WHOLE RECOMBINANT YEAST IMMUNOTHERAPEUTIC VACCINE EXPRESSING HUMAN MART-1 ELICITS 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).
  - The 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).

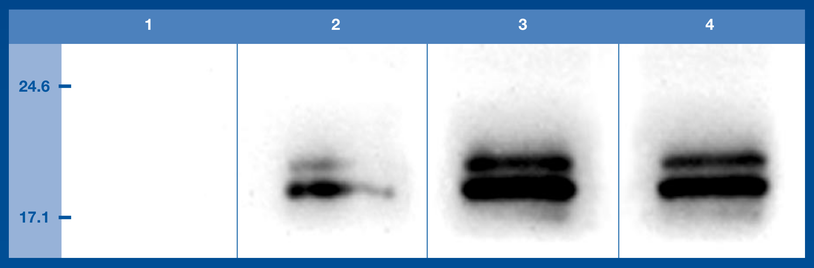


Fig. 1. hMART-1 expression in recombinant yeast. The cDNA of full-length human MART-1 was inserted into pYEX-BX vector and the transformed yeasts were selected on media lacking uracil. Expression of the protein was induced with copper sulfate at various incubation periods (from 4 hours to overnight).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

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

2) hMART-IT with copper induction for 4 hours

3) hMART-IT with copper induction for 8 hours

4) 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

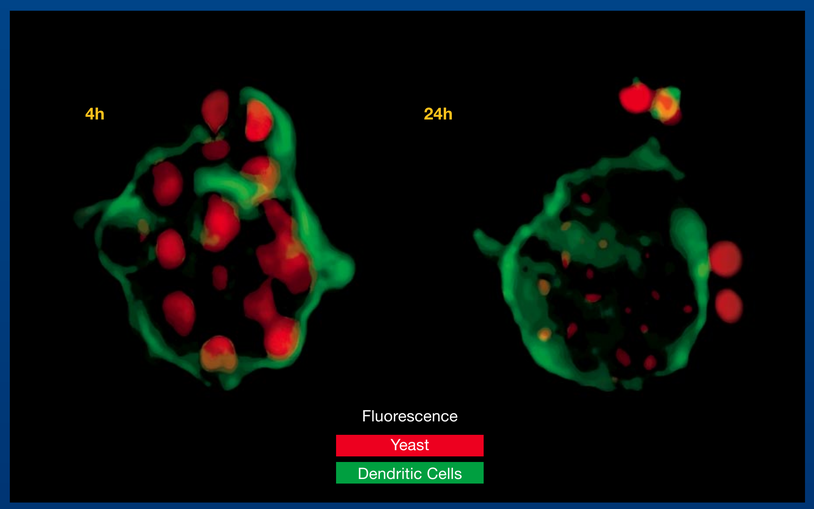


Fig. 2. Internalization of yeast by DCs. Immature DCs from day 5 bone-marrow cultures were co-incubated with yeast stained with MitoTracker Red at 10 yeast cells per DC for 4 or 24 hours. DCs were then stained with FITC-conjugated antibodies for CD11c or MHC class II.

Conclusion: Yeasts were avidly phagocytosed by dendritic cells.

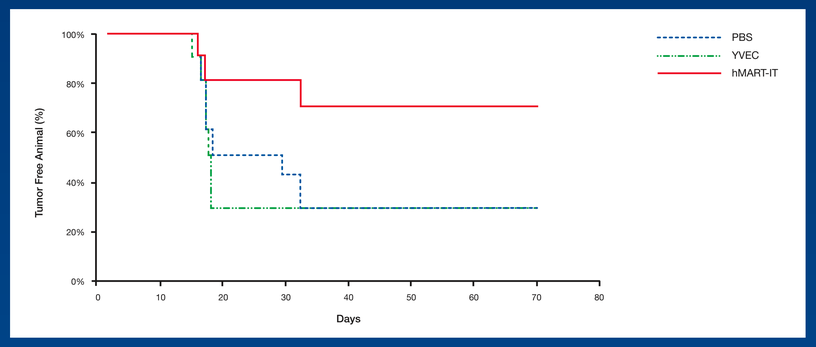


Fig. 3. Impact of hMART-IT on control of tumor development. C57BL/6 female naïve mice (ten animals per group) were immunized and challenged as described in Table 1. Animals were assessed every 2-3 days.

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

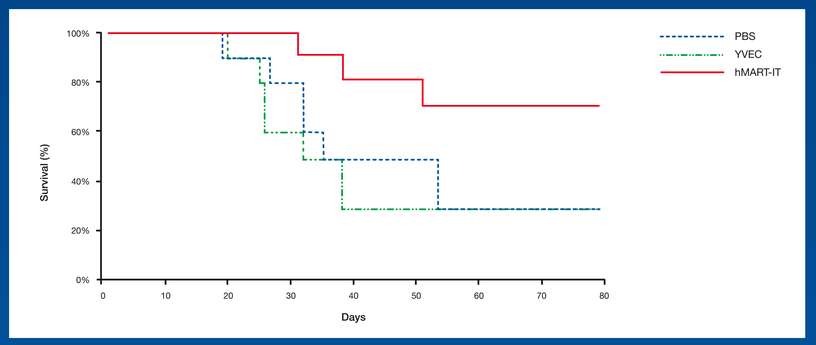


Fig. 4. Impact of hMART-IT on survival of mice. C57BL/6 female naïve mice (ten animals per group) were immunized and challenged as described in Table 1. Animals were assessed every 2-3 days.

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

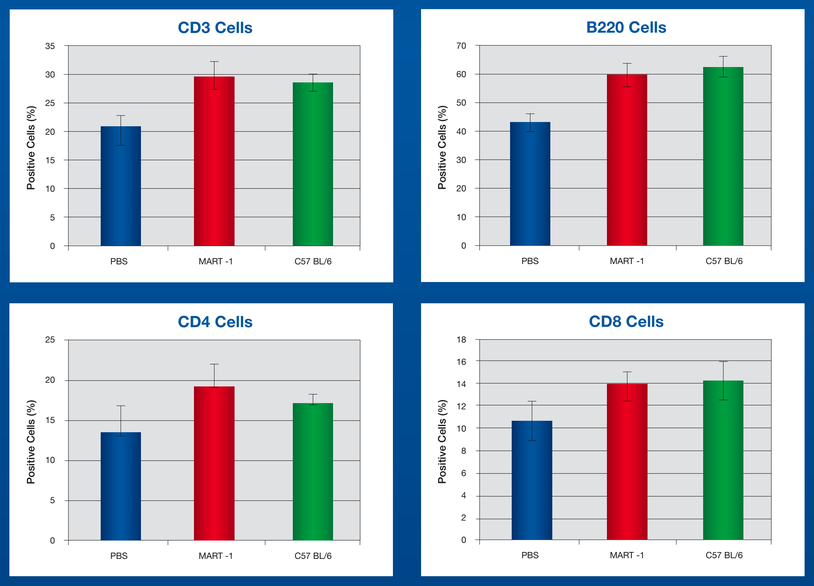


Fig. 5. Impact of hMART-IT on splenocytes after tumor challenge. C57BL/6 female naïve mice (five animals per group) were immunized with PBS or hMART-IT, and challenged by SC injection of B16F10. Spleens were harvested one month after the tumor challenge and analyzed for CD3 (A), B220 (B), CD4 (C), and 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.

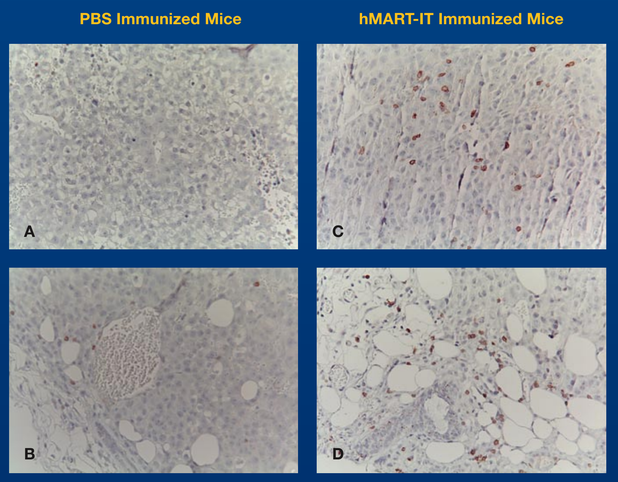
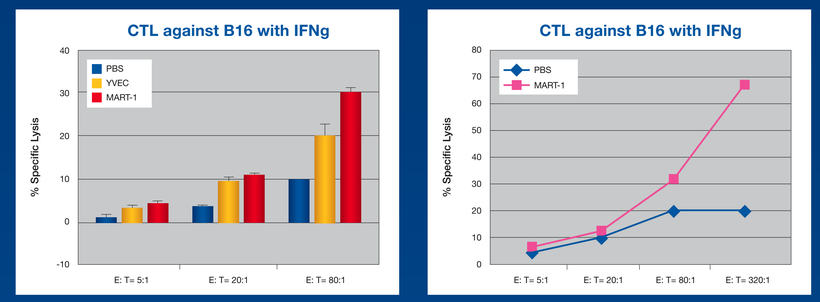


Fig. 8. Tumor infiltrating lymphocytes following hMART-IT immunization. C57BL/6 female naïve mice were immunized with PBS or hMART-IT and challenged with 104 B16F10 murine melanoma cells. Tumors developed from PBS immunized mice (A, B) and hMART-IT immunized mice (C, D) were immunostained with anti-CD3 antibody. (A-D) x 200.

Conclusion: hMART-IT induced tumor infiltrating CD3 cells.



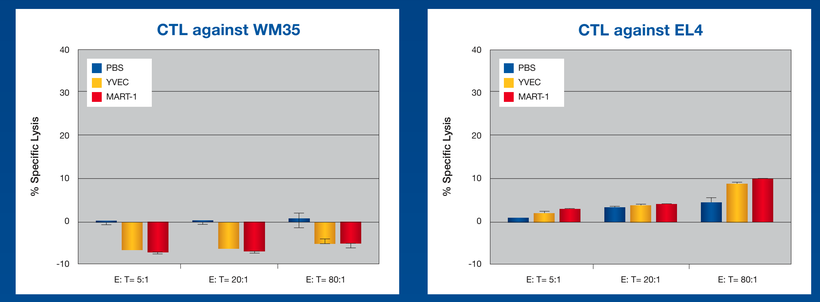


Fig. 6. Induction of CTL activity in mice after whole yeast immunization. Splenocytes from mice immunized with PBS, YVEC or hMART-IT were restimulated in vitro with hMART-1-IT. CTL activity against B16F10, WM35 (human melanoma cell line expressing hMART-1) or EL4 was determined using lactate dehydrogenase (LDH) release assay. E:T, effector:target.

Conclusion: The administration of hMART-IT resulted in an antigen-specific, MHC-class I restricted cytotoxicity.

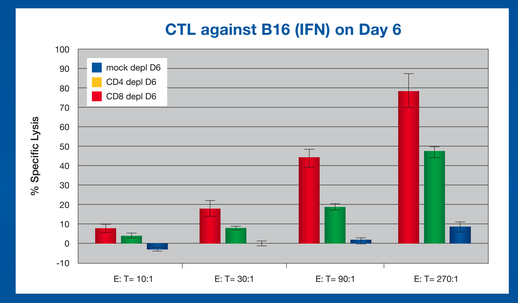


Fig. 7. CTL activity after depletion of T cell subsets. Splenocytes from mice immunized with hMART-IT were restimulated in vitro with hMART-1-IT. CTL activity against B16F10 was determined after depletion of T cell subsets using CD4 or CD8 microbeads (Miltenyi Biotech). LDH release assay was used. E:T, effector:target.

Conclusion: Cytotoxic activity was predominantly from CD8+ T cells.

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
- Antigen-specific, MHC-restricted CTL activity in vitro, predominantly by CD8 cells
- Tumor infiltrating lymphocytes

References

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Acknowledgment

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