5-aza-2’-Deoxycytidinedeoxycytidinedeoxycytidinedeoxycytidine 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

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.
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**

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**β-actin**

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RT-PCR of mRNA - multiple melanoma specific genes

Melan-A/MART-1

Tyrosinase

MITF-M
5aza in combination with IFN-β restores expression to antigen negative cells.
Dose response and Kinetics of 5aza effect

MART-1 promoter driven reporter expression

Days

5aza (uM)
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.