

5-aza-2'-Deoxycytidine Treatment Increases Expression of Melanoma Tumor Specific Antigens.

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CytoCure

Introduction

- Heterogeneous expression of tumor associated antigens represents a serious obstacle to specific immunotherapy.
- Loss of antigen expression is often the result of gene regulatory events rather than mutation and thus is reversible.
- Several different pathways simultaneously impact numerous tumor associated antigens in melanomas.

Immunoselection with Melan-A/MART-1 Therapy

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



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**Tumor Before
Immunotherapy**

**Immunotherapy
Eliminates Ag+
Tumor Cells**

**Eventual outgrowth
Of Ag-Negative
Tumor Cells**

Treatment of Heterogeneous Melanoma Stimulates Antigen Expression in Previously Antigen-Negative Tumor Cells

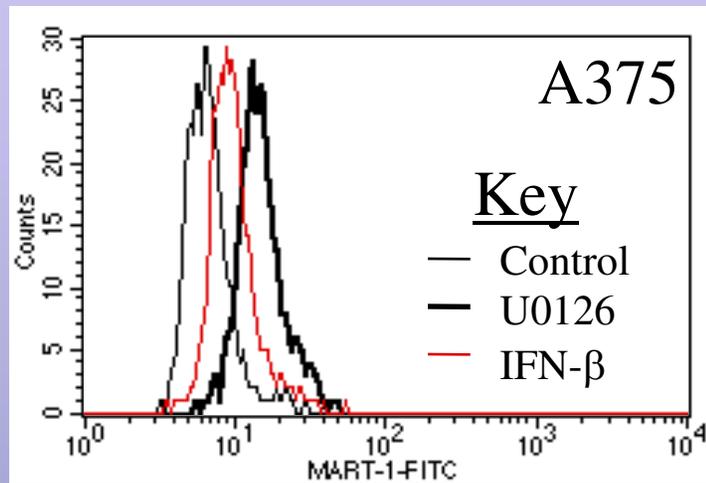


Heterogeneous Ag
Expression in
Melanoma

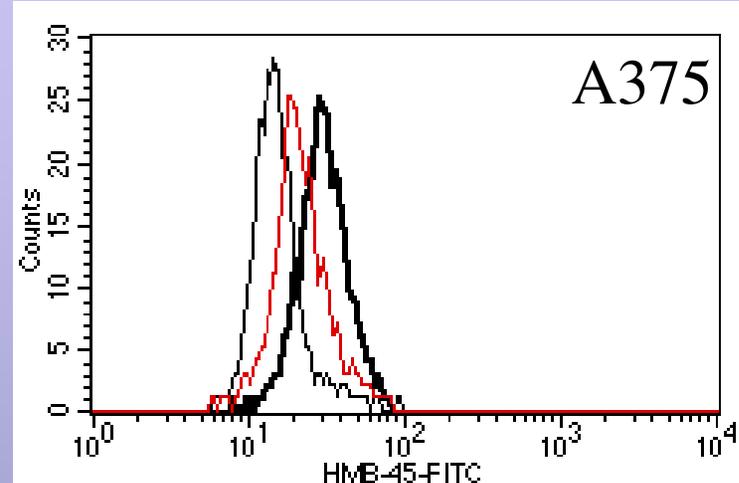


Treatment of Melanoma
Stimulates Ag Expression
To enhance Recognition

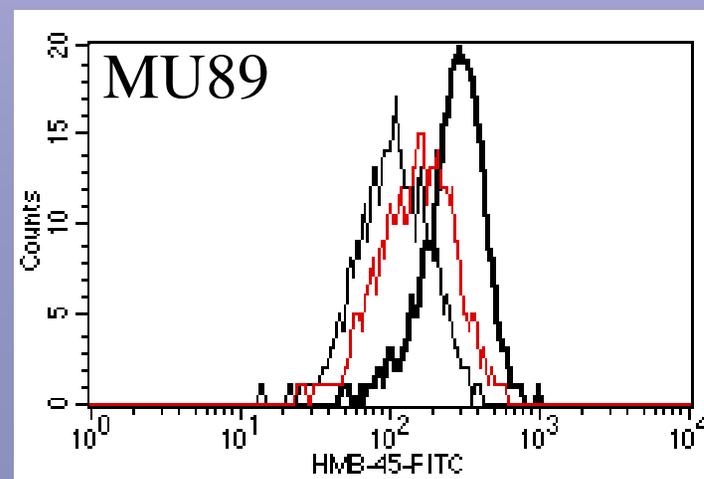
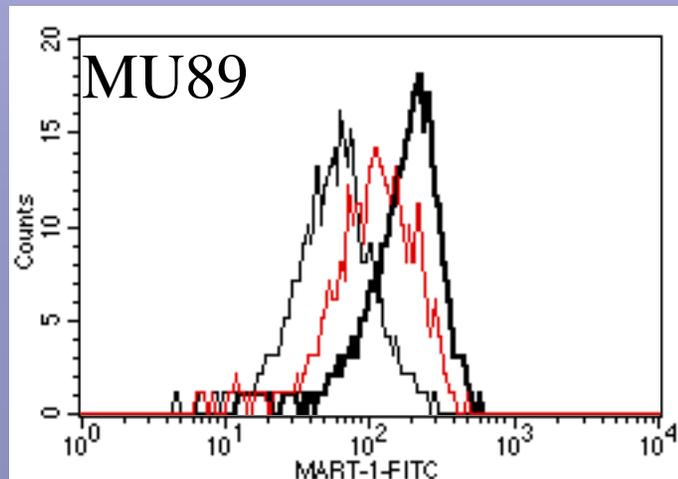
Chemical and biological agents can increase melanocyte gene expression



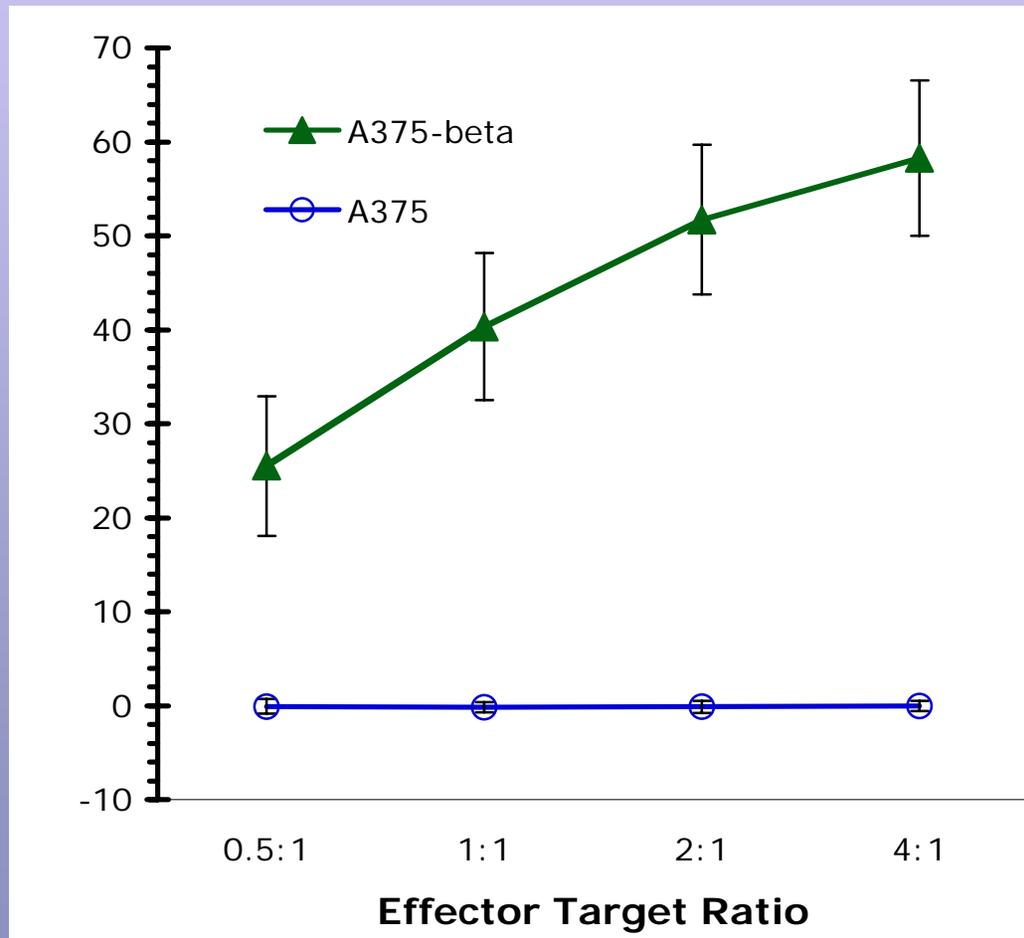
Melan-A / MART-1



gp100



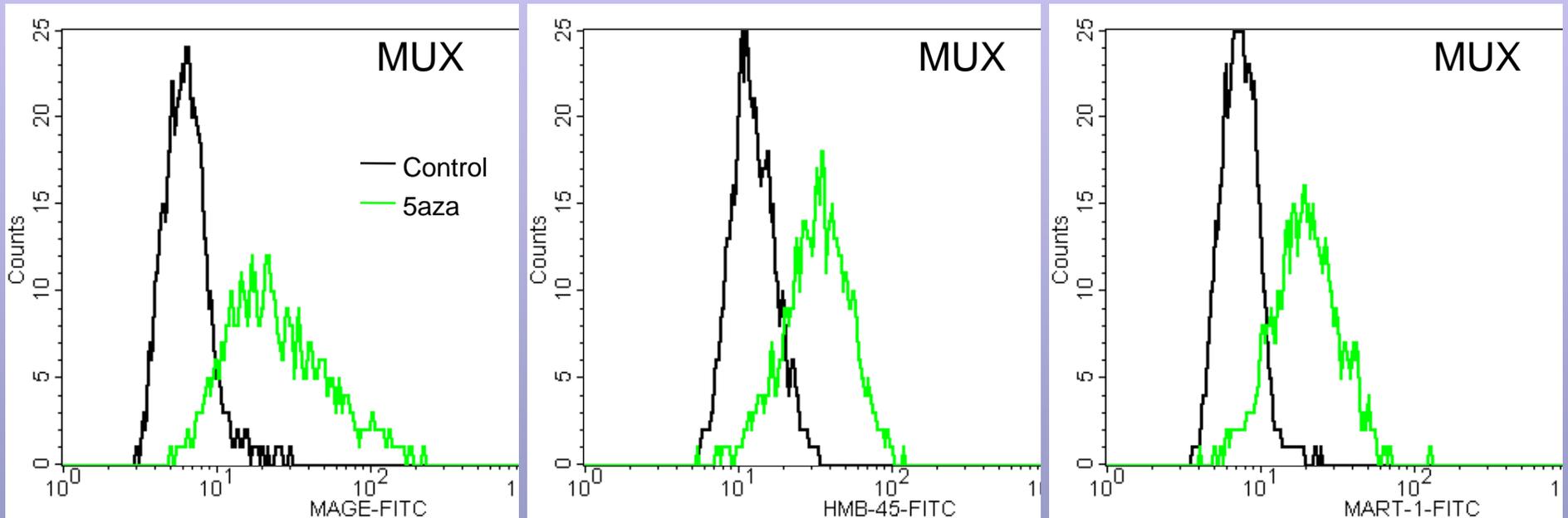
Increased CTL killing of IFN- β treated melanoma



Properties of 5aza

- Nucleoside analog incorporated into DNA during replication
- Blocks activity of DNA methyltransferase
- Cytotoxic and antiproliferative
- Known to reverse epigenetic silencing of gene expression

5aza exposure increases tumor antigen gene expression



MAGE-A1

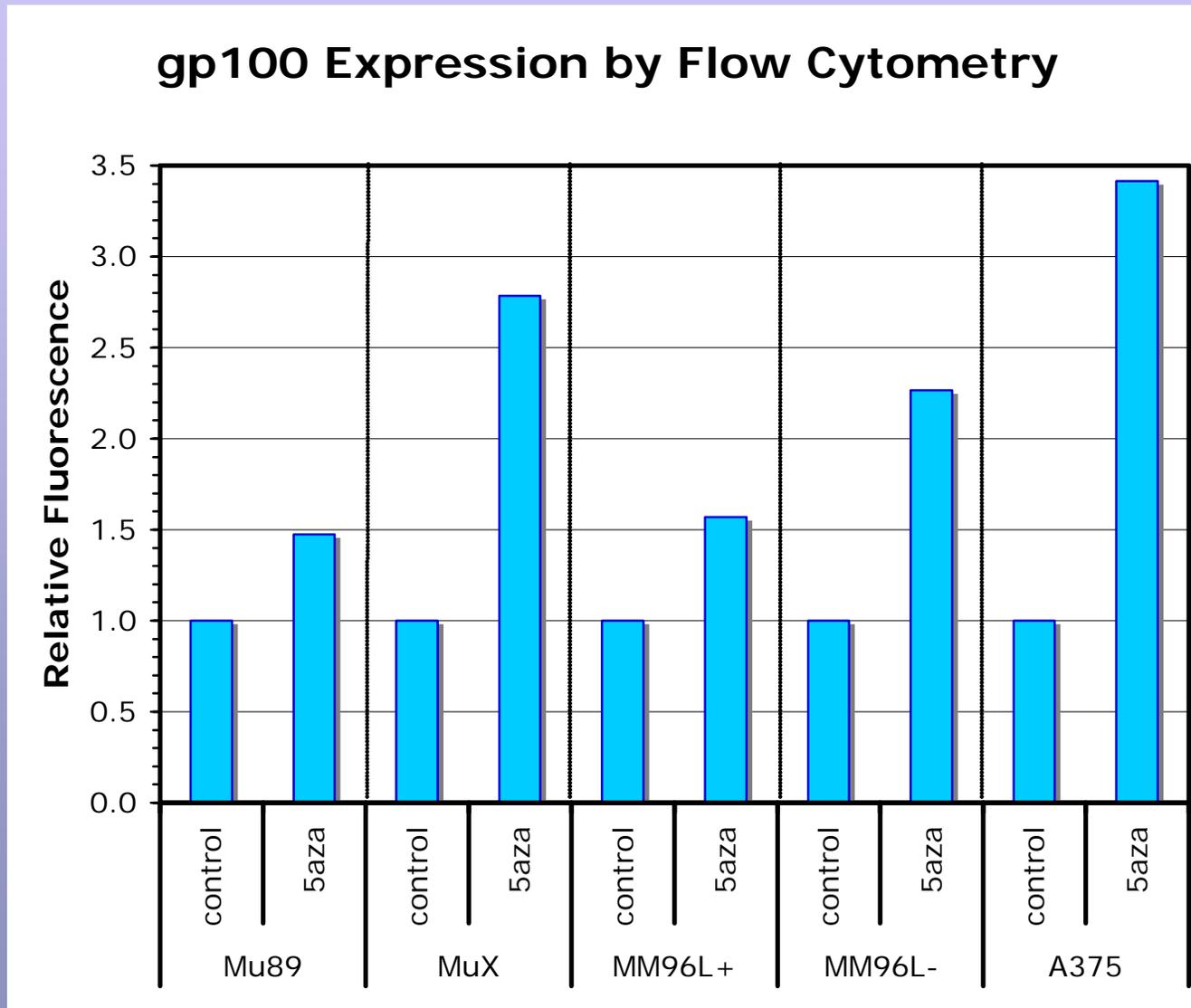
Cancer Testis Antigen

gp100

Melanocyte differentiation genes

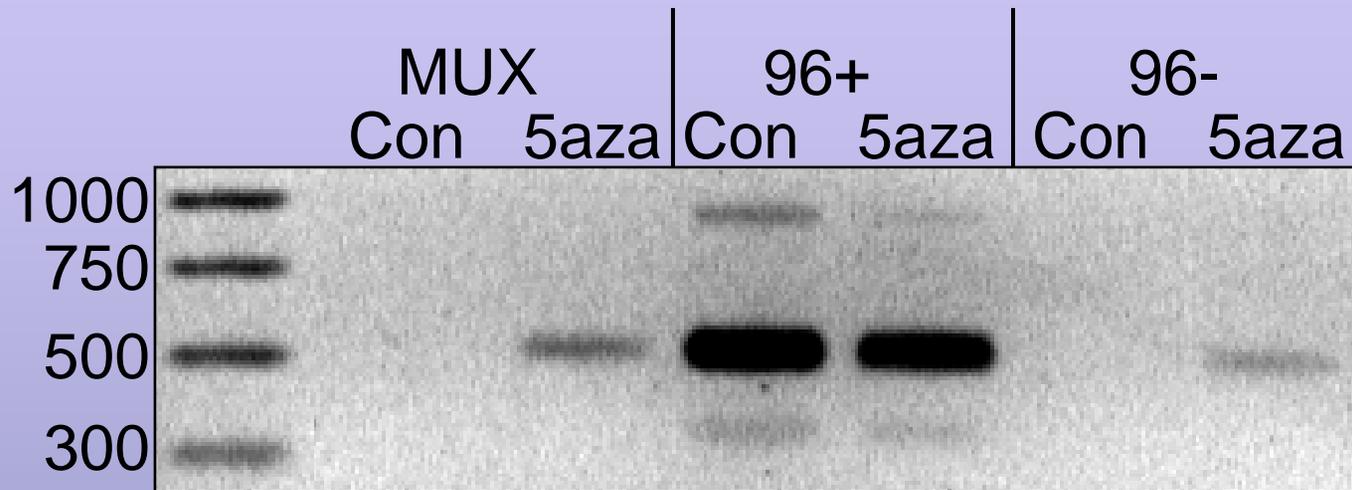
Melan-A/MART-1

5aza increases expression in multiple cell lines



RT-PCR of mRNA - multiple cell lines

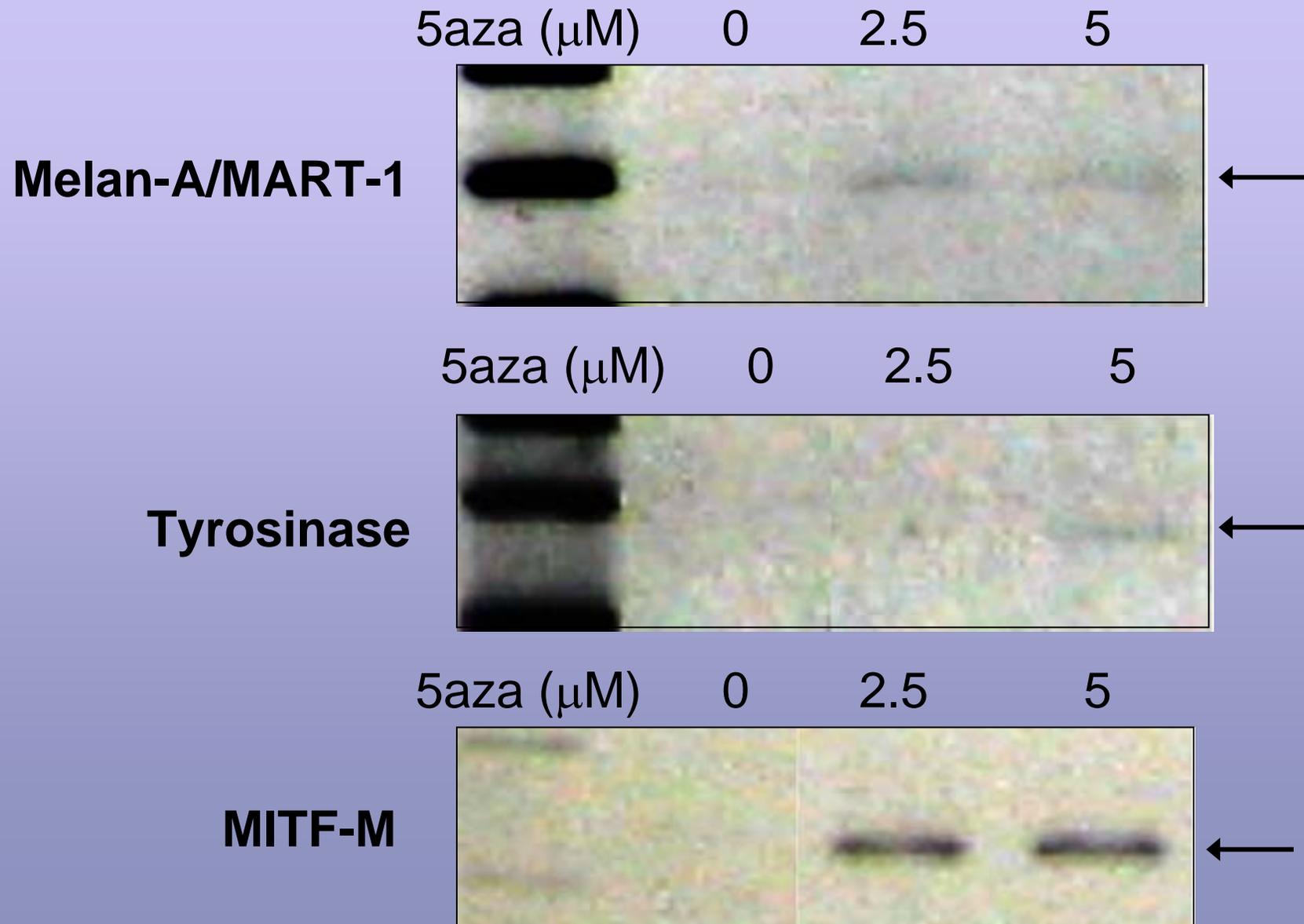
gp100



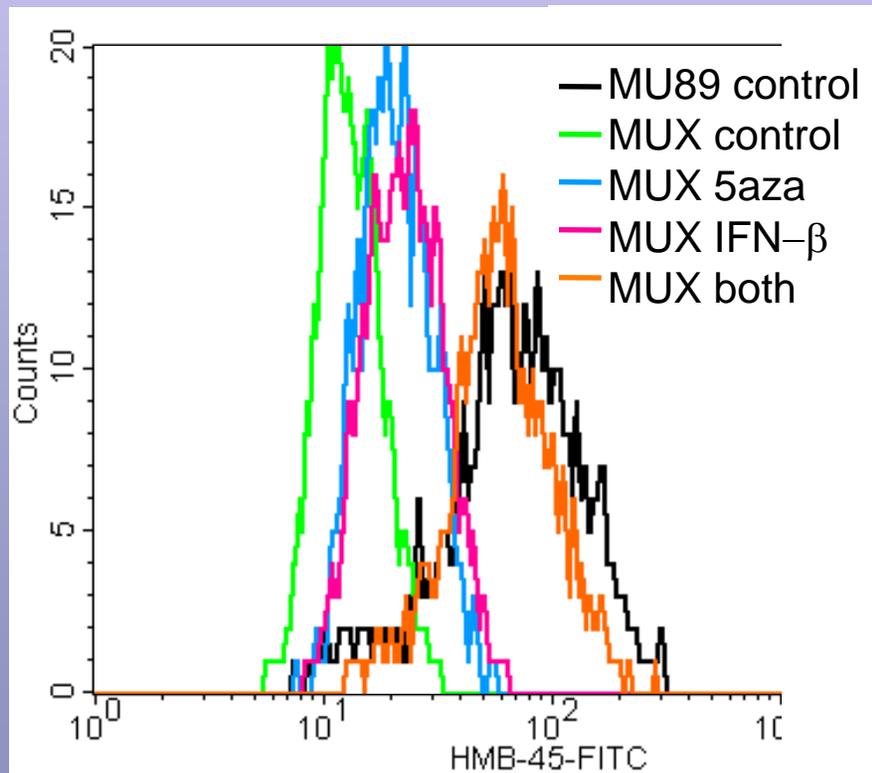
β -actin



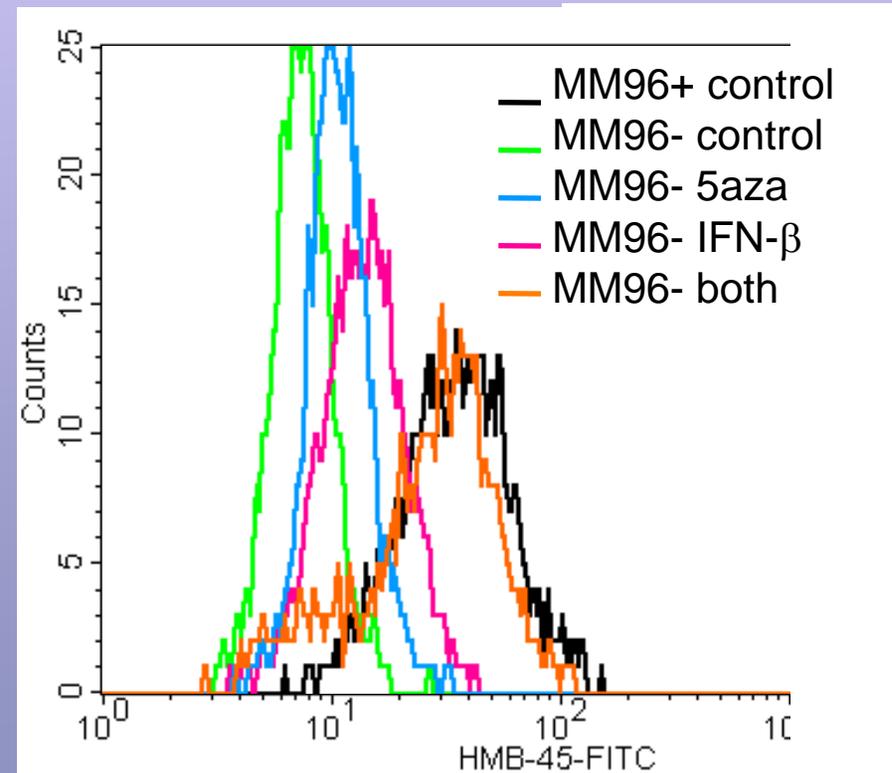
RT-PCR of mRNA - multiple melanoma specific genes



5aza in combination with IFN- β restores expression to antigen negative cells



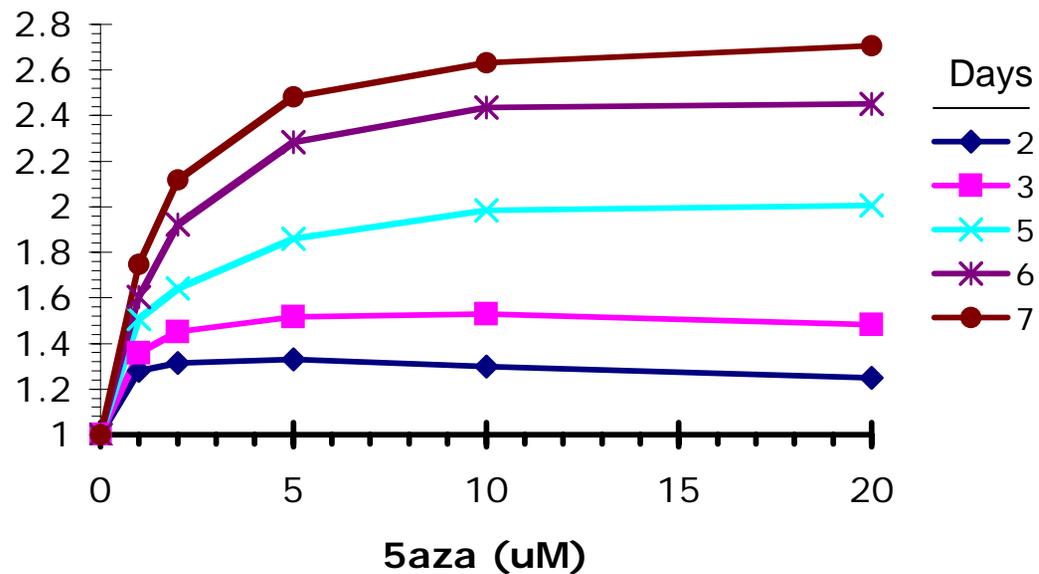
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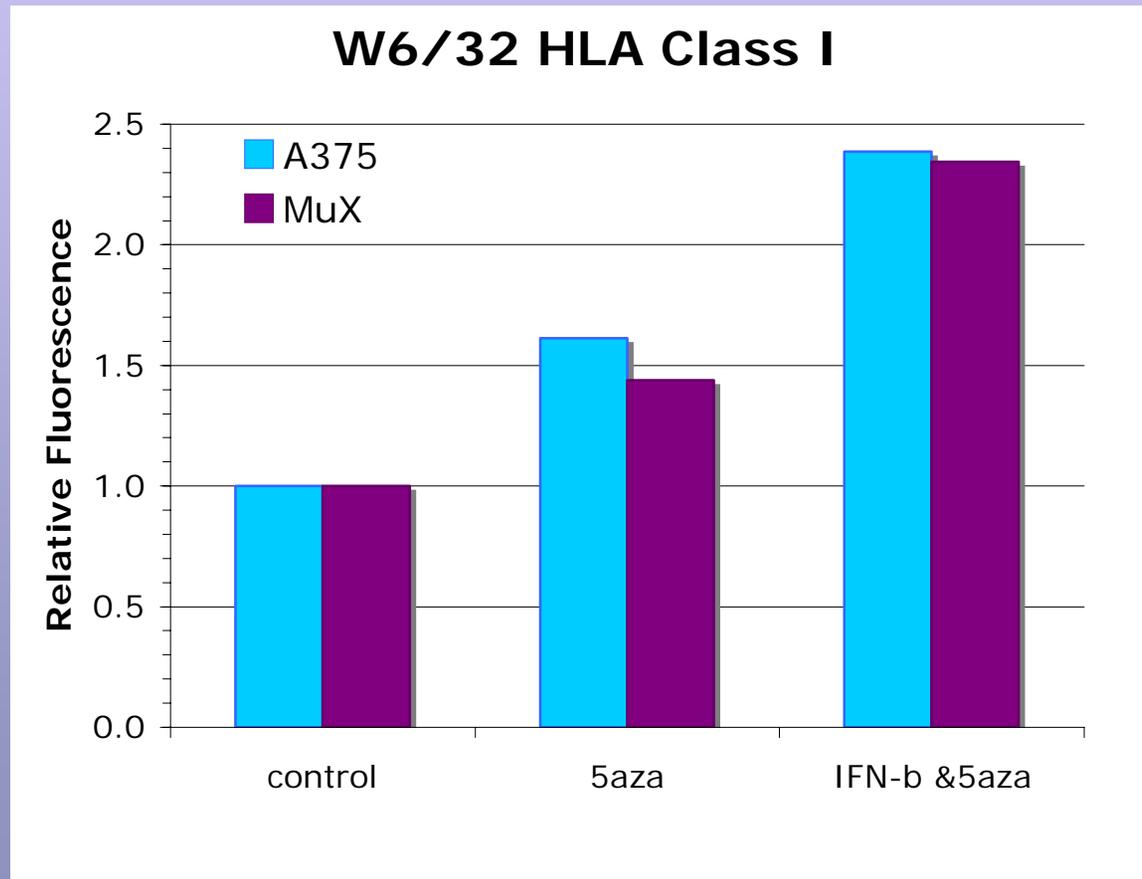
gp100

Dose response and Kinetics of 5aza effect

MART-1 promoter driven reporter expression



5aza also increases expression of Class I MHC



Conclusions

- 5aza treatment increases melanoma antigen expression for multiple genes including differentiation antigens and cancer testis antigens, and MHC class I in multiple cell lines.
- The combination of 5aza and IFN- β treatment consistently shows synergism for increasing antigen expression.

Implications for Immunotherapy

- Tumor specific antigens are often coordinately regulated.
- Antigen down regulation is often a reversible gene regulation event.
- Specific T cell targeting of tumors cells can be improved by combining agents that stimulate target antigen expression.
- As more potent specific immunotherapies are utilized, it becomes increasingly important to address the problem of immune escape via antigen loss. Therefore, we are developing strategies to assure the continued (or renewed) presence of the target antigens.