Biomedicine Advances

Biomedicine Advances. 2025;2(4):183-194
doi: 10.34172/bma.39
https://biomedad.ae

Review Article

Engineering the 'one-for-all' cell: Molecular approaches to universal hypoimmunogenic pluripotent stem cells

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Summary

The ground-breaking discovery of induced pluripotent stem cell (iPSC) technology has revolutionized regenerative medicine by providing an unlimited source of patient-specific cells capable of differentiating into any cell type. However, immune rejection following allogeneic transplantation remains a fundamental barrier to the widespread clinical implementation due to human leukocyte antigen (HLA) mismatches between donors and recipients. While current clinical studies are limited to HLA-matched approaches using autologous or HLA homozygous iPSC-derived grafts, practical constraints of time, cost, and scalability necessitate developing truly universal hypoimmunogenic iPSCs. This review examines molecular strategies for engineering universal iPSCs that can evade immune recognition through targeted genetic modifications. HLA class I elimination via B2M knockout effectively prevents CD8+T cell activation but renders cells vulnerable to NK cell-mediated "missing self" recognition. Alternative approaches include selective HLA targeting with HLA-C retention, which maintains NK cell inhibition while achieving substantial T cell evasion, and HLA class II elimination through CIITA knockout to prevent CD4+T cell activation. Addressing NK cell-mediated rejection requires complementary strategies including the introduction of NK inhibitory ligands such as HLA-E, HLA-G, and CD47. The most promising approaches utilize comprehensive combination strategies that simultaneously eliminate HLA class I and II molecules while incorporating multiple immune evasion mechanisms. Future directions include advanced gene editing technologies, inducible safety systems, and sophisticated computational modeling to optimize immune compatibility. These molecular engineering approaches represent a transformative pathway toward truly universal donor cells capable of transplantation without immune rejection, potentially revolutionizing the accessibility and clinical impact of iPSC-based regenerative medicine.

Keywords: Induced pluripotent stem cells (iPSCs), Universal donor cells, Hypoimmunogenic cells, HLA editing, Immune evasion, Regenerative medicine

Received: July 14, 2025, **Revised:** August 27, 2025, **Accepted:** September 18, 2025, **ePublished:** October 20, 2025

Introduction

The groundbreaking discovery of induced pluripotent stem cell (iPSC) technology by Yamanaka and Takahashi in 2006 fundamentally transformed regenerative medicine by enabling the reprogramming of adult somatic cells into pluripotent stem cells through the introduction of four transcription factors: Oct4, Sox2, Klf4, and c-Myc.[1] This discovery circumvented the ethical concerns surrounding embryonic stem cells while providing an unlimited source of patient-specific cells with the capacity for self-renewal and differentiation into all three germ layers.[1,2] The remarkable capacity of iPSCs for unlimited expansion, genetic engineering, and differentiation into diverse somatic cell types has positioned them as a cornerstone technology for treating numerous degenerative diseases, including Parkinson's disease, macular degeneration, heart failure, and diabetes.[3,4] iPSC technology has made substantial clinical progress as part of the broader human pluripotent stem cell field, which as of 2024 encompasses 115 clinical trials testing 83 distinct products,[3] with iPSC-derived therapies representing a significant and growing portion of this landscape.[3,5,6] Current therapeutic applications span ophthalmology, neurology, cardiovascular medicine, and oncology, with promising efficacy signals emerging from completed trials including ~50% symptom reduction in Parkinson's disease patients[3] and 60% survival rates in graft-versus-host disease treatment.[7] However, current clinical studies are limited to HLA-matched approaches, with only autologous iPSC-derived grafts or HLA homozygous iPSC-derived grafts being transplanted into patients following HLA matching, highlighting the urgent need for truly universal strategies.[8]

Despite these remarkable advances in iPSC technology, a fundamental immunological barrier continues to limit the widespread clinical implementation of iPSC-based regenerative medicine: immune rejection following allogeneic transplantation.[9,10] The human leukocyte antigen (HLA) system, representing the most polymorphic region in the human genome with over 33,490 documented alleles, presents the primary obstacle to successful stem cell transplantation.[11] While autologous transplantation utilizing patient-specific iPSCs avoids immunological

incompatibility, the practical constraints of time, cost, and scalability make this approach unsuitable for emergency treatments and broad clinical deployment.[12] Allogeneic iPSC-derived cell products, though offering significant advantages as "off-the-shelf" therapeutics, face substantial immunological hurdles due to HLA mismatches between donors and recipients.[3,12]

The HLA system, comprising highly polymorphic class I (HLA-A, -B, -C) and class II (HLA-DR, -DQ, -DP) molecules, represents the primary barrier to successful allogeneic transplantation. HLA class I molecules present endogenous peptides to $CD8^+$ T cells and regulate natural killer cell activation through killer immunoglobulin-like receptor interactions, while HLA class II molecules present exogenous antigens to $CD4^+$ helper T cells.[13,14] Immune rejection occurs through multiple pathways: direct recognition where recipient T cells recognize intact donor HLA molecules, indirect recognition involving donor antigen processing by recipient antigen-presenting cells, and natural killer cell-mediated "missing self" responses when HLA class I expression is absent or reduced.[15,16] Even in HLA-matched allogeneic iPSC transplantation models, immune rejection has been observed due to minor histocompatibility antigen mismatches, necessitating long-term immunosuppressive therapy with its associated complications.[17,18] Furthermore, even autologous iPSCs can trigger immune responses due to reprogramming-induced mutations, particularly mitochondrial DNA alterations that create novel antigens capable of triggering rejection.[19] Current matching requirements demand 8/8 high-resolution HLA compatibility for optimal outcomes in hematopoietic stem cell transplantation,[20] creating significant disparities in access across ethnic populations due to the extreme diversity of HLA haplotypes.[21]

To address these immunological challenges, HLA haplobanking has emerged as a pragmatic strategic approach, involving the establishment of repositories of iPSC lines from donors with homozygous HLA haplotypes to maximize recipient matching potential across broader patient populations.[22,23] Japan's pioneering iPSC Stock Project at CiRA represents the world's most successful operational haplobank, comprising 27 clinical-grade iPSC lines from 7 donors that provide HLA-A, -B, and -DRB1 compatibility for approximately 40% of the Japanese population.[23] These lines have supported over 10 clinical trials since 2015, demonstrating the feasibility of population-level coverage through strategic donor selection. Similar initiatives have emerged globally, with South Korea developing 10 clinical-grade haplolines covering 41% of the Korean population [24], Spain creating 7 haplolines covering 21% of Spanish patients [22], and other countries establishing national programs.[25] However, mathematical modeling reveals substantial limitations that constrain the haplobank approach. While Japan requires only 50 haplolines for 90% population coverage due to relative genetic homogeneity,[26] more genetically diverse populations face exponentially greater challenges requiring significantly more haplotypes for comparable coverage.[27,28] Global analysis indicates that even coordinated international haplobanking efforts using the 180 most frequent haplotypes would provide coverage ranging from only 19.5% to 81.7% of national populations, with substantial disparities between ethnically homogeneous and diverse populations.[25] Additional constraints include significant manufacturing costs, regulatory harmonization challenges across borders, limited donor consent for commercial applications, and the fundamental reality that even HLA-matched products may require immunosuppression due to minor histocompatibility antigens.[24,25,29]

The inherent limitations of haplobanking approaches—including variable population coverage, substantial infrastructure requirements, and persistent immunosuppression needs—have motivated the development of alternative strategies focused on generating universal hypoimmunogenic iPSCs (Figure 1). These engineered cell lines aim to evade immune recognition entirely through targeted genetic modifications that eliminate or modify key immunogenic molecules while preserving essential cellular functions and pluripotency characteristics.[10,30] This approach represents a transformative departure from population-specific matching strategies toward truly universal donor cells capable of transplantation into any recipient without immune rejection, enabled by recent advances in CRISPR/Cas9 genome editing technologies.[10]

Current molecular engineering strategies demonstrate remarkable success in creating "off-the-shelf" cellular products through sophisticated gene editing approaches targeting key immunological pathways.[31] Beta-2-microglobulin (B2M) knockout eliminates all HLA class I surface expression by disrupting the essential structural component required for MHC class I stability, achieving reduction in $CD8^+$ T cell responses but rendering cells vulnerable to natural killer cell-mediated lysis through "missing self" recognition.[9,32] More refined approaches involve selective targeting of HLA-A and HLA-B genes while retaining HLA-C expression, capitalizing on HLA-C's lower polymorphism and essential role in natural killer cell inhibition through killer immunoglobulin-like receptor interactions—modelling suggests 12 HLA-C-retained lines could cover > 90% of the global population.[10,33] Class II transactivator (CIITA) knockout eliminates HLA class II expression entirely, preventing $CD4^+$ helper T cell activation and antibody-mediated rejection.[10] Advanced strategies combine these approaches with immune checkpoint molecule expression including PD-L1, CD47, HLA-E, and HLA-G to create comprehensive immune evasion platforms that achieve a reduction in adaptive immune responses while maintaining tolerance

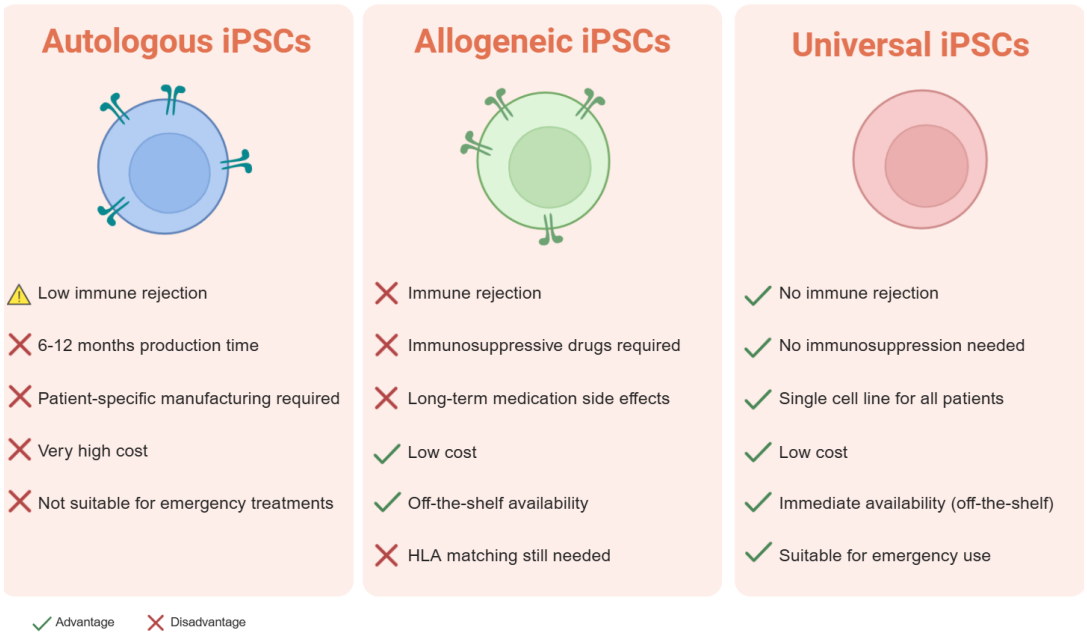

Figure 1. Comparison of iPSC-based therapeutic approaches (Created in BioRender. Elmusa, F. (2025) https://BioRender.com/ciuxnxp)

from innate immune cells.[34,35] Recent innovations include single-chain trimeric constructs incorporating HLA-E for universal natural killer cell inhibition,[36] and suicide gene switches using rapamycin-activated caspase 9 for enhanced safety.[9]

This review examines the current landscape of molecular approaches to engineering universal hypoimmunogenic pluripotent stem cells (Figure 2), synthesizing recent advances in gene editing technologies, immune evasion strategies, and clinical translation efforts.

HLA class I elimination strategies

Engineering pluripotent stem cells to evade immune recognition represents a critical frontier in regenerative medicine, with HLA Class I elimination emerging as the predominant strategy for creating "universal donor" cell therapies. Two principal approaches have dominated recent research: complete β2-microglobulin (B2M) knockout and selective HLA targeting with HLA-C retention. Each strategy offers distinct advantages and faces unique immunological challenges that fundamentally shape their therapeutic potential.

The B2M targeting approach

B2M serves as the invariant light chain essential for HLA Class I complex assembly and stability.[37] This 12-kDa protein forms non-covalent heterodimers with polymorphic HLA heavy chains (45 kDa) and 8-9 residue antigenic peptides, creating stable MHC Class I complexes. Without B2M, HLA Class I molecules cannot assemble properly in the endoplasmic reticulum, preventing their transport to the cell membrane and subsequent T cell recognition, resulting in minimal or absent surface expression of class I molecules.[38,39]

B2M targeting studies have demonstrated both the potential and limitations of this approach for creating universal iPSCs. Several research groups have successfully generated B2M-knockout iPSCs using various gene editing techniques, including CRISPR/Cas9, TALEN, and shRNA-mediated suppression.[8,10,40] Wang et al were among the first to demonstrate that B2M knockout in human ESCs could minimize immunogenicity by eliminating HLA class I surface expression, effectively preventing CD8+T cell recognition.[41] This approach was subsequently validated in multiple differentiated cell types, including cardiomyocytes, endothelial cells, and hematopoietic lineages. Wang et al further demonstrated the efficacy of B2M knockout using CRISPR/Cas9 in human iPSCs, showing that $B2M^{-/-}$ iPSCs maintained pluripotency while achieving partial reduction in HLA class I expression (HLA-A mRNA reduced by 43.3%, HLA-B by 72.7%, and HLA-C by 46.3%). In their xenogeneic monkey transplantation model, B2M knockout iPSCs showed improved teratoma formation rates (3/7 versus 2/7 for wild-type) and reduced $CD20^{+}$ B cell infiltration, though the effect on $CD3^{+}$ T cell infiltration was limited. The iPSCs demonstrated enhanced survival (3.24-fold increase compared to wild-type) in monkey wound healing models while maintaining therapeutic efficacy.[42] More sophisticated approaches have emerged to address the incomplete elimination of HLA expression. Karabekian et al demonstrated that partial B2M suppression using shRNA, which reduced B2M expression by 90% rather than complete elimination, could maintain some residual HLA class I expression sufficient to prevent NK cell activation while still achieving substantial T cell evasion.[43] This finding suggested that complete HLA class I elimination might not be necessary for immune evasion,

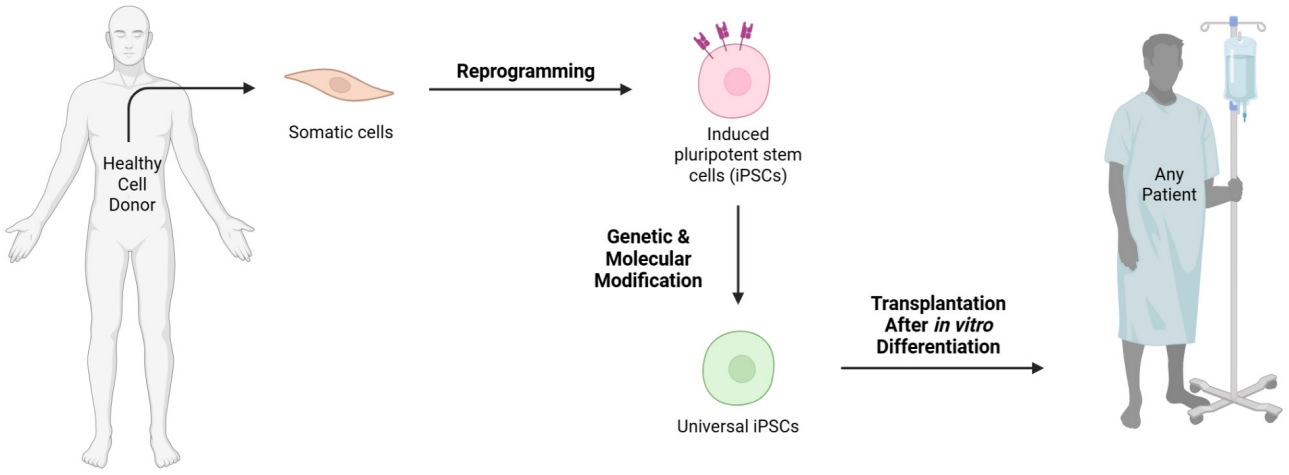

Figure 2. Overview of universal iPSC engineering strategy (Created in BioRender. Elmusa, F. (2025) https://BioRender.com/vayst0x)

and that maintaining minimal expression levels could provide protection against NK cell-mediated lysis.

The primary advantage of B2M targeting lies in its ability to achieve comprehensive elimination of HLA class I-mediated T cell responses. By disrupting the common subunit required for all HLA class I molecules, B2M knockout creates cells that are essentially invisible to CD8 + T cells, representing the most thorough approach to T cell evasion currently available.[40] This strategy offers broad applicability across multiple cell types derived from iPSCs, as the loss of B2M expression is maintained throughout differentiation processes. However, the fundamental limitation of B2M targeting is the activation of NK cell-mediated "missing self" recognition. NK cells are educated to tolerate cells expressing self HLA class I molecules, and the absence of these molecules triggers NK cell cytotoxicity through loss of inhibitory signaling.[8] Multiple studies have confirmed that B2M-deficient cells become highly susceptible to NK cell killing both *in vitro* and *in vivo*. This creates a critical therapeutic challenge, as NK cells represent a major component of innate immunity and are particularly active in transplant rejection scenarios.

In summary, B2M knockout completely eliminates HLA Class I surface expression by disrupting the essential β2-microglobulin subunit required for MHC Class I stability, achieving complete CD8 + T cell evasion (< 1% cytotoxicity) as demonstrated by Wang et al,[41] but simultaneously triggering intense NK cell activation through "missing self" recognition with a 28.4% increase in NK cell killing, requiring teratoma formation only in NK cell-depleted mice (Figure 3). While CD4 + T cell responses are expected to persist due to intact HLA Class II molecules, this has not been directly validated in B2M knockout studies, representing a knowledge gap requiring further investigation. This strategy exemplifies the fundamental trade-off between achieving robust adaptive immune evasion and maintaining protection against innate immune surveillance, necessitating complementary approaches such as NK inhibitory ligand introduction to create truly universal iPSCs.

Selective HLA targeting: HLA-C retention strategy

The HLA-C retention strategy emerged from the recognition that not all HLA class I molecules contribute equally to immune recognition and rejection responses. HLA-C displays significantly lower polymorphism compared to HLA-A and HLA-B, with fewer allelic variants present in human populations, making it more feasible to achieve population-level coverage with a limited number of cell lines.[8] More importantly, HLA-C serves as the primary ligand for killer immunoglobulin-like receptors (KIRs) on NK cells, providing essential "self" recognition signals that prevent NK cell activation through missing self-mechanisms.[40] This dual advantage-reduced polymorphism and critical NK inhibitory function-made HLA-C an attractive candidate for selective retention strategies. Population genetics analyses have revealed that only 12 common HLA-C alleles could provide coverage for over 90% of global populations across different ethnic groups, dramatically reducing the number of iPSC lines required for universal application compared to maintaining all HLA class I molecules.[10,33] The strategic elimination of highly polymorphic HLA-A and HLA-B molecules while preserving HLA-C allows for a substantial reduction in T cell recognition while maintaining NK cell tolerance, representing an elegant compromise between immune evasion and cellular protection.

Selective HLA-A and HLA-B targeting represents a sophisticated approach that balances immune compatibility with preserved antigen presentation capabilities by maintaining HLA-C expression to address the "missing-self" problem inherent in complete HLA Class I elimination. The conceptual framework for this strategy recognizes that HLA-C serves as the predominant ligand for inhibitory KIR receptors on NK cells, providing essential "don't-kill-me" signals, while HLA-A and

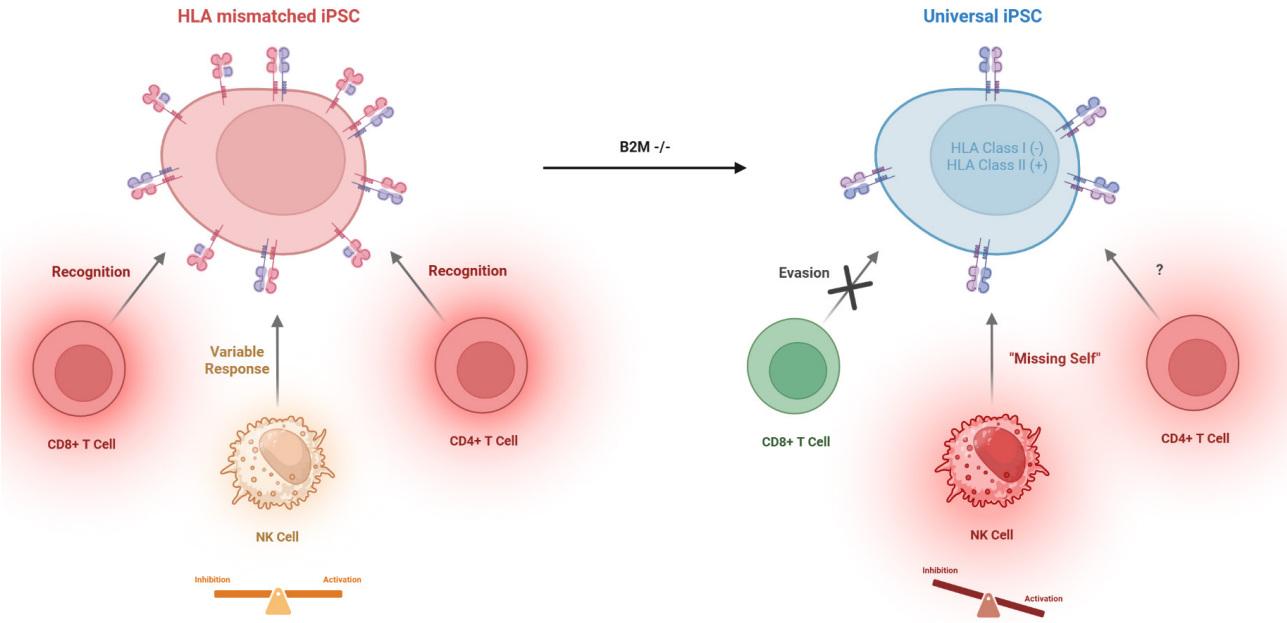

Figure 3. B2M Knockout Strategy: Complete HLA Class I Elimination and Resulting Immune Cell Responses (Created in BioRender. Elmusa, F. (2025) https://BioRender.com/5dhdj60)

HLA-B represent the primary targets of alloreactive T cell responses. Xu et al pioneered the systematic development of HLA-A/B knockout iPSCs while retaining HLA-C expression using CRISPR/Cas9 gene editing technology. Their approach involved creating customized guide RNAs specifically targeting HLA-A and HLA-B loci while leaving HLA-C expression intact. The resulting iPSCs demonstrated successful elimination of HLA-A and HLA-B surface expression while maintaining HLA-C and minor HLA class I molecules (HLA-E, -F, -G). When differentiated into CD43+hematopoietic cells, these edited cells showed a significant reduction in CD8+T cell activation and cytotoxicity when challenged with allogeneic peripheral blood mononuclear cells.[33] *In vivo* studies using mouse models have demonstrated that HLA-A/B knockout, HLA-C retained iPSCs show better protection compared to complete B2M knockout cells, confirming the protective effect of maintained HLA-C expression against NK cell-mediated rejection.[10]

The HLA-C retention strategy offers significant advantages over complete HLA elimination approaches by maintaining NK cell inhibitory signaling while achieving substantial T cell evasion. The preservation of HLA-C expression, along with minor HLA class I molecules, provides multiple inhibitory signals to NK cells through KIR and CD94/NKG2A receptors, effectively preventing the missing self-response that compromises B2M knockout approaches.[10,40] Additionally, this approach reduces the genetic modification burden compared to combination strategies requiring multiple immune evasion genes, potentially improving safety profiles and regulatory approval pathways. However, the semi-universal nature of this approach represents its primary limitation. While dramatically reducing the number of required iPSC lines compared to complete HLA matching, HLA-C compatibility between donors and recipients remains necessary for optimal outcomes. This requirement means that approximately 12 different HLA-C homozygous iPSC lines would still need to be maintained to achieve global population coverage, creating logistical and economic challenges for clinical implementation.[8,10] Furthermore, the approach may not eliminate all T cell responses, as HLA-C can still present antigens to CD8+T cells, albeit with lower efficiency than HLA-A or HLA-B. Long-term studies are necessary to determine whether residual T cell activation might lead to chronic rejection or require immunosuppressive therapy in clinical applications.

In summary, HLA-A/B knockout with HLA-C retention represents a sophisticated compromise strategy that eliminates the highly polymorphic HLA-A and HLA-B molecules responsible for the majority of T cell alloreactivity while preserving HLA-C expression to maintain NK cell inhibitory signaling through KIR interactions. Xu et al demonstrated that this approach achieves major CD8+T cell evasion and maintains NK cell protection, with *in vivo* survival exceeding one week against both T cells and NK cells (Figure 4). However, this strategy does not eliminate all CD8+T cell responses, as HLA-C can still present antigens to CD8+T cells with lower efficiency than HLA-A or HLA-B, and the potential for residual T cell activation leading to chronic rejection remains uninvestigated. CD4+T cell responses theoretically persist due to intact HLA Class II molecules, though this has not been experimentally validated in HLA-A/B knockout studies. The approach requires HLA-C compatibility between donors and recipients, necessitating approximately 12 different HLA-C

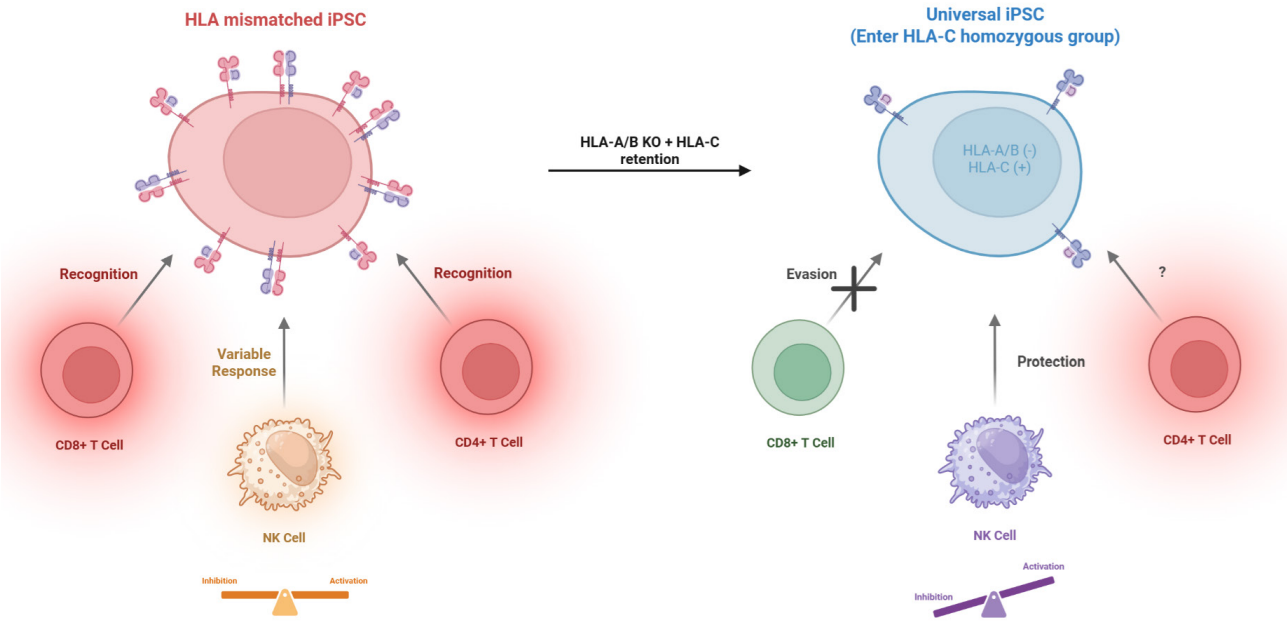

Figure 4. HLA-A/B Knockout with HLA-C Retention Strategy: Balanced CD8+T Cell Evasion and NK Cell Protection (Created in BioRender. Elmusa, F. (2025) https://BioRender.com/vhxicp8)

homozygous iPSC lines to achieve global population coverage, rather than a truly universal application.

HLA class II elimination strategies

While HLA class I molecules represent the primary target for T cell-mediated rejection through CD8+cytotoxic T cell activation, HLA class II molecules play equally critical roles in orchestrating immune responses by presenting extracellular antigens to CD4+helper T cells and initiating antibody-mediated rejection pathways. HLA class II elimination has emerged as an essential component of comprehensive immune evasion strategies, with CIITA targeting representing the most efficient approach due to its role as the master transcriptional regulator controlling all HLA class II gene expression.

The CIITA targeting approach

Class II major histocompatibility complex transactivator (CIITA) serves as the master regulator controlling transcriptional activation of all HLA class II genes, making it an attractive single-target strategy for eliminating HLA class II-mediated immune responses.[40] CIITA functions as a transcriptional coactivator that is essential for both constitutive and interferon-γ-inducible expression of HLA class II molecules, including HLA-DR, HLA-DQ, and HLA-DP. Unlike HLA class I molecules that are expressed on all nucleated cells, HLA class II expression is normally restricted to antigen-presenting cells such as dendritic cells, macrophages, and B cells, though it can be induced in other cell types including iPSC derivatives upon inflammatory stimulation.[13,44] CIITA knockout provides a single-gene target approach to eliminate all HLA class II expression, as it is required for the transcription of all class II genes across different loci.[45] This regulatory architecture makes CIITA targeting particularly attractive compared to the multiple gene modifications required for HLA class I elimination, as disruption of this single master regulator can completely abolish HLA class II surface expression.

Multiple research groups have successfully demonstrated effective HLA class II elimination through CIITA knockout in various iPSC systems using CRISPR/Cas9 technology. Romano et al generated homozygous CIITA knockout iPSCs using CRISPR/Cas9 with a single guide RNA targeting the third exon of the human CIITA gene, resulting in a frameshift mutation that led to a premature stop codon.[46] The resulting CIITA-/- iPSCs maintained typical pluripotent stem cell morphology, normal karyotype, expression of pluripotency markers, and retained differentiation capacity into all three germ layers, demonstrating that CIITA knockout does not compromise fundamental stem cell characteristics. Similarly, Wang et al successfully generated CIITA knockout iPSCs that effectively diminished expression of all MHC II alleles (HLA-DQA, HLA-DQB, HLA-DRA, HLA-DRB, HLA-DPA, HLA-DPB) while preserving pluripotency markers and differentiation capacity. In their xenogeneic monkey transplantation model, CIITA-/- iPSCs demonstrated reduced immunogenicity and enhanced therapeutic efficacy in wound healing applications compared to wild-type cells.[42]

The primary advantage of CIITA targeting lies in its efficiency as a single-gene modification that achieves complete elimination of HLA class II expression across all variants and cell types. This approach eliminates the need for multiple gene targeting and provides robust, sustained suppression of class II expression that persists through differentiation and inflammatory stimulation.

CIITA knockout effectively prevents CD4+T helper cell activation, which plays crucial roles in orchestrating adaptive immune responses and chronic rejection processes.[40] However, CIITA targeting addresses only HLA class II molecules and has no direct effect on HLA class I expression, which mediates the majority of acute rejection responses through CD8+T cell activation. This limitation necessitates combination approaches with B2M knockout or selective HLA class I targeting when comprehensive immune evasion is required.[8]

In summary, CIITA knockout eliminates all HLA Class II expression by disrupting the master transcriptional coactivator essential for MHC Class II gene activation, achieving complete CD4+T helper cell evasion as demonstrated by Romano et al[46] and Wang et al[42] with enhanced iPSC survival (Figure 5). However, this strategy preserves HLA Class I molecules intact, theoretically maintaining CD8+T cell recognition capacity, though this has not been directly validated in CIITA knockout studies, representing a critical knowledge gap requiring experimental verification (Table 1). NK cell responses remain unchanged since the HLA Class I-KIR inhibitory pathway is preserved, highlighting that CIITA targeting provides selective rather than comprehensive immune evasion and must be combined with HLA Class I elimination strategies for universal iPSC applications.

Addressing NK cell-mediated rejection

The elimination or reduction of HLA class I molecules through B2M knockout or selective HLA targeting strategies creates a fundamental immunological challenge by triggering NK cell-mediated "missing self" recognition, necessitating complementary approaches to maintain NK cell tolerance while preserving T cell evasion capabilities. NK cells are educated during development to recognize self HLA class I molecules through inhibitory receptors,

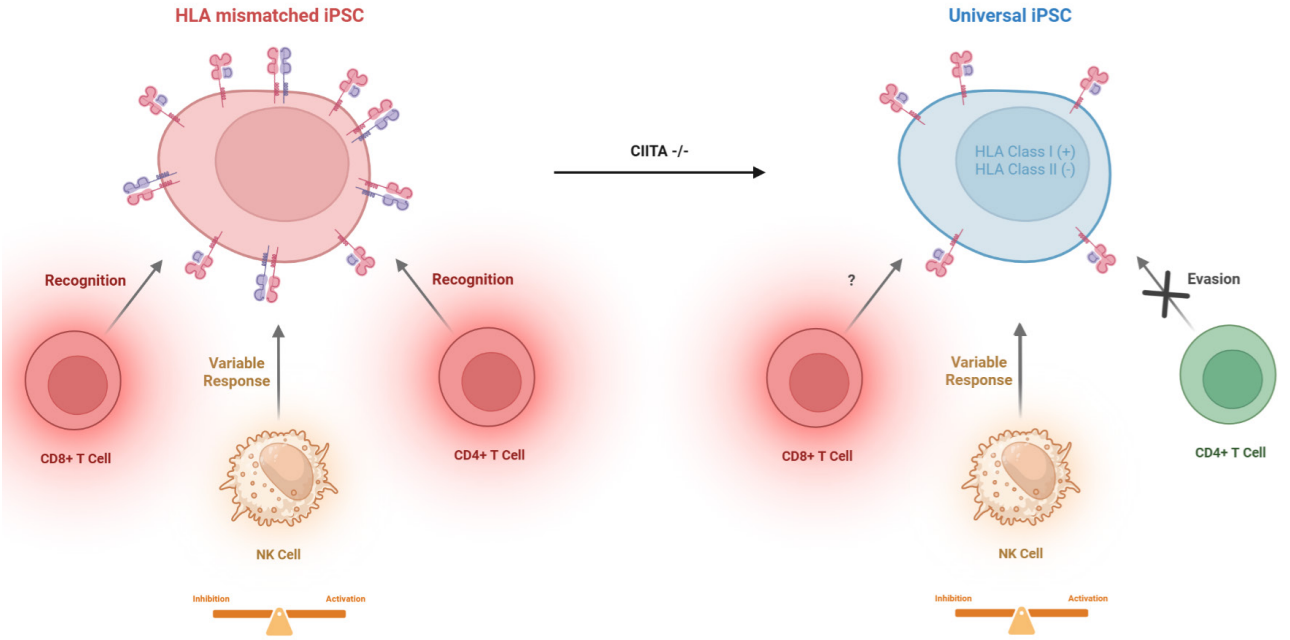

Figure 5. CIITA Knockout Strategy: Complete HLA Class II Elimination and Selective CD4+T Cell Evasion (Created in BioRender. Elmusa, F. (2025) https://BioRender.com/jkj73qi)

Table 1. Comparative analysis of immune cell responses to different HLA modification strategies

Strategy	CD8+T cell response	CD4+T cell response	NK cell response
HLA-mismatched iPSCs	✗ Active Recognition	✗ Active Recognition	Variable Activation
HLA I elimination (B2M knockout)	✓ Immune Evasion [41]	?	✗ Intense Attack [41]
HLA I elimination (Partial B2M suppression)	✓ Immune Evasion [43]	?	✓ Protection [43]
HLA I elimination (HLA-A/B knockout+HLA-C retention)	✓ Major Evasion [33]	?	✓ Protection [33]
HLA II elimination (CIITA knockout)	?	✓ Immune Evasion [42,46]	?
Combination strategies (B2M+CIITA double knockout)	✓ Immune Evasion [31,42]	✓ Immune Evasion [31,42]	✓ Partial Protection [31]
Combination strategies (HLA-A/B/C+CIITA KO+checkpoint)	✓ Major Evasion [34]	?	✓ Protection [34]
Combination strategies (HLA-A/B/C+RFXANK KO+multi-checkpoint+safety)	✓ Major Evasion [9]	✓ Major Evasion [9]	✓ Protection [9]

"?": indicates knowledge gaps where immune cell responses have not been experimentally validated.

and the absence of these molecules removes inhibitory signaling, leading to NK cell activation and cytotoxic responses against HLA-deficient cells.[40] This challenge was clearly demonstrated by Wang et al, who showed that complete B2M knockout resulted in significantly increased NK cell susceptibility despite achieving effective CD8+T cell evasion, with teratoma formation only possible in NK cell-depleted immunocompetent mice.[41] Several strategies have emerged to address this challenge, with varying degrees of success. Previous studies have demonstrated that maintaining at least 10% of initial B2M expression levels can be sufficient to prevent NK cell activation while still achieving substantial immune evasion, suggesting that complete HLA class I elimination may not be necessary for effective T cell evasion strategies. Karabekian et al explored this partial suppression approach through shRNA-mediated B2M reduction, which achieved 90% reduction in B2M mRNA levels while maintaining some residual HLA class I expression that could potentially provide protection against NK cell-mediated lysis while still reducing T cell proliferation to background levels.[43] More sophisticated approaches involve the preservation of specific HLA molecules with strong NK inhibitory function, such as HLA-C retention strategies that maintain killer immunoglobulin-like receptor signaling. Xu et al demonstrated that selective knockout of HLA-A and HLA-B while retaining HLA-C expression effectively maintained NK cell protection while achieving substantial T cell evasion, with HLA-C retained cells showing significantly better NK cell resistance compared to complete B2M knockout approaches in both *in vitro* and *in vivo* studies.[33] Alternative strategies focus on introducing NK inhibitory ligands including HLA-E, HLA-G, and CD47. HLA-E has proven particularly effective as it binds to CD94/NKG2A receptors expressed on most NK cells, while HLA-G provides inhibitory signaling through multiple receptors and is naturally expressed at the maternal-fetal interface during pregnancy, offering a biologically validated immune evasion mechanism.[8,10] Han et al showed that engineered expression of HLA-G significantly reduced NK cell degranulation from 13.51% to 5.43% in comprehensive combination strategies.[34] Tsuneyoshi et al further refined this approach by demonstrating that co-expression of HLA-G with B2M enhanced surface HLA-G expression and provided effective protection against both NK cells and macrophages.[9] CD47 functions as a "don't eat me" signal that inhibits phagocytosis by macrophages and also provides some NK cell inhibition, making it valuable for comprehensive immune evasion strategies.[10] The optimal approach appears to involve combination strategies that maintain or introduce multiple NK inhibitory pathways, as reliance on single inhibitory mechanisms may be insufficient given the heterogeneous expression patterns of NK cell receptors and the redundant nature of NK cell activation pathways, with successful implementations requiring careful balance between achieving T cell evasion and maintaining adequate NK cell tolerance for long-term graft survival.

Combination strategies for comprehensive immune evasion

The complexity of immune recognition mechanisms has driven the development of sophisticated combination strategies that simultaneously address multiple pathways of rejection, typically involving coordinated targeting of both HLA class I and class II molecules alongside introduction of immune inhibitory signals to create truly universal iPSCs. Early combination approaches focused on dual targeting of HLA expression pathways, with Mattapally et al demonstrating successful dual B2M and CIITA knockout using CRISPR/Cas9 technology, which reduced T-cell activation from 75% in wild-type cells to 24% in knockout cardiomyocyte spheroids while maintaining normal electrophysiological properties and preserving HLA-G expression for NK cell protection.[31] Wang et al further validated this dual targeting approach in iPSCs, showing that combined B2M and CIITA knockout achieved superior immune evasion compared to single gene modifications. Their $B2M^{-/-}$ and $CIITA^{-/-}$ iPSCs demonstrated dramatically enhanced survival (11.17-fold increase versus wild-type) in xenogeneic monkey wound healing models and achieved the highest teratoma formation rates (6/7 in first injection, 4/7 in second injection), indicating reduced immune rejection. Importantly, the dual knockout cells showed significant reduction in T-cell proliferation from 75% to 24% and substantially decreased $CD3^{+}$ T cell infiltration in transplanted tissues while maintaining pluripotency and therapeutic efficacy.[42] Building on these foundational approaches, more comprehensive strategies have emerged that integrate multiple immune evasion mechanisms. Han et al demonstrated a particularly sophisticated strategy involving knockout of HLA-A, HLA-B, HLA-C, and CIITA, followed by knock-in of PD-L1, HLA-G, and CD47 at the AAVS1 safe harbor locus, creating hypoimmunogenic iPSCs that showed minimal activation of T cells, NK cells, and macrophages both *in vitro* and in mouse models.[34] This approach achieved substantial reductions in CD8+T cell proliferation (14.32% to 5.95%) and NK cell degranulation (13.51% to 5.43%) while providing protection against macrophage engulfment. Recent advances have incorporated safety mechanisms alongside immune evasion strategies. Tsuneyoshi et al developed hypoimmunogenic iPSCs featuring a unique approach that selectively knocked out HLA-A, HLA-B, and HLA-C genes while preserving B2M expression, combined with RFXANK knockout for HLA class II elimination and introduction of HLA-G, PD-L1, PD-L2, and a rapamycin-activated caspase 9 (RapaCasp9) safety switch.[9] This strategy enhanced HLA-G surface expression through co-expression with

B2M and demonstrated effective protection against both adaptive and innate immune responses while providing an inducible elimination mechanism for safety. These combination strategies require careful consideration of the genetic modification burden, potential off-target effects, and long-term stability of multiple gene modifications, with successful implementations demonstrating that comprehensive immune evasion is achievable but necessitates sophisticated multi-gene engineering approaches that balance efficacy with safety concerns for clinical translation (Table 2).

Future directions and emerging technologies

The field of universal iPSC engineering stands at the threshold of significant technological advances that promise to address current limitations and expand clinical applicability through more sophisticated gene editing platforms, enhanced safety mechanisms, and improved understanding of immune evasion strategies. Next-generation gene editing technologies, including base editors and prime editors, offer the potential for more precise modifications with reduced off-target effects compared to current CRISPR/Cas9 systems, while advances in delivery methods such as lipid nanoparticles and adeno-associated virus vectors may enable safer and more efficient genetic modifications without the integration risks associated with current approaches. The development of inducible safety switches, including suicide gene systems and controllable expression platforms, represents a critical advancement for clinical translation by providing mechanisms to eliminate transplanted cells if adverse effects occur, while temporal control systems could allow for staged immune evasion that adapts to the recipient's immune status over time. Emerging strategies include the engineering of synthetic biology circuits that can respond to inflammatory signals by upregulating immune evasion genes, the development of tissue-specific immune evasion approaches that tailor modifications to particular therapeutic applications, and the investigation of epigenetic modifications that could provide reversible immune evasion without permanent genetic alterations. Additionally, advances in computational modeling and artificial intelligence are enabling better prediction of immune compatibility and optimization of gene modification strategies, while the development of more sophisticated humanized animal models and organ-on-chip technologies promises to improve preclinical testing and reduce the uncertainty associated with clinical translation of these complex engineered cell therapies.

Conclusion

The development of universal hypoimmunogenic iPSCs

Table 2. Summary of HLA Modification Studies in iPSCs: Strategies, Techniques, and Key Findings

Strategy	Approach	Technique	Results	Ref.
HLA class I elimination	B2M Knockout	Replacement targeting (homologous recombination)	• Complete HLA-I elimination • CD8+T cell cytotoxicity < 1% • NK cell killing increased: 28.4% • Teratoma formation only in NK-depleted mice	41
	Partial B2M Suppression	shRNA (lentiviral)	• B2M mRNA reduced by 90% • T-cell proliferation to background levels • May retain NK protection via residual HLA-I	43
	HLA-A/B KO + HLA-C Retention	CRISPR/Cas9	• CD8+T cell evasion achieved • NK cell protection maintained • *In vivo* survival > 1 week vs T cells/NK cells	33
	B2M Knockout	CRISPR/Cas9	• HLA-A mRNA ↓43.3%, HLA-B ↓72.7%, HLA-C ↓46.3% • CD20+B cell infiltration reduced • Teratoma formation: 3/7 vs 2/7 (WT) • T cell proliferation reduced	42
HLA class I elimination	CIITA Knockout	CRISPR/Cas9	• Complete HLA class II elimination	46
	CIITA Knockout	CRISPR/Cas9	• Complete HLA class II elimination • All MHC II alleles (DQA, DQB, DRA, DRB, DPA, DPB) suppressed • Teratoma formation: 4/7 vs 2/7 (WT) • iPSC survival: 2.31 × vs WT in wound model	42
Combination strategies	B2M + CIITA Double Knockout	CRISPR/Cas9	• T-cell activation: 75% → 24% • Normal spheroid size/function maintained • HLA-G preserved (NK protection), HLA-E/F lost	31
	B2M + CIITA Double Knockout	CRISPR/Cas9	• T-cell proliferation: 75% → 24% reduction • iPSC survival: 11.17 × vs WT • Teratoma formation: 6/7 (first injection), 4/7 (second) • CD3+infiltration significantly reduced	42
	HLA-A/B/C + CIITA KO + Immune Checkpoint	CRISPR/Cas9 + knock-in	• CD8+T proliferation: 14.32% → 5.95% • NK degranulation: 13.51% → 5.43% • Reduced macrophage engulfment • In vivo teratoma protection demonstrated	34
	HLA-A/B/C + RFXANK KO + Multi-checkpoint + safety switch	CRISPR/Cas9 + piggyBac	• Enhanced HLA-G expression with B2M co-expression • CD4+/CD8+T cell evasion achieved • NK cell and macrophage protection • Functional RapaCasp9 based suicide gene	9

represents a critical frontier in regenerative medicine, promising to overcome the fundamental immunological barriers that limit the clinical application of pluripotent stem cell therapies. The molecular strategies reviewed demonstrate that immune evasion is achievable through sophisticated genetic engineering approaches, though each strategy presents distinct advantages and limitations that must be carefully considered for clinical translation. B2M targeting provides complete HLA class I elimination and robust CD8 + T cell evasion but creates vulnerability to NK cell-mediated rejection through missing self-recognition. The HLA-C retention strategy offers an elegant compromise that maintains NK cell tolerance while achieving substantial T cell evasion, though it requires maintaining multiple iPSC lines for population coverage. CIITA knockout effectively eliminates HLA class II-mediated CD4 + T cell responses but requires combination with HLA class I targeting for comprehensive immune evasion. The most promising approaches utilize combination strategies that address multiple immune recognition pathways simultaneously, incorporating both HLA elimination and immune inhibitory signals to create truly universal cells. However, these comprehensive modifications increase the genetic engineering burden and potential safety concerns, necessitating careful optimization of modification strategies and rigorous safety evaluation. The field must also address challenges, including long-term stability of genetic modifications, potential off-target effects, and the development of more sophisticated preclinical models for evaluating immune compatibility. Current clinical applications remain limited to HLA-matched approaches, highlighting the urgent need for universal strategies that can provide off-the-shelf therapeutic products. The continued advancement of gene editing technologies, combined with improved understanding of immune evasion mechanisms and enhanced safety systems, positions the field to achieve truly universal iPSCs that can revolutionize regenerative medicine accessibility. Success in this endeavor will transform iPSC-based therapies from personalized treatments requiring extensive matching and preparation time to immediately available therapeutic interventions capable of treating patients regardless of their HLA haplotype, ultimately democratizing access to regenerative medicine across diverse patient populations worldwide.

Authors' Contribution

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Competing Interests

The authors declare no competing interests or financial conflicts related to this work.

Ethical Approval

Not applicable.

Funding

None.

Intelligence Use Disclosure

The authors used Claude 4 Sonnet (Anthropic) for grammar correction and language editing to improve manuscript readability. All AI-generated language suggestions were reviewed and edited by the authors.

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