Cytotherapy, 2018; ■■: ■■–■■

International Society for Cellular Therapy
ISCT®

Stromal progenitor cell modulation by thalidomide in the treatment of oral chronic graft-versus-host disease

LINDSAY C. DAVIES[1], EMMA BOARD-DAVIES[2], BERFIN SHAMLOU[1], ERIK BOBERG[1,3], KARIN GARMING-LEGERT[4] & KATARINA LE BLANC[1,3]

[1]Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden, [2]School of Dentistry, Cardiff University, Cardiff, United Kingdom, [3]Divisions of Clinical Immunology and Transfusion Medicine, Karolinska University Hospital, Stockholm, Sweden, and [4]Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden

Key Words: *graft-versus-host disease, oral mucosa, progenitor cell, stem cell, stroma, wound healing*

Chronic graft-versus-host disease (cGvHD) is a complication after hematopoietic stem cell transplantation (HSCT). The oral cavity is the second most commonly affected site, resulting in severe mucosal ulceration and significant patient morbidity [1]. We have previously published the potential importance of stromal dysregulation in oral cGvHD and how local injection of mesenchymal stromal cells (MSCs) can reduce inflammation and improve healing [2].

The oral mucosa in healthy individuals exhibits preferential healing, with minimal scar formation [3]. This regenerative response is partially attributed to a progenitor cell (PC) population within the lamina propria [4] with immunomodulatory and antibacterial potential [5,6]. Thalidomide's use as an immunomodulatory agent for oral ulceration has been reported with varying efficacy [7,8]. Cellular targets remain largely undefined, although anti-angiogenic (down-regulation of vascular endothelial growth factor A [VEGF-A] and basic fibroblast growth factor [bFGF] [9,10]) and anti-inflammatory effects have been described [11]. *In vitro* analyses investigating thalidomide's effects on different stromal cells suggest alterations to cytokine secretion, including interleukin (IL)-8 [12,13]. No profiling of the oral stroma or its endogenous PC population in response to systemic thalidomide treatment has been previously published. Within this case study we present data supporting the potential orchestrating role of oral PCs in regulating wound healing and novel evidence warranting further investigation for thalidomide in impaired oral healing.

The patient was male, 63, and had been previously treated with allogeneic HSCT for myelofibrosis. He developed cGvHD affecting the lung, skin and oral mucosa and at the time of thalidomide treatment was receiving systemic cyclosporin A, corticosteroids, prophylactic posaconazol, Fragmin (7500E) and topical tacrolimus. On commencement of thalidomide, the patient presented with ulceration and erythema of the buccal and labial mucosa, hard palate and tongue (Figure 1A(i)). Oral intake was limited to liquids due to pain from the lesions. He was classified as having severe cGvHD using global severity scoring (National Institutes of Health Consensus Working Group for Diagnosis and Staging of cGvHD), with a cGvHD organ score of 3 within the oral cavity [14,15]. Clinical oral microbiology results indicated increased prevalence of *Staphylococcus aureus* compared with the normal range, but no fungal infections. A 5 mm punch biopsy of buccal mucosa adjacent to the lesions was taken prior to and 11 weeks after commencing thalidomide treatment. The patient received 50 mg of oral thalidomide for 9 days, with no clinical improvement. The dose was increased, over 2 weeks, to 200 mg and the patient was monitored for 2 months. The study was approved by Karolinska University Hospital, Stockholm Ethical Committee, and was conducted in accordance with the Declaration of Helsinki. Buccal mucosal biopsy specimens were taken from healthy controls (HCs; n = 6) undergoing elective dental procedures or oral surgery at the Department of Oral and

Correspondence: **Lindsay Davies**, PhD, Department of Laboratory Medicine, Karolinska Institutet, Plan 8, Alfreds Nobel Álle 8, SE-14132, Stockholm, Sweden. E-mail: Lindsay.Davies@ki.se

(Received 3 November 2017; accepted 17 February 2018)

https://doi.org/10.1016/j.jcyt.2018.02.370

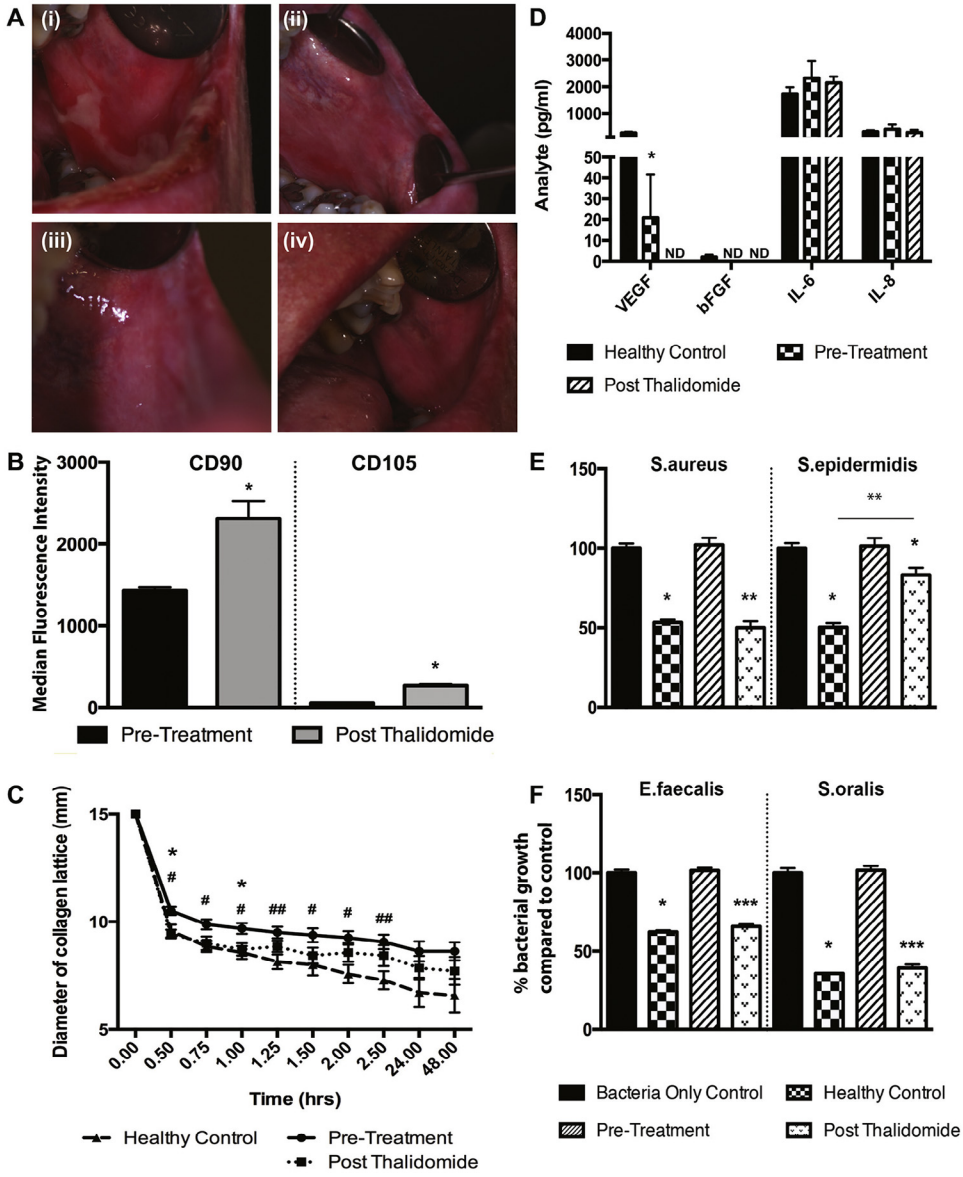

Figure 1. Clinical improvement of oral cGvHD symptoms correlates with oral PC phenotype. (A(i)) Severe ulceration and surrounding erythema was evident within the buccal mucosa prior to thalidomide treatment. Buccal mucosa at (ii) 3 weeks, (iii) 6 weeks and (iv) 11 weeks after commencing systemic thalidomide treatment. Significant reduction in ulceration and erythema is evident. Oral PCs were isolated from buccal mucosa biopsy specimens taken before and after thalidomide treatment. Statistically significant up-regulation of the cell surface markers (B) CD90 and CD105, as assessed by median fluorescent intensity using flow cytometry, was evident in oral PCs isolated post-treatment of the patient. Oral PCs derived from healthy control donors were compared against those of the cGvHD donor pre-treatment and post-treatment. (C) Oral PCs' reorganization of extracellular matrix was impaired in cGvHD cells and could be restored back to levels comparable with healthy controls, post-treatment. $*P \leq 0.05$ comparison between pre-treatment and post-treatment PCs. $\#P \leq 0.05$ $\#\#P \leq 0.01$ comparison between pre-treatment and healthy controls. Levels of the pro-angiogenic associated growth factors (D) VEGF-A, bFGF, IL-6 and -8 were measured within cell culture supernatants using enzyme-linked immunosorbent assay (ELISA). Notably VEGF-A was significantly lower ($*P \leq 0.05$) in the cGvHD cells and undetectable (ND) after the patient was treated. (E–F) Antibacterial testing confirmed a loss of antibacterial function in the cGvHD oral PCs against (E) Gram-positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and (F) Gram-negative (*Enterococcus faecalis* and *Streptococcus oralis*) bacterial strains that could be completely or partially restored to levels comparable with healthy controls after treatment. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$.

Maxillofacial Surgery, Karolinska University Hospital, Huddinge, Sweden, for comparative analysis. All donors provided written consent.

Multiple colonies of oral PCs, from the GvHD patient and HCs were isolated and expanded *in vitro* as previously described [4], with population doublings and colony-forming units recorded. Flow cytometry analysis confirmed oral PCs were CD90+, CD105+, CD73+, CD29+, human leukocyte antigen (HLA) I+, CD34-, CD45- and HLA II-. Osteogenic and