Robust human regulatory T cell expansion with fusion proteins HCW9302 and HCW9213 circumvents need for magnetic-bead or feeder cell approaches for adoptive cell therapy

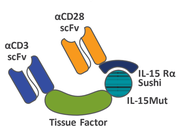
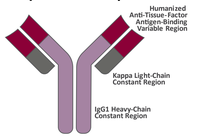
Niraj Shrestha, Varghese George, Michael J. Dee, Pallavi Chaturvedi, Xiaoyun Zhu, Nicole Encalada, Catherine Spoundis, Bai Liu, Lin Kong, Christian A. Echeverri, Lijing You, Jack O. Egan, Jin-an Jiao, Peter R. Rhode, Hing C. Wong

HCW Biologics Inc., Miramar, Florida

Abstract

Regulatory T cells ($CD4^+CD25^+FoxP3^+$) (Tregs) are a subset of CD4 T cells that suppress the activities of other immune cells and have applications in the treatment of autoimmune and inflammatory diseases. Their use as an adoptive cell therapy has been limited by the practicality of expanding and purifying clinically sufficient numbers of cells. HCW9213 and HCW9302 are fusion proteins based on HCW Biologics' TOBI™ technology platform, consisting of anti-CD3/anti-CD28 antibody domains and IL-2 domains, respectively. When used in combination, these fusion proteins were capable of expanding human Treg cells in vitro without the use of anti-CD3/CD28 magnetic beads and/or feeder cells, improving the overall yield, process efficiency and overcoming regulatory hurdles in manufacturing. Tregs generated with these molecules displayed similar phenotypes and suppressive cytokine production as Tregs expanded with recombinant human IL-2 (rhIL-2). Using a proprietary anti-CD39 antibody (HCW9108) to isolate ($CD39^+$)Tregs, we have been able to generate a Treg population with twice the suppressive activity against $CD4^+$ T responder cells as traditional $CD4^+CD25^+CD127^{low}$ Tregs. Thus, using its novel fusion proteins, HCW Biologics has been able to develop a superior Treg cell product ideal for the use in adoptive cell transfer. Additionally, this Treg platform can potentially be further optimized with addition of disease-targeted chimeric antigen receptors (CAR).

HCW Treg Cell Therapy Platforms

HCW9302: IL-2 Fusion Protein	HCW9213: Anti-CD3/CD28	HCW9108 (CD39 Ab)
		
Expansion Molecule	Activation Molecule	Highly Suppressive Treg Cells Purification Molecule

Superior expansion of $Foxp3^+$ Treg cells by HCW9302 compared to commercial rhIL-2

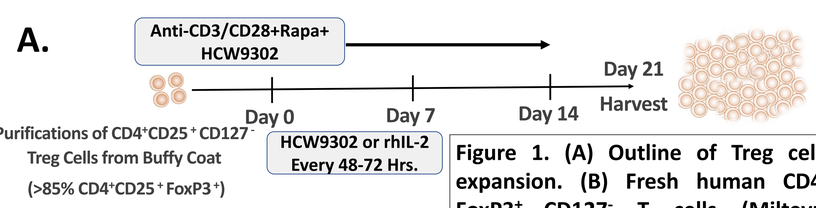


Figure 1. (A) Outline of Treg cells expansion. (B) Fresh human $CD4^+$ $FoxP3^+$ $CD127^-$ T cells (Milteyni Biotech) were purified from healthy donor buffy coat and stimulated with Dynabeads Human T-Activator CD3/CD28 beads (Invitrogen), Rapamycin (Sigma) and HCW9302 or rhIL-2 (Proleukin). Cells were stimulated in fresh media with Rapamycin and HCW9302 or rhIL-2 every 48 to 72 hours. Cells were stimulated with Dynabeads Human T-Activator CD3/CD28 beads with fresh media on day 7 and day 14. Cells were harvested on day 21 to calculate the fold expansion.

HW9302 expanded Treg cells express FoxP3, suppress T responder cells and express surface makers comparable to rhIL-2 expanded Treg cells

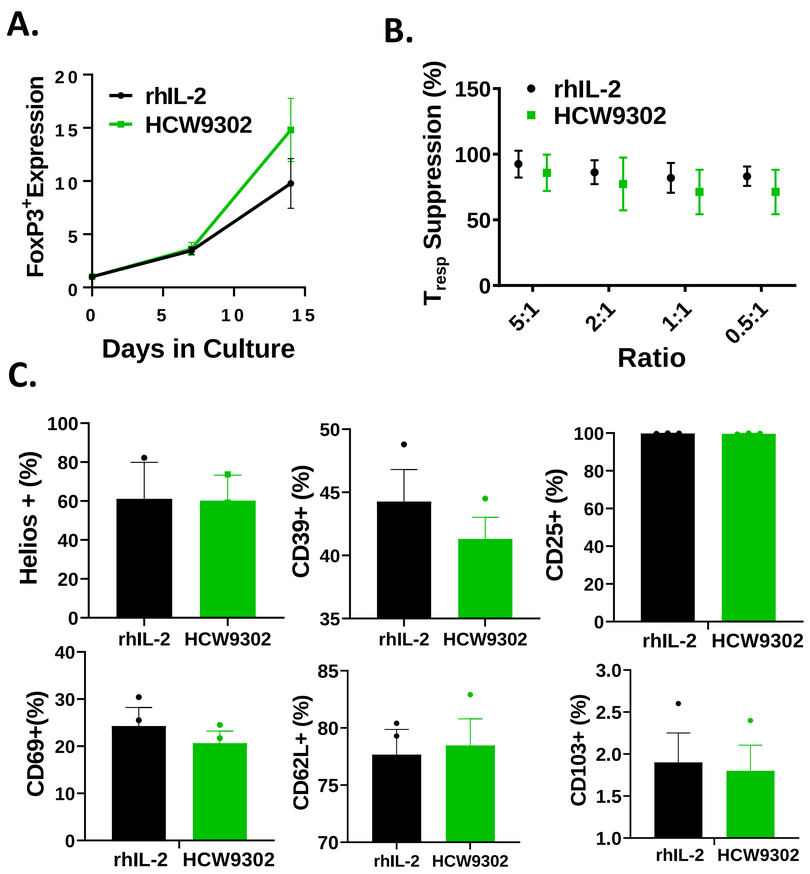


Figure 2. (A) Expression of $Foxp3^+$ in HCW9302 (with CD3/CD28 Beads with Rapamycin) expanded Treg cells. (B) For suppression assay, frozen autologous T responder (Tresp) cells were thawed and labelled with Cell Trace Violet (Sigma). Expanded Tregs cells from culture were washed, counted and added to T responder cells at various ratios as indicated. Anti-CD3/CD28 beads (Bead: Cell 1:70) and HCW9302 (25 nM) were added to the cultures and kept for 5 days. Cells were analyzed by flow cytometry to measure ability of Tregs to suppress proliferation of T responder cells by dye dilution. (C) Expression of different surface markers in HCW9302 expanded Treg cells compared with rhIL-2 expanded Treg cells.

HCW9213 bypasses magnetic beads-based expansion of $Foxp3^+$ Treg cells

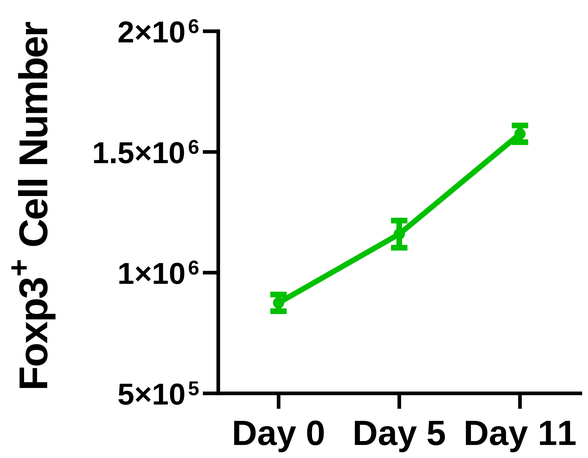


Figure 3. Fresh human $CD4^+FoxP3^+CD127^-$ T cells were purified from healthy donor buffy coat and stimulated with HCW9213 with Rapamycin and HCW9302. Cells were stimulated fresh media with Rapamycin and HCW9302 every 48 to 72 hours. $Foxp3^+$ cell number were calculated at different days.

HCW9108 (CD39 Ab) can purify highly suppressive $CD39^+$ $Foxp3^+$ Treg cells

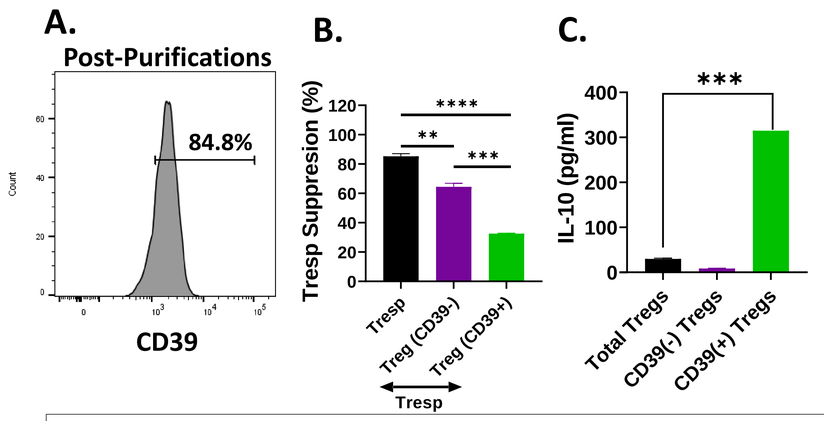


Figure 4. (A) Isolation of $CD39^+$ $Foxp3^+$ regulatory T cells from previously HCW9302 expanded $CD4^+CD25^+CD127^{low}$ T regulatory cells using biotinylated HCW9108 (αCD39) (StemCell Technology). Cells were stained to assess the purity of $CD39^+$ $Foxp3^+$ regulatory T cells by flow cytometry. (B) Purified $Foxp3^+CD39^+$ or $Foxp3^+CD39^+$ Treg cells were cultured with Autologous T responder (Tresp) cells were labelled with Cell Trace Violet (Invitrogen) (1:1 regulatory T cell: T responder cells). Cells were re-stimulated with HCW9302 and cultured for 5 days. Cells were harvested, washed and analyzed by Flow Cytometry. (C) Supernatant was collected and measured secreted IL-10 by ELISA (R&D System).

Proprietary CD26 CAR-Treg

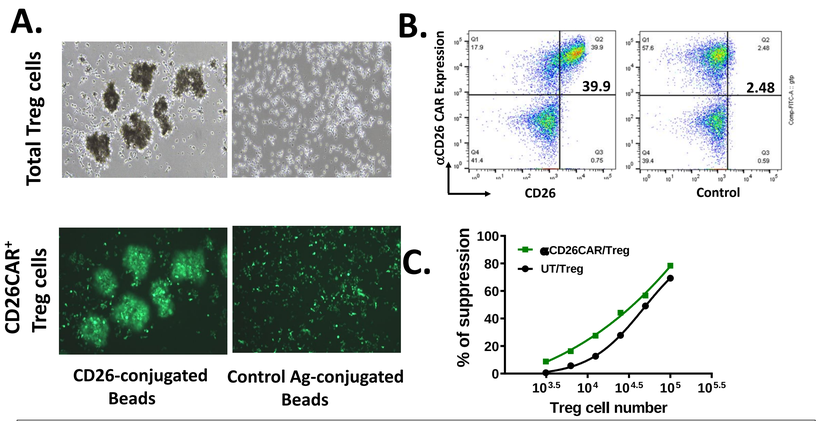


Figure 5. (A) Treg or CD26CAR-Treg cells were stimulated with either control antigen-conjugated beads or CD26-conjugated beads for 3 days. (B) The αCD26CAR/Treg cells were positively stained with the specific antigen (CD26) but not with the non-specific antigen (TF). (C) Suppression activity of αCD26CAR/Tregs and un-transduced Tregs.

Summary and Conclusions

- **HCW9302 can expand Treg cells *ex vivo* superior to rhIL-2.**
- **HCW9213 can bypass the magnetic-bead-based stimulation and expansion process.**
- **HCW9108 (CD39 Ab) can purify highly suppressive Treg cells from expanded Treg cells for adoptive cell therapies.**
- **HCW Treg cell therapy platform molecules:**
  - ✓ **Functionality:** Produce highly suppressive Treg cells.
  - ✓ **Yield:** Expand to support multiple infusions as well as expansion of CD26CAR-Tregs and CD36CAR-Tregs which is in discovery phase.
  - ✓ **Cost/Process:** Reduce manipulation/steps, eliminate magnetic beads for stimulation and expansion.

Niraj Shrestha, PhD
2929 N Commerce Pkwy, Miramar, FL 33025
Phone 954-842-2024 x208 Fax 954-842-2037
Email nirajshrestha@hcwbiologics.com

HCW Biologics