Increasing CSPG4/NG2 Expression on Several Tumor Cell Lines.

James G. Issa and Barbara Savoldo MD, PhD
Cancer Cell Biology, Lineberger Comprehensive Cancer Center

Background

- Following heart disease, **cancer is the second leading cause of death worldwide**. Specifically, head and neck squamous cell carcinoma (HNSCC) is the sixth most observed malignancy worldwide, and approximately 600,000 new cases are diagnosed each year in the United States[1]. HNSCC develops in the mucous membranes of the mouth, nose, and throat[1]. HNSCC has the ability to metastasize to various parts of the body, leaving a worse prognosis and an increased likelihood of death. While cytotoxic chemotherapy exists as a method of treatment, clinical options are limited and survival is poor. Thus, new therapies are needed[2,3].
- **The novel proteoglycan CSPG4** (Chondroitin sulfate proteoglycan-4, also referred to as NG2) has been the forefront of squamous cancer cell research. Increasing the expression of CSPG4 on tumor cells allows for CAR T-cells, a laboratory modified immune cell, to better target the tumor cells through increased recognition in order to treat head and neck squamous cell carcinoma more efficiently[2]. Since HNSCC expresses CSPG4 at low levels, we hypothesize that the epigenetic drugs will upregulate CSPG4.

Methods & Procedures

- **PCI-30, HTB-1, and SCC25** were the tumor cells lines examined to test for increased NG2 expression. Each cell line was treated with several epigenetic drugs, (in separate dishes) including DMSO (control), Tazemetostat, 5-aza-2'-deoxycytidine, and TNFα. These drugs were introduced to each cell line every 2-3 days. Over the course of 21 days in 7 day intervals, a flow cytometer analysis was performed to monitor the expression of CSPG4. MG63, an osteosarcoma cell line known to express CSPG4 was used as positive control.
- **Prior to running flow,** we assayed antigen expression on tumor cells by staining the cells with with IgG1 and NG2 (in their independent flow tubes). IgG1 acts as a control, while NG2 allows the flow cytometer to detect potential expression of NG2/CSPG4 within the cells. Simultaneously, the expression of the CAR molecule on T-cells will be tested.

Results

- Each cell line was treated with two **epigenetic drugs** or the cytokine TNFα twice a week for the 21 day span. The DMSO is a diluent for these drugs and thus acts as our negative control. The isotype acts a negative control for the antibodies used during flow cytometry, ruling out any nonspecific binding. The remainder of the epigenetic drugs act as our experimental factors.
- **After 1 week** of treatment MG-63, as expected, expressed CSPG4 at high level. In contrast, HTB-1 cell line (day 7) did not express NG2 and there was no upregulation regardless of the treatment (Figure 2).
- **After the second** week (day 14), there was minimal increased expression of NG2 for the cells treated with EZH2 (Tazemetostat) and 5-aza-2'-deoxycytidine, respectively. The cells treated with TNFα showed almost 100% of NG2 expression compared to the DMSO and isotype controls **(Figure 2)**. Surprisingly, there was no NG2 expression for the MG-63 cell line after the second week.
- **After the third and final week** (day 21), both cell lines treated with TNFα showed almost 100% of NG2 expression compared to the DMSO and isotype controls. The MG-63 cell line treated with 5aza showed near 100% of NG2 expression, whereas the HTB-1 cell line treated with 5aza kept NG2 expression consistent between week 2 and week 3 **(Figure 2)**.

Future Directions

- Determine the accurate effect of the epigenetic drugs, by testing more tumor cell lines. **Analysis for PCI30 and SCC25 are ongoing.**
- Directly test CAR-T of their ability and efficacy to recognize the tested lines of tumor cells treated with TNFα, Tazemetostat, and 5-aza-2'-deoxycytidine, compared to the same cell lines with no added drugs.

Treatment of tumor cells with epigenetic drugs, cell counting, staining, and flow cytometer

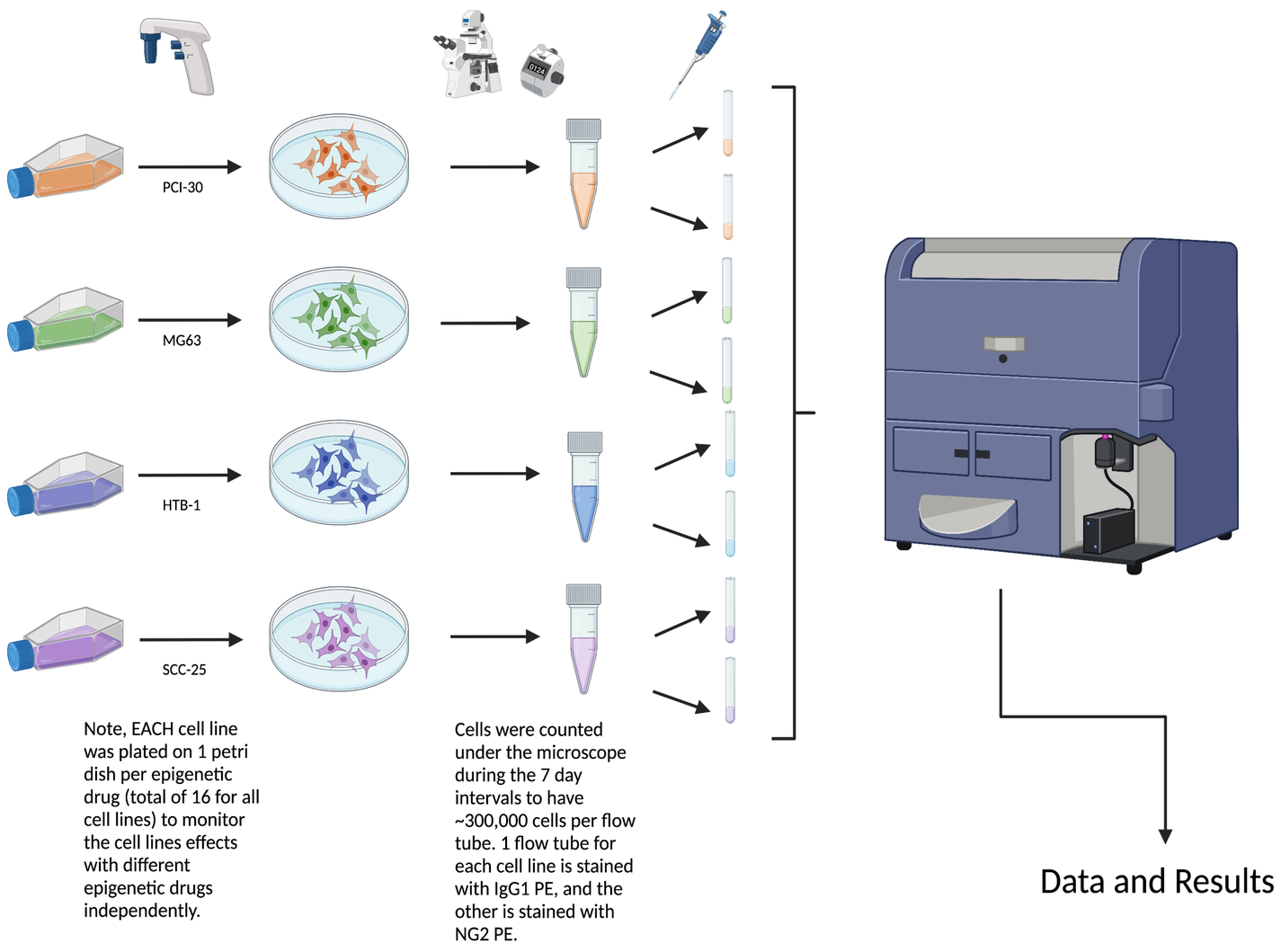

Figure 1. Procedure and treatment of each cell line with corresponding epigenetic drugs. This includes plating, cell counting, staining, and use of flow cytometer.

Data: HTB-1 & MG63 Cell Lines

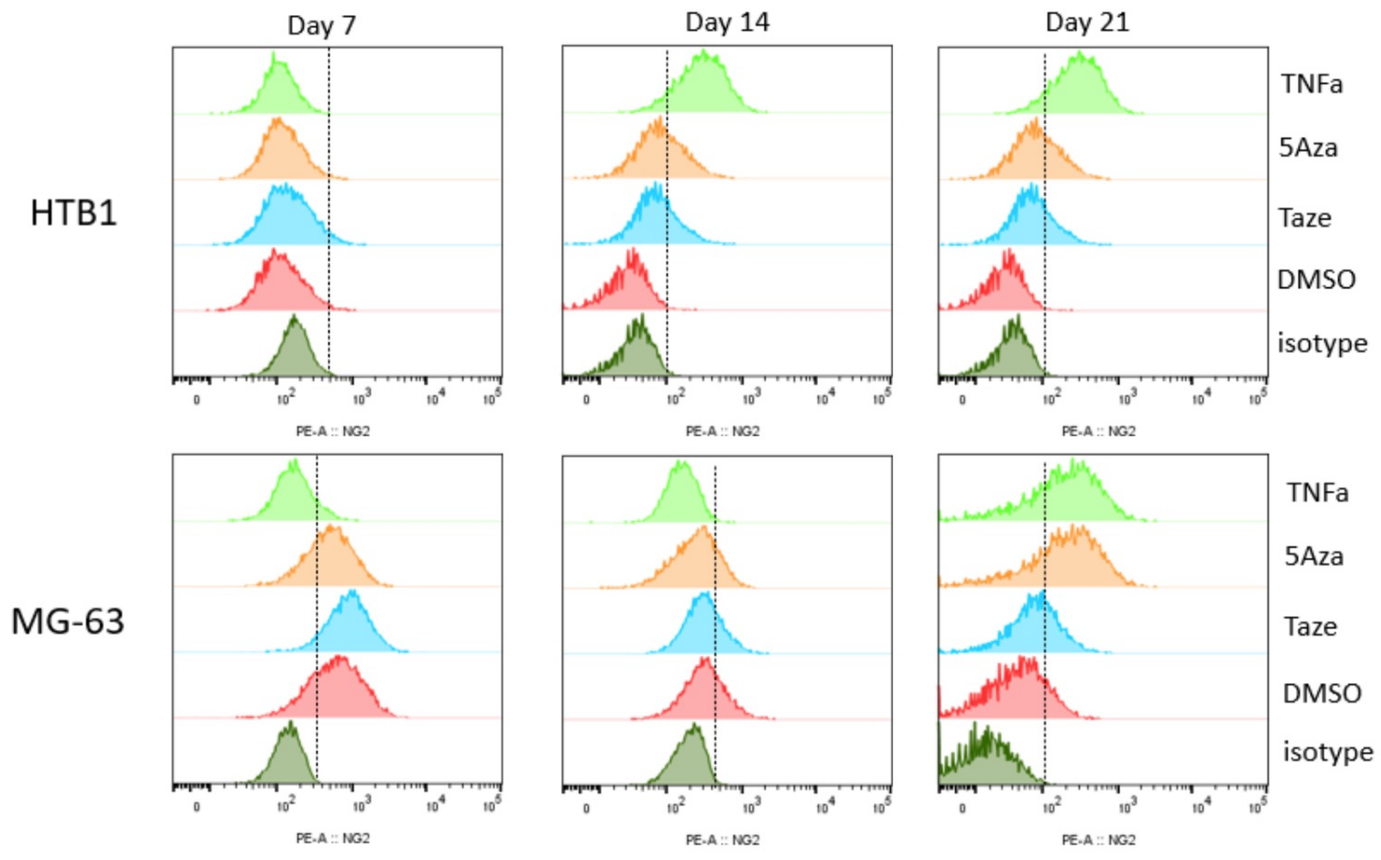

Figure 2: Data shown from the FORTESSA flow cytometer during 7 day intervals. Data shown for isotype control, as well as data from the addition of epigenetic drugs including DMSO, EZH2 (Tazemetostat), 5-aza-2'-deoxycytidine, and TNFα for 2 cell lines.

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References

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