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# Current and Recent Advances in the Treatment of Steroid Refractory Acute Graft versus Host Disease

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## ABSTRACT

Acute Graft versus Host Disease (aGVHD) is a life-threatening complication that occurs within the first 100 days after an allogeneic hematopoietic stem cell transplantation (allo-HSCT). In order to treat aGVHD, corticosteroids are commonly used as first-line therapy. Nevertheless, their complete response rate is only around 50%. The rest of the patients became steroid-refractory (SR), and the death rate of steroid-refractory aGVHD (SR-aGVHD) patients was more than 90%, which shows that there is a pressing need for a cure for this condition. Hence, this review aims to highlight the currently available and recent advancements in the field of treatments for SR-aGVHD. Despite the deaths, relapses and infections caused by SR-aGVHD, there are significant innovations, specifically in the area of using stem cells as a treatment. Ultimately, this literature has reflected on the development of SR-aGVHD treatments over the years, indicated by the rise in the number and the quality of ongoing and completed trials.

## Introduction

Acute Graft versus Host Disease (aGVHD) is a life-threatening complication that occurs within the first 100 days after an allogeneic hematopoietic stem cell transplantation (allo-HSCT).[1] An allo-HSCT involves taking a stem cell source from various sources (such as bone marrow) from a person who is not genetically identical to the recipient and infusing it to cure a variety of blood and immune system disorders.[2-3] Even though an allo-HSCT can destroy cancerous cells, approximately 30-50% of patients developed aGVHD.[4]

In order to treat aGVHD, corticosteroids are commonly used as first-line therapy.[5] However, treating aGVHD with corticosteroids is ineffective, as only 50% of the patients responded completely due to the variety of side effects.[6-7] The other 50% became steroid refractory (SR), meaning that they do not respond to steroids, or they responded at first, but GVHD still recurs.[8] The death rate for patients with steroid-refractory aGVHD (SR-aGVHD) is more than 90%,[9] which indicates that there is an extremely high demand for the effective treatment of SR-aGVHD. Therefore, this review will discuss the currently available regiments and introduce five novel therapies for treating steroid-refractory aGVHD that hold significant merit.

## Results

### GVHD Pathophysiology

T-lymphocytes (T-cells) in the stem cell graft have a role in the immune system to fight against pathogens and foreign substances.[10] However, instead of just attacking the cancerous cells alone in several blood and immunology disorders (such as leukaemia and lymphoma),[11-12] they also attack the healthy host cells.[13] It leads to GVHD that can be characterised by the inflammation of the liver, skin, gastrointestinal (GI) tract tissue.[14] That

deterioration causes the patient to suffer from jaundice, distressing rash, and debilitating diarrhoea – making this condition extremely lethal with a less than 10% survival rate.[9,15]

According to Ferrara and Reddy (2006),[16] the pathophysiology of GVHD occurs in a process with three steps. Step 1 starts with the host, where the body tissues are damaged before stem cell transplantation by toxic regimens in radiotherapy or chemotherapy to treat cancer. The patients who were going to receive an allo-HSCT must undergo chemotherapy or radiotherapy, as it can help provide space for the about-to-be-transplanted stem cells and reduce the chance of graft rejection.[17] After the patient is exposed to those toxic regimens, the stem cell graft will be infused intravenously and travel across the bloodstream, reaching the bone marrow to generate healthier blood cells.[18] Step 2 starts when the donor graft's T-cells start to activate and expand in the host after graft infusion. A type of signalling protein called cytokines (an example would be interleukins) will be secreted by the donor's T-cells and attack the host's tissues. Finally, the last step is where inflammation occurs, with the graft's intense secretion of inflammatory cytokines, thus leading to severe tissue destruction in the recipient's body.[16]

## Overview of Current Treatments

The standard treatment for aGVHD is corticosteroids; however, many patients do not respond, and the survival rate is incredibly low.[9] Therefore, several treatments have been widely analysed and used in various clinical trials, resulting in a range of overall survival (OS) from 20%-80% across all ages and aGVHD grades.[19-20] They are also called SR-aGVHD conventional treatments. On the other hand, lethal complications still developed, with the most common being bacterial and fungal infections due to the drugs themselves. A summary of the current conventional treatments for SR-aGVHD, their mechanism and explanations can be found in Table 1.

**Table 1.** Currently available treatments for SR-aGVHD with their mechanisms and explanations.

| Mechanism | Explanation and Treatment |
|---|---|
| Interleukin receptor inhibitors | These drugs compete with interleukins to bind with their receptors, leading to a reduction in inflammation.[21]<br>1) Inhibiting IL-2: Basiliximab,[22] Inolimomab,[23] Daclizumab.[24]<br>2) Inhibiting IL-6: Tocilizumab.[25] |
| Anti T-cells proliferation | These drugs can suppress T-cell growth through various ways.<br>1) Preventing guanine synthesis:[26] Mycophenolate mofetil.[27]<br>2) Cell-cell contact:[28] Bone marrow-derived mesenchymal stem cells.[29]<br>3) Preventing calcium to bind to T-cells' receptors:[30] Tacrolimus.[31] |
| Anti apoptosis of healthy cells | Some drugs do not target T-cells directly. Instead, they focus on maintaining the survival of healthy tissues in SR-aGVHD:[32] Etanercept,[33] Infliximab.[34] |
| Anti T-cell differentiation | These drugs interfere with the pathways that are associated with T-cell differentiation. [35-36] This prevents the naïve T-cells from changing and developing into cytotoxic T-cells.[35-36]<br>1) Mammalian target of rapamycin pathway: Sirolimus,[37] Everolimus.[38]<br>2) Janus kinase 1/2 pathway: Ruxolitinib.[22] |

| | |
|---|---|
| Complement-dependent lysis of T- cells | These drugs will only attack the certain types of T-cells that the immune system considers harmful, not all types of T-cells:[39-40] Anti-thymocyte globulin,[23] Alemtuzumab.[19] |
| Adenosine deaminase inhibitor | An enzyme inhibitor that causes the build-up of deoxyadenosine (a derivative of adenosine, which is part of the DNA, that can be very toxic in large quantity) and induce apoptosis of harmful donor T-cells:[41-42] Pentostatin.[20] |
| Anti T-cell homing | Prevent T-cells from localising to certain healthy tissues (e.g. gastrointestinal tract) and attack the healthy cells:[43-44] Vedolizumab.[45] |
| Light therapy | Using ultraviolet light for the apoptosis of white blood cells:[46] Extracorporeal photopheresis.[47] |

## Recent Advances

Regardless of the constant effort to find the best cure for SR-aGVHD, complications such as infections, pneumonia, relapse, and deaths still occur due to the treatments themselves. Thus, that has led to new initiatives by researchers, which include using stem cells derived from other parts of the human body besides the bone marrow (mentioned in Table 1), new cellular pathway inhibitors, faecal microbiota, and introducing a drug that can enhance gut function in SR-aGVHD patients.

### *MaaT013 Transplantation*

Faecal microbiota transplantation (FMT) is commonly used to treat *Clostridioides difficile* - a disease when bacteria cause diarrhoea.[48] Studies have shown that FMT can rebalance the diversity of gut bacteria when the patient has GI tract diseases, and it can also reduce the amount of inflammatory cytokines being produced.[49] Thus, FMT can be essential to reduce the severity of GI tract SR-aGVHD, as it can help strengthen the gut and reduce the inflammation caused by cytokines secreted by T-cells in GVHD. However, little research has been done to evaluate FMT's efficacy in treating SR-aGVHD. Hence, Malard et al. conducted the first clinical trial to evaluate the efficacy of the faecal microbiota MaaT013 to treat gastrointestinal SR-aGVHD in 2023.[50] MaaT Pharma manufactured MaaT013 from healthy donors' faecal materials containing ButycoreTM - a bacteria that can reduce inflammation.[51] Usually, an FMT only involves taking the stool (faecal) sample from one healthy donor of the patient's choice.[52] MaaT013 is unique in that it uses stool samples from 3-8 donors, which is known as "pooling technology" that aims to "provide greater bacterial diversity, in a standardised and scalable approach, to safely improve patients' outcomes".[53]

The researchers delivered MaaT013 to patients with lower GI SR-aGVHD by using a rectal catheter, and the OS was 25% after one year. The conductors observed increased richness and diversity of beneficial bacteria in the gut after the MaaT013 administration. Ultimately, their results suggested that MaaT013 might be effective for patients with GI SR-aGVHD, as the allogeneic gut microbiota MaaT013 is crucial for strengthening the patient's immune system.[50] In addition, a search on clinaltrials.gov indicated that the trial NCT04768907 is currently recruiting for an early-access program with MaaT013 to treat GI SR-aGVHD.

### *PI3K Inhibitors*

The phosphoinositide 3 kinase/protein kinase B/mechanistic (or mammalian) target of rapamycin (PI3K/Akt/mTOR) pathway is a network that has a role in T-cells activity and a significant pathway in the

progression of cancer.[54] Naïve T-cells can use this pathway to differentiate and proliferate into helper, regulatory, cytotoxic or memory T-cells, also known as "T-cell fate decision".[55] Hence, blocking this pathway in GVHD can interfere with “T-cells' fate” and reduce the severity of SR-aGVHD. While many studies have been done on mTOR inhibitors sirolimus and everolimus (Table 1), very few were done on PI3K inhibitors.

The most recent study was published by Herrero-Sánchez et al. in 2016.[56] They used the PI3K inhibitors BEZ235 (dactolisib) and BKM120 (buparlisib) on T-cells isolated from the donor's blood *in vitro* and *in vivo*. *In vitro*, T-cell apoptosis was shown better in BEZ235 than BKM120; both inhibit cytokine production in T-cells, with BEZ235 showing a better effect than BKM120. Ultimately, BEZ235 was selected for i*n vivo* experiments on mice with GVHD. This is due to the fact that more preferable results were shown, as the number of toxic T-cells and inflammatory cytokine reduced more than BKM120, which is necessary for GVHD to diminish fast. The severity of GVHD in mice did reduce, yet they also observed tissue damage and toxicity caused by the inhibitor drugs, not GVHD.[56]

### *Placenta-Derived Decidua Stroma Cells*

Placenta-derived decidua stromal cells (DCS) are a type of stem cells that is a component in a pregnant woman's decidua (a layer in a woman's endometrium that can form the base of the placenta during pregnancy).[57-58] Numerous studies have shown that DCS has better activity in inhibiting T-cells *in vitro* as it expresses more programmed death-ligand 1 than other types of stem cells.[59] This is advantageous over other kinds of stem cells because more programmed death-ligand 1 is crucial to an increase in the reduction of T-cell homing, activation and proliferation.[60]

A pilot study by Ringdén et al. in 2016 used DCS to treat aGVHD.[59] The patients were divided into 3 groups: group 1 received DSC in addition to 10% AB plasma, group 2 received DSC with 5% albumin (AB plasma and albumin were used as supplements for the DSC infusion process), and group 3 is the control group. After one year, the OS of SR patients were 31% in group 1 and 73% in group 2, which were significantly higher than the OS of 3% of the group 3. Severe adverse events (SAEs) due to the stem cells still occur, the most common being relapse, pneumonia and infections. Nevertheless, with the OS being relatively high, DCS can effectively treat SR-aGVHD, especially when combined with albumin.[59]

### *Human Umbilical Cord-Derived Mesenchymal Stem Cells*

Human umbilical cord-derived mesenchymal stem cells (hUC-MSC) are stem cells derived from the umbilical cord that can renew and differentiate themselves into different kinds of cells like muscle cells, liver cells and nerve cells.[61] hUC-MSC has been reported that it can renew itself quicker than stem cells from other sources (such as bone marrow); they are more ethically accepted and less destructive to healthy host cells.[62] Due to those benefits, the patients who received hUC-MSC as a treatment for SR-aGVHD might recover faster, the rate of mortality and the grade of SR-aGVHD could decrease. However, the field of study surrounding hUCB-MSCs to treat SR-aGVHD still has limited evidence, and there is a lack of large- scale studies. Hence, Ding et al. and Donadel et al. conducted studies with a larger population in 2023 to evaluate the effectiveness of hUC-MSC in SR-aGVHD patients.[62-63]

The mean age for both trials is 12.5 years old. Ding et al.'s trial reported that after one year, the overall OS was 60.9%.[62] Donadel et al.'s trial reported that the OS on day 100 was 51.9%, and on day 180 was 38.5%.[63] One patient in Ding et al.'s trial had an allergic reaction that recovered quickly, while 17 in Donadel et al.'s trial experienced SAEs. Donadel et al. explained that the high number of SAEs might result from "patients [having] more advanced disease". In addition, both trials concluded that children tend to have a better OR and OS than adults. In conclusion, using hUC-MSC is effective in patients of all ages, with children and patients with lower grades SR-aGVHD receiving more benefits.

### *Apraglutide*

Apraglutide is a novel glucagon-like peptide 2 receptor agonist (GLP2) that can provide constant GLP2 exposure in the gut.[64] This is very beneficial for patients with GI tract SR-aGVHD because GLP2 is an amino acid peptide secreted in the gut, that promotes nutrient absorption and enhancing gut function.[65] Hence, the constant secretion of GLP2 may mitigate the GI impairments caused by SR-aGVHD. A search on clinicaltrials.gov reveals that the trial NCT05415410 is currently evaluating the safety and efficacy of the drug apraglutide in patients ≥ 12 years old with grade II-IV GI tract SR-aGVHD, estimated to be completed in 2025.

## Discussion and Future Directions

Steroid-refractory acute graft versus host disease is so severe that infections, relapses and deaths still occur despite the advances in researching the best available cure. Acknowledging the severity of SR- aGVHD, even treatments with an OS of 25% are already up-and-coming and can result in treatment success. We can also see the innovation specifically in the area of stem cells. From using bone marrow originally (mentioned in Table 1), researchers have found other alternate sources, such as the placenta and umbilical cord, that resulted in a higher overall survival after treatment (bone marrow was 45%,[29] umbilical cord was 51.9-60.9%,[62-63] and the placenta was 73%).[59]

In addition, other novel drugs have been reviewed by other literature, such as begelomab, neihulizumab, brentuximab, itolizumab;[66] itacitinib and anti-CD3/CD7 immunotoxins that are worth mentioning.[67] Several papers have shown the efficacy of combination therapies of the current treatments. For instance, Tan et al., 2017 combined etanercept and basiliximab to alleviate SR-aGVHD, and the 1-year survival rate was 62%.[68] Etanercept alone can stop the healthy host cells from apoptosis which has a treatment success of 59% in 12 months (mentioned in Table 1),[33] and basiliximab inhibits inflammatory cytokines activities with a treatment success of 50% in 12 months (mentioned in Table 1).[22] Therefore, there is an increase in the effectiveness of combined therapy, as the overall survival after one year was much higher than its standalone treatments. Thus, it might be beneficial for future research to combine the newly-introduced therapies in this literature with the conventional, widely used therapies to evaluate their safety profile.

There are some limitations in this review, specifically in terms of the studies selected. Firstly, most of the experimental studies have a relatively small sample size, and the PI3K inhibitors trial was not done in a human setting. Secondly, the finished trials of hUC-MSC mentioned are retrospective, meaning that they only used existing data which causes selection bias.[69] From the limitations that arose from the selected trials, the result of this review can only represent the population that has been included in the study, not every single patient who had SR-aGVHD. However, since those were experimental, those small trials will serve as a stepping stone for more prominent, more extensive trials. Aside from combination therapy, future research that are randomised and include a larger population using the newly reviewed drugs is also encouraged. Therefore, that can help future results to be unbiased, knowing that the results of smaller, non-randomised trials are successful.

## Conclusion

All in all, this review has presented five new treatments for SR-aGVHD and re-addressed the conventional therapies that are widely used to treat SR-aGVHD. Even though all of the treatments showed positive responses and survival rates, SR-aGVHD is still fatal; the variety of different initiatives has shown that there is a pressing need for a more potent cure for SR-aGVHD. Ultimately, this literature has reflected on the advancements of SR-aGVHD treatments over the years, indicated by the rise in the number and the quality of ongoing and completed trials.

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