ELICITING CALRETICULIN-MEDIATED "EAT ME" PHAGOCYTIC SIGNAL IS ADDITIVE/SYNERGISTIC WITH CD47 BLOCKADE IN ENHANCING TUMOR ASSOCIATED MACROPHAGE PHAGOCYTOSIS OF TUMOR CELLS AND DECEASING XENOGRAFT TUMOR GROWTH IN EWING SARCOMA

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Background Tumor associated macrophages (TAM) are abundant in Ewing sarcoma (ES), a malignant pediatric bone and soft tissue tumor. Macrophages are known to harbor phagocytic activity against cancer cells, yet they fail to phagocytose ES cells and confer a poor prognosis. Cancer cells may utilize dual mechanisms, up-regulation of “don’t eat me” signal mediated by CD47 and down-regulation of “eat me” signal mediated by cell surface calreticulin (csCRT), to resist TAM-mediated phagocytosis. To overcome TAM resistance, both enhancing csCRT expression and blocking CD47 signal will be required. Chemotherapy is known to enhance translocation of CRT to the cell surface during the process of apoptosis. Magrolimab is a humanized, monoclonal antibody that blocks CD47 and is currently in phase III trials for AML/MDS.

Methods Expression of CD47 and csCRT on ES cells was examined by flow cytometry and western blotting. To elucidate whether csCRT increase is associated with apoptosis of ES cells, annexin V assays were carried out. Macrophages were derived from human peripheral blood. In vitro phagocytosis assays were performed to evaluate the efficacy of doxorubicin (DOX) and magrolimab, generously provided by Gilead, alone or in combination, in enhancing macrophage phagocytosis of ES cells. The efficacy of DOX combined with magrolimab in limiting ES tumor growth and prolonging animal survival in vivo was assessed in an ES xenograft NSG mouse model.

Results We found that most of the tested ES cell lines (A673, EWS502, SKNMC, RDES, TC32 and TC71) express high levels of CD47 and low levels of csCRT. The csCRT levels in A673, TC32, and EWS502 cells were increased by DOX treatment in a dose dependent manner (figure 1) and this increase in csCRT level was associated with apoptosis. DOX or magrolimab alone significantly enhanced, and the combination of the two further significantly enhanced, phagocytosis of ES cells by macrophages. The increased phagocytosis induced by magrolimab was due to CD47 blockade because CD47 KO rendered ES cells resistant to magrolimab induced increase in phagocytosis (figure 2). In ES xenografted NSG mice, DOX alone had no effect while magrolimab alone had a moderate effect on decreasing ES xenograft tumor growth. Importantly, DOX combined with magrolimab had an additive/synergistic effect on decreasing ES tumor growth and significantly prolonged animal survival compared to control and single agent treatment (figure 3).

Conclusions Our data demonstrate an additive/synergistic effect of DOX and magrolimab in vitro and in vivo against ES and provide a rationale to move this combinatorial therapy to clinical investigation.

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