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# POSTER ABSTRACTS

## 703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

### Trogocytosis May Attenuate the Efficacy of Anti-BCMA CAR T Cells Administered in Combination with $\gamma$-Secretase Inhibitor

*Pinar Ataca Atilla, MD*[1], *Sylvain Simon, PhD*[1], *Erden Atilla, MD*[1], *Melissa L Comstock*[1], *Andrew J. Cowan, MD*[2,3,4], *Geoffrey R Hill, MD*[1,2], *Stanley R. Riddell, MD*[1,5], *Damian J Green, MD*[1,5]

[1] Translational Science and Therapeutics Division, Fred Hutchinson Cancer Center, Seattle, WA
[2] Division of Medical Oncology, Department of Medicine, University of Washington, Seattle, WA
[3] Clinical Research Division, Fred Hutchinson Cancer Center, Seattle, WA
[4] Division of Medical Oncology, Fred Hutchinson Cancer Research Center, Seattle, WA
[5] University of Washington, Seattle, WA

**Introduction:**
$\gamma$-Secretase inhibitors (GSIs) are proven to reduce shedding and increase BCMA surface density on plasma cells thus enhancing anti-multiple myeloma (MM) efficacy of BCMA CAR T cells in preclinical systems (Pont, Blood 2019). Collectively, our recent findings indicate that BCMA density on malignant plasma cells following GSI administration is predictive of improved responses, progression free survival and overall survival in the Phase I trial. Despite that, most patients eventually relapse and 80% have no evidence of CAR T cells by qPCR at the time of disease progression (Cowan, Lancet Oncology, 2023). Trogocytosis involves stripping of the target antigen and incorporating it into the CAR T cell membrane. This phenomenon has been described as one of the causes of relapse following CAR T cell therapy. In this study, we aimed to investigate the effect of GSI on trogocytosis and determine if trogocytosis facilitates fratricide among anti-BCMA CAR T cells thus contributing to relapse.
**Material and Methods:**
Healthy donor T cells were activated and transduced with fully human anti-BCMA lentiviral CAR with 4-1BB/CD3z signaling domain. After 10 days of expansion, CAR T cells were sorted (BD FACSymphony S6) following staining with anti-recombinant BCMA/Fc-allophycocyanin (APC) (Creative Biomart). H929, MM.1R and MOLP 8 (MM cell lines) were obtained from ATCC. Cells were incubated with GSI, LY3039478-crenigacestat (Eli Lilly) 0.1 $\mu$M for 24h and mean fluorescence intensity (MFI) of BCMA was defined as previously described (Pont, Blood 2019). To identify trogocytosis, samples were analyzed by flow cytometry (BD Symphony 4, BD Celesta) after combined MM cell line and CAR T cell co-culture for 48 hours (h), 24h, 6h, 2h, 1h, 30 minutes (min), 10 min (effector to target ratio=1:1). For fratricide assays, CAR T cells with trogocytosis were sorted following 6h of co-culture with cell lines and re-plated with fresh CAR T cells and were analyzed by flow cytometry after 24h (effector to target ratio=1:1). Soluble BCMA (sBCMA) ELISA was performed according to the manufacturer's recommendations (R&D) Systems.
**Results:**
The BCMA MFI increased ~8-fold in MM cell lines-H929 and MM.1R after GSI incubation. In comparison, MOLP8 BCMA increased 3-fold (Figure 1a). BCMA expression on CAR T cells following 10 min, 30 min, 1h, 2h, 6h, 2h and 48h co-culture was increased in both H929 and MOLP8 cell lines consistent with trogocytosis. After GSI administration, using 30 min ($P<0.0001$), 1h ($P<0.0001$), 2h ($P=0.001$), 6h ($P<0.0001$), 24h ($P=0.0003$) and 48h ($P=0.02$) co-cultures, there was more trogocytosis detected with the high antigen density H929 cell line, whereas with MOLP8 significant trogocytosis was only observed at 30 min ($P=0.002$), 1h ($P=0.0007$) and 2h ($P=0.002$) (Figure 1b). When CAR T-trogo cells were sorted and re-challenged with naive CAR T cells, over 90% fratricidal killing was detected in the high BCMA expressor cell lines-H929 and MM1.R (Figure 1c). On the tumor side of the immune synapse, the expression of surface BCMA on H929 with, or without, GSI was decreased over time consistent with antigen downmodulation. sBCMA levels were significantly lower in the H929 cell line when GSI was added ($P=0.001$). Confocal microscopy and live imaging studies of the immune synapse are ongoing.
**Conclusion:**
GSI increases BCMA surface density on MM cells and promotes CAR T cell trogocytosis which results in fratricide that appears proportional to tumor cell BCMA density. Trogocytosis and the ensuing fratricide following anti-BCMA CAR T cell therapy may

contribute to reduced persistence of CAR T cells and relapse. This data may facilitate the optimal timing and frequency of GSI administration after BCMA CAR T-cell administration in future clinical trials.

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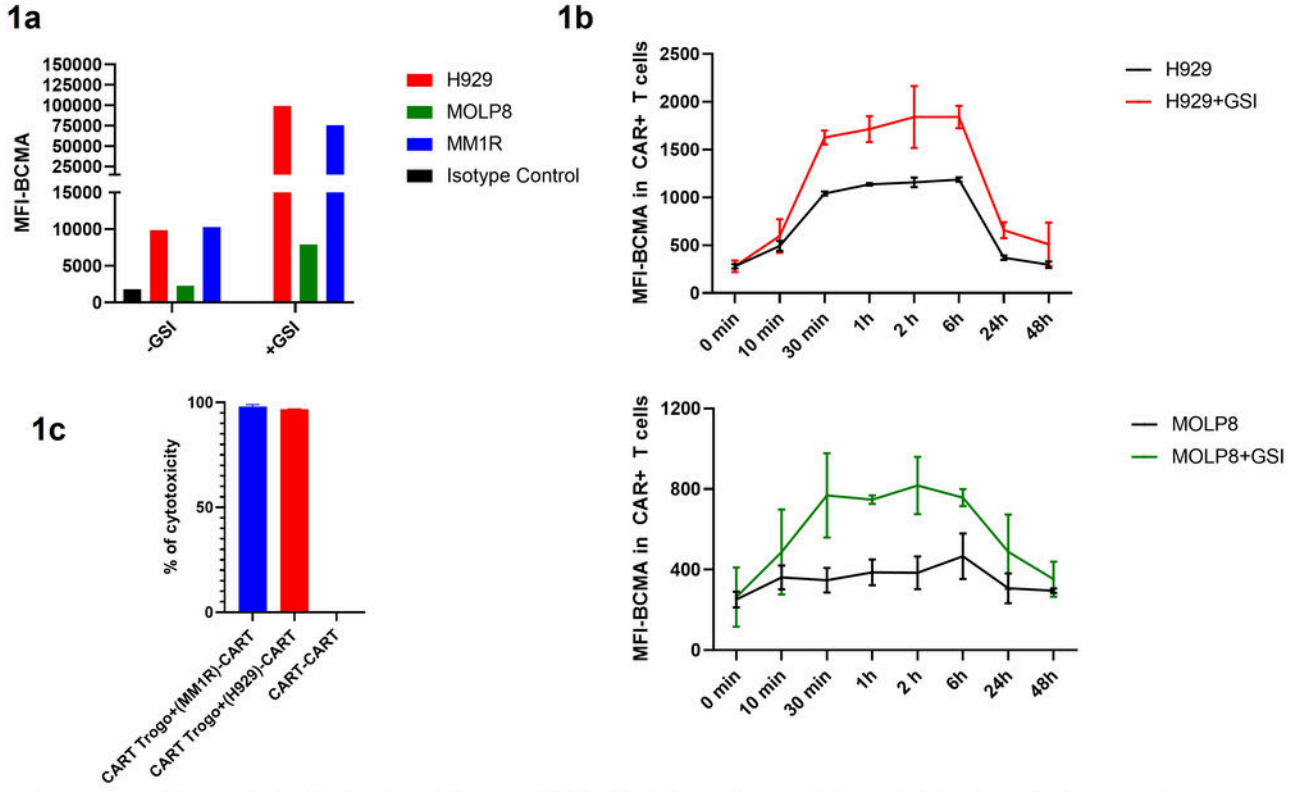


**Figure 1a.** The MFI-BCMA of cell lines +/-GSI **1b.** The BCMA-MFI in CAR+ T cells in co-cultures with H929 and MOLP8 cell lines +/-GSI (E:T=1:1) **1c.** The % of cytotoxicity with CART trogo+/CART (E:T=1:1)

**Figure 1**

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