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Chronic Graft-versus-Host disease: second line therapy review

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Orientado por:

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Resumo

A Doença de Enxerto-vs-Hospedeiro (DEVH) é uma complicação decorrente do transplante de medula óssea. A DEVH pode ser dividida em forma crónica e aguda sendo que neste trabalho apenas iremos desenvolver a apresentação crónica (DEVHc). Esta é uma complicação não só extremamente frequente (até 50% dos recetores de transplante) mas também muito importante, dada a sua grande mortalidade, sendo a principal causa de morte nestes doentes, excluindo a recorrência da doença de base.

Apesar de o nosso conhecimento sobre a DEVH ainda estar incompleto, já estão a ser feitos avanços. Em primeiro lugar, a própria conceção da DEVH alterou-se, passando de uma mera divisão temporal entre a forma aguda (caso se manifestasse nos primeiros 100 dias após o transplante) e a forma crónica (após o centésimo dia) para uma divisão baseada nas manifestações clínicas, que são diferentes entre ambas. Ainda mais, surgiram recentemente novos critérios de avaliação das manifestações e gravidade da DEVHc que refletem este avanço na compreensão da doença e possibilitam uma melhor avaliação dos doentes.

O tratamento da DEVHc é complexo e longe se ser o ideal. Atualmente, a terapêutica inicial consiste em Corticoterapia associada a um inibidor da Calcineurina, no entanto, esta opção apresenta apenas uma eficácia limitada (com 10% dos doentes sem qualquer resposta e menos de 50% apenas com melhoria parcial). Assim, muitos doentes necessitam de terapêutica de segunda linha. No entanto, esta também não é perfeita pois, embora exista uma variedade considerável de terapêuticas possíveis e em constante atualização, estas estão pouco estudadas e muitas vezes apresentam resultados pouco satisfatórios, sendo a escolha entre estas difícil e muitas vezes baseada numa abordagem de "tentativa e erro". Assim, é neste contexto que é desenvolvido este trabalho, com o objetivo de desenvolver cada uma destas terapêuticas de segunda linha e tecer considerações sobre estas.

Palavras-chave: DEVH crónica; DEVH cortico-resistente; terapêutica de 2ª linha

Abstract

Graft-versus-Host disease (GVHD) is a complication of bone marrow transplant. It has two forms: acute and chronic. In this work we'll only address the chronic one (cGVHD). cGVHD is a very frequent complication of the transplant (up to 50% of the patients) and a major one, given its high mortality rate, being the main cause of death in these patients, apart from the recurrence of the primary malignancy.

Even thought our understanding of GVHD is far from complete, some progresses are being made. Firstly, the disease itself is being defined differently, being no longer just a temporal division between the acute GVHD (if occurred in the first 100 days after the transplant) and the chronic form (after the 100 day mark) but a more precise distinction based on the clinical manifestations, which are different between both forms. Even more, new criteria to evaluate the manifestations and the overall severity of the cGVHD have been developed, reflecting the advances in this area and enabling a better management of the patients.

cGVHD treatment is very complex and far from perfect. Nowadays, the first approach relies on Corticosteroids plus a Calcineurin inhibitor. However, this treatment option has a limited efficacy (with 10% of the patients without any improvement and less than 50% only with a partial response). So, many of the patients will require second line therapy, however this isn´t perfect either. Even though there is a vast amount of options and in constant update, the number of studies is small and in many times the results aren´t satisfactory, making the clinical decision very hard and relying in a "trial and error" approach. So, with all this in mind, we develop this work in order to address every single one of these second line therapies and make some conclusions regarding them.

Key-Words: chronic GVHD; steroidrefractory cCVHD; 2nd line therapy

O Trabalho Final exprime a opinião do autor e não da FML

“Resumo alargado”

A DEVH é uma complicação decorrente do transplante de medula óssea. Existem dois tipos de DEVH: a forma crónica e a forma aguda sendo que neste trabalho apenas iremos desenvolver a apresentação crónica. Esta é uma complicação não só extremamente frequente (podendo ocorrer até 50% dos recetores de transplante) mas também muito importante, devido à sua grande mortalidade uma vez que é principal causa de morte nestes doentes, excluindo a recorrência da doença de base.

O conhecimento que possuímos sobre a DEVH está gradualmente a avançar, levando a profundas alterações na abordagem desta doença. Em primeiro lugar, a própria divisão entre forma crónica e aguda já não é tão simplista como outrora, em que a divisão era apenas feita consoante se já tinham decorridos 100 dias desde o transplante no momento em surgiu a doença (forma crónica) ou se ainda não tinham decorrido 100 dias (forma aguda). Atualmente, a divisão é baseada nas próprias manifestações clínicas da doença uma vez que são diferentes entre os dois subtipos. Assim, segundo os novos critérios diagnósticos, existem manifestações consideradas de diagnósticas (que nos permitem de imediato afirmar que estamos perante um caso de DEVHc), características (que embora altamente sugestivas da forma crónica não nos permitem afirmar esse diagnóstico, motivando a realização de uma biópsia do tecido afetado) e ainda as manifestações indeterminadas (dada a sua raridade) e as comuns (presentes em ambas as formas). Para além destes critérios de diagnóstico, foram criados novos critérios de avaliação das manifestações da doença para cada órgão específico (avaliado de 0-3) e, decorrente da sua avaliação conjunta, um sistema de avaliação global da DEVHc que permite dividir os doentes entre os que apresentam DEVHc ligeira, moderada e grave. Para além destes avanços na abordagem aos doentes, também o nosso entendimento sobre a fisiopatologia da DEVHc tem evoluído, mantendo-se no entanto incompleto. Atualmente encara-se esta doença como uma disfunção generalizada do sistema imunitário, havendo uma excesso de células ativadas, tanto células B como T, e um decréscimo das células T reguladoras (Treg). Assim existe um desequilíbrio da resposta imunitária que impede o desenvolvimento da tolerância imunitária para as próprias células do recetor, o que resulta nas manifestações da doença.

O tratamento da DEVHc é bastante complexo logo a começar pelo seu princípio teórico. A DEVHc pode ser encarada como um processo teoricamente "autolimitado" uma vez que, com o decorrer do tempo, será sempre atingida a tolerância imunitária das células provenientes do enxerto contra o recetor sem a intervenção médica, apenas pela atuação das células Treg. No entanto, acontece que na prática essa atitude expectante não é aceitável pois até se desenvolver a tolerância, vão decorrer desse desequilíbrio imunitário diversas agressões ao hospedeiro, potencialmente fatais. Assim, existem duas abordagens possíveis. A primeira, e a que está na base da terapêutica atual, passa por utilizar imunossupressores de modo a silenciar as agressões imune-mediadas até estar estabelecida a tolerância. Outra hipótese, seria induzir a tolerância de modo a provocar uma cura precoce da doença. Convém referir que a abordagem terapêutica varia consoante as manifestações apresentadas, sendo que apenas está preconizada a utilização de terapêutica sistémica perante casos de doença moderada e grave.

A terapêutica sistémica inicial é igual em todos os doentes, constituindo numa associação de Corticosteroides (maioritariamente Prednisona) e um inibidor de Calcineurina (Ciclosporina ou Tacrolimus), principalmente utilizado para permitir uma redução da dose dos corticoides e assim diminuir os efeitos adversos destes. É importante salientar que várias outras associações foram tentadas (e.g. Azatioprina e Micofenelato de Mofetil(MMF)), mas mostraram ser inferiores, ou pelo menos não superiores à atual. No entanto, esta primeira abordagem terapêutica está longe de ser perfeita, estando associada a uma taxa de remissão completa inferior a 60% e em 10% dos doentes a doença permanece totalmente inalterada, motivando assim a necessidade de outro tratamento em quase metade dos casos.

Assim, perante a falência da terapêutica de primeira linha, definida como progressão da doença mesmo em doses máximas de Prednisona (1mg/kg/dia) , estabilização apenas com doses altas de Prednisona (superiores a 0,5mg/kg/dia) ou incapacidade de utilizar doses inferiores a 0,5mg/kg/dia de Prednisona, é necessário recorrer à terapêutica de segunda linha. No entanto, a abordagem a seguir encontra-se pouco regrada e apesar de haver muitas alternativas terapêuticas a linha de ação normalmente acaba por ser uma abordagem empírica de "tentativa e erro" até se atingir remissão da doença.

Como já foi dito, existem diversas opções terapêuticas, com diferentes mecanismos de ação. Em primeiro lugar, podem ser utilizados outros imunossupressores não específicos, cujo princípio de ação é semelhante ao dos corticoides: silenciar o sistema imunitário de

modo a que não haja manifestações da doença até ser atingida a tolerância imunitária. Dentro deste grupo as principais opções terapêuticas são o Sirolimus, a Pentostatina e MMF que nos estudos já realizados apresentaram taxas de resposta, no mínimo com melhoria sintomática aceitáveis (aproximadamente de 50% no caso da Pentostatina e MMF, chegando aos 80% no caso do Sirolimus).

Outra hipótese terapêutica passa por realizar uma imunossupressão dirigida contra um interveniente específico do sistema imunitário que se sabe estar envolvido na DEVHc, os linfócitos B. Assim, com a utilização de Rituximab (anticorpo monoclonal dirigido contra CD20, apenas expresso nestas células) é possível fazê-lo, tendo sido atingida nos diversos estudos já realizados uma taxa total de resposta (completa e parcial) entre 50%-80%. Para além de ser possível realizar uma inibição dirigida contra um interveniente celular do sistema imunitário, atualmente também é possível inibir especificamente uma via de sinalização imunitária, conseguindo igualmente um efeito imunossupressor sendo que já foi estudado no contexto da DEVHc a utilização de Imatinib (fármaco desenvolvido para a leucemia mieloide crónica, que é um inibidor de tirosina-cinase que poderá bloquear vias de sinalização expressas na DEVHc), Ruxolitinib (inibidor de JAK cinase 1 e 2, que teoricamente influencia vias de activação do sistema imunitário) e Bortezomib (inibidor da via NF-kB). Estes fármacos demonstraram ter efeitos na DEVHc bastante significativos, sendo que no caso do Imatinib, associa-se o seu efeito a uma diminuição da produção de fibrose, estando associado a melhorias especialmente evidentes das manifestações pulmonares, que são particularmente refratárias aos outros tratamentos. No caso do Bortezomib e do Ruxolitinib, observou-se um reequilíbrio no sistema imunitário com diminuição dos níveis de citocinas envolvidas e de células ativadas e inclusive um aumento de células Treg. Estes fármacos, apesar de avaliados num número reduzido de doentes, apresentaram altas taxas de eficácia apresentando uma resposta, no mínimo parcial, em aproximadamente 80% dos doentes.

Para além destas opções terapêuticas cujo principal mecanismo de ação é o bloqueio do sistema imunitário, existem outras que atuam principalmente (pelo menos em teoria) pela indução do reequilíbrio imunitário. Neste grupo inclui-se a fotoquimioterapia extracorporal (FQE) e a utilização de IL-2. A FQE consiste na remoção de sangue do doente e irradiação deste (*ex vivo*) levando a apoptose das células imunitárias. Esta acção, por motivos não totalmente esclarecidos leva a uma diminuição da resposta inflamatória tanto por um efeito imunossupressor (via produção de citocinas anti-inflamatórias) mas

também por efeitos no equilíbrio das células T, aumentando as células Treg apresentando assim uma possível contribuição para a indução direta de tolerância. A FQE já foi avaliada num ensaio randomizado, tendo apresentado melhores taxas de resposta do que a terapêutica convencional particularmente no caso de manifestações cutâneas e da mucosa oral sendo assim uma ótima escolha caso o doente apresente envolvimento destes tecidos. Já a IL-2, atua como um fator de crescimento para as células Treg, tendo sido já comprovado o seu efeito em doentes, nos quais elevaram drasticamente esta população celular e provocaram também melhoria clínica em aproximadamente 60% dos doentes, provando assim que a IL-2 pode ser tida em conta como uma opção terapêutica para a DEVHc.

Podemos assim concluir que existem diversas opções terapêuticas e que, nenhuma delas se sobressai perante as outras como uma verdadeira alternativa à Corticoterapia e assim, perante um doente que apresente uma DEVHc refractária, a decisão terapêutica deverá ser dirigida ao doente em específico e às manifestações deste, uma vez que diferentes opções terapêuticas apresentam diferentes taxas de resposta para cada órgão/tecido envolvido. Outra conclusão evidente é a grande necessidade em realizar novos estudos referentes à utilidade e eficácia das terapêuticas de segunda linha. Para isso, as novas definições e critérios da doença são altamente importantes pois só agora é que é possível fazer um diagnóstico correto e uma avaliação objetiva destes doentes, possibilitando assim um avanço não só no conhecimento desta patologia mas principalmente na nossa abordagem clínica à mesma.

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Introduction

Allogenic hematopoietic stem cell transplantation (HSCT) is nowadays the best treatment option, potentially curative, for a diversity of hematological malignancies and nonmalignant disorders such as Acute myeloid leukemia, acute lymphoblastic leukemia and others.(1)

However, the HSCT doesn't come without risks, and within the variety of possible complications of this procedure one of them stands out, the "Graft-versus-host-disease" (GVHD). This disorder can be divided in two types of GVHD: Acute (aGVHD) and Chronic (cGVHD) and the distinction between them isn´t simple and it will be discussed in another section of this work. But since the focus of this work is just the chronic form this will be the only one that it's going to be detailed throughout the work.

This disease (cGVHD), occurs in a large proportion of the patients submitted to the HSCT.(2) Also, besides being a frequent side effect it is as well a major one with severe implications.(3) The full extent of this disorder will be shortly addressed. However, despite the fact that cGVHD is not only a frequent but also a serious complication of cGVHD, the management of this disease is less than desirable, with a first line therapy made of Corticosteroids and Calcineurin inhibitors (CNI) that shows to be insufficient in a considerable amount of patients and a wide variety of "salvage therapies", from which the knowledge is less than perfect. (4)

So, the goal of this review is to briefly address cGVHD in what comes for its main features regarding epidemiology, pathophysiology and the new diagnosis criteria and also, as the main focus of this work, its treatment, starting with an overview of the standard first line therapy and afterwards address each one of the alternative treatment options.

cGVHD – Overview

Epidemiology

cGVHD is a very important complication of the HSCT, and just as was mentioned before, it's a very frequent one and illustrating that fact, Wingard et al., in their study, analyzed the outcome of 10,632 patients who received HSCT from all around the world during 1980 and 2003, being this the largest retrospective study regarding HSCT ever(2). In this study, the patients were submitted to the transplant in the setting of Acute myeloid leukemia (AML); acute lymphocyte leukemia (ALL); Myelodysplastic syndromes(MDS); Lymphoma and Severe aplastic anemia (SAA). The grafts specimens were mainly from the bone marrow (between 74% and 95%) but there was some discrepancy about the donor type for each disorder: with the percentage of HLA-identical siblings donors being between 81% and 52%. It's important to highlight that the vast majority of the patients performed prophylaxis against cGVHD (around 97% and 85% of the patients). The incidence of cGVHD varied from 33% to 56%, according to the primary hematological disease that led to the transplant being less frequent in the setting of severe aplastic anemia (33%) and more frequent in the setting of myelodysplastic syndrome (56%). The same results were observed in another studies like the retrospective study from Abou-Mourad et al. (5) were they analyzed 361 long-term survivors of HSCT and cGVHD was diagnosed in 193 patients (53,1%) with similar epidemiologic conditions.

But the cGVHD is a very important complication of the HSCT not only due to its high incidence , but also because it's the leading cause of late mortality after HSCT (excluding recurrence of the primary malignancy) as observed by Bhatia et al. in a study where there were analyzed 1479 patients who were long-term survivors of the HSCT (3) and where 241 of these patients had died. cGVHD was identified has the cause of death in 53 of these patients (22%) only after the recurrence of the primary malignancy which accounted for 29% of the deaths. These results are similar to the ones found by Wingard et al. in their analysis.(2) Besides all of this, cCHVD is also a relevant cause of morbidity, and decrease in the quality of life of the patients (6).

Diagnostic Criteria and classification scores

As previously stated, GVHD can be of two types: Acute and Chronic. The past definition was only based in time criteria, being the aGVHD when it occurred in the first 100 days after the transplant and the cGVHD when it developed after this 100-day mark. However, nowadays it has been established that this definition is incorrect and now the criteria resides in the clinical manifestations, which are different for the acute and the chronic form (7), making the diagnosis of cGVHD a clinical one. This manifestations can occur in diverse organs and sites, like skin, hair and nails, mouth, eyes, genitalia, Gastrointestinal (GI) tract, liver, lungs, musculoskeletal system, hematopoietic and immune system and others. The disease has a very variable expression and in some patients it can be present in all, or many, of these organs and in other cases, only one organ or site is involved. (8)

Because of this, and in order to establish the diagnosis and differentiate it from aGVHD, the NIH criteria of 2014 categorized these manifestations by their specifity to cGVHD. So, there are the "diagnostic manifestations" which by themselves can make the diagnosis of cGVHD, like the lichen-planus lesions in the skin, and the lung involvement as a bronchiolitis obliterans syndrome and others. Then, there are the "distinctive manifestations" which although they are quite specific of cGVHD, this specificity is not enough to make an immediate diagnosis and so, confirmation with biopsy of the affected tissue is needed, to look for histopathological features of cGVHD. Examples of these manifestations are alopecia, xerostomia, xeroftalmia and others.

There are also the " unclassified manifestations" which are very rare and therefore it's diagnostic value is reduced and even the "common manifestations" that are present both in the chronic and acute type so, the diagnostic value of these last is null.(8)

Besides this diagnostic evaluation, the NIH also created an evaluating score in order to assess the severity of the disease. So, for each organ or site that could theoretically be involved, there is a scale from 0, meaning no disease, to 3, meaning severe involvement of this organ/site. For example, in order to assess the involvement of the skin, the criteria is to calculate the total body area with lesions.

With this severity classification of the disease we can create the overall score or classification of the cGVHD. This overall classification will therefore be based in the number of organs involved and the severity of the involvement of each one of them and contemplates the evaluation of eight distinct organs: skin, mouth, eyes, gastrointestinal tract, liver, lungs, joint and fascia, and genital tract.

So, we can classify cGVHD as mild, moderate and severe disease: Mild cGVHD is defined when the patient has involvement of only one or two organs with no more than a severity score of 1 in each of these and neither of those organs could be the lungs. Therefore, moderate cGVHD are the situations where the patient has any kind of minor involvement of the lungs (classified as 1 in the severity score), when he has minor involvement (score 1) in three or more organs; or when there is a moderate involvement (not higher than score 2) of at least one organ/site besides the lungs even if it is the only manifestation. Severe cGVHD are the situations of moderate and severe involvement of the lungs (score 2 and 3) and the situations where there is at least a severe involvement of one organ (score 3) even if it is the only manifestation of the disease.(8)

This new, more complete and objective evaluation of cGVHD poses some advantages that deserve consideration. First of all, the new definition of acute vs chronic is more adjusted to the reality. The clinical reality isn´t as simple as it was implied in the 100 day-mark classification: there are some cases of manifestations of aCVHD that only develops after the 100^{th} day and previously were falsely classified as chronic forms. This patients are now defined as having late-onset aGVHD. Furthermore, there are patients that have manifestations of both acute and chronic forms: the overlap GVHD, which couldn´t be correctly evaluated in the previous model when only the date of the manifestations was accounted for(8).

In second place, the new criteria for the diagnosis and evaluation of the disease have major implications. Firstly, now we can diagnose and classify the patients equally when in the previous years that wasn´t possible. With that, we can have more precise prognostic information and are capable to provide a more appropriate treatment to the disease. Secondly, with the standardization of the severity index for each organ, there are advantages both in the clinical practice, in order to evaluate a concrete patient correctly and his response to the treatment but also, and very importantly, there are major

advantages in the studies regarding this disease: with the same classification tools the results will be more reliable and more conclusions can be taken which could lead to many advances regarding the treatment of cGVHD.

Pathophysiology

The cGVHD is the result of a very complex and, therefore, not yet fully understood interactions of several cell types of our immune system such as B cells, T cells and others and, we can talk about an overall dysfunction of the immune system. The immune system is in fact so altered that at the same time we observe a potentially generalized and massive attack to the host, this same person may suffer opportunistic infections associated with his immunodeficiency, such as *Pneumocystis Jirovecii*, or an increased susceptibility to regular pathogens, being this one of the main causes of death in these patients(7).

Firstly, the donor B cells produce an exaggerated amount of antibodies against the antigens of the patient causing direct damage. There's even a more specific antibody in circulation in cGVHD, the anti PDGFR (platelet-derived growth factor receptor), stimulating this receptor and therefore resulting in the extensive fibrosis usually seen in cGVHD. Besides the antibody production, there are other involvements of this cell type, although without certainty, like chemokine production and antigen presentation(7). Besides all this hypotheses we know for sure that the homeostasis of these cells is altered in cGVHD because of the high levels of BAFF (B-cell activation factor) resulting in an elevated replication rate and activity.(9)

The T cells are also implicated in the cGVHD pathology. We know that the donor T cells are key effectors of the immune response present in cGVHD against the patient. And yet again, the homeostasis of this cells is impaired resulting this in a small amount of Treg cells. This Treg cells modulate and control the activity of our immune system; if they're diminished, so is the control over our immune response, leading to an over activation and eventually damage against the host, which is exactly what occurs in cGVHD. This change in the populations of T cells is expressed by a low Treg/Tcon (conventional) ratio meaning that we have few Treg cells to control our immune system, a common finding in these patients.(7) This control of the immune system is also called tolerance, which is the ability to don't respond, and therefore tolerate, a given stimulus, in this case the host cells. This is a gradual process that will occur in these patients and it's mediated by the

Treg cells, but given their decreased numbers it takes even longer and, during that time the rest of our immune system, left uncontrolled, produces the effects and manifestations of cGVHD, as previously described.(7)

Management of cGVHD

The treatment of cGVHD is quite complex, being its basic premise immediately more complex than the average disease. So, explaining the theory behind the practice. As previously stated, cGVHD is, or it could be considered if we didn't have to intervene, an auto-limited process in which the deranged immune system will develop tolerance toward the host, stopping the damage. However, until that happens the patient will suffer from this immune mediated damage. So, there can be two ways to approach this problem. In the first, we only suppress the activity of the immune system controlling the manifestations and wait while the tolerance develops by itself, this is our current line of action. Another possibility is to enhance and accelerate this process in order to cause full remission earlier. This effect is not achieved with the traditional therapy and even with the new options, lacks information if that is happening at all.(7)

Regarding the treatment itself, the approach is different based on the severity of the disease. In the presence of a mild cGVHD it's only indicated to perform topical therapy, if the involved organ is accessible, and avoid the systemic one. However, in the presence of a moderate or severe cGVHD, and only in these two situations, systemic therapy should be offered to the patients(8)(4). This systemic therapy can be divided in first line therapy, taken by all patients when diagnosed with cGVHD: prednisone plus a calcineurin inhibitor; and the second line therapy, which is taken when the first option fails in achieving control of the disease, which consists in a vast option of treatments.(7)(4) These different treatment regimens will be shortly discussed

However, even if it isn't the focus of this work, two small considerations must be made regarding topical treatment in the setting of cGVHD. First, their main application as the frontline therapy in the milder forms of cGVHD where systemic therapy isn't indicated, as previously stated. And also, topical treatment has an important use as adjunct therapy, in addition to the required systemic therapy, in the moderate and severe forms of cGVHD

in order to help the management of skin and other accessible manifestations, like the eyes and genital tract, and also to try to reduce the dosage of the systemic drugs. (10)

First line therapy

About the first line therapy, it relies on corticosteroids as the basis of the treatment. The treatment begins with prednisone at high doses, 1,0 mg/kg, in order to induce remission of the manifestations(10) due to its immunosupressor effect that blocks the inadequate response. However, this initial dose could be, and normally is, exaggerated. So, the amount of the drug should be reduced, especially if one considers its side effects. This decreasing of the dose is called tapering. The tapering of the prednisone will enable us to give the minimal effective dose (full effect with the less toxicity possible) which should be 0,1 mg/kg(4) and eventually the complete removal of the drug. However this process can´t be made immediately and the standard tapering regimen indicates a reduction of 20%-30% of the dose every two weeks, although other options are equally valid.(4) It's important to highlight that with the reduction of the steroid treatment the manifestations can reoccur and should be promptly controlled with the reinstitution of the previous prednisone dose or even a higher one.(4)

Besides the prednisone, patients are also given a Calcineurin inhibitor: Cyclosporine or tacrolimus. This association has proven to not be inferior to the prednisone alone in the trial performed by Koc et al.(11) where 287 patients were randomized to receive prednisone alone or prednisone with cyclosporine. In this study the patients within each arm had similar median age (30,0 vs 30,8) similar non-related donor percentage (10% vs 13%). In this study they found that there was similar incidence of death, similar incidence for the need of secondary treatment, similar survival at 5 years. (11) Although there was a tendency favoring the prednisone alone, these results weren't statistically significative. But another finding was made regarding the incidence of avascular necrosis, which happened in 32 patients (22%) with prednisone alone and in 18 patients (13%) with the association of prednisone with cyclosporine, demonstrating an important effect of the cyclosporine in reducing the side effects of the steroid therapy, being this the main theoretical justification to use a CNI along the prednisone, to protect the patients of the possible corticoid induced toxicity, and also facilitating the tapering.

Other possible associations of prednisone with azathioprine, mycophenelate mofetil (MMF) and hydroxicloroquine have been studied, but not a single one of them proved to be beneficial, and in some cases had with worse results. (12)(13)(14)

So, according to our current knowledge, the first line treatment for cGVHD continues to be the prednisone plus a CNI. This treatment can have a long duration, needing to be continued for a long period of time. This fact was illustrated by Stewart et al. (15) in a retrospective study where they analyzed the data of 751 patients and found out that the average time until discontinuation of the immunosuppressive therapy was 23 months and at the end of their 7-year study, still 15% of the patients were under therapy (15). In this study there were some factors identified with the duration of the treatment that could justify this variability between the patients and also to let us know what to expect when a new diagnosis is made. This factors are grafts derived from peripheral blood; male patients with female donors; patients with HLA disparity with the donors and also, patients who have a more systemic disease.(15)

However, this therapy does not treat every case. In another study, performed by Flowers et al.(16), 668 patients with cGVHD and treated with initial systemic therapy were analyzed. In these patients, more than half of the donors weren't related to the patient (53%) with a mismatch in 16%, and the most part of the grafts was from peripheral blood (80%). In these 668 patients, 56% of them needed to change the initial therapy showing the high inefficacy of the frontline therapy. Similar results were found by Pérez-Simon et al. (17) where there was made an analysis of 91 patients with cGVHD of all severities (mainly moderate with 42 patients (46%) but with 24 (26%) with mild cGVHD and 25 (27,5%) with severe cGVHD), the percentage of patients achieving a partial response (at least a decrease of 1 point in a single organ) was 90% and only in 58% of the case a complete response was seen (total remission), meaning that 1 in each 10 patients will maintain is disease unaltered by the treatment and that 42% will continue to have symptoms, illustrating the unsatisfactory performance of the first-line therapy.

In order to surpass these unsatisfactory results, there has been some recent activity regarding new first-line therapies such as rituximab and bortezomib. Regarding Rituximab, in a recent single-arm study(18), 25 patients with the first episode of cGVHD

in which 22 (88%) of them had a peripheral blood graft, were treated immediately with rituximab in a 375 mg/m2 dose, weekly on day 1, 8, 15, and 22, and then 1 dose every 3 months until one year was completed. The disease in these patients was moderate in 56% of the cases and severe in 32%. The author found out that it was achieved clinical response in 88% of the patients and a complete one in 84% of the total patients which was considered very positive and worthy of future studies, given the observed superiority against the standard first-line therapy described in the literature.

Regarding Bortezomib, in another single-arm study(19) 22 patients with the first episode of cGVHD were treated with prednisone 0,5-1mg/kg with 1.3 mg/m2 bortezomib intravenously on days 1, 8, 15, and 22 of each 35-day cycle for 3 cycles. The grafts in these patients were all from peripheral blood with only 22,9% of the grafts coming from a matched relative. The authors found a partial response in 70% of the participants plus a complete response in 10% which he considered to be an encouraging result that justified future studies with Bortezomib.

Second line therapy

Regarding the second line therapies, as it was just said, they're extremely important due to the fact that the frontline treatment for cGVHD isn't perfect, with an important portion of the patients needing an alternative. Those cases are referred to as steroid-refractory or steroid-dependent cGVHD due to the inability of prednisone to resolve the disease. These situations are defined by three situations: when occurs progression (worsening) of the disease while taking prednisone at 1 mg/kg/day for at least 2 weeks; when the disease is stabilized for at least 1-2 months while taking prednisone at higher doses than 0,5 mg/kg/day; or when it's impossible to taper prednisone to smaller doses than 0,5 mg/kg/day. (20)

So, in these situations it's necessary to use an alternative to prednisone, but the recurrent problem in cGVHD management is that there isn't an official procedure or a guideline in what to do next. The choice of the therapy then relies on the experience and preference of the physician/center and safety for the patient, and is determined by an empirical "trial

and error approach" until response is achieved.(4)(20) Because of this uncertainty, is very important the reevaluation of the patients after 2-3 months of therapy in order to asses if response was achieved and, in the negative cases, alter the treatment option to another one and see if this new one can cause the remission of the symptoms.(20)

There are many different treatment options with different mechanisms of action and here we'll make a short review of the most important ones.

Nonspecific immunosuppressive agents

In first place we shall consider other immunosupressor drugs with the same theoretical principal of the steroid therapy: to blunt the immune system, in an unspecific way, while the tolerance develops by itself.

Firstly, we'll address **Sirolimus,** a macrolide antibiotic produced by a bacteria: *Streptomyces hygroscopicus*(21). However, Sirolimus has other properties in humans since it binds and inhibits the mammalian target of rapamycin (mTor), blocking the proliferation of the cells of the immune system, therefore producing an immunosuppressive effect(21)(22).

This drug was studied for its utilization in cGVHD in a single-arm study by Jurado et al.(23), where it was given to 47 patients as a salvage therapy in a 2mg/day orally regimen. In that study, only 25% of the patients had only performed frontline therapy before (in the other 75%, sirolimus was third-line therapy or higher). 55% of these patients had progressive disease (worsening) with previous drugs or just had relapse in 17%. The graft was from peripheral blood in 87% of the cases and in 81% was from matched relatives and in the other 19% from matched unrelated donors. The authors found out that Sirolimus induced a response rate of 81% (38 patients) and in 38,3% (18 patients) of the patients a complete response. The authors considered those results as very positives.

However, Sirolimus showed an important toxicity profile, being the main adverse effect renal impairment, (23), which occurred in 29,8% of the patients (n=14) and in some cases there was even thrombotic microangiopathy and hemolytic uremic syndrome (HUS)(23). This high adverse effect rate was particularly observed with the association of Sirolimus with a CNI, and if this CNI was reduced or removed the adverse effects were mitigated(23).

Sirolimus can therefore be considered as a valid option for refractory-cGVHD due to its efficacy observed in the referred study as well in others(22), however it has a more dangerous toxicity profile than other drugs, meaning that it needs some limitations in its usage, like a tight control of its serum levels and to never associate it with a CNI.(20)(22)(23)

Besides this drug, two more immunosuppressive agents deserve consideration in the setting of cGVHD. Both of them act by inhibiting the DNA synthesis and therefore blocking the proliferation of all the immune system cells.

Firstly, there is **pentostatin**, a nucleoside analog that inhibits the activity of adenosine deaminase and, as mentioned, blocks the replication of immune cells, leading to a decrease in both T cells and B cells.(20)(24)(25)

Although it isn´t the most studied drug in the setting of cGVHD, one single-arm study was already performed, by Jacobson et al. (26), in which 58 patients whith refractory cGVHD to an elevated number of previous treatment alternatives (the median was 4 previous drugs) were studied. The grafts were in the majority of the cases from the bone marrow (79,3%). The authors observed that pentostatin produced response in 55% of the patients being that response complete 10,3% of the cases. It's important to refer that in the study, very low responses were observed in liver involvement, being the organ with best response rate the skin .(26) It was also registered a decrease in the corticosteroid dose in the patients (from 0,25mg/kg average to 0,05mg/kg average) meaning that pentostatin enables the tapering of prednisone(26). It was detected nausea as the main adverse event after the administration, and 29% of the patients had a serious adverse event, being severe infections by far the most important one, which occurred in 11 patients. (26).

Regarding all of this, we can say that pentostatin is a valid alternative option, with slightly more than half of the patients achieving some results, therefore it's worth a try when other options have already failed. Also the toxicity observed is not impeditive to its use. However it's very important to highlight that for patients with liver and pulmonary manifestations, pentostatin is not the best alternative option.(20)

At last, the third immunosupressor worth mentioning is **MMF**. This drug, when taken, is rapidly converted to its active metabolite, mycophenolic acid. This metabolite inhibits an enzyme called inosine monophosphate dehydrogenase, and as mentioned blocks the DNA synthesis and therefore the replication of T and B cells involved in the disease.(27)

Like pentostatin, there isn't many information regarding the use of MMF as salvage therapy for cGVHD but in a study(28), MMF was used as salvage therapy in 24 patients; where 54,1% of the cases were only of mild cGVHD and 42% had moderate cGVHD, the response rate of MMF observed in the study was 54,1% of partial responses plus 20,8% of complete responses.(28), which the authors considered encouraging. Besides the response rate, the authors documented a reduction in the steroid dosage in 73% of the patients. However, MMF doesn't have the best safety profile of these drugs. Lopez et al. in this article(28) described several infectious episodes (twelve events) as well hematological toxicity with occurrence of neutropenia in one patient.

In regard of all of this facts, MMF can be an alternative option because of its favorable response rate profile but due to its toxicity, physicians must be advised and consider it before starting the treatment with this drug.(20)

B-Cell specific inhibition

As the knowledge regarding the pathophysiology of cGVHD disease evolved, another treatment options emerged. In fact, with the discovery that B cells are greatly involved in the process, therapies directed against these cells began to make sense. So **Rituximab**, a monoclonal antibody against CD20, which is only present in B cells, became a treatment option in cGVHD by eliminating and mitigating the activity of these cells and therefore, cutting off an important intervenient in cGVHD contributing to the suppression of the immune system like the previous drugs, but in a more specific way.

The biggest prospective study with Rituximab, performed by Cutler et al.(29) in which 21 patients with steroid-refractory cGVHD were treated with 375mg/m^2 per 4 consecutive weeks per cycle (more cycles were administered if needed). The grafts in this cases were 71% from peripheral blood and 29% from the blood marrow. There was a match between the donor and the host in 100% of the cases, even if related (67%) or unrelated (33%).

The authors observed a partial response in 60% of the patients plus a complete response in two patients (10%).(29) Two more findings were made in this study: it was observed a significant steroid-sparing effect, with a reduction in the dose in 68% of the cases being the initial average dose of 0,4mg/kg and in the end it was 0,10mg/kg. Even more significant, is that the individuals who had complete response (10%) were able to discontinue all immunosuppressive therapy illustrating the lasting immunomodulatory effect of rituximab.(29) There were also been made diverse retrospective studies that support this findings, with a response rate between 50%-80%(20).

In the same study, the authors however reported some adverse effects of Rituximab that deserve consideration, mainly infectious episodes, being described a hepatitis B reactivation. Also in that study, an extensive hematological study of the patients was made revealing no hematological toxicities(29) with platelets and red blood cells unaltered. Leukocytes were slightly diminished but it wasn't significant.

The authors concluded that Rituximab can be considered safe and that, associated with a response rate constantly higher than 50% and a complete remission in 10% of these patients make Rituximab a valid option for steroid-refractory cGVHD, when other therapies have already failed.(20)(29)

Specific pathway inhibitors

As we all know, the inflammation in general is a very complex process of an interaction between diverse cell types, and an even bigger amount of cytokines and other signaling mechanisms, such as specific cellular receptors to regulate the actions of the former ones. As previously referred, many of this mechanisms are altered in the process of cGVHD. And, if one possible management approach was to directly block these cells (either specifically or not), another hypothesis is to block these signaling pathways and therefore, interfere with the disarranged immune system, in a less direct way.

This theoretical principle has already shown to be effective in many hematological diseases such as Chronic Myeloid Leukemia (CML) and other myeloproliferative syndromes and given the lack of an effective salvage therapy for Steroid refractory-GVHD (SR-GVHD), this principle begun to be studied in this setting, in order to attempt to restrain the immune system activity. This can be accomplished with many different

drugs.

In this context, the first drug we'll analyze is **Imatinib**, which is a tyrosine kinase inhibitor, created for the management of CML(30). In the setting of the cGVHD, Imatinib was thought to be a possible treatment option because it blocks the PDGF-R pathway and the TGF-B (transforming growth factor beta) pathway, which are determinant for the fibrotic manifestations of diverse immunological diseases,(31) just like it happens in cGVHD, due to an over activity of the PDGF-R pathway that occurs in some of the patients due to the production of an activating antibody by the dysregulated immune system. By blocking this pathway, Imatinib decreases this fibrotic process and diminishes disease progression and also its manifestations(30).

Imatinib has already been the focus of some studies. In the first one, performed by Olivieiri et al.(30), 19 patients with refractory cGVHD were submitted to treatment with imatinib, first at 100mg per day, increasing until 400 mg per day. This patients were 100% matched whit the donor even if it was unrelated in 15,6% of the patients. The grafts were mainly from the bone marrow in 58% of the cases and from peripheral blood in 35% (the other patient had a graft from cord blood). The authors observed an overall response ratio at 3 months of 63% (58% - 11 patients plus 5% - 1patient) which further increased at 6 months to an overall response ratio of 79% (42% of partial responses+37% of complete responses). Besides this response ratio, it deserves to be highlighted a particularly high response ratio in the lung involvement. There were 11 patients with lung involvement and 6 had significative response(30)(32) particularly with mild to moderate involvement (the author hypothesized that when severe involvement is present, due to the high damage, imatinib couldn't change the progression). Similar results were again found by Olivieri et al in another study(31) where 39 patients with steroid-refractory cGVHD were studied and treated with imatinib, first with 100mg per day and gradually increasing doses until 400mg per day. 75% of the grafts came from peripheral blood samples (25% from blood marrow) and there was a match between the donor and the host in all of the cases (20% were unrelated). Response was achieved in 35,9% of the patients. The more responsive organs were the GI tract with 50% of response and the lungs with 35% of response. Besides the clinical response, it was registered tapering effect in the prednisone dose, diminishing from an average dose of 0,16mg/kg to 0,03mg/kg.(31) Regarding of Imatinib toxicity, it was reported fluid retention in 2 cases in the first study (19 patients) and 4

cases of hematological toxicity (one case of severe anemia).(30) Similar toxicities were observed in the second study but with higher frequencies, for example with mild anemia present in 64% of the patients.(31)

So, Imatinib can be considered a valid option for steroid-refractory cGVHD particularly in the setting of pulmonary involvement, where the majority of the other second-line therapies usually perform badly as opposed to imatinib which is capable of producing response in a considerable portion of the patients and so, it should be the preferable treatment option. In the other situations it remains a possibility after the failure of other possible therapies(31)(32)

As it was just stated, there is a vast amount of signaling patways in our immune system. Of note, is the family of the JAK kinases which are fundamental to the activation of the immune cells and the response to a diversity of cytokines like IFN-γ (Interferon gamma) and others (33). With that in mind, it is reasonable to attempt to block this important signaling pathway in order to suppress the inflammatory pathway. But besides its activity in inflammatory processes, JAK kinases are also involved in cell proliferation and mutations in these genes are a known cause of myeloproliferative disorders such as myelofibrosis and their blockade is a therapeutical option for those diseases. That is achieved with **Ruxolitinib**, a specific JAK 1/2 inhibitor which was first created for that purpose but, in the light of the role these kinases play in inflammatory processes their were hipothized to have some activity in cGVHD.(33)

Firstly, Ruxolitinib did show to have immunomudolatory proprieties, with a reduction in the IFN-γ levels, TCD4+ cells and an increase in Treg cells count.(33). Therefore it was reasonable to be tried in cGVHD patients. That was conducted by Zeiser et al.(34) in 41 patients all with moderate or severe disease and steroid refractory and even refractory to other second line therapies as well. In his study, patients were treated with 5mg or 10mg of Ruxolitinib. It was achieved an overall response ratio of 85,4% (in 35 of the 41 patients), with the vast majority being partial responses (78%-32 patients) and 3 cases of complete responses (7,3%). Even more, these patients were assessed during 6 months and the relapse of cGVHD was only of 5,7% of the responsive patients, showing a very desirable lasting effect.

However, there were identified some hazards regarding Ruxolitinib usage, like reactivation of CMV (Cytomegalovirus) infection, induction of citopenias (an expectable effect considering the usage of this drug in myeloproliferative disorders) although this particular side effect was difficult to evaluate given the fact that some patients already had them prior to the treatment. Very important to mention is that there was only 1 case of relapsing malignancy showing, accordind to the authors, that there wasn't loss of the graft-versus-leukemia effect .

So taking all of this into account Ruxolitinib has to be considered an option for the treatment of SR-cGVHD given to its considerable response ratio and manageable side events, particularly if surveillance measures for infections or hematologic abnormalities are implemented

Also in this group of drugs, we must adress **Bortezomib,** a proteasome inhibitor and also an inhibitor of NF-kB pathway which, due to this last effect, could produce immunoregulatory effects.(35) About its use in cGVHD very little is known but there were already made some experiments that encourage further study of this drug.

In a molecular study it was observed that Bortezomib, via the blockade of the NF-kB, caused direct apoptosis of T cells, especially the ones that were already activated as also a decrease in their cytokines. (36) In another study(37), with mice, several positive results were found. First, it inhibited B cells by inducing apoptosis and also by decreasing BAFF levels(37). Similarly with the previous study, there was an effect on T cells, since Bortezomib induced the reconstitution of the Treg cell population.(37) It was also observed that Bortezomib has a narrow effective concentration because if very low, it doesn't exert enough activity and if very high will causes tissue necrosis, being the best effective concentration 0,1mg/kg in mice(37) With all these findings, the authors considered that Bortezomib should be thought as an hypothesis, or a future one at least since it produce many laboratorial and hematological effects desired to treat cGVHD.

With this findings, the same authors initiated a study in 10 patients with moderate-severe cGVHD refractory to other therapies already tried. These patients were submitted to an escalating dosage of Bortezomib which started at 0,2mg/m^2 and was increased another 0,2mg/m^2 per 2 weeks, until there was response or toxicity. Only 6 were available for evaluation of response. In these, partial response was present in five (83,3%).(37) These

results are promising due to the high response rate but, regarding the very low number of patients, conclusions cannot be yet made. Also, this study showed to be too small to make definitive conclusions regarding the safety of Bortezomib in humans, although some adverse effects were already found like neuropathy and increase in the infectious risk in the patients.(37)

The only conclusion that the can be taken for the usage of Bortezomib in cGVHD is that deserves and needs more studies before any other steps be taken.

Immunoregulatory treatments – induction of tolerance

However, there are other lines of action than to block the immune system and wait for the normal development of tolerance. This tolerance can (at least theoretically) be induced, and in that way we could treat cGVHD. That could be performed in more than one way, given the complexity of the control of the immune system.

First, and more important, of all, there are the Treg cells which, as previously stated, are fundamental to the regulation of our immune system, and when their numbers are diminished, so is the control over our immune system. And that's exactly what happens in cGVHD,(7) resulting this in an attack from the donor cells towards the patient. One possible treatment approach is therefore to restore their numbers and with it, the balance in our immune system. But besides this major regulatory intervenient, there are other less studied immunoregulatory processes, such as anti-inflammatory cytokine secretion, resulting this in more than one treatment option.

Firstly, a treatment which already has been used for quite some years in the setting of cGVHD may have this mode of action, since it's precise mode of action and the full range of effects aren't entirely known is ECP, also called **extracorporeal photochemoterapy** because, it not only appears to have an immunosupressor effect but also, what seems to be an immunoregulatory effect. This technique consists of an irradiation of the leukocytes of the patient. This process, as the names suggests, is made *ex vivo*, so blood is taken from the patient and then exposed to UVA light plus a photosensitizer agent, 8-methoxyprosalen. This will induce apoptosis of these cells which then are reintroduced in the patient. (20)(38) As it was just stated, the precise mechanism by which the ECP induces the remission of cGVHD is not fully understood. However, some advances are

already been made. First of all, ECP does not treat cGVHD by destroying every single leucocyte responsible for the reaction, because of the simple fact that not all of these cells are in fact exposed do the radiation so, the effect is due to the reintroduction of these apoptotic cells in the patient, and there are many possible explanations for that. Firstly, inhibition of the production of pro-inflammatory cytokines and also the stimulating of anti-inflammatory ones: the possible immunosupressor effect of ECP, and as a second explanation, ECP appears to have a deep effect in the T cells homeostasis: it seems that it reduces the stimulation of T cells, it deletes T cells and even stimulates the Treg population, restoring (or at least helping to restore) the control over the immune system, which theoretically could lead to the development of tolerance against the host .(39) About its regimen, there isn't at the time a uniform opinion for ECP usage. However, the more used one is 3 times per week and after that 2 times per week, if tolerated. If response is observed it's continued 2 time a week until complete response is reached, as long if it's tolerated. (38)

ECP has already been evaluated in a randomized controlled trial (RCT), by Flowers et al.(40). This trial was performed with 95 patients where 48 were in the treatment arm (in which the patients were treated with ECP for 12 weeks and the conventional treatment) and 47 were allocated in the control arm in which only the conventional treatment was made. All the patients were under steroids and another immunosupressor therapy like MMF, tacrolimus or Cyclosporin A. The grafts were mainly from peripheral blood (65% in the treatment arm and 68% in the control arm) and there was a match, even if from an unrelated donor, in the majority of the cases (10% of mismatch in the treatment arm and 12% in the control arm). The authors analyzed the effect that ECP produced in the organs/sites involved separately, reporting a more favorable evolution of the skin manifestations (40% of the patients improved in the treatment arm vs 10% in the control arm) of cGVHD as well in other organs like in the eye manifestations (30% vs 7%) and the oral mucosa (52% vs 27%). The authors concluded that the results weren´t that great due to the duration of the treatment which was only for 12 weeks which may be too short to produce drastic improvement (the trial treatment was continued until 24 weeks, with far better results but were impossible to asses against the control arm). A far more positive result was the steroid-sparing effect that was seen in that same trial: with 25% of the patients at week 12 (against 12,8% of the control patients) and 39,6% at week 24, these

patients has a sustained response with a decrease of at least 50% of the corticosteroid dosage.(40)

In the same study, Flowers et al. reported serious adverse events in 28,6% of the patients in the treatment arm, but that wasn't significative because this same serious adverse events occurred in 26% of the control patients. This serious events were mainly severe infections. However, even if it wasn't an increase in serious adverse events, there were some toxicities worth mentioning. Hematological toxicity as anemia occurred in 24,5% of the patients against 6% of the control arm, being the major adverse event. Nausea also occurred with a slight tendency in the treatment arm patients (18,4% vs 12%) and also, there was a slight increase in the incidence of infection in the treatment arm (53,1% against 44%). (40)

Therefore, ECP can be considered as an important salvage therapy for cGVHD steroid-refractory particularly in the patients who have cutaneous involvement, and in these cases it may have a very significative effect. In other situations the response ratio is inferior and ECP may indeed not cause remission or reduction of the symptoms and, therefore, another therapy should be given before, however, in the absence of alternatives, ECP remains a viable option.(38)(41)

It deserves to be noticed that, although ECP is a viable option, there are been made advances to this technique. It's currently under development a new photosensitizer agent: dibromorhodamine (TH9402), which appears to have an increased specificity in eliminating activated Tcon cells while preserving Treg cells, producing better results than the actual option. The clinical application of this innovation is yet under study.(42)

Besides ECP, there is another possible intervention to induce immune tolerance and that is, to act directly and specificly in the Treg cell population (which in the case of ECP is only a theoretical explanation for its effects). this can be accomplished with**: IL-2**, which is known for many years to be a growth factor for Treg cells(43).

This molecule had already been analyzed in two studies. In the first, a phase I study performed by Koreth et al.(44), realized in 29 patients, determined that the most effective and safe dosage of IL-2 is 1x10e6 IU due to persistent constitutional symptoms with a dosage of 3x10e6 IU which needed to be reduced. Also in this study, it was observed an

increase of 8 times in the Treg cell population, in every patient, from the baseline value associated with very few changes in any other cell type, including the "regular" (Tcon) cells which were at same level in the beginning and in the end of the IL-2 therapy(44). This leads to an increase in the Treg/Tcon ratio, an important measure of the immune control. So, the theoretical effects of IL-2 were observed in the patients.

In another study, a phase II trial, realized by Koreth et al.(45) was conducted in 35 patients, with the majority of the grafts coming from peripheral blood (94,1%) with only 8,6% of mismatched donors. These patients were submitted to a course of IL-2 with daily subcutaneous administrations per 12 weeks. The same hematological effects were observed, with a drastic increase in the Treg cell population (8 times) with little changes in the other cells. In this study it was made a clinical evaluation of the patients which demonstrated a partial response in 60%. A similar result was found on the first study(44) with a partial response of 52,2%. Besides this effectiveness in symptom control, it was also registered a steroid-sparing effect with a decrease, in average of 60%, of the prednisone dose. (44)

However, some adverse effects of IL-2 were found, like constitutional symptoms, renal impairment and thrombocytopenia (all in just one patient each). More severely, there were seen two cases of thrombotic microangiopathy (TMA) with renal dysfunction, but in those cases the patients were taking both sirolimus and tacrolimus, which reverted and didn't happened again without this combination.(44) In the second study the adverse events were quite similar with 6 patients with constitutional effects, and three infectious episodes. There wasn't any cases of TMA, but the combination of tacrolimus and sirolimus was prohibited from the start.(45)

IL-2 is therefore a very good example of the importance of knowing every detail of the pathophysiology of the conditions to provide us with more therapeutic options. The results were considered promising by the authors and worthy of future studies.

Although it´s beyond the focus of this work, it deserves to be mentioned that this immunoregulatory perspective in the management of GVHD is gaining relevance, not only with the treatment with IL-2, as previously addressed, but also as a new prophylactic option with a direct infusion of Treg cells or with a transplant specimen enriched with Treg cells, representing this a change in the paradigm of the prophylactic regimes, since they were only formed by immunosuppressive drugs.(7)(46)

Discussion

There are many different therapies that can be given to a patient who has a cGVHD that doesn't respond to steroids or depends on high doses of these drugs in order to stay in remission. But, despite the great variety of options, it can be concluded that there isn't one that emerges as better than the rest, meaning that when steroid therapy fails there isn't a "next-in-line" treatment which is taken by all these patients. However, the choice between the diverse options should be guided and tailored to the specific patient that is being treated, since different therapies produce distinct effects on the many manifestations of the disease, and therefore should be chosen the treatment that has a better chance of inducing a response in that specific organ instead of the other options. For example, if the patient has a significant involvement of the skin, maybe the first alternative after failure with steroids should be ECP, which has shown a major activity in these manifestations but, in the other hand, if a patient has major involvement of the lungs, ECP shouldn't be used since it doesn't exert much activity, and another therapy should be given, like imatinib who showed activity in this particular setting.

There are also some other considerations worth doing. Firstly, the definition of the refractory state or, more specifically, the time that it takes to a patient to be defined like that deserves an highlight because, nowadays it's speculated that when the disease is more advanced, the outcome and the response to therapy tend to be worse, even if it is other drug with a different mode of action, because the damage is already so severe that very little can be done and so, if the patients were considered sooner to be refractory and therefore starting earlier the salvage therapies, better results could be achieved. Secondly, the utility of a new frontline therapy in order to substitute to the corticosteroids is debatable, since many of the patients that respond with these new drugs were also going to be successfully treated with the standard steroid therapy and even if the response ratio is superior, maybe this drugs are better used only as salvage therapy, specially if we consider the costs of treating all the cGVHD patients with these new drugs.

Another consideration is the good future perspective with new drugs being constantly discovered, or at least new applications of previously discovered drugs, like Bortezomib, giving hope to the physicians and patients alike. However, the scientific advances in this area must be made with caution since, as observed in many of the studies utilized to this work, there may be many limitations in these which sometimes may impede us to take

many conclusions and progress, like for example, the small number of patients enrolled in the studies and that many of them are either retrospective or prospective ones, with less evidence power than RCTs in order to take conclusion and also, and the most important limitation in many of the past studies, is that there was made a non-standardized evaluation of the patients, being therefore impossible to compare results and explaining the big variety of responses. However, with the new NIH classification this problem should disappear.

In summary, there are many options to treat a steroid-refractory cGVHD patient and the treatment should be tailored to the specific patient. Moreover, there is a considerable urge to perform more and better clinical trials in order to consolidate the current knowledge and even to make advances in the cGVHD treatment.

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Bibliography

1. Majhail NS, Farnia SH, Carpenter PA, Champlin RE, Crawford S, Marks DI, et al. Indications for Autologous and Allogeneic Hematopoietic Cell Transplantation: Guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant [Internet]. 2015 Nov [cited 2016 Oct 10];21(11):1863–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26256941

2. Wingard JR, Majhail NS, Brazauskas R, Wang Z, Sobocinski KA, Jacobsohn D, et al. Long-Term Survival and Late Deaths After Allogeneic Hematopoietic Cell Transplantation. J Clin Oncol [Internet]. 2011 Jun 1 [cited 2016 Dec 29];29(16):2230–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21464398

3. Bhatia S, Francisco L, Carter A, Sun C-L, Baker KS, Gurney JG, et al. Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. Blood [Internet]. 2007 Nov 15 [cited 2016 Oct 10];110(10):3784–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17671231

4. Flowers MED, Martin PJ. How we treat chronic graft-versus-host disease. Blood. 2015;125(4).

5. Abou-Mourad YR, Lau BC, Barnett MJ, Forrest DL, Hogge DE, Nantel SH, et al. Long-term outcome after allo-SCT: close follow-up on a large cohort treated with myeloablative regimens. Bone Marrow Transplant [Internet]. 2010 Feb [cited 2016 Oct 10];45(2):295–302. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19597425

6. Pidala J, Kurland B, Chai X, Majhail N, Weisdorf DJ, Pavletic S, et al. Patient-reported quality of life is associated with severity of chronic graft-versus-host disease as measured by NIH criteria: report on baseline data from the Chronic GVHD Consortium. Blood [Internet]. 2011 Apr 28 [cited 2016 Oct 10];117(17):4651–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21355084

7. Socié G, Ritz J. Current issues in chronic graft-versus-host disease. Adv Hematop Cell Transplant [Internet]. 2014;124(3):374–84. Available from: http://www.bloodjournal.org/content/124/3/374.long?sso-checked=true

8. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant [Internet]. 2015 Mar [cited 2016 Oct 11];21(3):389–401.e1. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25529383

9. Latour P De, Maki G, Robin M. Impact of acute and chronic graft-versus-host disease on human B-cell generation and replication. Blood. 2015;124(15):2459–63.

10. Wolff D, Gerbitz A, Ayuk F, Kiani A, Hildebrandt GC, Vogelsang GB, et al. Consensus conference on clinical practice in chronic graft-versus-host disease (GVHD): First-line and topical treatment of chronic GVHD. Biol Blood Marrow Transplant [Internet]. 2010;16(12):1611–28. Available from: http://dx.doi.org/10.1016/j.bbmt.2010.06.015

11. Koc S, Leisenring W, Flowers MED, Anasetti C, Deeg HJ, Nash RA, et al. Therapy for chronic graft-versus-host disease: a randomized trial comparing cyclosporine plus prednisone versus prednisone alone. Blood [Internet]. 2002 Jul 1 [cited 2016 Oct 18];100(1):48–51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12070007

12. Sullivan KM, Witherspoon RP, Storb R, Weiden P, Flournoy N, Dahlberg S, et al. Prednisone and azathioprine compared with prednisone and placebo for treatment of chronic graft-v-host disease: prognostic influence of prolonged thrombocytopenia after allogeneic marrow transplantation. Blood [Internet]. 1988 Aug [cited 2016 Oct 18];72(2):546–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3042041

13. Martin PJ, Storer BE, Rowley SD, Flowers MED, Lee SJ, Carpenter PA, et al. Evaluation of mycophenolate mofetil for initial treatment of chronic graft-versus-host disease. Blood [Internet]. 2009 May 21 [cited 2016 Oct 18];113(21):5074–

82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19270260

14. Gilman AL, Schultz KR, Goldman FD, Sale GE, Krailo MD, Chen Z, et al. Randomized trial of hydroxychloroquine for newly diagnosed chronic graft-versus-host disease in children: a Children's Oncology Group study. Biol Blood Marrow Transplant [Internet]. 2012 Jan [cited 2016 Oct 18];18(1):84–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21689773

15. Stewart BL, Storer B, Storek J, Deeg HJ, Storb R, Hansen JA, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. Blood [Internet]. 2004 Dec 1 [cited 2016 Oct 19];104(12):3501–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15292060

16. Flowers MED, Storer B, Carpenter P, Rezvani AR, Vigorito AC, Campregher P V, et al. Treatment change as a predictor of outcome among patients with classic chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2008 Dec [cited 2016 Dec 30];14(12):1380–4. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1083879108004163

17. Pérez-Simón JA, Encinas C, Silva F, Arcos MJ, Díez-Campelo M, Sánchez-Guijo FM, et al. Prognostic factors of chronic graft-versus-host disease following allogeneic peripheral blood stem cell transplantation: the national institutes health scale plus the type of onset can predict survival rates and the duration of immunosuppressive therapy. Biol Blood Marrow Transplant [Internet]. 2008 Oct [cited 2016 Oct 18];14(10):1163–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18804047

18. Solomon SR, Sizemore CA, Ridgeway M, Zhang X, Smith J, Brown S, et al. Corticosteroid-Free Primary Treatment of Chronic Extensive Graft-versus-Host Disease Incorporating Rituximab. Biol Blood Marrow Transplant [Internet]. 2015;21(9):1576–82. Available from: http://dx.doi.org/10.1016/j.bbmt.2015.04.023

19. Herrera AF, Kim HT, Bindra B, Jones KT, Alyea EP, Armand P, et al. A phase II study of bortezomib plus prednisone for initial therapy of chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2014;20(11):1737–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25017765

20. Wolff D, Schleuning M, von Harsdorf S, Bacher U, Gerbitz A, Stadler M, et al. Consensus conference on clinical practice in chronic GVHD: Second-line treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2011;17(1):1–17. Available from: http://dx.doi.org/10.1016/j.bbmt.2010.05.011

21. Kirken RA, Wang YL. Molecular actions of sirolimus: sirolimus and mTor. Transplant Proc [Internet]. 2003 May [cited 2016 Oct 25];35(3 Suppl):227S–230S. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12742500 (abstract only)

22. Johnston LJ, Brown J, Shizuru JA, Stockerl-Goldstein KE, Stuart MJ, Blume KG, et al. Rapamycin (sirolimus) for treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2005 Jan [cited 2016 Oct 24];11(1):47–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15625544

23. Jurado M, Vallejo C, Pérez-Simón JA, Brunet S, Ferra C, Balsalobre P, et al. Sirolimus as part of immunosuppressive therapy for refractory chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2007 Jun [cited 2016 Oct 24];13(6):701–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17531780

24. Higman M, Vogelsang GB, Chen A. Pentostatin - pharmacology, immunology, and clinical effects in graft-versus-host disease. Expert Opin Pharmacother [Internet]. 2004 Dec [cited 2016 Oct 23];5(12):2605–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15571477 (abstract only)

25. Jacobsohn DA, Gilman AL, Rademaker A, Browning B, Grimley M, Lehmann L, et al. Evaluation of pentostatin in corticosteroid-refractory chronic graft-versus-host disease in children : a Pediatric Blood and Marrow Transplant Consortium study. Clin Trials Obs. 2009;114(20):4354–60.

26. Jacobsohn DA, Chen AR, Zahurak M, Piantadosi S, Anders V, Bolaños-Meade J, et al. Phase II study of pentostatin in patients with corticosteroid-refractory chronic graft-versus-host disease. J Clin Oncol. 2007;25(27):4255–61.

27. Furlong T, Martin P, Flowers MED, Carnevale-Schianca F, Yatscoff R,

Chauncey T, et al. Therapy with mycophenolate mofetil for refractory acute and chronic GVHD. Bone Marrow Transplant [Internet]. 2009;44(11):739–48. Available from: http://dx.doi.org/10.1038/bmt.2009.76

28. Lopez F, Parker P, Nademanee A, Rodriguez R, Al-Kadhimi Z, Bhatia R, et al. Efficacy of mycophenolate mofetil in the treatment of chronic graft-versus-host disease. Biol Blood Marrow Transplant [Internet]. 2005 Apr [cited 2016 Oct 23];11(4):307–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15812396

29. Cutler C, Miklos D, Kim HT, Treister N, Woo SB, Bienfang D, et al. Rituximab for steroid-refractory chronic graft-versus-host disease. Blood [Internet]. 2006;108(2):756–62. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16551963

30. Olivieri A, Locatelli F, Zecca M, Sanna A, Cimminiello M, Raimondi R, et al. Imatinib for refractory chronic graft-versus-host disease with fibrotic features. Blood [Internet]. 2009 Jul 16 [cited 2017 Jan 2];114(3):709–18. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2009-02-204156

31. Olivieri A, Cimminiello M, Corradini P, Mordini N, Fedele R, Selleri C, et al. Long-term outcome and prospective validation of NIH response criteria in 39 patients receiving imatinib for steroid-refractory chronic GVHD. Blood [Internet]. 2013 Dec 12 [cited 2017 Jan 2];122(25):4111–8. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2013-05-494278

32. Olivieri A, Cimminiello M, Locatelli F. Response: Imatinib in lung cGVHD of mild to moderate severity. Blood. 2009;114(17):3719–20.

33. Spoerl S, Mathew NR, Bscheider M, Schmitt-Graeff A, Chen S, Mueller T, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. Blood [Internet]. 2014 Jun 12 [cited 2017 Feb 5];123(24):3832–42. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2013-12-543736

34. Zeiser R, Burchert A, Lengerke C, Verbeek M, Maas-Bauer K, Metzelder SK, et al. Ruxolitinib in corticosteroid-refractory graft-versus-host disease after allogeneic stem cell transplantation: a multi-center survey. Leukemia [Internet].

2015 Jul 31 [cited 2017 Feb 5];29(10):2062–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26228813

35. Moreau P, Richardson PG, Cavo M, Orlowski RZ, San Miguel JF, Palumbo A, et al. Proteasome inhibitors in multiple myeloma: 10 years later. Blood [Internet]. 2012 Aug 2 [cited 2016 Oct 24];120(5):947–59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22645181

36. Blanco B, Pérez-Simón JA, Sánchez-Abarca LI, Carvajal-Vergara X, Mateos J, Vidriales B, et al. Bortezomib induces selective depletion of alloreactive T lymphocytes and decreases the production of Th1 cytokines. Blood. 2006;107(9):3575–83.

37. Pai C-CS, Chen M, Mirsoian A, Grossenbacher SK, Tellez J, Ames E, et al. Treatment of chronic graft-versus-host disease with bortezomib. Blood [Internet]. 2015;124(10):1677–89. Available from: http://www.bloodjournal.org/content/124/10/1677.abstract\nhttp://www.ncbi.nlm.nih.gov/pubmed/25009225\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4155274

38. Abu-Dalle I, Reljic T, Nishihori T, Antar A, Bazarbachi A, Djulbegovic B, et al. Extracorporeal Photopheresis in Steroid-Refractory Acute or Chronic Graft-versus-Host Disease: Results of a Systematic Review of Prospective Studies. Biol Blood Marrow Transplant [Internet]. 2014;20(11):1677–86. Available from: http://dx.doi.org/10.1016/j.bbmt.2014.05.017

39. Peritt D. Potential mechanisms of photopheresis in hematopoietic stem cell transplantation. Biol Blood Marrow Transplant [Internet]. 2006 Jan [cited 2016 Oct 21];12(1 Suppl 2):7–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16399596

40. Flowers MED, Apperley JF, van Besien K, Elmaagacli A, Grigg A, Reddy V, et al. A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease. Blood [Internet]. 2008 Oct 1 [cited 2016 Oct 21];112(7):2667–74. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18621929

41. Bittenberg, J; Reichrath, J. Extracorporeal Photopheresis for Non-skin GvHD.

2016;Anticancer research 36

42. Bastien J-P, Krosl G, Therien C, Rashkovan M, Scotto C, Cohen S, et al. Photodepletion differentially affects CD4+ Tregs versus CD4+ effector T cells from patients with chronic graft-versus-host disease. Blood [Internet]. 2010 Dec 2 [cited 2017 Feb 5];116(23):4859–69. Available from: http://www.bloodjournal.org/cgi/doi/10.1182/blood-2010-03-273193

43. Nelson BH. IL-2, regulatory T cells, and tolerance. J Immunol [Internet]. 2004 Apr 1 [cited 2016 Oct 25];172(7):3983–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15034008

44. Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med [Internet]. 2011 Dec 1 [cited 2016 Oct 25];365(22):2055–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22129252

45. Koreth J, Kim HT, Jones KT, Lange PB, Reynolds CG, Chammas MJ, et al. Efficacy, durability, and response predictors of low-dose interleukin-2 therapy for chronic graft-versus-host disease. Blood. 2016;128(1):130–7.

46. Cutler CS, Koreth J, Ritz J. Mechanistic approaches for prevention and treatment of chronic GVHD. Blood. 2016;129(1):1–21.