most of the chPD1 T cell receptor combinations secreted both pro-inflammatory (IFNγ, TNFα, IL-2, GM-CSF, IL-17, and IL-21) and anti-inflammatory cytokines (IL-10), chPD1 T cells containing a Dap10 costimulatory domain secreted high levels of proinflammatory cytokines but did not secrete a significant amount of anti-inflammatory cytokines. Furthermore, T cells expressing chPD1 receptors with a Dap10 domain also had the strongest anti-tumor efficacy in vivo. ChPD1 T cells did not survive for longer than 14 days in vivo, however treatment with chPD1 T cells induced long-lived protective host-anti-tumor immune responses in tumor-bearing mice.

Conclusions Therefore, adoptive transfer of chPD1 T cells could be a novel therapeutic strategy to treat multiple types of cancer and inclusion of the Dap10 costimulatory domain in chimeric antigen receptors may induce a preferential cytokine profile for anti-tumor therapies.

Ethics Approval The study was approved by Longwood University’s IACUC.

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**BCMA-TARGETING CAR-T CELLS EXPANDED IN IL-15 HAVE AN IMPROVED PHENOTYPE FOR THERAPEUTIC USE COMPARED TO THOSE GROWN IN IL-2 OR IL-15/IL-7**

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**Background** Chimeric antigen receptor-T (CAR-T) cells that target B cell maturation antigen (BCMA-CARs) have emerged as a promising treatment for multiple myeloma (MM). Despite impressive initial responses to BCMA-CAR therapy in clinical trials, relapse is common, signifying a need to improve the in vivo efficacy and persistence of BCMA-CARs.¹ The development of unfavourable differentiation or T cell dysfunction, such as exhaustion and senescence, during the ex vivo expansion of the BCMA-CARs could be limiting their therapeutic potential. For CD19-directed CARs, reduced dysfunction and differentiation and improved anti-tumour responses were achieved by expanding the cells with IL-15 instead of IL-2.² Therefore, in this study, our aim was to determine whether expanding BCMA-CARs with IL-15 or IL-15/IL-7 instead of IL-2 alters their levels of exhaustion, senescence, differentiation and activity.

**Methods** T cells stimulated with anti-CD3/anti-CD28-coated beads were supplemented with IL-2, IL-15, IL-15 + IL-7 or no cytokine and transduced with AR12h, a BCMA-CAR with a 4-1BB co-stimulatory domain produced at our institution.³ Expanded BCMA-CARs were analysed by flow cytometry for markers of T cell dysfunction, or challenged with MM cell line ARP-1 and then tested for cytokine production, cytotoxic ability and activation signals.

**Results** BCMA-CARs cultured in IL-15 or IL-15/IL-7 expanded similarly to those grown in IL-2, with comparable CAR transduction efficiencies, CD4:CD8 ratios and proliferation rates. BCMA-CARs grown in IL-15 had low expression of exhaustion marker LAG-3 and high expression of the costimulatory molecule CD27, which is important for T cell survival and persistence, when compared to BCMA-CARs cultured in IL-2. Moreover, BCMA-CARs grown solely in IL-15 were less differentiated than those supplemented with IL-7, and had higher expression of stem cell memory marker CXCR3 within the naïve population than those expanded with IL-2. When challenged with MM cell line ARP-1, IL-15-grown BCMA-CARs upregulated activation marker CD69, exhibited strong cytotoxicity and robust production of IFNγ and IL-2. However, in comparison to BCMA-CARs expanded in IL-2 or IL-15/IL-7, those grown with IL-15 had lower mTORC1 activity and p38 MAPK phosphorylation when activated by ARP-1 cells, suggesting differential regulation of key pathways for T cell metabolism and senescence, respectively.

**Conclusions** To summarise, BCMA-CARs expanded with IL-15 alone exhibited the most favourable phenotype for therapeutic use compared those grown with IL-2 or IL-15/IL-7. Future experiments using murine MM models will be critical in understanding the in vivo benefits or drawbacks of culturing BCMA-CARs in IL-15 compared to IL-2 or IL-15/IL-7.

**Ethics Approval** Research involving human material was approved by the Ethical Committee of Clinical Research (Hospital Clinic, Barcelona). Peripheral blood T cells were obtained from healthy donors after informed consent in accordance with the Declaration of Helsinki.

**REFERENCES**


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**A THIRD-GENERATION HUMAN GUICY2C-TARGETED CAR-T CELL FOR COLORECTAL CANCER IMMUNOTHERAPY**

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**Background** Colorectal cancer (CRC) presents a significant public health burden, responsible for the second most cancer-related deaths in the United States, with an increasing incidence in young adults observed globally.¹,² While the blockade of immune checkpoints received FDA approval as a CRC therapeutic, only patients with microsatellite instability, accounting for 15% of sporadic cases, demonstrate partial or complete responses.³ We present a third-generation chimeric antigen receptor (CAR)-T cell directed towards the extracellular domain of the mucosal antigen guanylyl cyclase C (GUICY2C), which is over-expressed in 80% of CRC cases, as a therapeutic alternative for late stage disease. Here, we demonstrate that human GUCY2C CAR-T cells can selectively kill GUCY2C-expressing colorectal cancer cells in vitro and produce inflammatory cytokines in response to antigenic stimulation.

**Methods** Peripheral blood mononuclear (PBMCs) cells were isolated from leukoreduction filters obtained from the Thomas Jefferson University Hospital Blood Donor Center (IRB #18D.495). Magnetic Activated Cell Sorting (MACS) technology was used to negatively select pan-T cells (Miltenyi Biotec), followed by activation and expansion using anti-CD3, anti-CD28, and anti-CD2 coated microbeads (Miltenyi Biotec) and supplemented with IL-7 and IL-15 (Biological Resources

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