WHOLE RECOMBINANT YEAST IMMUNOTHERAPEUTIC PROTECTION AGAINST MELANOMA

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Introduction

- Immunotherapy using whole recombinant yeast represents a new generation therapeutic cancer vaccine approach.
  - The administration of whole recombinant yeast delivers tumor antigens directly to antigen-presenting cells (APC), such as dendritic cells (DC), while simultaneously causing DC maturation and activation (1).
  - We demonstrated that therapeutic administration in mice of whole recombinant yeast expressing mutated Ras proteins caused the ablation of pre-existing, carcinogen-induced lung tumors (2).
- MART-1 (melanocyte/melanoma antigen recognized by T cells) is a nonmutated self-antigen, present in melanosomes of melanocytes and is overexpressed in most melanomas (3).
- In this study, human MART-1 was engineered as a tumor antigen for immunotherapy in *S. cerevisiae* yeast (yeast hMART-IT or GI-7001).

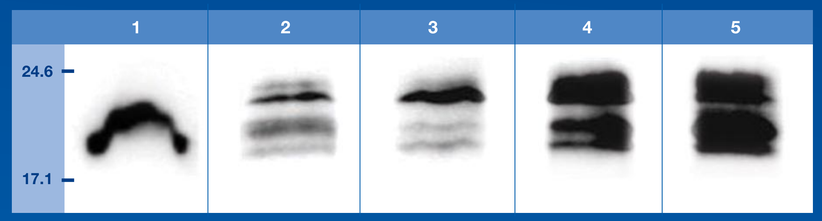

Fig. 1. hMART-1 expression in recombinant yeast. The cDNA of full-length human MART-1 was inserted into pYEX-BX vector and the transformed yeasts were selected on media lacking uracil. Expression of the protein was induced with copper sulfate at various incubation periods (from 4 hours to overnight). Lysates of yeast were separated by SDS-PAGE and transferred onto nitrocellulose membrane. Membranes were incubated with antibody to MART-1.

1) WM35 human melanoma cell line

2) hMART-IT (whole recombinant yeast expressing hMART-1) without copper induction

3) hMART-IT with copper induction for 4 hours

4) hMART-IT with copper induction for 8 hours

5) hMART-IT with copper induction for overnight

Conclusion: Western blotting analysis of yeast lysates confirmed hMART-1 expression from hMART-IT.

Table 1. Immunotherapy Schedule

- Animals
  - 7-9 week female C57BL/6 mice ($H2K^b$) (n=10)
- Vaccination schedule
  - SC injection at -4, -3, -1 weeks before SC tumor challenge
  - Three groups of mice:
    - PBS
    - 2 OD (5 x 10^7) whole yeasts with pYEX-BX (YVEC)
    - 2 OD (5 x 10^7) whole yeasts with hMART-1 (hMART-IT)
- Tumor challenge
  - SC injection of 10^4 B16F10 murine melanoma cells
- Assessment

Yeast are Avidly Phagocytosed by Dendric Cells

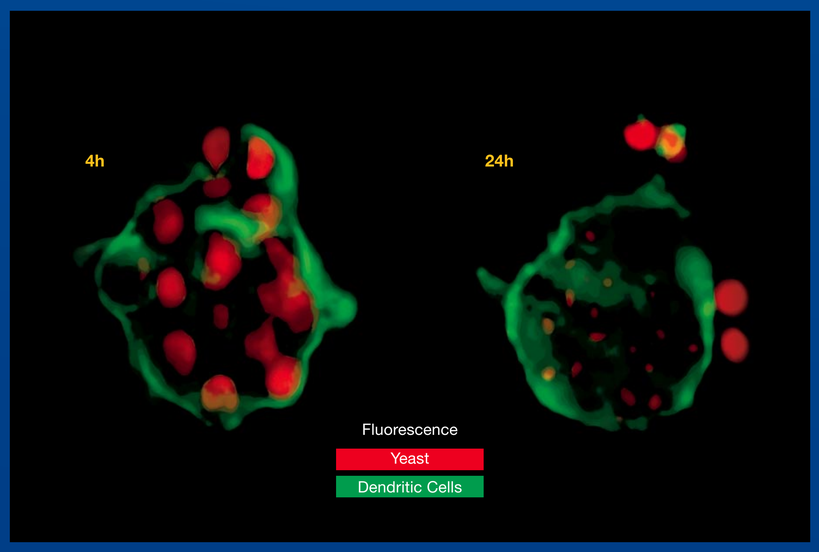

Fig. 2. Internalization of yeast by DCs. Immature DCs from day 5 bone-marrow cultures were co-incubated with yeast stained with MitoTracker Red at 10 yeast cells per DC for 4 or 24 hours. DCs were then stained with FITC-conjugated antibodies for CD11c or MHC class II.

Conclusion: Yeasts were avidly phagocytosed by dendritic cells.

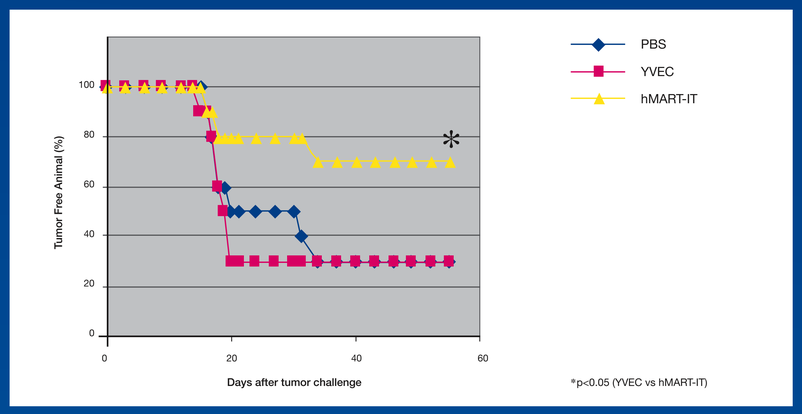

Fig. 3. Impact of hMART-IT on control of tumor development. C57BL/6 female naïve mice (ten animals per group) were immunized and challenged as described in Table 1. Animals were assessed every 2-3 days.

Conclusion: The administration of hMART-IT resulted in a statistically signif icant protection from tumor development ($p<0.05$).

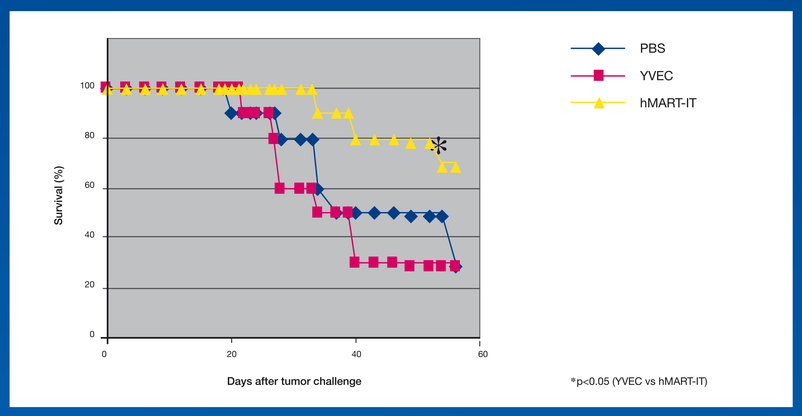

Fig. 4. Impact of hMART-IT on survival of mice. C57BL/6 female naïve mice (ten animals per group) were immunized and challenged as described in Table 1. Animals were assessed every 2-3 days.

Conclusion: The administration of hMART-IT resulted in a statistically significant survival of mice ($p<0.05$).

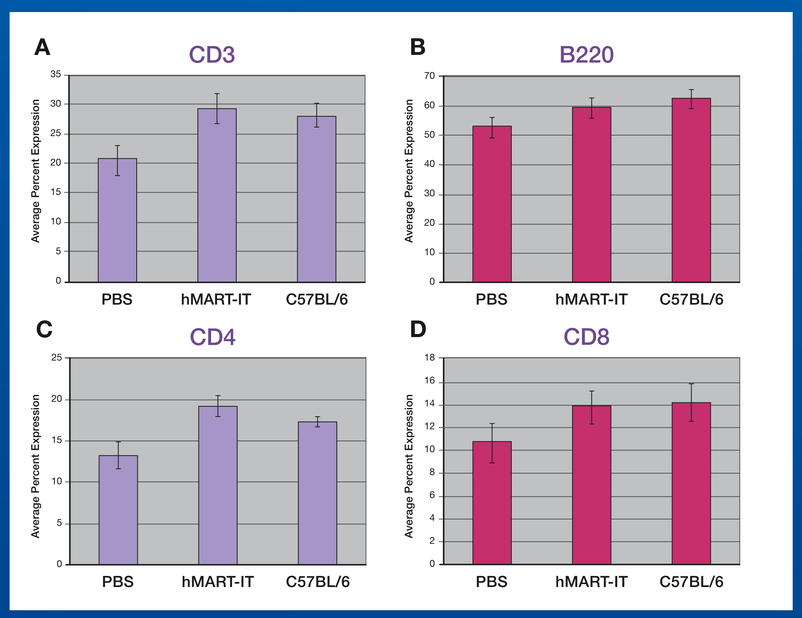

Fig. 5. Impact of hMART-IT on splenocytes after tumor challenge. C57BL/6 female naïve mice (five animals per group) were immunized with PBS or hMART-IT, and challenged by SC injection of B16F10. Spleens were harvested one month after the tumor challenge and analyzed for CD3 (A), B220 (B), CD4 (C), and CD8 (D). C57BL/6 female mice (five) without immunization or tumor challenge were analyzed as a control.

Conclusion: hMART-IT immunized mice showed protection from decline in CD3, CD4, and CD8 cells in spleen after tumor challenge.

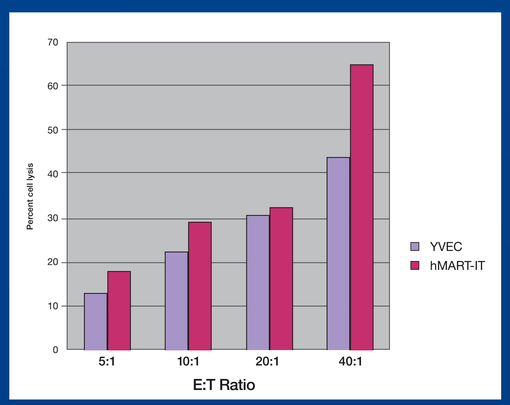

Fig. 6. Induction of CTL activity in mice after whole yeast immunization. Lymph nodes and spleen cells from mice immunized with YVEC or hMART-IT were restimulated in vitro with hMART-1 transfected mature DC. CTL activity against B16F10 was determined using lactate dehydrogenase (LDH) release assay. E:T, effector:target.

Conclusion: The administration of hMART-IT resulted in a greater lysis of B16F10 cells in vitro.

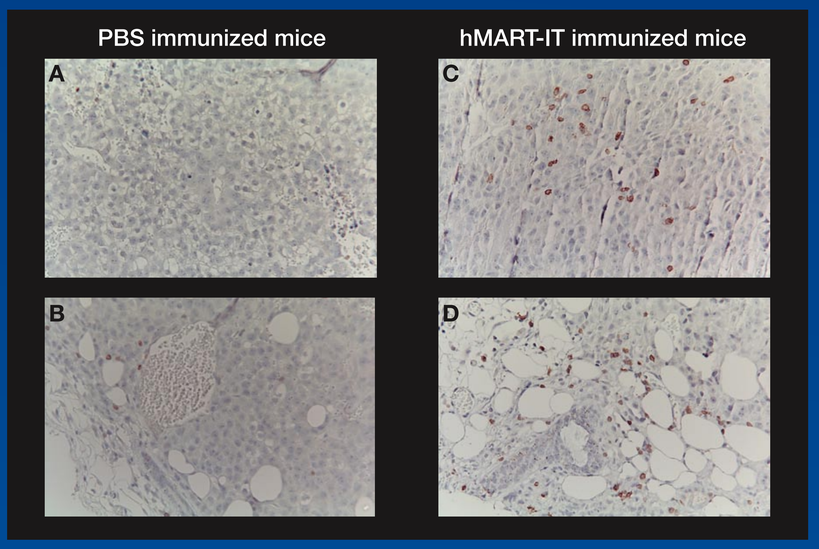

Fig. 7. Tumor infiltrating lymphocytes following hMART-IT immunization. C57BL/6 female naïve mice were immunized with PBS or hMART-IT and challenged with 10^4 B16F10 murine melanoma cells. Tumors developed from PBS immunized mice (A, B) and hMART-IT immunized mice (C, D) were immunostained with anti-CD3 antibody. (A-D) x 200.

Conclusion: hMART-IT induced tumor infiltrating CD3 cells.

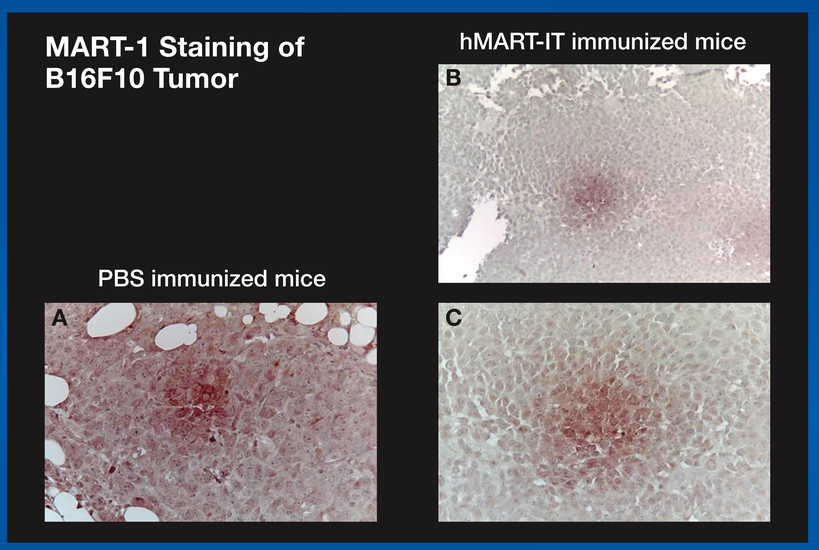

Fig. 8. MART-1 expression following hMART-IT immunization. C57BL/6 female naïve mice were immunized with PBS or hMART-IT and challenged with 10^4 B16F10 murine melanoma cells. Tumors developed from PBS immunized mice (A) and hMART-IT immunized mice (B, C) were immunostained with anti-mouse MART-1 antibody. (A) x 100, (B, C) x 200.

Conclusion: hMART-IT induced loss of antigen from escaped tumors.

Conclusion

- Immunization with whole recombinant yeast expressing human MART-1 elicited:
  - Protection against challenge with B16F10 tumors in vivo
    - MART-1 antigen in yeast elicits cross presentation against endogeneous mouse MART-1 protein in tumor.
  - Protection from the tumor-mediated decline in splenic CD4 and CD8 cells
  - CTL activity in vitro
  - Tumor infiltrating lymphocytes
  - Loss of antigen from escaped tumors

References

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3. Y Kawakami, YS Eliyahu, CH Delgado, PF Robbins, K Sakaguchi, E Appella, JR Yammelli, GJ Adema, SA Rosenberg. Identification of a human melanoma antigen recognized by tumor-infiltrating lymphocytes associated with in vivo tumor injection. Proc Natl Acad Sci USA 91: 6458, 1994.

Acknowledgments

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