Vaccine-associated immune and WT-1 responses are associated with better relapse-free survival in patients with AML in remission treated with a GM-CSF secreting leukemia vaccine and autologous stem cell transplant (ASCT)

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ABSTRACT

Background: Preclinical models have demonstrated the efficacy of GM-CSF secreting cancer vaccines (GVAX®) accompanied by vaccine-primed lymphocyte infusion following ASCT.

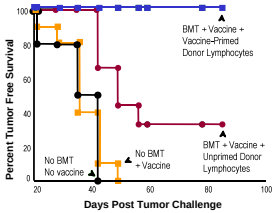
Methods: Patients (Pts) ≤ 60 years old with de novo AML (excluding M3) were enrolled. Leukemia cells were harvested at diagnosis followed by induction and consolidation chemotherapy and ASCT. A single pretransplant vaccine composed of irradiated autologous leukemia cells mixed with GM-CSF gene-modified K562 cells (CG9962) was given followed by collection of vaccine-primed lymphocytes that were reinfused with the stem cell graft. Posttransplant vaccinations were initiated at week 6 (or upon platelet engraftment) and given every 3 weeks ($10x10^6$ tumor cells + $4x10^6$ CG9962 cells) x 8 vaccinations.

Results: 54 pts were enrolled, 45 (83%) achieved a complete remission, and 27 (50%) proceeded to ASCT. Delayed-type hypersensitivity (DTH) reactions to autologous tumor were induced post vaccination in 43% of pts and this was associated with a trend toward longer relapse-free survival (RFS) (100% vs. 60%, p = 0.08) with a median follow up of 13 months. Antibodies reactive against autologous tumor were induced in 33% and against CG9962 cells in 93%, but no correlation with relapse was noted. Minimal residual disease (MRD) was monitored by quantitative analysis of peripheral blood (PB) and bone marrow (BM) for WT-1, a leukemia-associated gene, by RT-PCR. A decrease in WT-1 in PB (median ~ 1 log) was noted in 69% of pts following the single pretransplant vaccination and this was associated with longer RFS (80% vs. 0%, p = 0.02). Post transplant, 75% of pts demonstrated clearance of WT-1 from PB and this was also associated with improved RFS (90% vs. 20%, p = 0.002). WT-1 remained detectable in the BM in 63% of pts, most likely representing background expression in normal hematopoietic progenitors.

Conclusions: Induction of an immune response to vaccination and a vaccine-associated decrease in MRD as monitored by WT-1 is associated with longer RFS. Clearance of WT-1 from the blood following ASCT appears to predict for better outcome.

BACKGROUND

BMT + Adoptive Transfer of Vaccine-primed Lymphocytes Increases GVAX® Potency in Mice

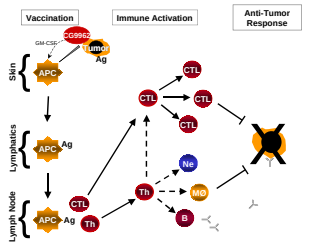


Borrello et al. Blood 2000; 95:3011

Balbic mice underwent myeloablative irradiation followed by infusion of syngeneic bone marrow and either naïve splenocytes or splenocytes from mice vaccinated two weeks prior with a GM-CSF producing lymphoma vaccine (A20/GM-CSF). 3 weeks post transplant, these mice as well as untransplanted mice were challenged with 10^5 A20 cells IV and vaccinated 5 days later with 10^6 A20GM-CSF. Mice were followed for tumor-free survival.

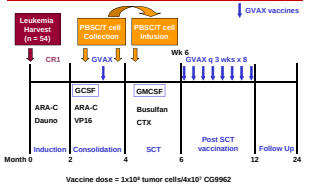
BACKGROUND

Proposed Mechanism of Action of GVAX®



METHODS

Mixed GVAX® for AML: Study Design



Leukemia Cell Processing and Vaccine Production

At diagnosis, leukemia cells (target collection of 2 x 10^9 cells) are harvested at the treatment center through peripheral blood draw, apheresis, or bone marrow aspirate (if insufficient circulating blasts). Mononuclear cells are isolated through density gradient separation, irradiated, aliquoted and stored frozen. CG9962 cells, produced at Cell Genesys, are also irradiated, aliquoted, and shipped frozen to the clinical site. At the time of vaccination, aliquots of leukemia and CG9962 cells are mixed at a ratio of 5:2 and delivered intra-dermally.

RESULTS

Study Status as of May 2005

54 – Enrolled/Leukemia Harvest

53 – Induction Chemotherapy

45 – Complete Remission

34 – Consolidation Chemotherapy

28 – Pre-transplant Vaccine

27 – Stem Cell Transplant

21 – Initiated Post-transplant Vaccines

Baseline Patient Characteristics

Characteristic	Enrolled (n=54)	Vaccine Treated (n=28)
Age: Median (range)	47 (21-60)	49 (24-59)
Gender – No. (%)		
female	31 (57)	10 (36)
male	23 (43)	18 (64)
Cytogenetics		
normal	27 (50)	15 (54)
complex	7 (13)	2 (7)
t (6;9)	2 (4)	0 (0)
-7	1 (2)	0 (0)
t (8;21)	4 (7)	1 (4)
Inv (16)	3 (6)	3 (11)
t (15;17)	1 (2)	0 (0)
other	9 (17)	7 (25)

Leukemia Cell Harvest

	Enrolled (n=54)
Target Leukemia Cell Dose	
Total	$2x10^9$
Dose/vaccination	$1x10^9$
Source of Leukemia Harvest – No. (%)	
Leukapheresis	28 (52)
Blood draw	22 (41)
Bone marrow	4 (7)
Leukemia Harvest Success – No. (%)	51 (94)*

*Two vaccines with insufficient dose, one sterility failure

Transplant Engraftment / Vaccine Safety

Vaccine injection site reactions grade 1/2 in all patients

Median time to neutrophil engraftment (ANC > 500) 15 days

Median time to platelet engraftment (plt > 50k) 20 days

No vaccine-related serious adverse events

No evidence of autoimmunity

RESULTS

RFS for Study Cohorts

Study Cohort	N	2 year RFS*	Median Follow-up
All Patients in CR	45	51%	16 mos.
Received Consolidation Chemotherapy	34	58%‡	20 mos.
Received Autologous Stem Cell Transplant	27	67%‡	22 mos.

*2 yr RFS from time of CR

‡2 yr RFS measured from time of initiating consolidation chemotherapy remains unchanged

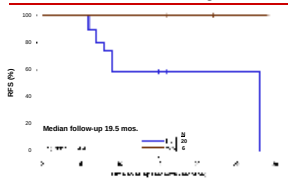
Interim Summary of Immune Response

Endpoint	No of Pts (%)
Vaccine Injection Reaction	26/27 (96)
Tumor DTH* Reaction (≥5 mm)	
baseline	2/26 (8)
following pre-transplant vax	0/26 (0)
following post-transplant vax	6/19 (32)†
Tumor-reactive Ab induction	
K562 cells	13/14 (93)
Autologous Tumor	5/15 (33)

*DTH = delayed-type hypersensitivity skin reaction to injection of autologous tumor cells

‡All 6 DTH (+) patients had undetectable WT-1 (4) or decrease in WT-1 (2) following pre-SCT vaccination

DTH Response to Vaccination Correlates with Prolonged RFS



Quantitative WT-1 as Marker of Minimal Residual Disease in AML

WT-1 is a tumor suppressor gene responsible for Wilm's tumors

Highly expressed in AML (> 95%), ALL, and CML

May play a role in leukemogenesis

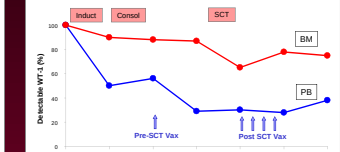
Candidate immunodominant antigen in AML

Increase in WT-1 levels associated with hematologic relapse

Quantification of WT-1 levels may serve as a tool to measure the impact of post remission therapies on minimal residual disease

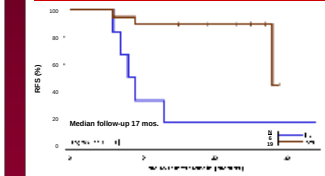
RESULTS

Percent Patients with Detectable WT-1 Throughout Study Treatment

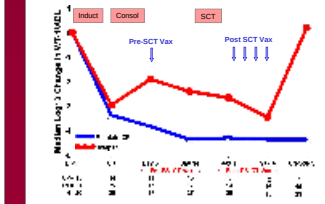


	Number Studied	Enrollment	CR	Day 0	Day 14	Vax 1	Vax 4	Off Study
PB	50	39	28	27	20	18	23	
Marrow	39	36	24	23	20	18	20	

Undetectable WT-1 Status Post-SCT Predicts for Improved RFS



Median Log Change in WT-1 (PB) for Patients in CR who Maintain CR vs. Relapse

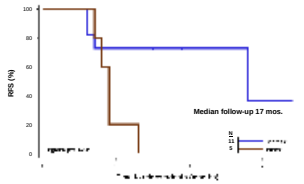


RESULTS

Vaccine-Associated Decreases in WT-1

	Bone Marrow	Peripheral Blood
Initial Decrease Following Pre-SCT Vaccine (D0-14)	12/20 (60%)	11/16 (69%)
Additional Decrease Following Post-SCT Vaccine (Vax 1-4)	2/12 (17%)	2/4 (50%)

WT-1 Decrease Following Pre-SCT Vaccine Associated with Longer RFS



CONCLUSIONS

Favorable safety profile; no evidence of autoimmunity

DTH immune response to vaccination associated with 100% RFS

Vaccine associated reductions in MRD by WT-1 PCR are associated with improved RFS (p = 0.029)

Achievement of undetectable WT-1 post-transplant is associated with improved RFS (p = 0.002)

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