AD_____

Award Number: DAMD17-01-1-0107

TITLE: Targeting Osteoblastic Bone Metastasis with a Novel Site Restricted Gene Therapy

PRINCIPAL INVESTIGATOR: Kenneth S. Koeneman, M.D.

CONTRACTING ORGANIZATION: The University of Texas Southwestern Medical Center at Dallas
Dallas, Texas 75390-9105

REPORT DATE: June 2004

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	June 2004	Final (1 Jun 2001 - 31 May 2004)

4. TITLE AND SUBTITLE
Targeting Osteoblastic Bone Metastasis with a Novel Site Restricted Gene Therapy

5. FUNDING NUMBERS
DAMD17-01-1-0107

6. AUTHOR(S)
Kenneth S. Koeneman, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas Southwestern Medical Center
at Dallas
Dallas, Texas 75390-9105

E-Mail: kenneth.koeneman@utsouthwestern.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Original contains color plates: All DTIC reproductions will be in black and white.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

Continuing from the previous two years work on this project, encouraging data was also obtained in year 3. Effectiveness of the Ad-BSP-E1a viral contrast was again demonstrated in the intraosseous model of prostate cancer bone metastasis. Further tests were completed and, as such, it appears that low-dose systemic taxotere administration in combination with the viral construct Ad-BSP-E1a may have better efficacy and long-term cancer control, based upon serum PSA as a biochemical recurrence marker. This metastasis model ***in vivo*** data corresponds nicely to the previously reported ***in vitro*** data which was presented in year 2 progress report. After much effort, we were able to construct the Ad-hTERT-E1a construct. The cloning data and purification analysis is presented in this report. This construct will be available for future testing and future attempts at funding. The most striking nature of this research is the apparent synergism with the "chemogene therapy" approach in the ***in vivo*** metastasis model. It appears that systemic Ad-BSP-E1a virus along with taxotere has a striking effect on control of the prostate intraosseous tumors. The likely most efficacious combination in humans afflicted with prostate cancer metastasis is Ad-BSP-E1a virus, and low-dose taxotere (with dexamethasone) as evidenced by our data presented herein.

14. SUBJECT TERMS
Prostate neoplasm, osseous metastasis, bone sialoprotein

15. NUMBER OF PAGES
20

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The purpose of the study is to improve the efficacy of prostate cancer gene therapy by constructing and assessing new replication competent adenoviral vectors using the tumor restricted promoter bone sialoprotein (BSP), and human telomerase catalytic subunit (hTERT). The first two years' work has revealed excellent efficacy of this Ad-BSP-E1 construct both in ***in vitro*** and ***in vivo*** models. In this report, year 3 and final installment, we present data to show that the concept of "chemogene therapy" is a viable one and likely will be a promising treatment for men with intraosseous prostate cancer metastasis. Systemic Ad-BSP-E1a in combination with low-dose taxotere appears to be the most efficacious regimen to control these prostate cancer tumors, and even cure some mice as evidenced by serum PSA, as a biochemical measurement of outcome. Data presented herein can be used as an initial step towards advancing these novel treatments and treatment regimens into humans. To build upon the data herein, pre-clinical formal toxicology testing can be done which then can initiate a new investigational drug application with the FDA.

RECENT PROGRESS

Year 3 of this research grant has progressed extremely well in most aspects. Remarkable effectiveness has been revealed in the prostate bone metastasis model using the therapeutic adenovirus Ad-BSP-E1a. Strikingly, the concept of chemogene therapy presented has become a viable option. It appears that combination of low-dose taxotere and intravenous Ad-BSP-E1a virus produces the best results in controlling these prostate cancer bone metastasis tumors in a mouse model. In humans, we would expect that this would also be true. We found that taxotere is the best agent to augment the effect of conditionally replication competent adenovirus. It is superior to Vitamin-C, Vitamin-D, and other inducing agents that we had previously tested in year 2 of the project.

Previously, we had encountered problems with the viral construction. In this year, we were successful at constructing Ad-hTERT-E1a. This is a conditionally replication competent adenovirus under the control of a 1.5 kg base pair promoter fragment which is the catalytic subunit of telomerase. This promoter fragment is termed hTERT. We believe that the hTERT directed promoter adenovirus, which is active in cancer cells which express telomerase, will be a great addition to our previously constructed Ad-BSP-E1a virus. In such, this virus may be used alone or in combination with the previously studied and constructed virus. Much work has been done upon the central nature of telomerase in prostate cancer, and we believe that a telomerase directed lytic adenovirus may be useful in the future for treating prostate cancer and other tumors.

Overall, the tasks proposed in this grant for all three years were accomplished. We have learned many new insights and believe our successes and failures laid a foundation for future research in prostate cancer, and even clinical trials directed at improving the lives of men afflicted with prostate cancer.

YEAR 3 PROGRESS SPECIFIC TASKS: OVERALL OBJECTIVE 3:

"Evaluation of Ad vector and chemotherapeutic synergism on CAR motif expression and histological and tumor-associated parameters both *in vitro* and *in vivo.*"

Technical objective 3 concentrated upon possible use on immunocompetent rats. The noble rat cell line was not used in this year secondary to the fact that Ad-BSP-E1a, the best viral construct, did not replicate in rat cell lines. As evidenced from the progress report in year 2, figures 16 to 19 E1a virus had slight cytotoxicity to the AIT noble rat cell line. However, it was not appreciably different than non-treated or dummy virus treated proliferation rate, as seen in figure 17 from last year. Because of this result and the known fact that in E1a conditional replication competent virus does not replicate in rat cell lines but does only in humans, we decided to change the course of this study. In addition, the mutant TK virus and the TK virus were judged to be not nearly efficient enough at cell killing to be effective in humans. This is seen in a number of papers evaluating thymidine kinase or TK adenovirus constructs. In addition, the TK virus was many log fold lower efficiency than the Ad-BSP-E1a virus at cell killing. Because of this fact, in year 3, we concentrated on the most efficacious "chemogene therapy" we could identify from previous experiments and papers published (reference 1). We identified that mini-dose taxotere would potentiate the effects of adenovirus from prostate cancer cells in at least an additive, if not a synergistic fashion. Based upon these previous results, we initiated a long-term experiment with the intraosseous model of C42B PSA producing cell line.

This model system was designed to test the efficacy of our aforementioned, and tested Ad-BSP-E1a virus alone or in combination with mini-dose taxotere (1 mg/kg), to ascertain if the clinical effects of biochemical PSA free recurrence were potentiated. A large mouse experiment was initiated to compare phosphate buffered saline, E1a virus alone, E1a virus plus mini-dose taxotere, and thymidine kinase (TK) dummy virus plus taxotere (which is a taxotere alone equivalent group). Please see figures 10 – 11 and the figure legends for the results. From the results demonstrated in the figures, it is apparent that mini-dose taxotere along with dummy TK virus has no statistically significant difference effect on the PSA production (and therefore tumor volume) than phosphate buffered saline. It is evident that the Ad-BSP-E1a virus has a significant effect however, the effects of PSA recurrence are prolonged with the addition of taxotere. Indeed, these results are significant because the small-dose taxotere (1mg/kg given in three doses over 10 days) really had no independent effect on the mice (as evidenced in the TK dummy virus plus taxotere group, which is not statistically significant different than the true control of saline-treated group). These results reveal that a conditionally replication competent adenovirus such as Ad-BSP-E1a, plus a small dose taxotere likely have synergism in killing human prostate cancer cells when in the metastatic bone microenvironment. This has great implications for the testing and treatment of humans afflicted with prostate cancer metastasis in the bone.

SPECIFIC TASKS

** All specific tasks 1-10 that were initially proposed were completed in one fashion or another, with progress yearly reports 1 and 2 containing the data for most of tasks 1-8.

Task 1, month 1-6: clone GRE_4 upstream of BSP promoter and construct virus.

We were unable to make this viral construct. In addition, *In vitro* data in year 2 revealed plasmid construct GRE-BSP was no better than BSP alone, so attempts were abandoned to make mature Ad virus.

Task 2: month 7-12: Production of Ad-hTERT-E1a virus.
We were successful in producing this construct using PCR amplification, and homologous recombination. Please see figures 1-8, with explanation. Three initial attempts to make Ad-hTERT-E1a virus failed. These failed attempts included the following procedures: clone plasmid hTERT into p Shuttle, and then blunt end clone to pBPA E1 II; clone hTERT to Bluescript SK, then blunt end clone to pBPA E1 II; and , TA clone to PCR out the hTERT and do ECORi single cutting site cloning. The **successful strategy used the plasmid p Shuttle-hTERT(from jery Shay, PhD) as a template to TOPO TA PCR out the hTERT 1.4 kb DNA fragment**. Multiple TOPO clones were correct, and were confirmed by enzymatic digestion and sequencing. The TOPO hTERT was then placed into the pBPAE1 II plasmid via directional enzymatic placement. Multiple pBPAE1-II –hTERT subclones were correct by digestion confirmation, and sequencing. Ad-hTERT-E1a was then made by homologous recombination via pJM17 in 293 cells using the single plaque purification technique. Expansion of Ad-hTERT-E1a will be done in 911 cells.

Task 8, month 14 – 20: Test various ad constructs *in vitro* on AIT rat cell line with and without Vitamin D and glucocorticoid.
This task was completed in year 2. Please refer to year 2 progress report for figures, legends, and descriptions.

Task 9, month 21-30: Test Ad constructs with enhancers and chemotherapeutics *in vitro* AIT rat cell line and correlate these results to tumor factors(PSA) and CAR motif expression.
This task was completed in year 2. Please refer to figures and legends in year 2 progress report.

Task 10, month 30-36: Test best combination of Ad vectors, inducers, and chemotherapeutics in *in vivo* AIT immunocompetent noble rat model.

Please refer to above description on the previous two pages. This last task was aborted since the best (most potent) virus made was the Ad-BSP-E1a construct- and this best virus did not **replicate** in rat cells and apparently only replicates in human cells. Previously, we had hoped that a mutant TK or a TK system could be tested in immunocompetent noble rats, however, the Ad-BSP-TK and mutant TK constructs have extremely low power to kill prostate cancer, compared to the replication competent construct Ad-BSP-E1a, and it was deemed that further testing of this system (Ad-BSP-Tk, and thus the noble rat line) for use in humans is not worthwhile. Instead, we developed and tested "chemogene" protocol regimen, consisting of Ad virus (Ad-BSP-E1a) and mini-dose taxotere (please see figures 10 and 11 and legends for data).

KEY RESEARCH ACCOMPLISHMENTS

1. Confirmation that Ad-BSP-E1a in an ***in vivo*** bone osseous model is extremely potent in controlling prostate cancer. Statistical significance was achieved. Please see figure 9 (Poster, AACR) for data. In some instances, it produces a cure in the mouse model.
2. Cloning of conditionally replication competent adenovirus termed, Ad-hTERT-E1a. This adenovirus is under the control of human telomerase catalytic subunit promoter and will be very useful for prostate cancer. It has the potential to be used alone or in combination with Ad-BSP-E1a virus to treat human prostate cancer.
3. Testing and confirmation in osseous bone metastasis model that systemic Ad-BSP-E1a adenovirus **plus** mini-dose taxotere has **synergism** in the controlling of human

prostate cancer as evidenced by PSA outcomes. Please see figures 10 and 11, and corresponding legends.

4. Confirmation of absence of BSP IHC staining in harvested femurs of Ad-BSP-E1a treated mice(see poster, figure 9 for data, and pictures).

REPORTABLE OUTCOMES

Intravenous Bone Sialoprotein Promoter-Based Replication Competent Adenovirus, Treatment of Bone Metastatic Prostate Cancer. Li, Hudson, Kacka, Baseman, Wilhelm, Elmore, and Koeneman presented at American Association of Cancer Research (AACR) Annual Meeting, Orlando, Florida, March 2004. (see figure 9)

CONCLUSIONS

Based upon the vigorous ***in vivo*** and ***in vitro*** studies conducted above from the last 3 years, Ad-BSP-E1a is a viable construct to treat advanced osseous hormone-resistant prostate cancer. Ad-hTERT –E1a needs to be further tested and should be the basis for further pre-clinical studies. The chemotherapeutic drug, taxotere, given in mini-dose fashion has synergism with Ad-BSP-E1a virus in the treatment of bone metastatic prostate cancer. The combination of taxotere and Ad-BSP-E1a virus should be moved forward to hopeful human testing in clinical trials.

REFERENCES

1. Yiming Li, T Okegawa, D. Lombardi, E. Frenkel, JT Hsieh. Enhanced transgene expression in androgen independent prostate cancer gene therapy by taxane chemotherapeutic agents. J. of Urology , volume 167, 339-346, jan 2002.

LIST OF PERSONNEL

Kenneth S. Koeneman, M.D. (PI)
Yingming Li, M.D. (Research fellow)
Melissa Hudson, B.S. (Technician)

LIST OF APPENDICES

Figure 1: This is a schematic of the base pairs of the promoter region of the hTERT promoter sequence. hTERT is the catalytic sub unit of human telomerase enzyme. The cloned promoter sequence is approximately 1.4 kilobase pairs long and corresponds to the sequence of DNA in this figure.

Figure 2: This is the cloning schemata of the human hTERT into the E1 Shuttle Vector and the homologous recombination with plasmid pJM 17, which is the "1st generation" Ad genome. This schemata was successful in producing appropriate virus.

Figure 3: This is the PCR gel confirmation of obtaining the correct hTERT 1.4 kilobase fragment. Using P Shuttle hTERT plasma obtained from Dr. Jerry Hsieh at UT Southwestern, the appropriate construct was obtained. P Shuttle hTERT was used as template to PCR out hTERT 1.4 kilobase pair fragment. In the gel/diagram, the appropriate fragment was seen with the arrow pointing to it.

Figure 4: Multiple subclones of TOPO cloning vector was obtained which contained hTERT subclones. Clone 2 is starred and this was picked for further investigation.

Figure 5: This is the sequencing data confirming that TOPO hTERT cloned 2 was the correct DNA sequence. This is seen for approximately 450 base pairs.

Figure 6: This is the schemata showing cloning of hTERT into the plasmid pBPAE1-hTERT shuttle vector. Included is the enzymatic digestion sites used.

Figure 7: This is a **PCR gel** confirming that the pBPAE1-hTERT subclones are correct. Enzymatic digestion with EcoR1 and Xho 1 confirmed the correct fragment size on multiple clones. Partial sequencing of this fragment for approximately 500 base pairs confirmed once again correct sequence was obtained(sequence data not shown).

Figure 8: Ad-hTERT-E1a virus was constructed using homologous recombination with the pJM17 adenovirus vector backbone. Clone 5 was a single clone and it was harvested and viral genomic DNA was extracted. PCR with primer A3A4 for the virus and hTERT primer A and B were used to confirm the virus contains the appropriate inserts. Depicted in the gel/diagram on the left is the viral (correct) insert sizes and on the right, the promoter (correct) insert sizes.

Figure 9: This reveals American Association Cancer Research abstract #1192 which was presented this spring in Orlando, Florida. This abstract serves the basis for a full manuscript which is in preparation. Over 10 months, the data from last year has been updated especially with the intraosseous results seen in row 3. We have added a number of animals to the control groups to **obtain statistical significance ($p< 0.05$).** On the right side on row 3 is a bar graph which reveals Ad-BSP-E1a treated group had a complete response of 8 and a partial response of 2, whereas the control PBS and Ad-BSP-TK(dummy virus) groups had minimal to no response. Immunohistochemistry was also performed revealing difference in bonesialo protein (BSP) expression. In the cured and Ad-BSP-E1a treated animals, little BSP expression was obtained, which was hoped for and expected. **This poster serves as one of the the major outcomes of this grant. Development of this construct can now be entertained to enter human trials.**

Figure 10: This is a line graph of PSA versus time in 4 treatment groups. The data revealed the combination of Ad-BSP-E1a with mini dose Taxotere improves PSA outcome in an osseous model. Controls groups are PBS, and TK with Taxotere. TK is a dummy virus and Taxotere in mini dose fashion reveals it is no different than PBS or saline treated. The Ad-BSP- E1a group at 10 weeks with 7 animals has a higher PSA, than E1a plus Taxotere. The average PSA for the E1a group is 8.14 ng/ml plus or minus the standard error(SE) of 0.86. The E1a -Taxotere group average PSA is 6.57 ng/ml plus or minus 0.78. In comparison, the control groups PBS is 22.27 ng/ml plus or minus 1.25, and TK plus Taxotere is 96.32 ng/ml plus or minus 5.25 PSA. Over the next 6 weeks, we will continue these animals out further, drawing PSA every 2 weeks. **There should be increased difference(separation of curves) in the E1a versus E1a-Taxotere groups, based upon previous studies with E1a in that some animals will recur late (week 12-20).** As such, the synergism of Taxotere plus Ad-BSP-E1a virus could be a major finding and a major advance for treatment in men with human prostate cancer.

Figure 11: This reveals a bar graph showing E1a plus Taxotere group has lower PSA values than control or virus alone groups. Standard error bars are on the graph however, the standard errors in each group are quite small, and barely discernable. Please refer to data explanation in Figure 10.

Figure 1: hTERT promotor fragment with "active sites"

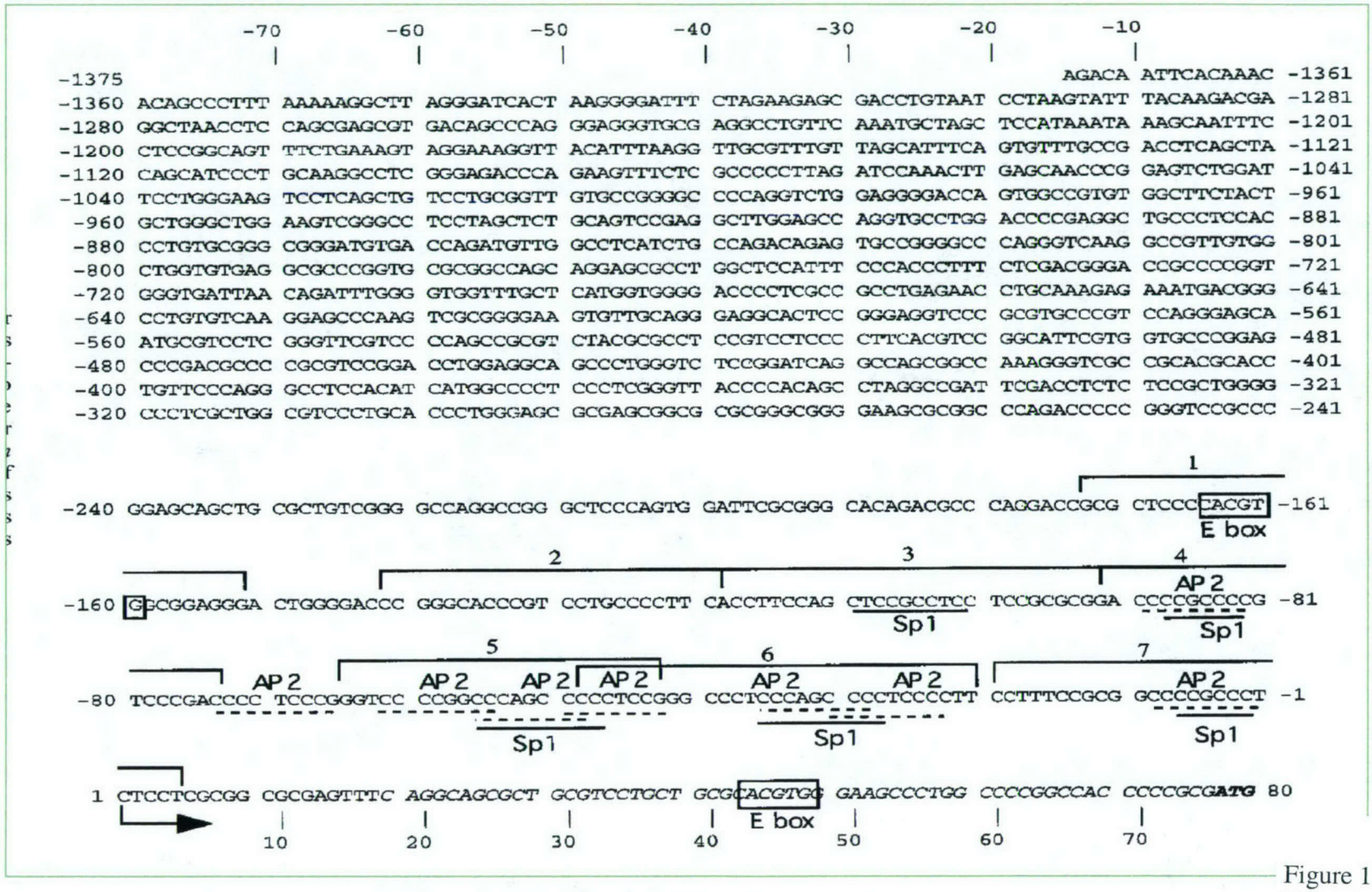


Figure 1
K.S. Koeneman
DAMD17-01-1-0107

Figure 2:Cloning schemata- the Human hTERT Promoter into E1 Shuttle Vector

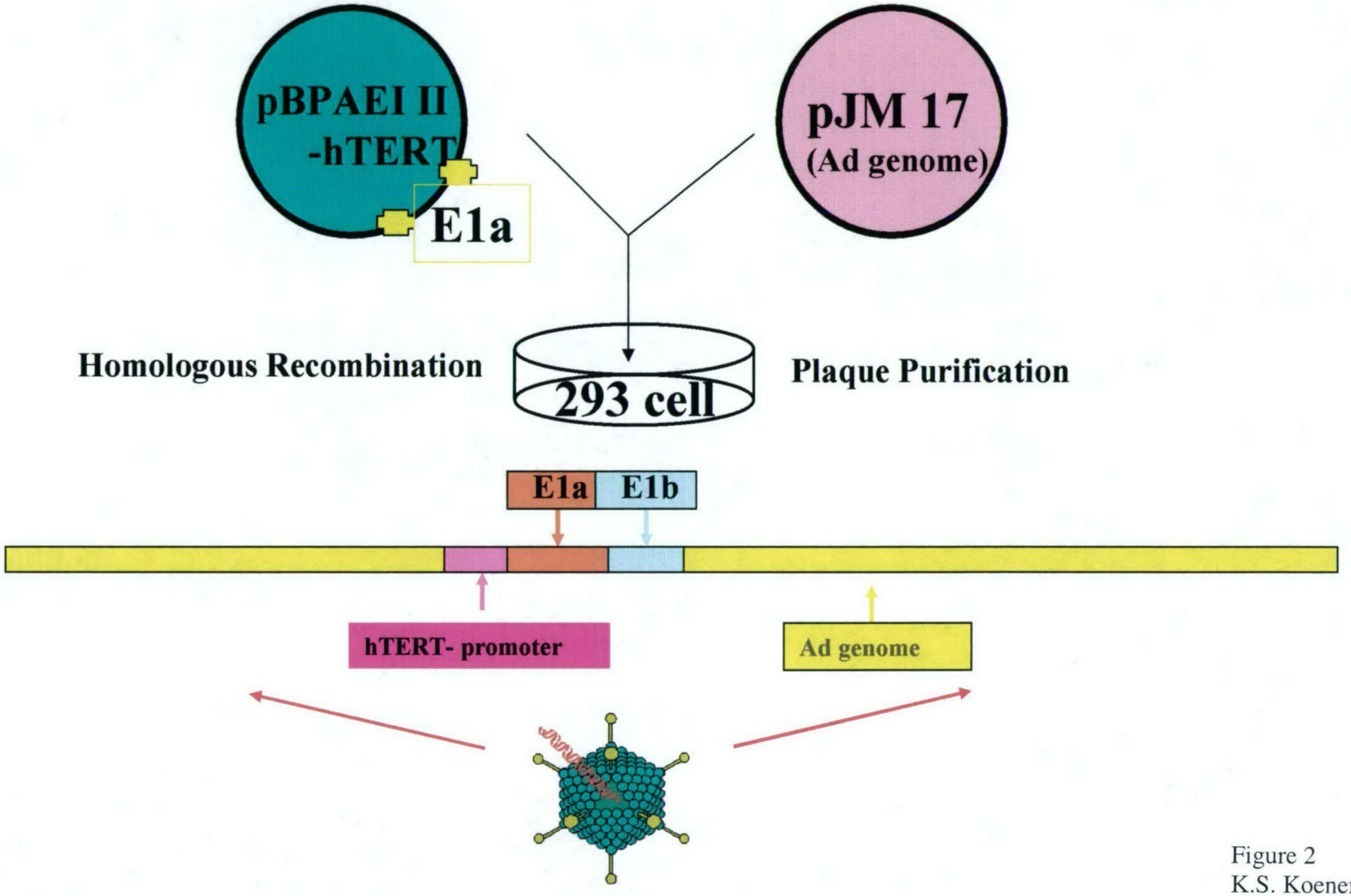


Figure 2
K.S. Koeneman
DAMD17-01-1-0107

Figure 3:Using pShuttle-hTRET as template to PCR hTERT 1.4kB fragment

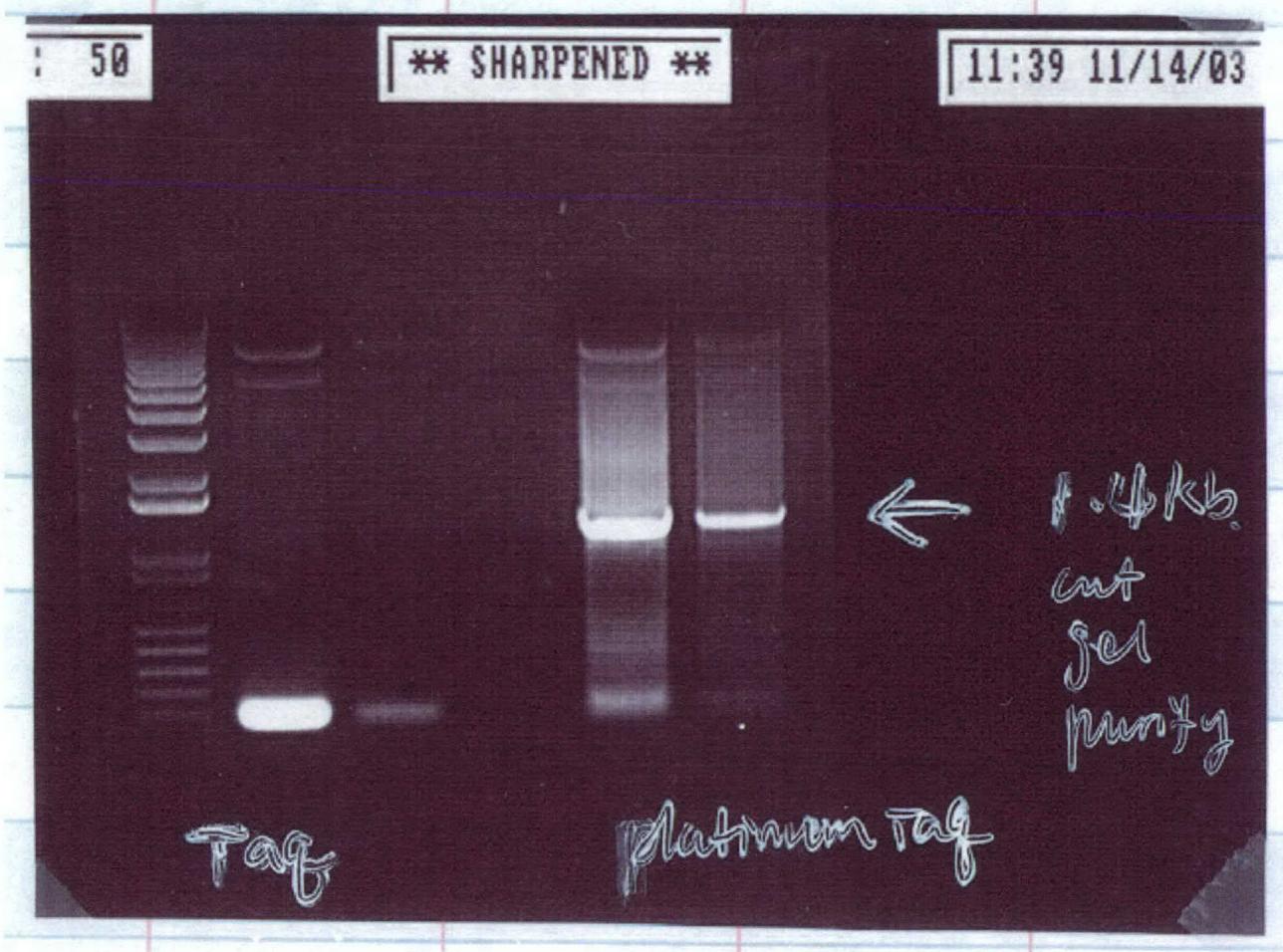


Figure 3
K.S. Koeneman
DAMD17-01-1-0107

Figure 4:TOPO-hTERT subclones obtained,-clone 2 picked for further use

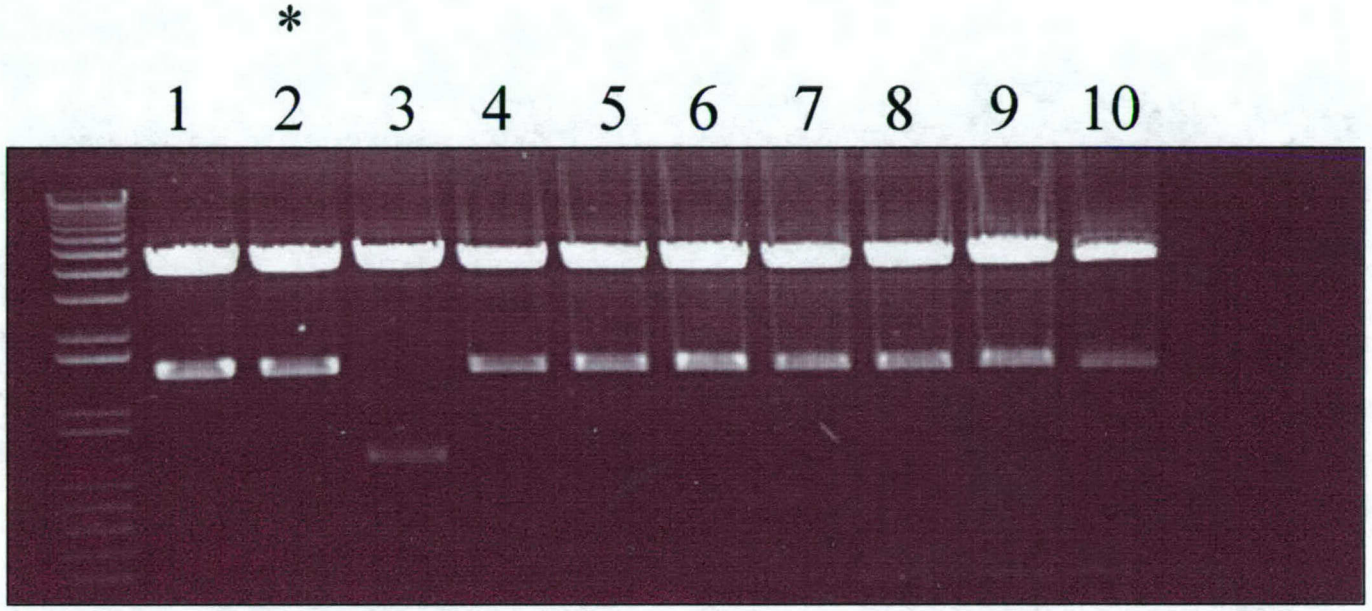


Figure 4
K.S. Koeneman
DAMD17-01-1-0107

Figure 5 : sequencing confirms correct hTERT promoter fragment

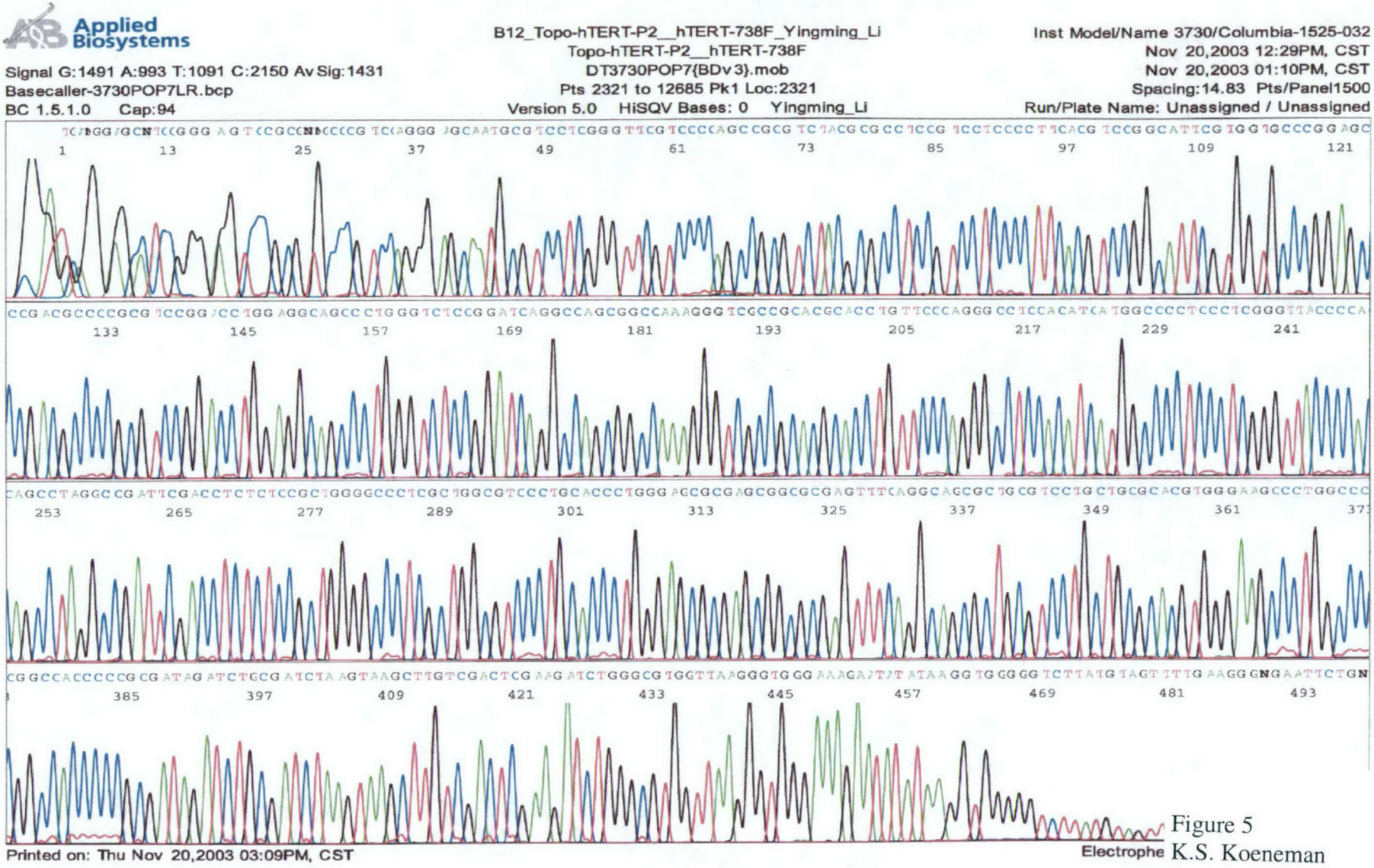


Figure 5
K.S. Koeneman
DAMD17-01-1-0107

Figure 6: cloning scheme for shuttle vector with enzyme sites

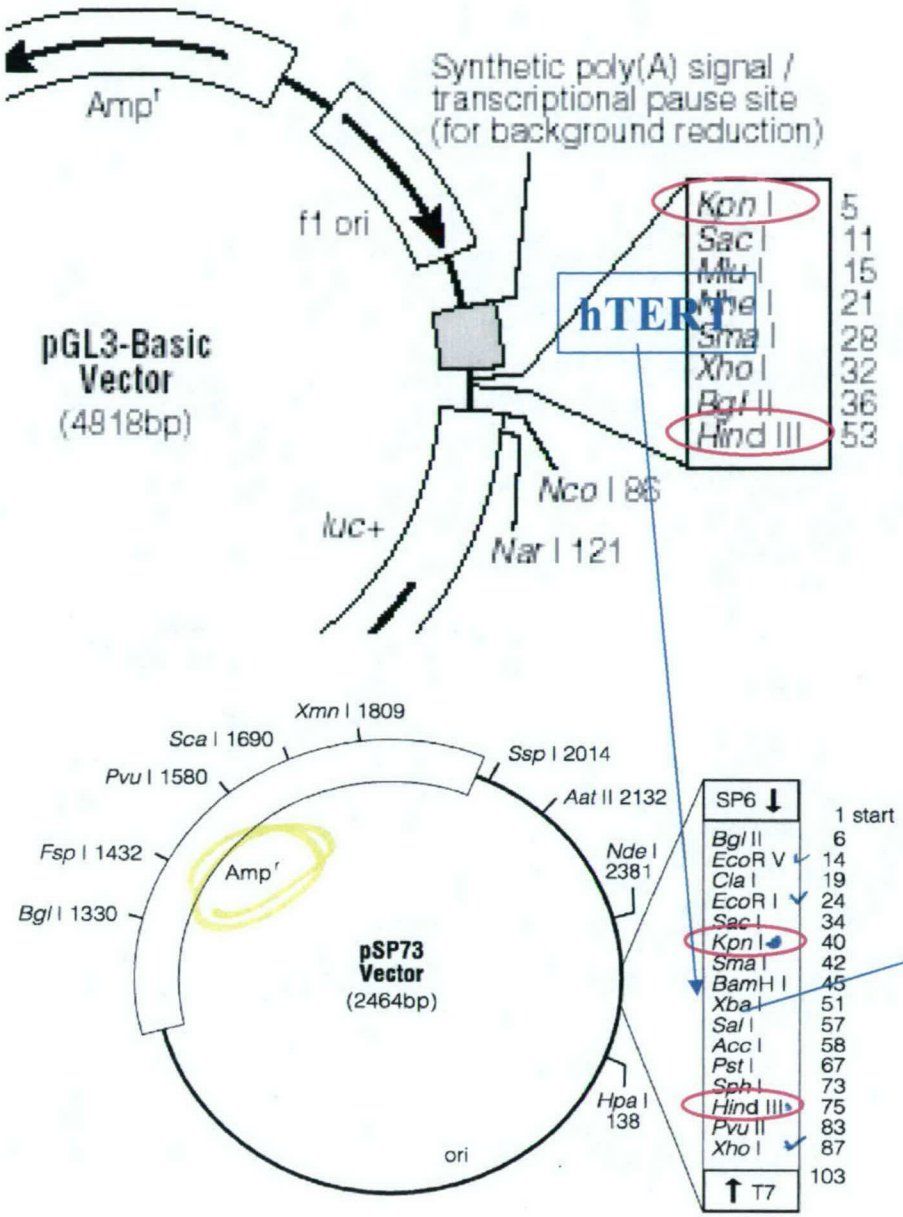
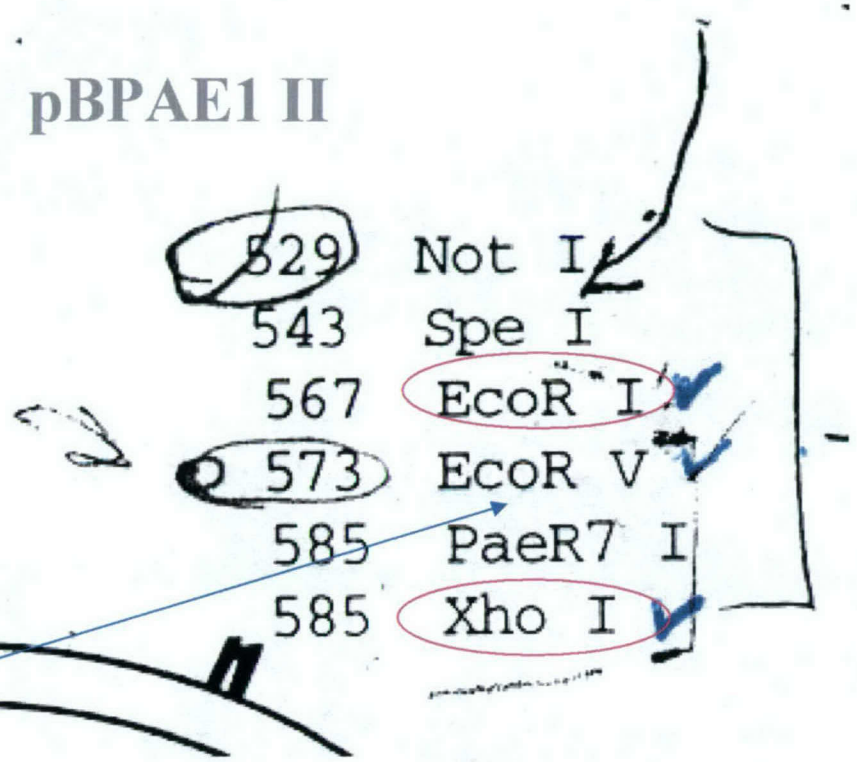


Figure 2. pSP73 Vector circle map and sequence reference points.



pBPAE1-hTERT shuttle vector

Figure 6
K.S. Koeneman
DAMD17-01-1-0107

Figure 7: pBPAE1-hTERT Subclones Digested With EcoR I and Xho I--Multiple correct clones obtained.

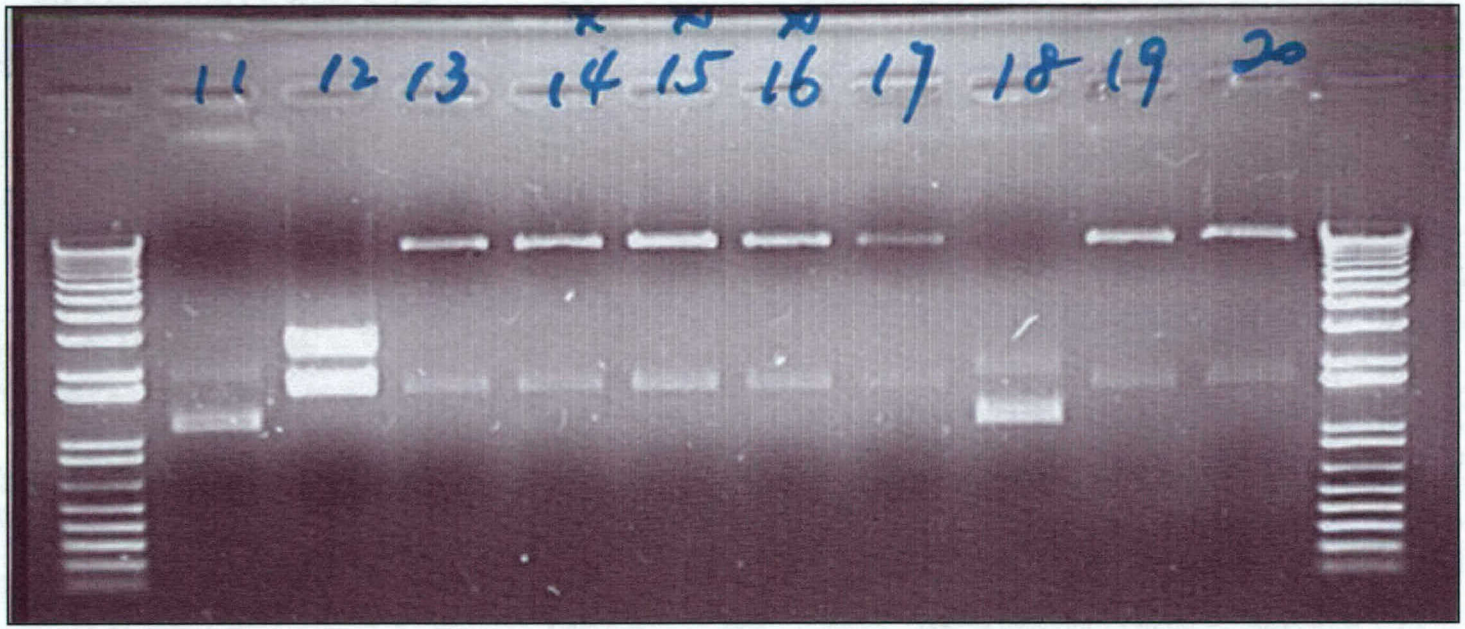
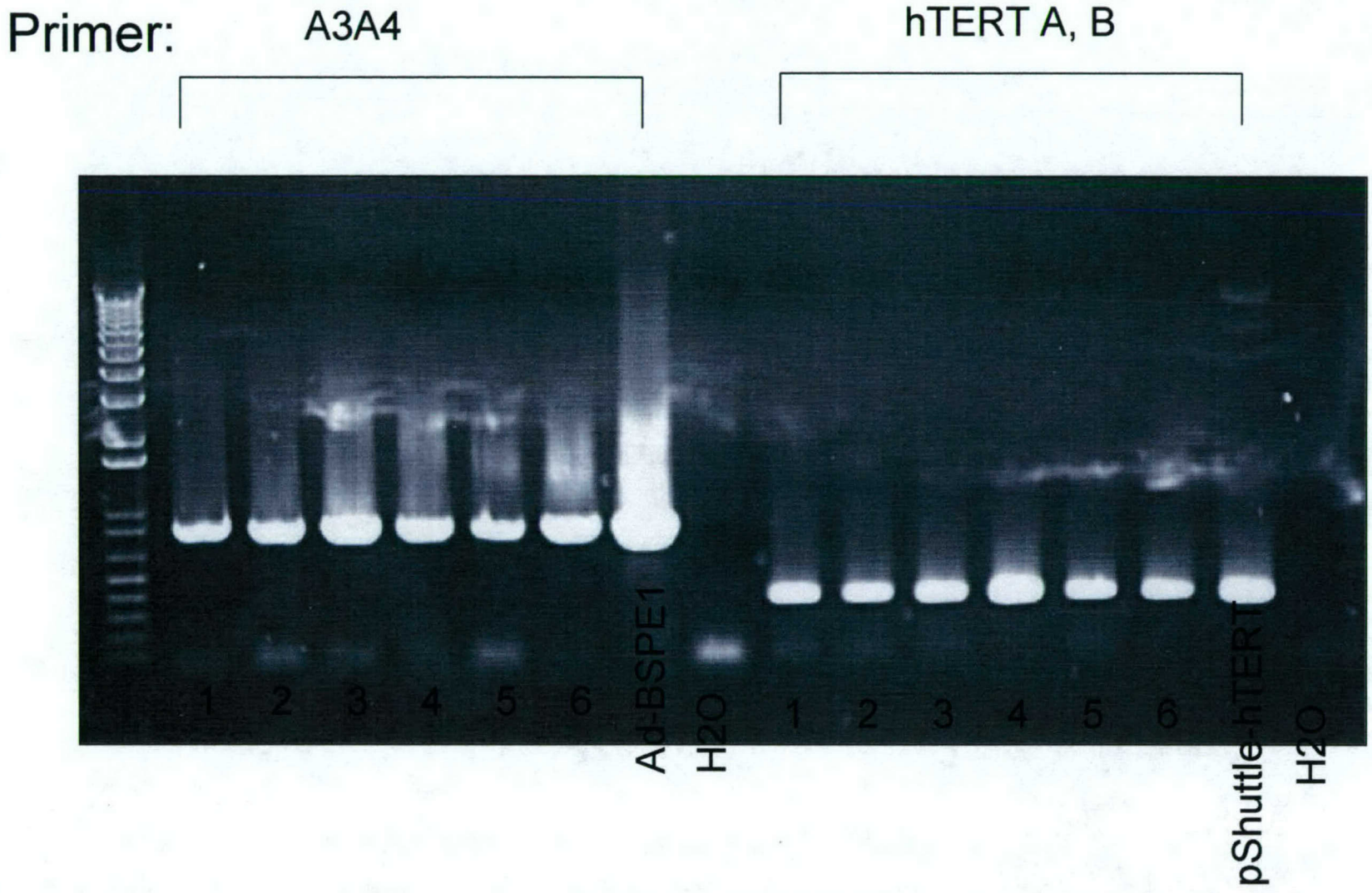


Figure 7
K.S. Koeneman
DAMD17-01-1-0107

Figure 8: RT-PCR Confirms the Virus and correct hTERT Insert are present- viral sequences on left, and promoter on right match controls for all 6 constructs



Abstract # 1192

Intravenous Bone Sialoprotein Promoter-Based Replication Competent Adenovirus Treatment of Bone Metastatic Prostate Cancer

Yingming Li, Melissa Hudson, Michael Kacka, Adam G. Baseman, David M. Wilhelm, James M. Elmore, and Kenneth S. Koeneman

University of Texas Southwestern Medical Center, Dallas, TX

Abstract

Introduction: Bone sialoprotein (BSP)-based promoter-driven toxic adenovirus has been shown to limit the growth of prostate cancer cells in vitro and in vivo. Replication-competent adenovirus (Ad-BSP-E1a) was used to treat xenograft bone tumors established in mice via the intravenous (IV) route. BSP expression is limited to calcifying tissues, as well as osteotropic tumors, such as prostate cancer.

Methods: A replication-competent adenovirus, Ad-BSP-E1a, was constructed, and *in vitro* cytotoxicity assays were used to evaluate sensitivity to the virus and promoter-specificity. Subcutaneous (SC) xenografts of C4-2B prostate cancer cells were injected in nude mice with a dose of Ad-BSP-E1a (2.5×10^9 pfu) or control. The *in vitro* and *in vivo* control used was Ad-BSP-TK (without ganciclovir). Intraosseous (IO) femoral xenografts were created in mice with PSA-producing C4-2B prostate cancer cells and were treated IV with one or more doses of Ad-BSP-E1a (2×10^9 pfu) via tail vein.

Results: Prostate cancer cell lines C4-2 and C4-2B expressed BSP and were very sensitive in vitro to Ad-BSP-E1a (MOI < 0.1). SC xenografts revealed that C4-2B cell lines can be completely cured by intratumoral injections of Ad-BSP-E1a. The average volume of Ad-BSP-TK treated tumors increased 227% from week 0 to week 4, while E1a treated tumors decreased 81.8 %. The pattern and scale for the average serum PSA for each treatment group varies in comparison to the average tumor volume, but still has a similar trend. Ad-BSP-E1a tumors show a generally decreasing PSA from week 2 to week 10, and the average PSA for the Ad-BSP-E1a treated mice remains lower than Ad-BSP-TK treated mice. Nine of 11 femoral C4-2B xenografts were found to be sensitive to IV Ad-BSP-E1a therapy. We found a 82% PSA cure (PSA ≤0.1) and 18% non-response. Ad-BSP-TK (without ganciclovir) treated animal groups showed a 70% non-response rate. All 8 mice in the PBS treated group were non-response. Chi-square analysis of serum PSA in the C4-2B IO mice at week 8 post treatment showed a significant difference between Ad-BSP-E1a and PBS or Ad-BSP-TK treated groups (P<0.05). IHC of both PSA and BSP showed decreased staining in the Ad-BSP-E1a treated group, indicating the tumor cells were eradicated by the treatment.

Conclusions: IV Ad-BSP-E1a is a tumor-specific modality for treating intraosseous prostate cancer. The observed response was "all or none," with no evidence of a dose dependent response.

Materials and Methods

Generation of Tissue Specific, Replication Competent Adenovirus, Ad-BSP-E1a: The virus was engineered by inserting the BSP promoter into the shuttle vector with the E1a region of the adenovirus genome. The BSP-E1a shuttle vector and the pJM17 vector were cotransfected into 911 embryonic kidney cells to generate the recombinant virus Ad-BSP-E1a. PCR and sequencing were used to confirm the creation of the desired clone. The Ad-BSP-E1a was amplified and purified by cesium chloride gradient ultra centrifugation.

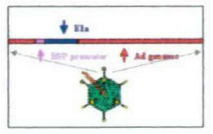
***In Vitro* Cell Proliferation Studies:** Prostate cancer cell lines C4-2 and C4-2B demonstrated BSP. For each cell type, 5,000 cells per well were plated on 12-well plates and treated with Ad-BSP-E1a, Ad-BSP-TK without ganciclovir, or PBS. Cells were fixed and stained with crystal violet on days 1, 3, 5 and 7.

***In Vivo* Subcutaneous Prostate Cancer Xenografts:** C4-2B prostate cancer cells (1×10^6) were mixed with Matrigel (BD Biosciences) and injected into the subcutaneous tissue on the flank of nude, irradiated mice. Once measurable tumors had been established, intratumoral injections of Ad-BSP-E1a, Ad-BSP-TK without ganciclovir, or PBS were given. A second viral injection was given 3 weeks after the first. Tumor size and serum PSA were measured on a weekly basis.

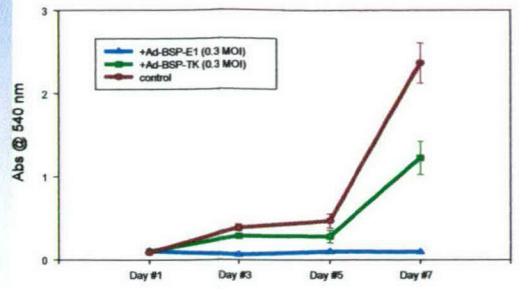
***In Vivo* Intraosseous Prostate Cancer Xenografts:** C4-2B prostate cancer cells (1×10^6) were injected into the right femur of nude, irradiated mice. Bilateral orchiectomy was performed 2 weeks after tumor cell inoculation. Detectable PSA levels were found at 40 days following injection. Ad-BSP-E1a or control virus or PBS was injected into the tail vein (2×10^9 pfu) twice at week0 and week 3. Serum PSA was measured on a weekly basis. Radiographic and histologic analysis of the bone tumors were performed.

Results

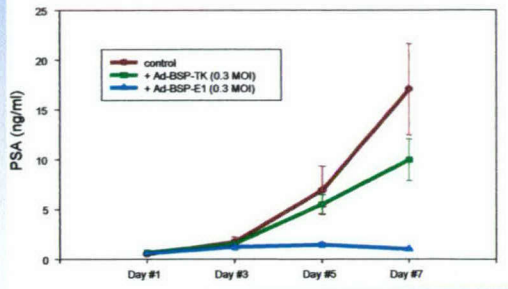
Ad-BSP-E1a Construct



C4(2) Cell Proliferation Assay

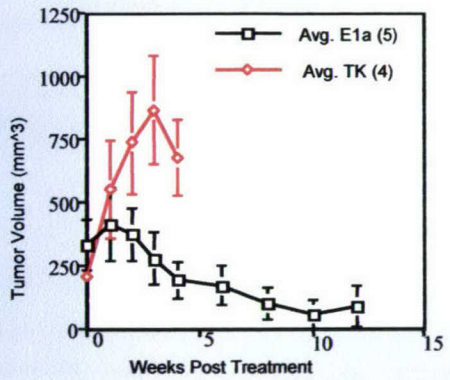


C(4)2 Cell Line PSA Production

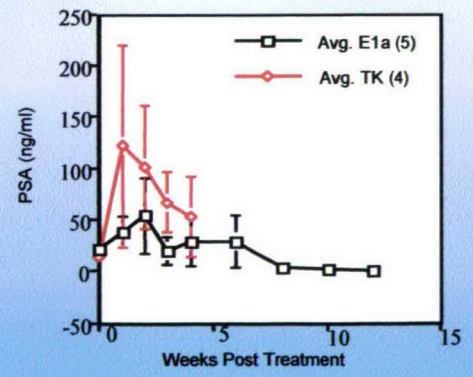


Tumor volume and PSA changes were correlated in response to E1 virus treatment

C4(2)B S.C. Tumor Volume Following Treatment

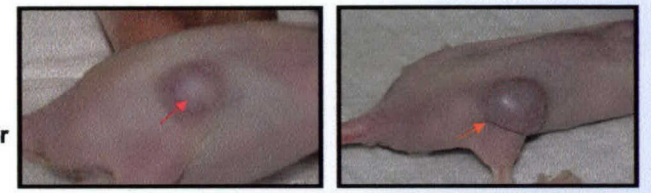


C4(2)B S.C. PSA Values Following Treatment



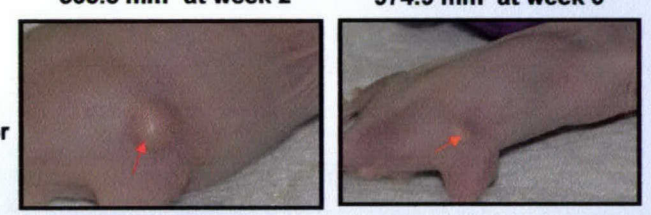
C4-2B Subcutaneous tumor volume of viral treatment

Ad-BSP-TK treated tumor



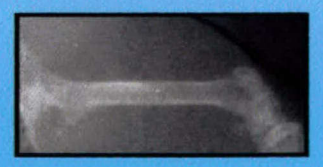
360.5 mm³ at week 2 — 974.9 mm³ at week 5

Ad-BSP-E1a treated tumor



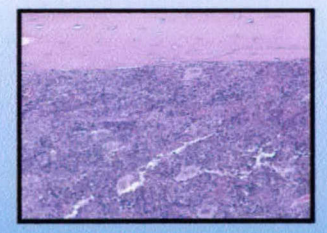
177.9 mm³ at week 2 — Too small to measure at week 5

Normal Femur

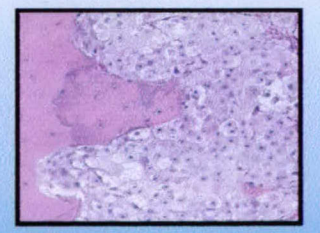


Evidence of Osteoblastic Prostate Bone Cancer

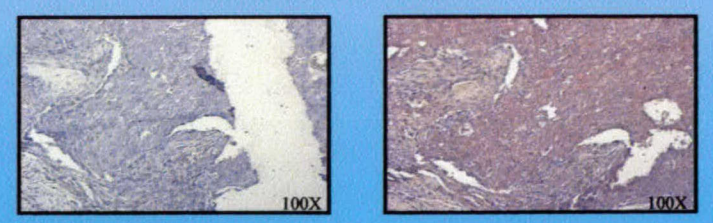
Normal Bone Control H & E Stained



C4-2B Cells Within Right Femur – H & E Stained



PSA & BSP Double Stain

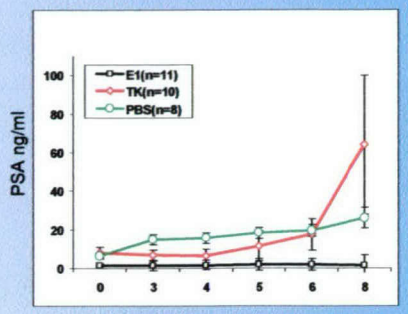


C4(2)B I.O. #30, PBS Treated, 24 weeks post treatment Final PSA=399.9 ng/ml

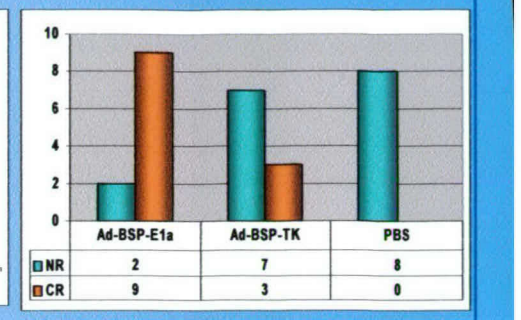
PSA=Red
BSP=Brown

Results

C4(2)B Intraosseous PSA Following Treatment Over Time



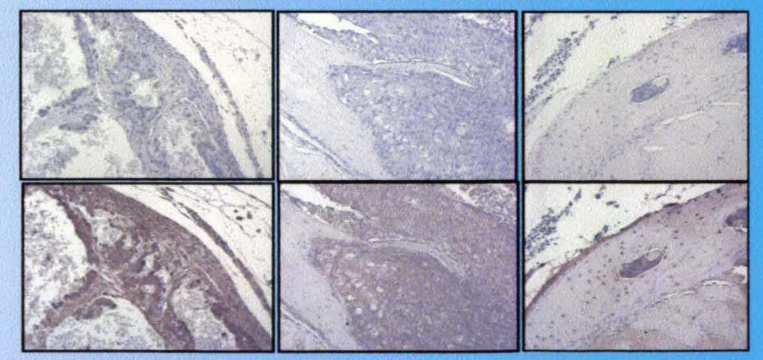
C4-2B Intraosseous Tumors Treated IV PSA Response to Treatment at Week 8



Chi-square analysis with 1 degree of freedom
Ad-BSP-E1a VS Ad-BSP-TK, P=0.017
Ad-BSP-E1a VS PBS, P<0.001
Ad-BSP-TK VS PBS , P=0.09

CR: number of mice with complete response, PSA<1.0ng/ml at the end (week 8) of treatment
NR: number of mice with no response, PSA>1.0ng/ml at the end (week 8) of treatment

C4(2)B Intraosseous Expression of BSP Among Different Treatment Groups



100X #30 PBS — #31 Ad-BSP-Tk — #37 Ad-BSP-E1a

Conclusion

Intravenous Ad-BSP-E1a is a tumor-specific modality for treating intraosseous prostate cancer. The observed response was "all or none," with no evidence of a dose dependent response. Future work includes immune invasive and ligand-targeting capacities which will hopefully make this therapy feasible for humans affected with metastatic osseous prostate cancer.

Figure 9
K.S. Koeneman
DAMD17-01-1-0107

Figure 10:Combination of Ad-BSP-E1a with minidose taxotere improves PSA outcomes in osseous model

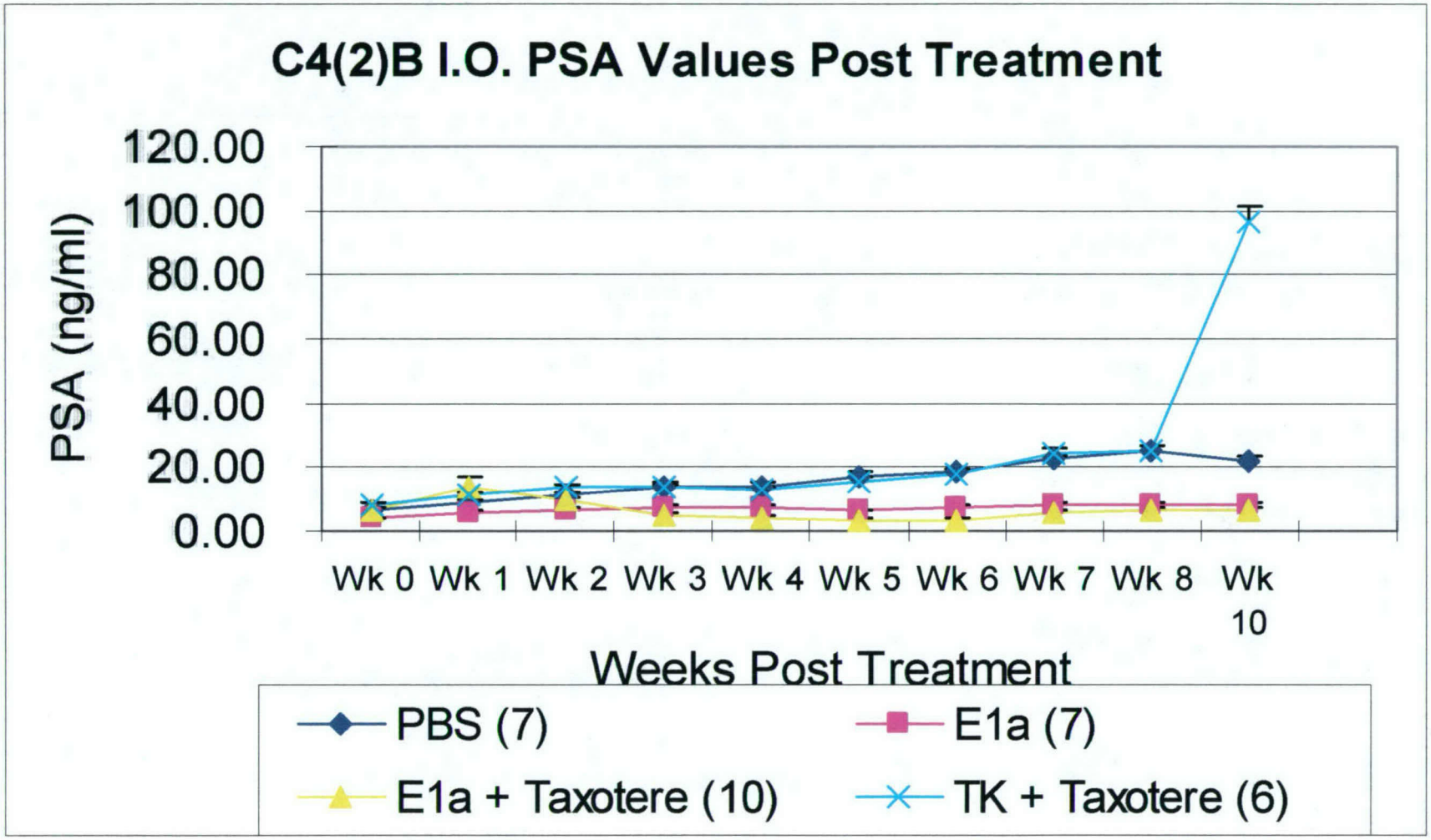


Figure 10
K.S. Koeneman
DAMD17-01-1-0107

Figure 11:E1a plus taxotere group has lower PSA values than controls(PBS, TK+taxotere), and Virus alone group(E1a)

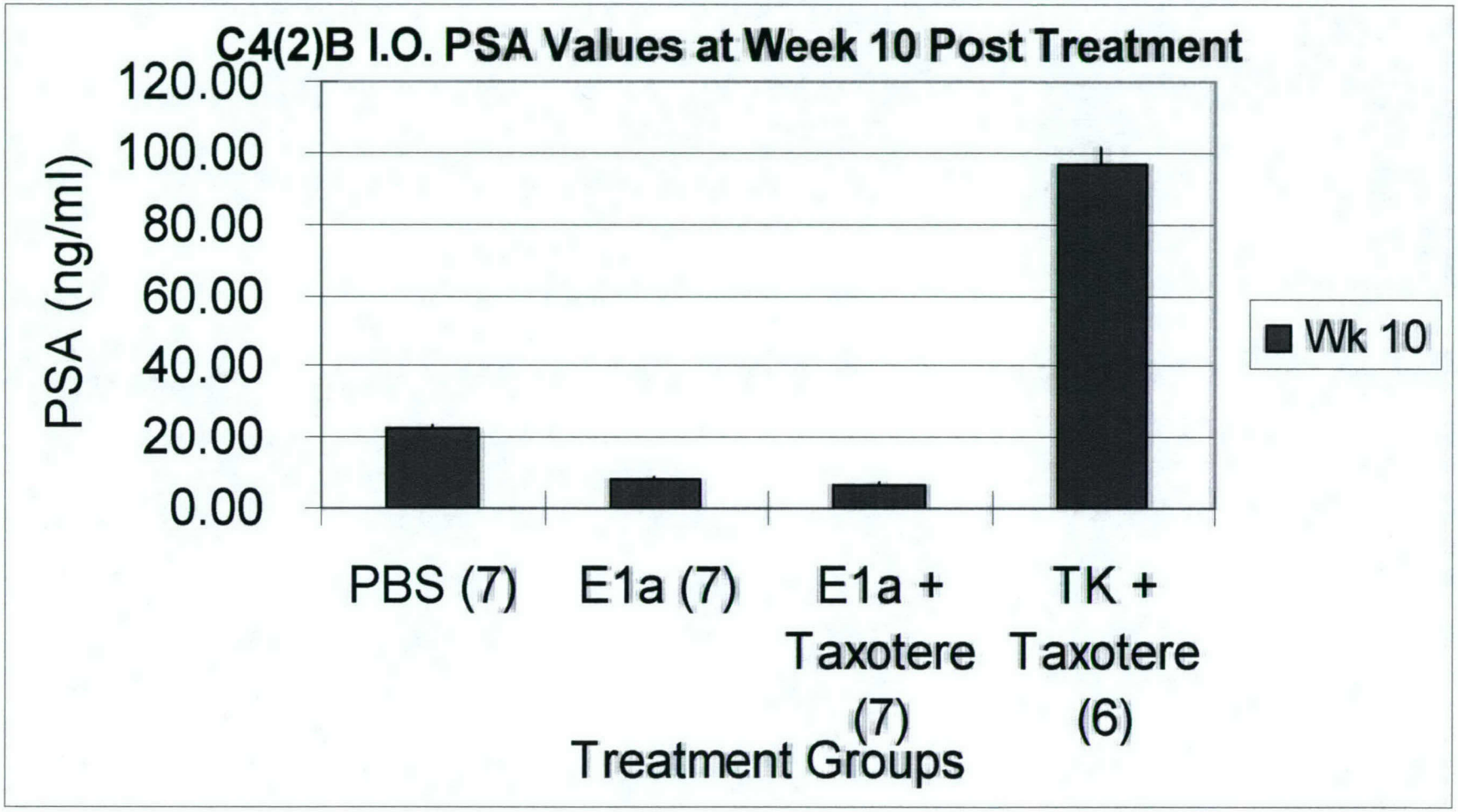


Figure 11
K.S. Koeneman
DAMD17-01-1-0107