# Effects of Epigenetic Modifiers on HLA-ABC in a Human Breast Cancer Cell Line

Andrew Kareeparampil and Dr. Robert O'Donnell

DEPARTMENT OF Biology

## Abstract

Human leukocyte antigen (HLA) is a group of proteins coded by the major histocompatibility complex (MHC) and is a vital part of the human immune response. HLA allows abnormal cells to be destroyed by cytotoxic T-cells by presenting antigens to the cell surface. In tumors, however, there is diminished HLA expression, allowing cells to bypass the immune system. Experiments were conducted to determine the effects of various drug epigenetic modifiers on HLA expression in MDA-MB-231 cells, a human breast cancer cell line. HLA expression was compared between control and drug treatments using flow cytometry. Initial results found that HLA-ABC expression is increased in cells treated individually with the drugs 5-Azacytidine and Vorinostat. We are continuing to test combinations of these drugs, along with the addition of gamma-Interferon, an immune cytokine known to upregulate HLA-ABC expression. Furthermore, we would like to study how two other HLA proteins, HLA-E and HLA-G, can be affected by these epigenetic modifiers. Effective increases in HLA expression can be beneficial to cancer immunotherapy.

## Introduction

- Previous experimentation in cancer immunotherapy have demonstrated that tumor cells are able to proliferate via downregulation of MHC class I, thus bypassing a T cell-mediated immune response [1].
- Vorinostat and interferons have been shown to restore HLA expression in Merkel cell carcinoma [2].
- DNA methyltransferase (DNMT) inhibitors like 5-Azacytidine have been found to lack curative effects but still enhance the activity of cytotoxic drugs for treating acute myeloid leukemia and lung cancer [3]

## Methods

**MDA-MB-231 Cell Culture**
Cells grown in 25 $cm^2$ with Iscove's Modified Dulbecco's Medium

↓

**Treatment Selection**
Control and experimental flasks made

↓

**Treatment Marking**
Anti-HLA Class I antibodies added to each treatment for visualization of HLA

↓

**Flow Cytometry**
HLA-ABC analyzed via flow cytometry

## Results

| Experiment | Control (no drug) | 5-Aza | Vorinostat | IFNγ | IFNγ + 5-Aza | Vorinostat + IFNγ |
|---|---|---|---|---|---|---|
| 1 | 83.38 | 453.77 (444% increase) | – | – | – | – |
| 2 | 53.25 | – | 92.90 (74.5% increase) | – | – | – |
| 3 | 217.32 | – | – | 350.70 (61.4% increase) | 293.46 (35.0% increase) | – |
| 4 | 124.66 | – | 201.51 (61.6% increase) | 181.40 (45.5% increase) | – | 298.99 (139.84% increase) |
| 5 | 98.63 | 111.59 (13.1% increase) | – | 161.58 (63.8% increase) | 129.17 (31.0% increase) | – |

**Table 1. Flow Cytometry Fluorescence of HLA-ABC, shown as mean log values.** A variety of treatments were done across 5 separate experiments. Treatments that were not applied are noted as a dash (–). Every trial shows a clear increase in HLA expression from each experimental treatment. For each drug treatment, percent increase relative to the control is shown.

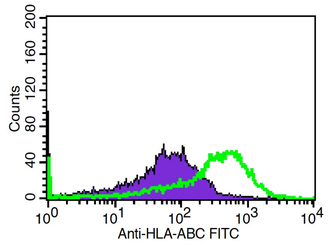


**Figure 1. Flow cytometry fluorescence of HLA-ABC in MDA-MB-231 treated with 5-Azacytidine.** A strong increase in HLA expression is shown as a result of the drug treatment.

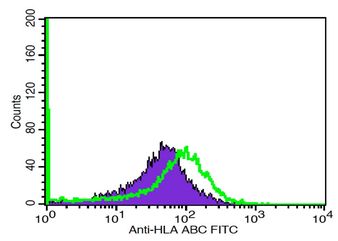


**Figure 2. Flow cytometry fluorescence of HLA-ABC in MDA-MB-231 treated with Vorinostat.** A noticeable increase in HLA expression is shown as a result of the drug treatment.

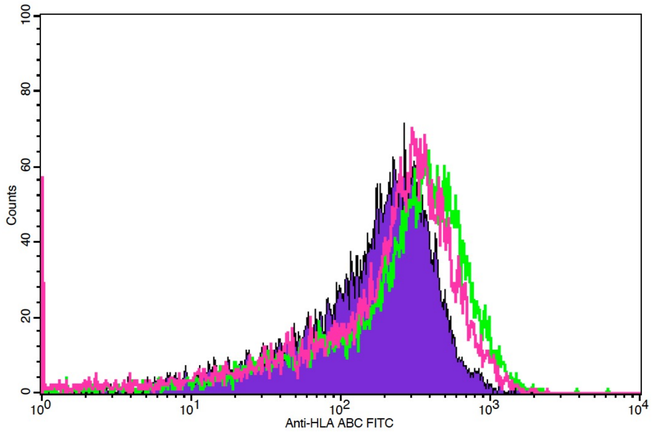


**Figure 3. Flow cytometry fluorescence of HLA-ABC in MDA-MB-231 under different conditions.** Control (no drug) is represented by purple, IFNγ is represented by light green, and IFNγ with 5-Azacytidine is represented by pink.

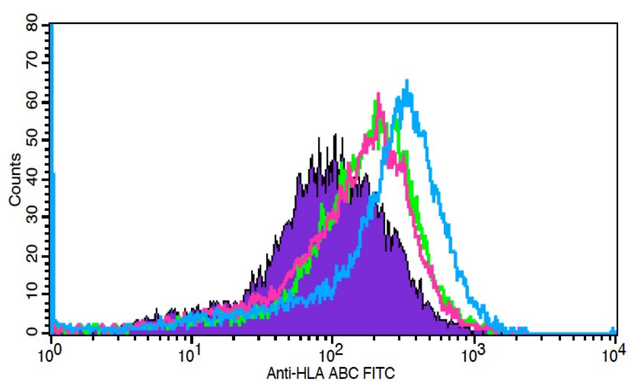


**Figure 4. Flow cytometry fluorescence of HLA-ABC in MDA-MB-231 under different conditions.** Control (no drug) is represented by purple, Vorinostat is represented by light green, IFNγ is represented by pink, and IFNγ with Vorinostat is represented by blue.

## Conclusions

- 5-Azacytidine and Vorinostat both individually upregulate HLA-expression in MDA-MB-231.
- Gamma Interferon also demonstrated a strong upregulation in HLA-ABC expression (Table 1).
- Although a combination of Gamma Interferon and Vorinostat was found to augment expression, a combination of Gamma Interferon with 5-Azacytidine lowered expression below those of individual drug treatments alone, while still above that of control conditions (Figures 3 and 4).

## Future Experiments

- We would like to investigate into other components of MHC Class I, such as HLA-G and HLA-E.
- Moreover, we are interested in investigating HLA expression of MHC Class II, as it has been speculated that the upregulation of MHC Class II in tumor cell lines may improve chances of recognition by the immune system [4]

## References

1. Bubenik J. Tumour MHC class I downregulation and immunotherapy (Review). Oncol Rep. 2003 Nov-Dec;10(6):2005-8. PMID: 14534734.
2. Ritter, C., Fan, K., Paschen, A. *et al.* Epigenetic priming restores the HLA class-I antigen processing machinery expression in Merkel cell carcinoma. *Sci Rep* **7**, 2290 (2017). https://doi.org/10.1038/s41598-017-02608-0
3. Füller M, Klein M, Schmidt E, Rohde C, Göllner S, Schulze I, Qianli J, Berdel WE, Edemir B, Müller-Tidow C, Tschanter P. 5-azacytidine enhances efficacy of multiple chemotherapy drugs in AML and lung cancer with modulation of CpG methylation. Int J Oncol. 2015 Mar;46(3):1192-204. doi: 10.3892/ijo.2014.2792. Epub 2014 Dec 10. PMID: 25501798.
4. Axelrod ML, Cook RS, Johnson DB, Balko JM. Biological Consequences of MHC-II Expression by Tumor Cells in Cancer. Clin Cancer Res. 2019 Apr 15;25(8):2392-2402. doi: 10.1158/1078-0432.CCR-18-3200. Epub 2018 Nov 21. PMID: 30463850; PMCID: PMC6467754.

## Acknowledgement

I would like to thank Dr. O'Donnell for his continuous help and mentorship throughout my research with him. I would also like to thank the other members of the O'Donnell lab for their assistance, as well as the SUNY Geneseo Biology Department for providing necessary materials for these experiments.