SELECTIVE BLOCKADE OF CD47-SIRP ALPHA "DON'T EAT ME" SIGNALLING WITH A HER2-CD47 BISPECIFIC ANTIBODY-DERIVATIVE

Yuzhu Qi*, Harm Lourens, Gerwin Huls, Edwin Bremer. University Medical Center Groningen, Groningen, Netherlands

Background Human epidermal growth factor receptor 2–positive (HER2+) breast cancer accounts for ~25% of breast cancer cases. Trastuzumab improves clinical outcomes by targeting HER2, but recurrences still occur. Targeting the CD47-SIRPα interaction alone or in combination with therapeutic antibodies such as Trastuzumab contributes to cancer cell elimination, but ubiquitous CD47 blocking associates with toxicity. Here, we designed a bispecific antibody fragment aimed to selectively block CD47 on HER2-positive breast cancer cells (BC) in order to reduce off-tumor binding and limit toxicity towards normal CD47-positive cells, while increasing efficacy against cancer cells.

Methods A novel HER2-CD47 bispecific single chain fragment (HER2-CD47 bs-scFv) antibody with HA tag was produced in HEK293 cells and purified using Anti-HA affinity purification. The HER2-CD47 bs-scFv comprised a CD47-targeting scFv and a Her2-targeting trastuzumab-derived scFv fragment. For binding experiments, BC cells were incubated with HER2-CD47 bsAb in the presence (or absence) of either trastuzumab or anti-CD47 or both, whereupon binding was detected using anti-HA staining. Her-2 restricted blocking activity of the HER2-CD47 bs-scFv was assessed using recombinant human SIRPα-Fc. For phagocytosis experiments, cancer cells were labelled with CSFE and then incubated with differentiated macrophages (derived from peripheral blood mononuclear cells). Phagocytosis was measured by flow cytometry.

Results The HER2-CD47 bs-scFv selectively bound to CD47+/Her2+ cells, but only marginally to single CD47 positive cancer cells. Binding of the HER2-CD47 bs-scFv was partially reduced by trastuzumab or epitope-competing CD47 antibody. Furthermore, incubation with HER2-CD47 blocked the binding of recombinant human SIRPα-Fc only on CD47+/Her2+ cells, demonstrating that functional blocking of SIRPα/CD47 interaction is restricted to HER2+ cells. Importantly, HER2-CD47 selectively enhanced macrophage-mediated phagocytic removal of CD47+/Her2+ BC cells cancer cells by up to 30%. In contrast, similar treatment did not significantly increase phagocytosis of CD47+/Her2- BC cells.

Conclusions The HER2-CD47 bs-scFv blocked CD47 “don’t eat me” signaling in a HER2-restricted manner with essentially only activity towards Her2 and CD47 double-positive cancer cells. This HER2-CD47 bs-scFv may provide a new strategy for the treatment of Her2+ breast cancers.