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Research Article

Drug delivery formulation impacts cyclosporine efficacy in a humanised mouse model of acute graft versus host disease

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ABSTRACT

Acute graft versus host disease (aGvHD) is an allogeneic T cell mediated disease which manifests as a severe inflammatory disease affecting multiple organs including the liver, skin, lungs and gastrointestinal tract. Existing prophylactic and therapeutic approaches in aGvHD include the use of cyclosporine A (CyA), however the currently approved CyA formulations which were designed to optimise systemic CyA bioavailability can have a number of side effects including nephrotoxicity as well as the potential to attenuate the beneficial Graft-versus-Leukemia (GvL) effect. An added complication with CyA is that it has a narrow therapeutic window, and following oral administration is absorbed only from the small intestine, with variable cytochrome P450 metabolism contributing to intra- and inter-patient variability. This study sought to investigate the efficacy of a novel CyA oral formulation enabled by the integrated SmPill® oral drug delivery platform in a humanised mouse model of aGvHD. The study compared the approved optimised CyA (Neoral®) with SmPill®-enabled CyA and a systemic intravenous CyA formulation. Our findings clearly demonstrate superior efficacy of the novel SmPill® CyA in prolonging survival in a clinically relevant humanised aGvHD model. SmPill® CyA significantly reduced pathological score in the small intestine, colon, liver and lung of aGvHD mice. In addition, SmPill® CyA significantly reduced the levels of pro-inflammatory cytokines in all the GvHD target tissues examined. Notably, SmPill® CyA was significantly more potent in reducing GvHD associated pathology and inflammatory cytokine production compared to the optimised approved oral CyA formulation, Neoral®.

1. Introduction

The development of graft versus host disease (GvHD) represents a life threatening complication following allogeneic hematopoieic stem cell transplantation (HSCT). The disease manifests as a severe inflammatory condition affecting multiple organs, including the gastrointestinal (GI) tract, the liver and in some cases the lungs. In particular, the GI tract is significantly impacted by conditioning regimens leading to breakdown of mucosal barriers and release of damage associated molecular patterns (DAMPs) and pathogen associated molecular patterns (PAMPs) like bacterial derived lipopolysaccharide which play a key role in priming and activation of the allogeneic immune cells [1]. Gastrointestinal GvHD (GI-GvHD) is a progressive process that can affect all sections of the alimentary tract, albeit the terminal ileum and the colon are predominant sites. The gut is one of the main targets of acute GvHD. Severe gastrointestinal (GI) GvHD remains a major issue after allo-HCT, since it is difficult to treat and involvement of the GI tract is reported in almost all fatal acute GvHD cases [2].

For prophylaxis of aGvHD, cyclosporine A (CyA) is administered for up to six months after allogeneic HSCT [3]. Recent studies demonstrate that high concentrations of CyA early after haploidentical haematopoietic cell transplantation reduces the risk of aGvHD without any detrimental effect on relapse [4,5]. CyA has also been reported to be effective in the treatment of established GvHD [3,6]. The optimal application of CyA is influenced by its narrow therapeutic index, its metabolism and its systemic bioavailability and differential tissue distribution following intravenous or oral administration [7]. Following oral administration, CyA is systemically absorbed from the small intestine with negligible absorption from the colon [7]. As a lipophilic cyclic peptide [8], use of microemulsion-based formulations has enhanced CyA absorption from the small intestine [9]. However, despite improved absorption, differential expression of cytochrome P450 enzymes in the

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upper GI of patients leads to variation in systemic bioavailability between patients [10]. The limited absorption and relatively short half-life results in a risk of systemic side-effects in susceptible organs such as the liver and kidneys [7]. Within the context of the gastrointestinal tract, existing oral formulations result in a short-burst of high-concentration exposure in the small intestine, but limited luminal exposure to active CyA in the ileum and colon [7].

The existing oral CyA formulations, Sandimmune® and Neoral® were designed to maximise systemic release from the small intestine, with Neoral® providing enhanced CyA oral bioavailability and reduced pharmacokinetic variability [11,12]. Despite the improved bioavailability that Neoral has enabled, high rapid peaks to trough CyA pharmacokinetic profile [13] is associated with unwanted systemic side effects and a potential risk that the beneficial Graft versus Leukemia (GvL) effect may be attenuated [11,14].

Sigmoid Pharma (renamed Sublimity Therapeutics) have developed a sophisticated drug delivery technology called SmPill® which encapsulates CyA into multiple minisphere format where the outer coating controls the release of CyA in the small intestine and/or permit release of solubilised, active CyA throughout the ileum and colon [15]. The minisphere cores are designed such that CyA is fully solubilised and remain soluble when released into the GI lumen. The ileal and colonic release profile is achieved by applying a polymer coating on the core minispheres. Pre-clinical studies using a porcine model demonstrated that CyA released into the colonic lumen is not systemically absorpbed and attains therapeutic concentrations within the ileal and colonic tissue [15]. Notably, the CyA SmPill® minisphere formulation had a lower systemic bioavailability than Neoral® CyA formulation (that is approved for oral administration) in a porcine study [15].

As aGvHD is a multi organ inflammatory disease with the GI tract having a primary role in initiation [16], this study sought to investigate the capacity for SmPill® mediated GI delivery of CsA to provide greater protection than the standard oral and CsA delivery approach using Neoral®. For comparison, systemic delivery using i.v. administration of SandImmune® was included. Previous work within our research group contributed to the establishment of a robust and reproducible humanised mouse model of aGvHD based on transfusion of peripheral blood mononuclear cells (PBMCs) to immunodeficient NOD-SCID IL-2 receptor gamma null (NSG) mice [17], modified from that first described by Pearson et al.[18]. Using this approach, the disease is generated and sustained by human immune cells offering a more clinically relevant model of disease than murine models of aGvHD. In this study, we demonstrate that the drug delivery formulation impacts CyA efficacy in aGvHD, with SmPill® mediated GI delivery of CsA providing greater protection against GvHD.

2. Materials & methods

2.1. Ethical approval

All procedures involving the use of animals or human materials were carried out by licensed personnel. Ethical approval for all work was granted by the biological research ethics committee of Maynooth University (BRESC-2013-13). Project Authorisation was received from the scientific animal protection unit of the health products regulatory agency (HPRA) under AE19124/P002 whereby the terms of the animal experiments within this project were outlined and adhered to. NOD.Cg-Prkdc^{scid}IL2^{tmlWjl}/Szj (Jackson Labs, Bar Harbour, Maine, USA) mice were used in these studies.

2.2. Acute graft versus host disease humanised mouse model

A humanised mouse model of acute graft versus host disease (aGvHD) was developed and optimised from a protocol described by Pearson et al. [18]. NOD.Cg-Prkdc^{scid}IL2^{tmlWjl}/Szj (NOD-Scid IL-2rγnull) (NSG) were exposed to a conditioning dose of 2.4 Gray (Gy) of whole body gamma irradiation. 8.0×10^5 g-1 freshly isolated human peripheral blood mononuclear cells (PBMCs) (isolated from buffy coat packs supplied by the Irish Blood Transfusion Service) were administered i.v. 4-6 h following irradiation [17]. Animals were returned to their cages where they were monitored closely for the first hour and at regular intervals thereafter for any signs of distress or ill health. Animals were weighed daily and weight loss was documented accordingly. Any animals which displayed greater than 15% total body weight loss were sacrificed humanely. In addition, an animal welfare score sheet was utilized throughout the study. aGvHD development was determined by examining features including weight loss exceeding 15% total body weight, ruffled fur, hunched posture and general activity.

2.3. Preparation and administration of cyclosporine formulations

SmPill® formulated cyclosporine (Sigmoid Pharma, Invent Centre DCU, Dublin) consisted of 2 distinct minispheres, uncoated minispheres for release in the small intestine and minispheres coated with an ethylcellulose-based coating for release in the ileum and colon. Each minisphere weighed between 2 and 3 mg and comprised ~10% CyA loading. The dose of CyA (25 mg/kg) was chosen based on published studies demonstrating efficacy of CyA in prolonging survival in murine GvHD models [19,20]. SmPill® minispheres and Neoral® were administered at a dose per animal of 25 mg/kg CyA via oral gavage. The Sandimmune® formulation of CyA (provided by Sigmoid Pharma) (25 mg/kg dose) was delivered i.v..

2.4. Cytokine analysis from acute graft versus host disease tissues

Facial bleeds were performed just before euthanisia of the mice on day 13. Following centrifugation, serum was removed and stored at −20 °C until a human TNFα ELISA (R&D Systems) was performed. After euthanasia, the small intestine, colon, lungs and liver were removed from mice and a section was immediately snap frozen and stored at −80 °C. Tissues were thawed and gut contents were removed from the small intestine and colon. The tissues were chopped finely and homogenised using an Ultra-Turrax homogeniser (IKA, Staufen, Germany) in 1 ml of chilled homogenisation buffer (PBS: 2% heat inactivated FBS supplemented with protease inhibitor cocktail (Roche, Dublin, Ireland)). The homogenate was microcentrifuged at 15,000 g for 15 min at 4 °C. The supernatant was removed and stored at −20 °C. The protein concentration of the tissue extracts were determined by Bradford assay. Protein extracts were analysed for human IL-1β, IL-2, IL-6, IL-17, IL-23 and IFNγ by ELISA (R&D Systems, Abingdon, UK, or eBioscience, Paisley, Scotland).

2.5. Histology

The lungs, liver, small intestine and colon were harvested from experimental mice at day 13 and fixed in 10% (v/v) neutral buffered formalin for at least 24 h. Samples were transferred to 70% ethanol for a further 24 h. Samples were processed for histology using an automated processor (Shandon Pathcentre, Runcorn, UK) which immersed the tissues in fixatives and sequential dehydration solutions including ethanol (70%, 80%, 95% x 2, 100% x 3) and xylene (x 2) (Sigma-Aldrich). After processing, tissues were embedded in paraffin wax (Sigma-Aldrich) and cut at 5 μm sections for Haemotoxylin and Eosin (H&E) staining. Following H&E staining, slides were coded without reference to prior treatment and examined in a blind manner. A semi-quantitative scoring chart was used to assess disease progression in the lungs, liver and GI tract [17].

2.6. Statistical methods

Mantel-Cox test (log rank test) were used to compare survival between treatment groups. The ratio for median survival was computed

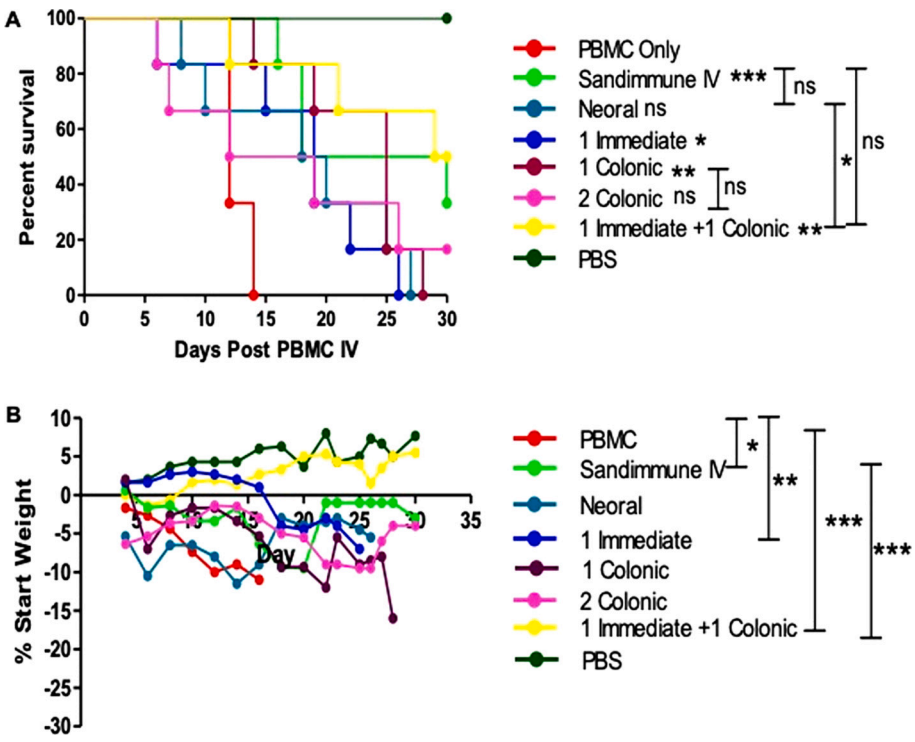

Fig. 1. Drug delivery formulation impacts cyclosporine efficacy in acute Graft versus host disease. A humanised mouse model of aGvHD was established by administering 8×10^5 human PBMC gram^{-1} (or PBS as a control) to irradiated (2.4Gy) NSG mice on day 0. CyA was delivered intravenously (Sandimmune® IV) or by oral gavage (Neoral®, SmPill® formulations consisting of 1 or 2 colonic minispheres or 1 immediate release microsphere or 1 immediate +1 colonic microsphere) for 5 doses (25 mg/kg per dose) every 2 days from day 4. A. Survival curve. B. % Weight change. $n = 6$ mice per group. Statistical analysis was carried out using a Mantel-Cox test for the survival curve and unpaired student *t*-test for weight change where * <0.05, ** <0.01 and *** <0.001. * with no bar are in comparison to the PBMC group.

using GraphPad Prism. For comparison between two groups, the *p* values were generated using nonparametric analysis using the Mann–Whitney U; $p < 0.05$ (*), $p < 0.01$ (**) or $p < 0.001$ (***) were considered statistically significant. All the analyses were performed using the GraphPad Prism 6 software (Graphpad Sofware, San Diego, California, USA).

3. Results

3.1. Drug delivery formulation impacts cyclosporine efficacy in a humanised mouse model of acute graft versus host disease

The SmPill® minispheres enable the release of solubilised CyA as an immediate release or a delayed, colonic release manner [15]. The

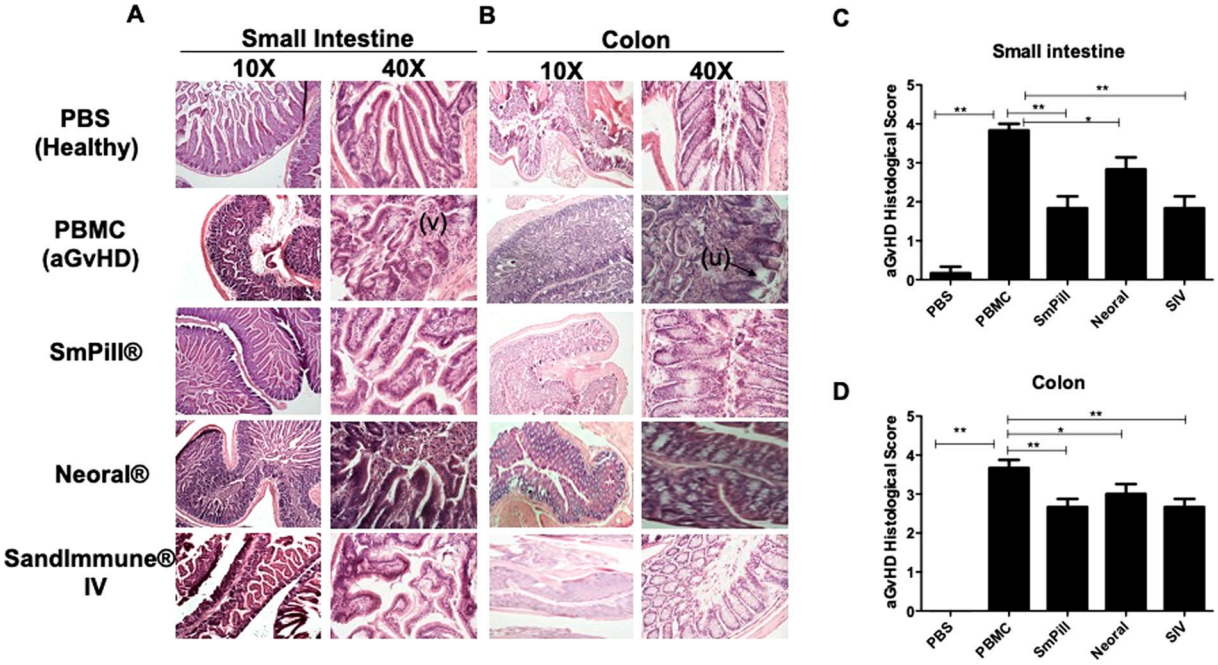

Fig. 2. SmPill® CyA therapy significantly decreased occurance of ulceration in the colon and reduced villi destruction in the small intestine of aGvHD mice. The aGvHD model was set up exactly as described in Fig. 1. Tissue samples were harvested on day 13, formalin fixed, paraffin embedded and stained with H&E (A). Representative images were analysed for lymphocyte infiltration, villi destruction (v) and ulceration of the colonic mucosa (u) and displayed for each group. Images were captured at 100× and 400×. aGvHD histological scoring was carried out blinded (B). 3 random areas in each section were scored from $n = 6$ mice per group. Statistical analysis was carried out using the Mann–Whitney *U* test to compare between two groups; where * signifies $p < 0.05$ and ** signifies $p < 0.01$.

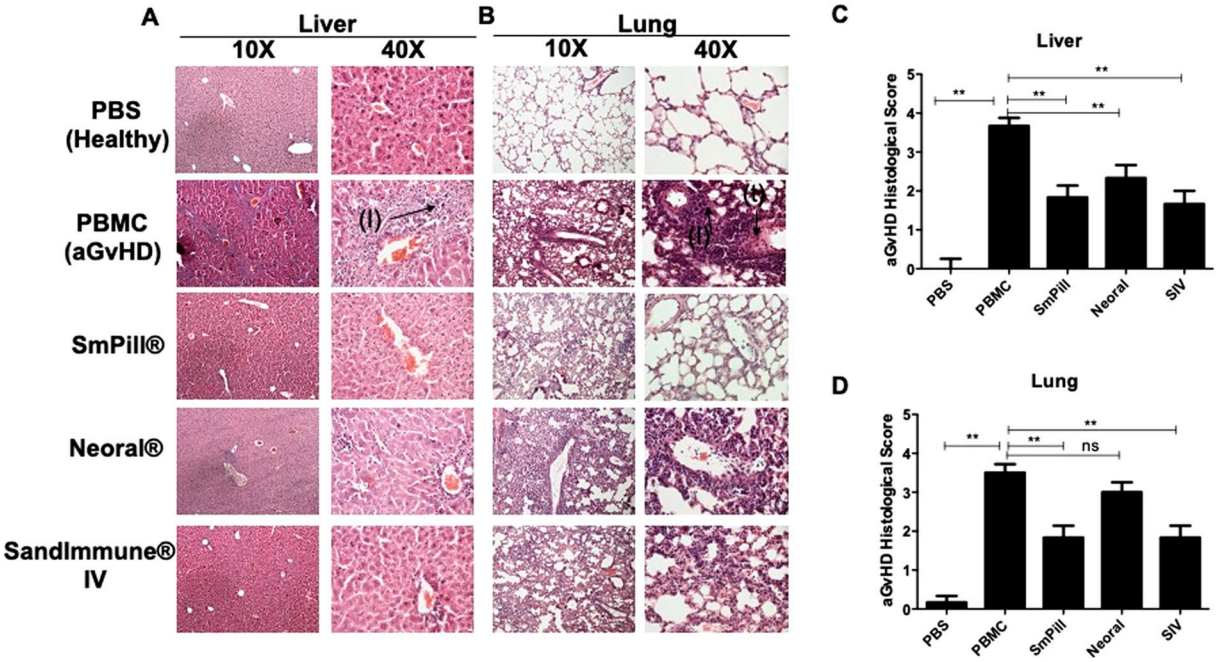

Fig. 3. SmPill® CyA therapy significantly reduced pathology and infiltration in the liver and lung of aGvHD mice. The aGvHD model was set up exactly as described in Fig. 1. Tissue samples were harvested on day 13, formalin fixed, paraffin embedded and stained with H&E (A). Representative images were analysed for lymphocyte infiltration (l) and thickening of epithelial airways (t) and displayed for each group. Images were captured at 100× and 400×. aGvHD histological scoring was carried out blinded (B). 3 random areas in each section were scored from $n = 6$ per group. Statistical analysis was carried out using the Mann–Whitney U test to compare between two groups; where ** signifies $p < 0.01$.

colonic release is enabled through the application of a pH-independent ethylcellulose-based polymer coating. The uncoated minispheres release CyA within the small intestine from where it is systemically absorbed, while the coated minispheres avoid release in the small intestine and permit controlled release throughout the ileum and colon. When combined, the release of CyA throughout the entire GI is permitted [15]. The immediate release SmPill® formulation provides a slower release than Neoral® which permits modulated pharmacokinetic (PK) profiles, with lower peak-to-trough ranges, thus permitting adequate trough levels to be attained with a lower peak level than that associated with Neoral® [15]. This study sought to investigate the optimal minisphere treatment protocol for enhanced protection of the GI tract as well as other target organs affected by GvHD. To achieve this, different immediate and or colonic release minisphere combinations were delivered (5 doses every 2 days from day 4 to day 12) to aGvHD mice to explore the systemic and GI effects mediated by these CyA loaded minispheres and compared with the approved oral (Neoral®) and i.v. (Sandimmune®) CyA formats.

Acute GvHD mice that received no CyA therapy significantly lost weight and succumbed to aGvHD with all mice humanely sacrificed from this group by day 14 (Fig. 1A). The combination of 1 immediate and 1 colonic release minisphere was the most effective of the various CyA bead formulation groups and had greater efficacy than Neoral® in significantly prolonging survival of the aGvHD mice. Neoral® therapy prolonged survival with a median survival time (MST) of 19 days although SmPill® (1 immediate +1 colonic) was significantly better in doing so with an MST of 29 days. 1 colonic minisphere significantly prolonged the survival of aGvHD mice to day 25 (MST) while 2 colonic minisphere only prolonged survival to day 15.5 (MST) (Fig. 1A). Sandimmune® IV significantly prolonged survival with a MST of 24 days, however there was no significant differences when compared to SmPill® (1 immediate +1 colonic) or Neoral® (Fig. 1A). Administration of CyA via 1 immediate release minisphere and via a combination of 1 immediate and 1 release minisphere significantly reduced % weight loss. While Sandimmune® IV significantly reduced weight loss in aGvHD mice, SmPill® (1 immediate +1 colonic) was significantly better in doing so (Fig. 1B).

For the remainder of the study, the efficacy of SmPill® (1 immediate +1 colonic release minisphere) was compared to Neoral® delivered by oral gavage or Sandimmune® delivered intravenously. These refined experimental groups were further probed to examine the efficacy of these CyA formulations in reducing GvHD pathology and proinflammatory cytokine production.

3.2. SmPill® delivery of CyA significantly reduced pathology in acute graft versus host disease target tissues

Our previous work using the humanised mouse model of aGvHD has demonstrated the pathogenic effects of xenogeneic human T cells in the mouse liver, lung and small intestine [17]. Histological analysis of small intestine and colon sections revealed characteristics of aGvHD which included lymphocyte infiltration and villous destruction or blunting (v) (Fig. 2A & B) in the PBMC (aGvHD) group that received PBMC only. SmPill® and Sandimmune® IV were shown to have similar effects in improving small intestine (Fig. 2A & C) and colon (Fig. 2B & D) pathology, significantly reducing not only the level of villi destruction, but also and lymphocyte infiltration into the lamina propria (Fig. 2). Although less significantly, Neoral® treatment resulted in less villi destruction with reduced signs of infiltrating lymphocytes in the small intestine (Fig. 2A & C). In this study, we extended our GvHD tissue targets to include the colon given the capacity the SmPill® colonic release formulation to release solubilised, active CyA throughout the entire colon [15]. GvHD pathology of the colon was determined by lymphocyte infiltration, crypt distortion and ulceration of colonic mucosa (u). Irradiated PBS control mice displayed an intact epithelium with well-defined gland lengths and no lymphocyte infiltration in the mucosa (Fig. 2). SmPill® therapy significantly reduced GvHD pathology in the colon with a significant reduction in lymphocyte infiltration and maintenance of a well-defined epithelium in comparison to untreated or placebo treated mice (Fig. 2B & D). The GvHD pathology in the colon of Sandimmune® IV treated mice displayed similar characteristics to

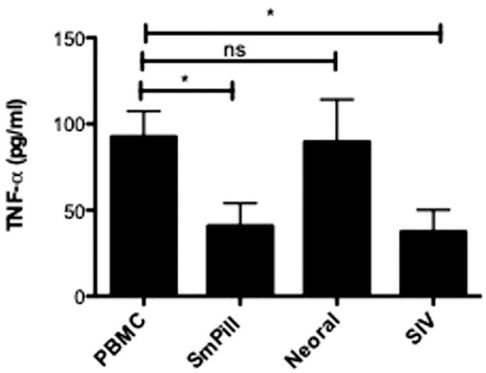

Fig. 4. SmPill® CyA but not Neoral significantly reduced the levels of TNFα in the serum of aGvHD mice. The aGvHD model was set up as described in Fig. 1. On day 13, facial bleeds were performed aGvHD mice and aGvHD mice that received different CyA therapies (SmPill®, Neoral® or SandImmune® IV). The total level of circulating human TNFα was analysed in the serum using ELISA. $n = 6$ per group. Statistical analysis was carried out in comparison to the PBMC group using the Mann–Whitney *U* test to compare between two groups; * signifies $p < 0.05$.

SmPill® treated mice, while Neoral® therapy also improved colon pathology but to a lesser extent (Fig. 2D).

After aGvHD development, untreated mice receiving PBMC only or PBMC and placebo had a significant increase in lymphocyte infiltration (l) and endothelialitis particularly in the hepatic ducts when compared to PBS control mice (Fig. 3A & 3C). SmPill® therapy significantly reduced liver pathology with a significant reduction in lymphocyte infiltration and endothelialitis of hepatic ducts (Fig. 3A&C). Neoral® and Sandimmune® IV had similar effects to SmPill® on alleviating signs of GvHD in the liver.

The characteristics of aGvHD lungs classified as lymphocyte infiltration (l) and thickening of epithelial airways (t) were significantly evident in the aGvHD mice in comparison to PBS controls (Fig. 3B & D). Following treatment with SmPill® and Sandimmune® IV, lymphocyte infiltration was substantially lowered with a marked reduction in the airway epithelium thickness (Fig. 3B & D). In comparison, Neoral® therapy did not improve lung pathology in aGvHD mice which resulted in no significant change in histological scoring of aGvHD (Fig. 3B & D).

3.3. SmPill® delivery of CyA significantly reduced proinflammatory cytokines in acute graft versus host disease tissues in a targeted manner

Acute GvHD is a disease driven by donor T cells following the recognition of foreign HLA antigen. The production of proinflammatory cytokines mediated by these effector T cells are a hallmark of aGvHD pathology. TNFα has been characterised as having a key role in the initiation and maintenance of aGVHD [21,22]. Its involvement in activation and proliferation pathways of T cells has made it a target in the treatment of steroid-resistant patients [23]. This study shows the level of circulating TNFα detected in the serum was significantly reduced by SmPill® therapy and Sandimmune® IV but not by Neoral® (Fig. 4).

There have been numerous studies showing how proinflammatory cytokines such as IL-1β, IFNγ, IL-2, IL-6, IL-17 and IL-23 contribute to the severity of aGvHD [24,25]. This study examined the effect of SmPill® therapy on the production of proinflammatory cytokines specifically in the small intestine and colon (GI tract) of aGvHD mice. In the small intestine, there was a significant increase in the levels of all the cytokines except IFNγ in the mice that received PBMC compared to the PBS control mice (Fig. 5). SmPill® therapy significantly decreased the levels of all proinflammatory cytokines except IFNγ in the small intestine of aGvHD mice when compared to untreated aGvHD (PBMC) mice (Fig. 5). In contrast Sandimmune® IV and Neoral® had less potent effects across all proinflammatory cytokine levels than SmPill®-CyA.

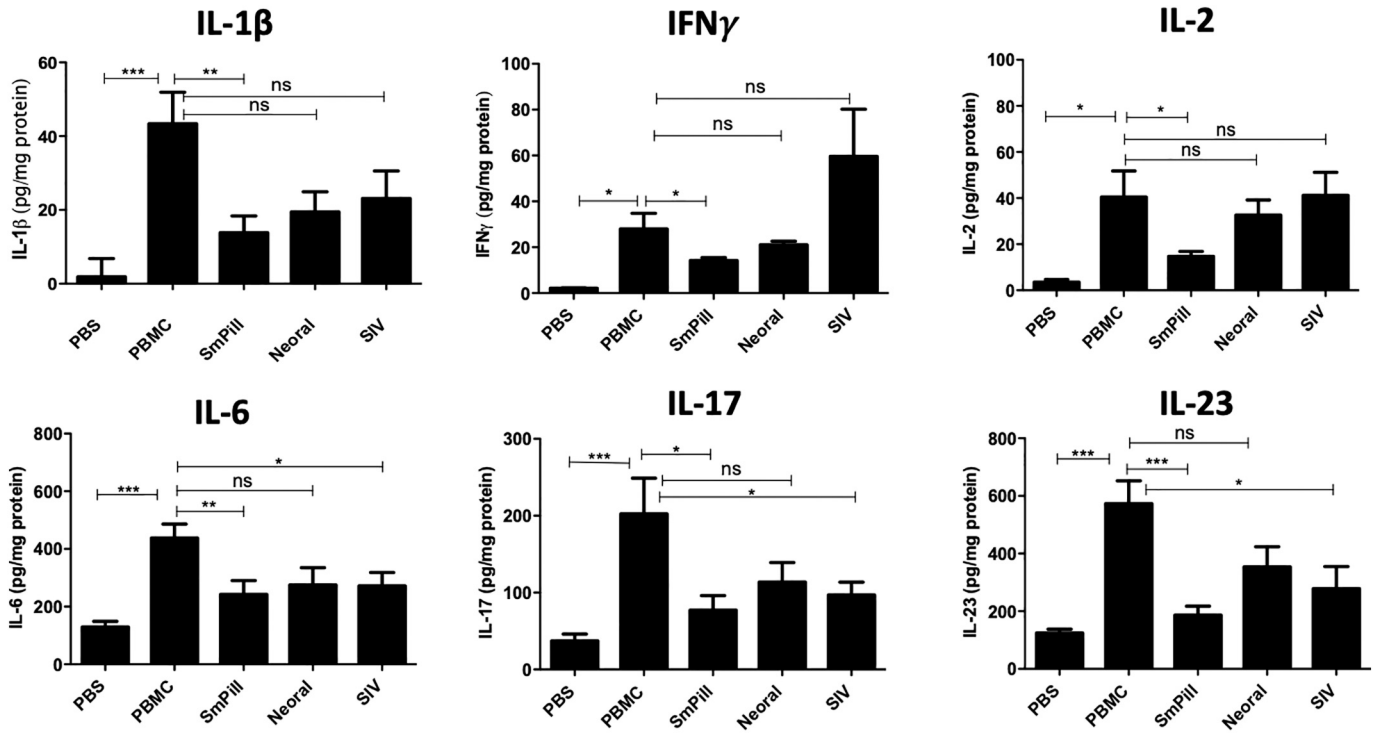

Fig. 5. SmPill® CyA significantly reduced proinflammatory cytokines detected in the small intestine of aGvHD mice. The aGvHD model was set up exactly as described in Fig. 1. Tissue samples were harvested on day 13, immediately snap frozen and stored at −80 °C. Homogenates were prepared and ELISA was used to detect proinflammatory cytokines (IL-1β, IFNγ, IL-2, IL-6, IL-17, IL-23). Concentration of cytokine is expressed as pg cytokine per mg tissue protein (normalised by bradford protein assay). $n = 3$ per group for IL2 and IFNγ, n = 6 per group for IL-1β, IL-6, IL-17 and IL-23. Statistical analysis was carried out using the Mann–Whitney U test to compare between two groups; where * signifies $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

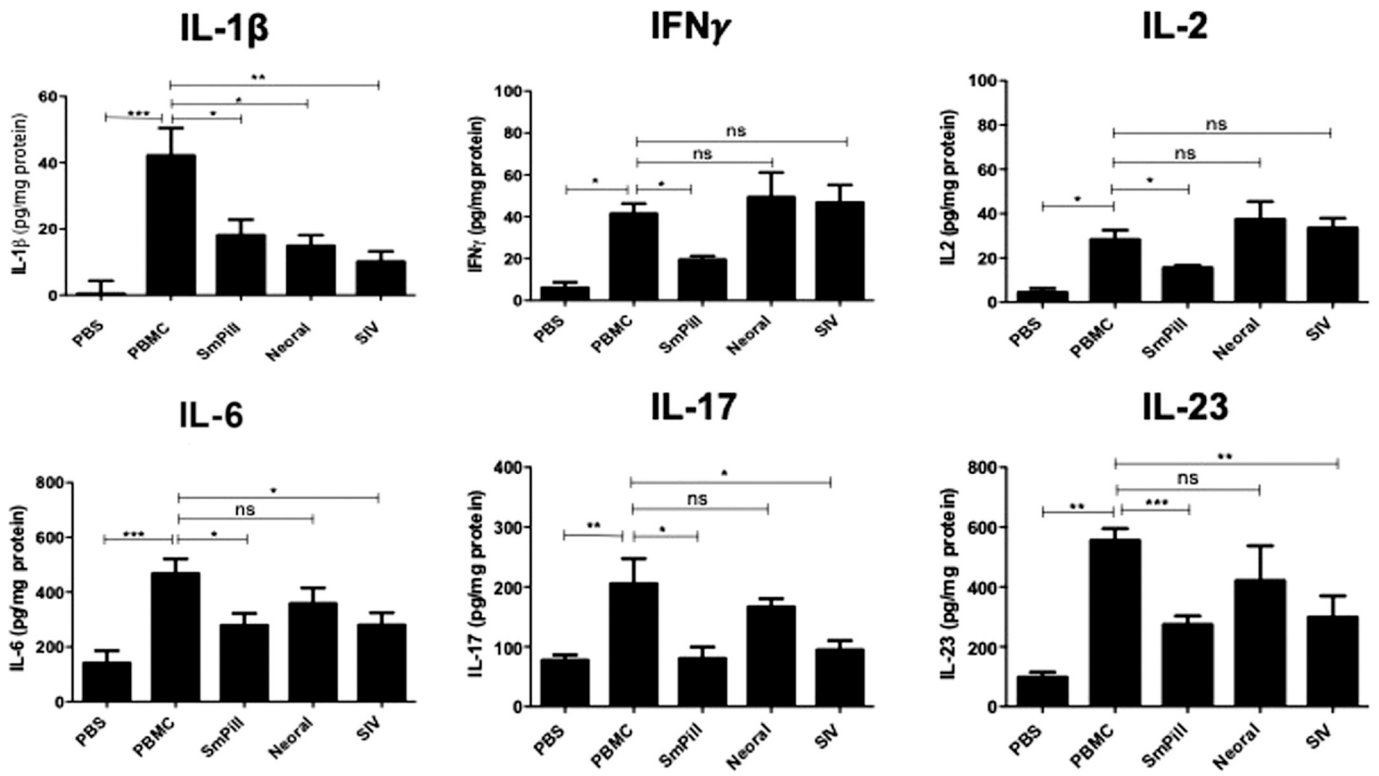

Fig. 6. SmPill® CyA significantly reduced proinflammatory cytokines detected in the colon of aGvHD mice. The aGvHD model was set up exactly as described in Fig. 1. Tissue samples were harvested on day 13, immediately snap frozen and stored at −80 °C. Homogenates were prepared and ELISA was used to detect proinflammatory cytokines (IL-1β, IFNγ, IL-2, IL-6, IL-17, IL-23). Concentration of cytokine is expressed as pg cytokine per mg tissue protein (normalised by bradford protein assay). $n = 3$ per group for IL-2 and IFNγ, $n = 6$ per group for IL-1β, IL-6, IL-17 and IL-23. Statistical analysis was carried out in comparison to the PBMC group using the Mann–Whitney *U* test to compare between two groups; where * signifies $p < 0.05$, ** <0.01 and *** <0.001.

Sandimmune® IV significantly reduced levels of IL-6, IL-17 and IL-23, while Neoral® did not significantly reduce any of the cytokines analysed in the small intestine (Fig. 5).

All cytokines were significantly increased in the colons of aGvHD mice in comparison to PBS controls (Fig. 6). Similar to the findings in the small intestine, SmPill® therapy significantly decreased the levels of all proinflammatory cytokines examined in the colon of aGvHD mice in comparison to untreated aGvHD mice (Fig. 6). Sandimmune® IV significantly reduced IL-1β, IL-6, IL-17 and IL-23, while Neoral® significantly reduced IL-1β levels in the colon of aGvHD mice (Fig. 6).

In liver and lung homogenates collected from aGvHD mice, the levels of all cytokines tested (IL-1β, IL-2, IL-6, IL-17 and IFNγ) were significantly reduced by SmPill® therapy (Supp. Figs. 1 & 2). In contrast Neoral® therapy significantly reduced IL-6 only, while Sandimmune® IV significantly reduced all cytokines except IL-1β in the liver (Supp. Fig. 1). In the lung, Neoral® therapy and Sandimmune® IV significantly reduced IL-1β, IL-2 and IL-6, but not IL-17 or IFNγ (Supp. Fig. 2).

To determine if the differential effects of the CyA formulations on cytokine levels in aGvHD organs was associated with reduced infiltration or engraftment of human T cells we examined engraftment levels of human CD45 + CD4+ and CD45 + CD8+ T cells. Significant levels of human T cells were detected in the GI (Supp. Fig. 3A&B), liver (Supp. Fig. 3C&D) and lung (Supp. Fig. 3E&F) of aGvHD mice compared to PBS control. Different formulations of CyA had no significant effect on the frequency of human CD45 + CD4+ and CD45 + CD8+ T cells in the GI, liver or lung of aGvHD mice (Supp. Fig. 3).

4. Discussion

Cyclosporine A remains a vital immunosuppressive agent for use both as a prophylactic to reduce the risk or severity of aGvHD as well as being integrated into aGvHD and cGvHD treatment protocols.

SmPill® drug delivery technology-enabled formulations of CyA have been proven to modulate systemic pharmacokinetics while also providing thorough distribution throughout the gastrointestinal tract, thus overcoming the limitations associated with existing oral and intravenous CyA formulations, each of which has been designed to maximise systemic bioavailability [15,26,27]. The study presented here sought to compare the therapeutic efficacy of a combination of small intestinal- and colonic-release SmPill® CyA minispheres against each of the approved orally administered immediate-release Neoral® soft-gel and the intravenous Sandimmune® formulations.

Using a humanised mouse model of aGvHD it was demonstrated that the optimised SmPill® format comprising a minisphere to release CyA throughout the small intestine and a delayed release minisphere to release CyA throughout the colon provided optimal aGvHD efficacy. In line with other studies using a mouse model of aGvHD [20], administration of 5 doses of CyA (25 mg/kg) in the SmPill® format or in the Sandimmune® format significantly prolonged survival. Based on histological evaluation, SmPill® was more effective than the approved Neoral® immediate release format, with SmPill® being significantly more effective in reducing aGvHD pathology in the small intestine, colon, liver and lungs. SmPill® was comparable to the approved iv Sandimmune® format in reducing aGvHD pathology in each of the above target organs. A recent study has shown that CyA reduces the levels of the pro-inflammatory cytokines IFNγ, IL-6, IL-17 and TNFα in the liver and intestine of acute GvHD mice [28]. Herein, we show that SmPill® was significantly more effective in reducing small intestine and colon levels of IL-1β, IFNγ, IL-2, IL-6, IL-17 and IL-23 when compared to Neoral® or Sandimmune® IV. Systemically, unlike Neoral®, SmPill® significantly reduced proinflammatory cytokine levels in the liver and lung of aGvHD mice. While equally efficacious to iv Sandimmune® in

significantly reducing serum TNFα levels, only SmPill® CyA significantly reduced IL-17 and IFNγ levels in the lungs of aGvHD mice.

The generally comparable effect observed between SmPill® CyA and Sandimmune® reflects the clinical setting in which Sandimmune® is superior to Neoral® as well as the recent finding that the colon-targeted SmPill® CyA permits comparable colon tissue levels to that attained by iv Sandimmune® [15].

Given that the GI is known to exert a significant role in the initiation and propagation of aGvHD, it seems plausible that the optimised CyA GI immunosuppression mediated by SmPill® CyA, as evidenced by the statistical reduction in GI proinflammatory cytokines as well as systemic TNFα, confers better initial as well as sustained control of the pathogenic T cell responses throughout the GI. It is important to note, that to our knowledge there is no comparable study that has investigated the efficacy of CyA delivered in different formats to a humanised mouse model of acute GvHD. As such this study provides novel insight to the differential efficacy of CyA depending on the delivery formulation.

Although CyA is more commonly used as prophylaxis, it has been used as in the treatment of acute and chronic GvHD [3]. Given the data presented in this study regarding SmPill®-enabled CyA as a prophylactic measure, it would be expected to carry the same delivery advantages for the treatment of GI aGVHD over other CyA formulations due to its enhanced bioavailability within the GI tract [15].

Nephrotoxicity associated with CyA remains an important consideration in its clinical application [29]. A limitation of this study is that we did not look at SmPill® CyA-associated toxicity and therefore cannot claim differential nephrotoxic and neurotoxic effects compared to other CyA formulations.

Based on the data presented here, the SmPill®-enabled CyA formulation approach may provide an optimised GI and systemic aGvHD prophylaxis and treatment approach for patients receiving cell-based immunotherapies, including allogeneic HSCT.

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Author's contributions

JC performed the studies, analysed the data and co-wrote the paper. IH analysed the data and co-wrote the paper. IC Contributed intellectually to the study, analysed the data and co-wrote the paper. KE designed and supervised the study, analysed the data and wrote the paper.

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Declaration of Competing Interest

IC is the named inventor of the SmPill® technology and SmPill®-enabled Cyclosporine A products, is the founder of Sigmoid Pharma Limited (renamed Sublimity Therapeutics) and remains a shareholder in Sublimity. JC was funded under an Irish Research Council Enterprise Partnership Scheme Scholarship part funded by Sigmoid Pharma. IH, MNC, CMF and KE declare that they have no competing interests.

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