507 SHIFTING THE PARADIGM IN CD47-MEDIATED THERAPY; EMPHASIZING DIRECT AGONISM OVER 'DON'T EAT ME!' INHIBITION

[1,2]Sittana Matar, [1]Seham Skah, [1]Liza E Diomande, [1]Kjetil Hestdal, [1]Rolf Pettersen, [1,3]Nina Richartz*. [1]*Caedo Oncology, Oslo, Norway*; [2]*University of Stavanger, Stavanger, Norway*; [3]*University of Oslo, Oslo, Norway*

Background The transmembrane protein CD47 plays a crucial role in immune surveillance by regulating phagocytosis, also known as the 'don't eat me' signal.[1] Cancer cells frequently overexpress CD47 to avoid host immune cell elimination and the protein has therefore become an attractive target for developing novel cancer therapies.[2] However, despite CD47 having been studied extensively, resulting clinical responses have been limited.[3] Moreover, the hematotoxicity associated with CD47 inhibition has raised concerns about its therapeutic viability.[4] Here, we present CO-1bi, which through its novel fusion design, opens the doors to a paradigm shift in cancer treatment targeting CD47.

Methods CO-1bi is a novel bivalent scFv-Fc fusion protein. The direct induction of programmed cancer cell death (PCCD) by CO-1bi was evaluated by staining the cells with Annexin V and 7-AAD. The enhancement of tumor cell phagocytosis was determined by co-culturing fluorescently labelled murine macrophages and target cell lines in the presence or absence of CO-1bi treatment. Phagocytosed cells were indicated by cells that stained double positive for the fluorescent tags.

Xenograft models were established by injecting lentivirally transduced cancer cells into NOD-*scid* IL2Rγ^{null} (NSG) mice. We demonstrated the mechanism for distinct modes of cancer cell elimination *in vivo* by administering various anti-CD47 candidates to xenograft mice, inducing either solely phagocytosis, PCCD, or both mechanisms.

Results CO-1bi demonstrated a remarkable specificity for malignantly transformed cells as no binding was observed to red blood cells or B cells from healthy human donors. Furthermore, CO-1bi induced swift and potent PCCD exclusively in cancer cells. This specific binding pattern suggests that CO-1bi can effectively distinguish between healthy and cancerous cells, allowing for targeted therapeutic intervention.

The ability of CO-1bi to induce both PCCD and phagocytosis proved to be a significant advantage over 'phagocytosis-only' anti-CD47 agents, which exhibited limited success in a xenograft model of B cell precursor acute lymphoblastic leukemia. Furthermore, CO-1bi also demonstrated significant efficacy in a xenograft model of Burkitt's lymphoma, where few injections at low doses lead to swift eradication of the cancer.

Conclusions CO-1bi represents a remarkable new approach in CD47-mediated therapy that goes beyond the conventional inhibition of the 'don't eat me' signal. By inducing direct PCCD in the cancer cells, while maintaining a high safety profile in normal cells, underscores the potential of CO-1bi to become a game-changing cancer therapeutic.

REFERENCES

1. Huang J *et al.* Role of CD47 in tumor immunity: a potential target for combination therapy, *Sci Rep* (2022)
2. Kaur S *et al.* CD47 (Cluster of Differentiation 47), Atlas Genet Cytogenet Oncol Haematol (2021)
3. Huang Y *et al.* Targeting CD47: the achievements and concerns of current studies on cancer immunotherapy, *J Thorac Dis* (2017)
4. Ma, L., Zhu, M., Gai, J. *et al*. Preclinical development of a novel CD47 nanobody with less toxicity and enhanced anti-cancer therapeutic potential. *J Nanobiotechnol* **18**, 12 (2020)

Ethics Approval The study was conducted under adherence with the Declaration of Helsinki. Animals were kept under appropriate housing conditions with food and water ad libitum. All animal experiments were approved by the Norwegian Food Safety Authorities.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0507