Novel Immunotherapy Targets Cathepsin G in Solid Tumors

Authors: N. Dawson[1], A. Gibson[2], P. Sukhumalchandra[1], G. Al Atrash[1], L. St. John[1]

[1] Department of Hematopoietic Biology & Malignancy, The University of Texas MD Anderson Cancer Center, Houston, TX

[2] Department of Pediatrics, The University of Texas MD Anderson Cancer Center, Houston, TX

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Making Cancer History®

Introduction:

- Cathepsin G (CG) is a serine protease found in Polymorphonuclear Neutrophils (PMNs).
- Research suggests some leukemia tumor cells can take up CG and present its respective peptide (CG1) on their surface.
- We hypothesize that CG can be taken up by Non-Small Cell Lung Cancer (NSCLC) cells and Osteosarcoma (OS) cells and be presented on their surface as novel immunotherapy targets.

Methods:

- Standard cell culture procedure was done for NSCLC, OS, U937 (histiocytic leukemia) and T2 (multiple myeloma) cell lines.
- Reverse transcriptase - polymerase chain reaction (RT-PCR) was performed to assess endogenous CG mRNA.
- Cells were pulsed with a 10:1 ratio of PMNs to malignant cell for 4 hours.
- Cells were stained with appropriate antibodies, and we then performed flow cytometry to determine CG uptake.
- Cytotoxic Assay was performed to assess percent killing when cells were co-incubated with CG1 specific cytotoxic lymphocytes (CTLs).

Results:

- RT-PCR showed no endogenous mRNA expression of CG in NSCLC/OS cells lines (Figure 1).
- Flow cytometry analysis in H1650 (NSCLC) showed CG uptake with a **1.25-fold increase in the CG-mean fluorescence intensity (**CG-MFI) vs. non-pulsed cells (Figure 2).
- Flow cytometry analysis in Saos2 (OS) showed CG uptake with a **two-fold increase in the CG-MFI** vs. non-pulsed cells (Figure 2).
- Cytotoxicity assay showed non-specific killing of both pulsed and control (T2) control cells (Figure 3).

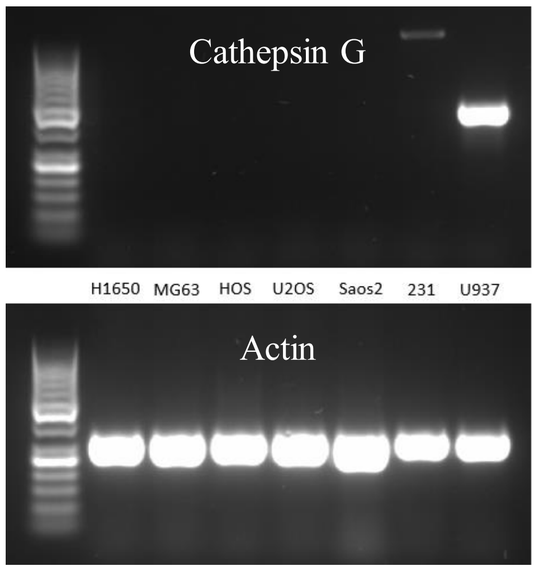

Figure 1: RT-PCR show no endogenous CG MRN in OS or NSCLC cell lines

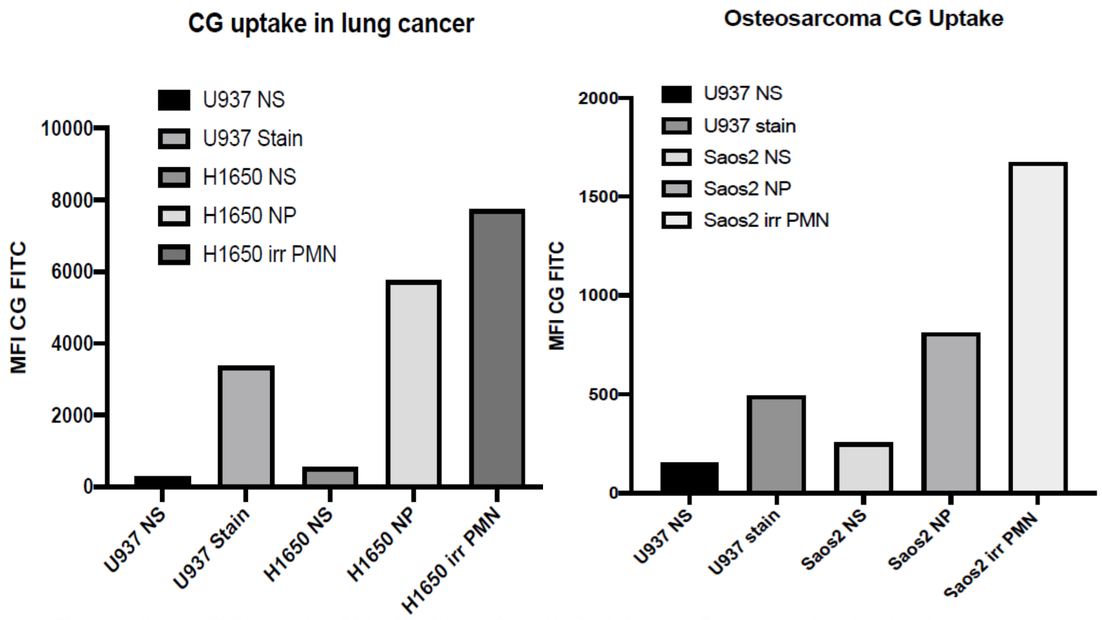

Figure 2: A. CG uptake H1650 Non-Small Cell Lung Cancer cells (pulsed at a ratio of 10:1). B. CG uptake in SaOS2 Osteosarcoma cells (pulsed at a ratio of 7:1)

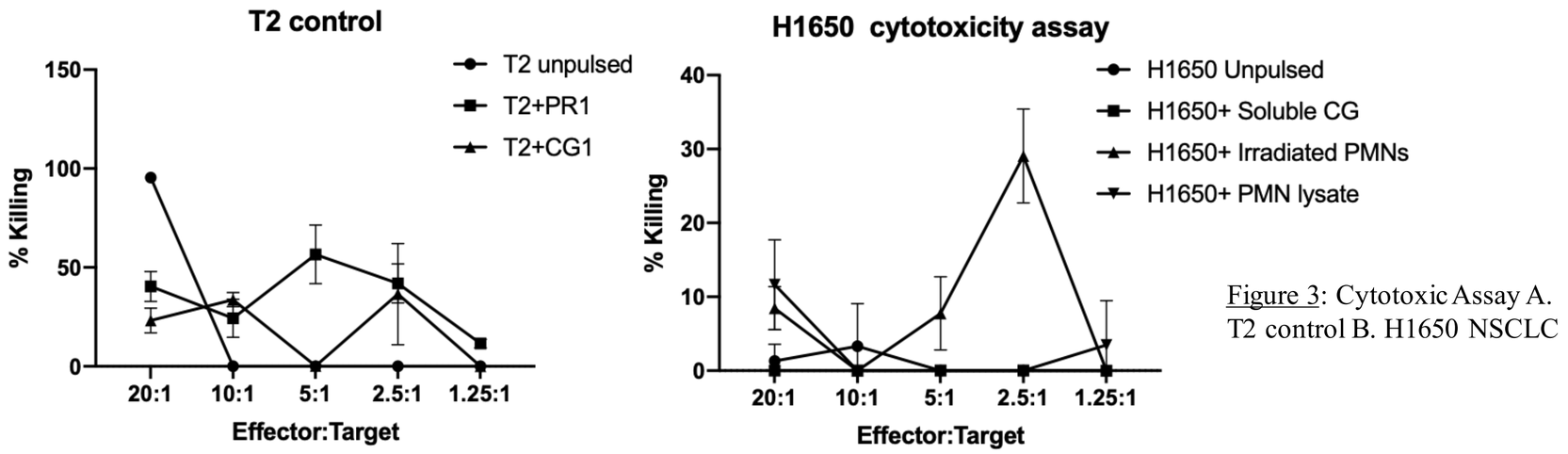

Figure 3: Cytotoxic Assay A. T2 control B. H1650 NSCLC

Conclusions:

- Both OS and NSCLC show no endogenously express CG
- OS and NSCLC can take up CG when pulsed with irradiated PMNs
- The cytotoxicity assay was inconclusive, likely due to a high concentration of CTLs.
- In the future, we will validate CG uptake in multiple OS and NSCLC cells lines and hope to demonstrate dose-dependent cytotoxicity of target cells.