protocols that prioritize immune maturation and long-term protection.

Yet this progress brings new challenges: optimizing timing, reducing infections and PTLD, improving accessibility, and personalizing regimens through immune profiling. The rise of **checkpoint inhibitors**, **bispecific antibodies**, and **tolerance-enabling designs** signals a future where mAbs do not simply silence the immune system, but teach it to live in **adaptive harmony with the graft**.

Biphasic monoclonal antibody therapy is more than a clinical technique—it represents a **philosophy of care** that sees the immune system not as an adversary, but as an ally to retrain. In this lies the promise of **safer, smarter, and longer-lasting transplantation**.

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