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TITLE: Combinational Targeting of Prostate Carcinoma Cells and Tumors Associated Pericytes with Antibody Based Immunotherapy and Metronomic Chemotherapy

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14. ABSTRACT The hybridoma secreting the HMW-MAA-specific mAb 225.28 which is used for immuno prevention of prostate carcinoma and the hybridoma secreting the isotype matched mAb F3C25 have been tested for activity. Ascitis has been prepared and monoclonal antibodies have been purified and monitored for purity and activity. The colony of TRAMP mice has been expanded to test the efficacy of mAb 225.28 plus cyclophosphamide metronomic therapy in the inhibition of progression of prostate cancer. Sixty-four TRAMP mice have been enrolled in the combinatorial treatment schedule. Animals are being screened 2 times a week for palpable tumors.					
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Combinatorial targeting of prostate cancer cells and tumor associated pericytes with antibody-based immunotherapy and metronomic chemotherapy.

PROGRESS REPORT

INTRODUCTION

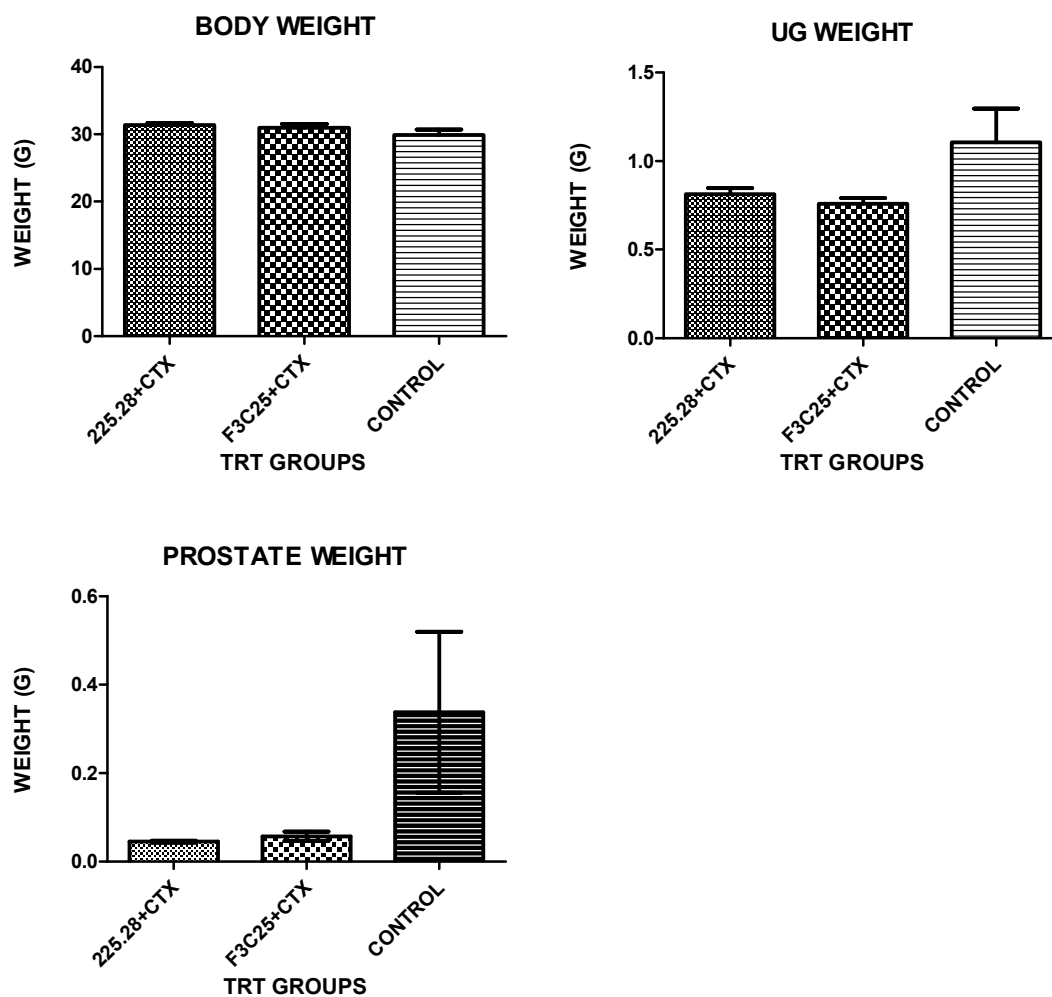
The lack of efficacy of conventional therapies in prostate cancer has stimulated interest in developing and implementing novel therapeutic strategies. Among them is immunotherapy. To immunize the negative impact of escape mechanisms on the outcome of immunotherapy, the present proposal aims at showing that the efficacy of immunotherapy of prostate cancer can be enhanced by targeting not only cancer cells, but also activated pericytes in the tumor microenvironment with a combinatorial immunotherapy. The latter includes AN-2-specific monoclonal antibody and continuous administration of low dose cyclophosphamide, i.e. metronomic chemotherapy.

BODY

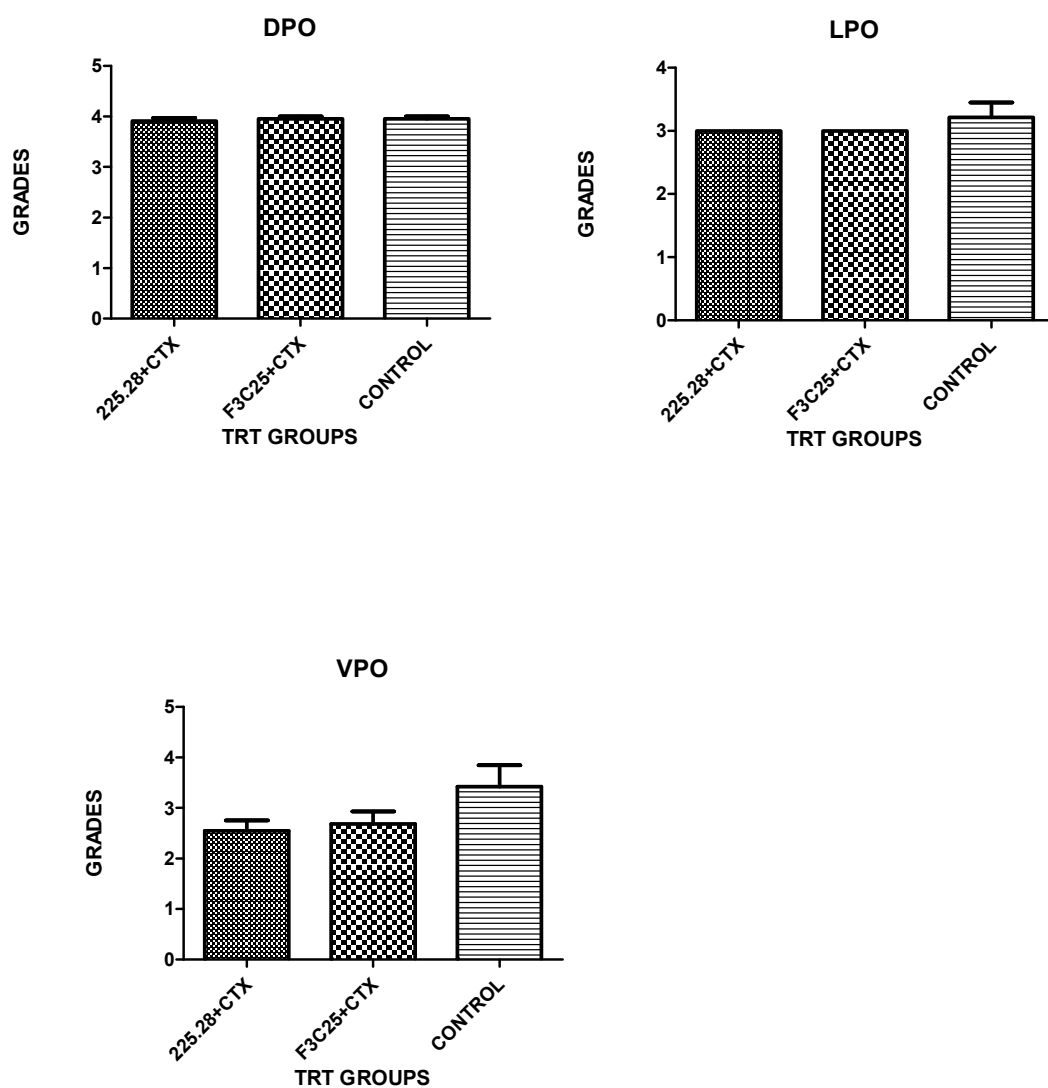
Delay of tumor progression in TRAMP mice by combinatorial targeting of prostate cancer cells and activated pericytes in the tumor microenvironment with AN2-specific mAb in combination with CTX metronomic chemotherapy. TRAMP mice were bred and enrolled on treatment starting at 10 weeks-of-age. Mice in cohort 1 (**225.28 + CTX**, n=27) received i.p. injections of AN2-specific purified mAb (225.28) at 100µg/injection/mouse in combination with oral administration of metronomic chemotherapy of cyclophosphamide (CTX) at a dose of 10 mg/kg/day in drinking the water throughout the experiment. Mice in cohort 2 (**F3C25 + CTX**, n=26) received i.p. injections of purified irrelevant isotype matched mAb (F3C25) at 100µg/injection/mouse in combination with oral administration of metronomic chemotherapy of cyclophosphamide at a dose of 10 mg/kg/day in drinking the water throughout the experiment. Mice in cohort 3 (**Control**, n=24) were left untreated as a reference for the natural course of the disease. Animals were observed 3 times a week (MWF) for signs of toxicity and tumor formation. Antibody injections were given on Tuesdays and Fridays. At 18 weeks-of-age the animals were euthanized and prostatic tissues and relevant data collected. Body weights, reproductive tract weights and prostatic weights were collected. (**Graph 1**) There was no difference in the body weights between the different cohorts indicating that treatment did not adversely impact the overall vigor of the animals. The UG and prostate weights were decreased in the treated animals compared to control with a trend towards improved response with combinatorial therapy as measured by prostate weight. The prostatic complex was microdissected under a stereomicroscope and the individual lobes of the prostate were weighed. The combination of dorsal (DP), lateral (LP) and ventral (VP) prostate weight was combined to give the prostate weight. Prostate tissues were for malin fixed and embedded in paraffin and processed for histological evaluation by hematoxylin and eosin staining. Histology was evaluated and each lobe of the prostate was given a tumor grade. Grade 1 represents normal prostate with secretory epithelial cells and an open lumen. Grade 2 has an open lumen but areas for transformed cells with a shift in the cytoplasmic to nuclear ratio and a piling up of the cells. In grade 3 all the epithelial cells have a transformed phenotype and the lumen is being filled with cells and a profound piling up of the cells. Grade 4 represents carcinoma in situ with cells invading through the basement membrane. Grade 5 represent solid tumors with a glandular architecture

and grade 6 are poorly differentiated tumors with sheets of anaplastic cells. Each lobe of the prostate was given three tumor grades representing the best grade, the worst grade and the overall grade. **Graph 2** is the overall grade for the dorsal, lateral and ventral prostate. The ventral prostate was examined more in depth because it has been shown to be the most responsive to therapeutic intervention and demonstrated the most response in this study. Graphs of the initial analysis of the data for tumor grade in the VP are presented in **Graphs 3 & 4**. The distribution of tumor grade was examined by low, intermediate and cancer. Low represents grades 1 and 2. Intermediate represents grade 3. Cancer presents grades 4, 5 and 6. The data for distribution is presented in **Table I and Graph 4**.

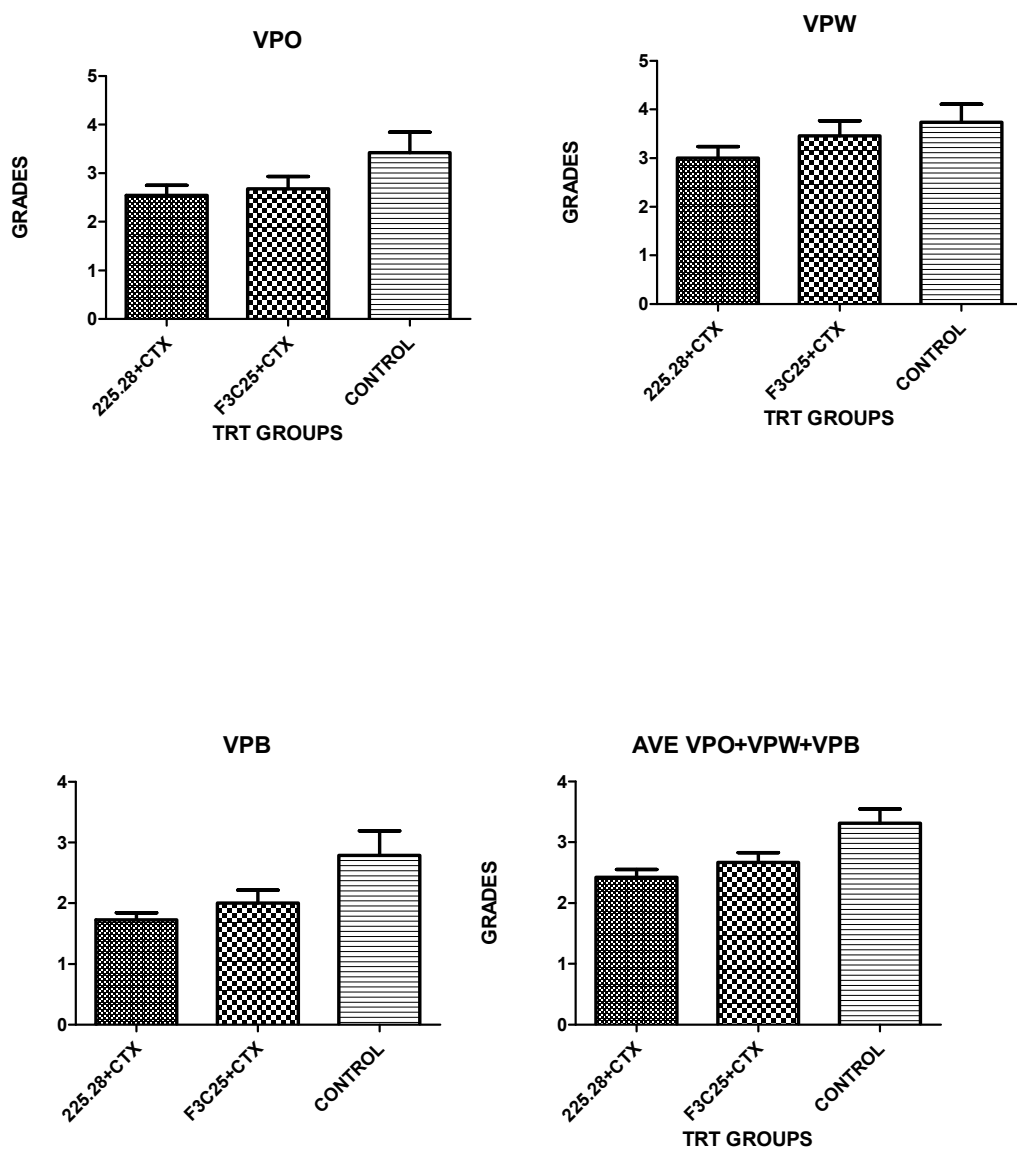
Additional slides have been cut and are being stained to examine the endothelial cells and pericytes of the prostatic vessels.



Graph 1. Body weight, UG weight and prostate weight of each cohort. Note that there is a trend towards further decrease in prostate weight in the combination therapy (225.28 + CTX). Bars are Standard Error



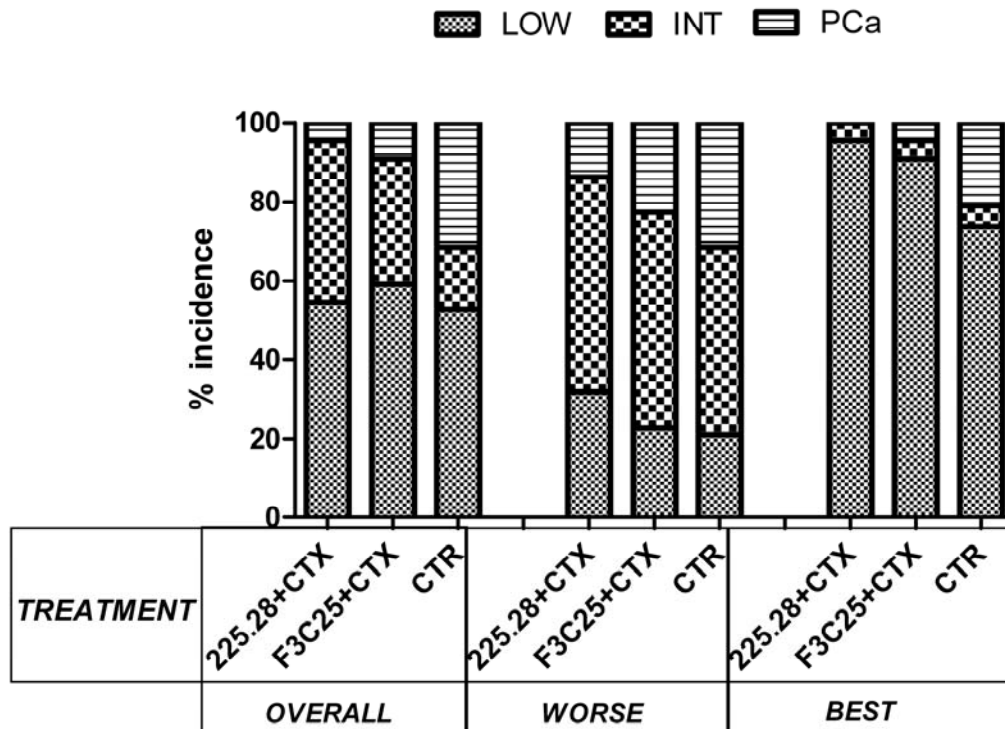
Graph 2. Overall tumor grade of dorsal prostate (DP), Lateral Prostate (LP) and ventral prostate (VP). Note that in the ventral prostate, which has been demonstrated to be the most responsive lobe of the prostate, there is a trend towards decreased tumor grade. Bars are Standard Error



Graph 3. Ventral Prostate Grades Overall (VPO), Worse (VPW), Best (VPB) and Average (VPO+VPW+VPB). Note the trend towards improved response with the combination therapy. Bars are Standard Error

Table I. Tumor Grades for Ventral Prostate

Grade VP overall	225.28 + CTX	F3C25 + CTX	Control
1	1	0	0
2	11	13	10
3	9	7	3
4	0	0	0
5	0	0	0
6	1	2	6
Grade VP worse	225.28 + CTX	F3C25 + CTX	Control
1	0	0	0
2	7	5	4
3	12	12	9
4	1	0	0
5	0	0	0
6	2	5	6
Grade VP best	225.28 + CTX	F3C25 + CTX	Control
1	7	5	2
2	14	15	12
3	1	1	1
4	0	0	0
5	0	0	0
6	0	1	4



Graph 4. Grade distribution for Ventral Prostate. Note that with treatment the prostate have lower grade with less incidence of cancer. There is a trend for improved response with combinational treatment versus CTX with control antibody.

KEY RESEARCH ACCOMPLISHMENT

- Treatment of twenty seven TRAMP male mice with AN2-specific mAb and with metronomic chemotherapy and of twenty six TRAMP mice with metronomic chemotherapy has shown that both treatments delay tumor progression. Studies are in progress to determine whether the results of the two treatments are significantly different.

REPORTABLE OUTCOMES

Administration of AN2-specific mAb with metronomic chemotherapy and of metronomic chemotherapy delays disease progression.

CONCLUSION

The results obtained thus far indicate that the therapeutic strategy we outlined in the grant application is effective. Experiments are in progress to determine whether the combination AN2-specific mAb and metronomic chemotherapy is more effective than metronomic chemotherapy alone. Furthermore the mechanisms underlying these effects have to be investigated.