

Antisense MDM2 Sensitizes Prostate Tumors to Radiation Therapy and Androgen Deprivation *in vivo*

Radka Stoyanova, Ph.D.¹, Paul Hachem, B.S.¹, Harvey H. Hensley, Ph.D.², HaeWon Kwon, B.S.¹, Sudhir Agrawal, D. Phil.³, Alan Pollack, M.D., Ph.D.¹

¹Department of Radiation Oncology and ²Basic Science Division, Fox Chase Cancer Center, Philadelphia, PA, USA, ³Hybridon, Inc., Cambridge, MA

Background: We have previously shown that antisense MDM2 (AS) sensitizes wild type LNCaP cells to androgen deprivation (AD), radiation therapy (RT) and the combination *in vitro*.

Purpose/Objective(s): In this report we test whether AS-MDM2 will sensitize androgen sensitive prostate cancer cells to AD and/or RT in an orthotopic *in vivo* model. Use of an orthotopic model is essential in prostate cancer because of tumor-stromal interactions which are particularly important to consider when treatments in combination with AD are being evaluated.

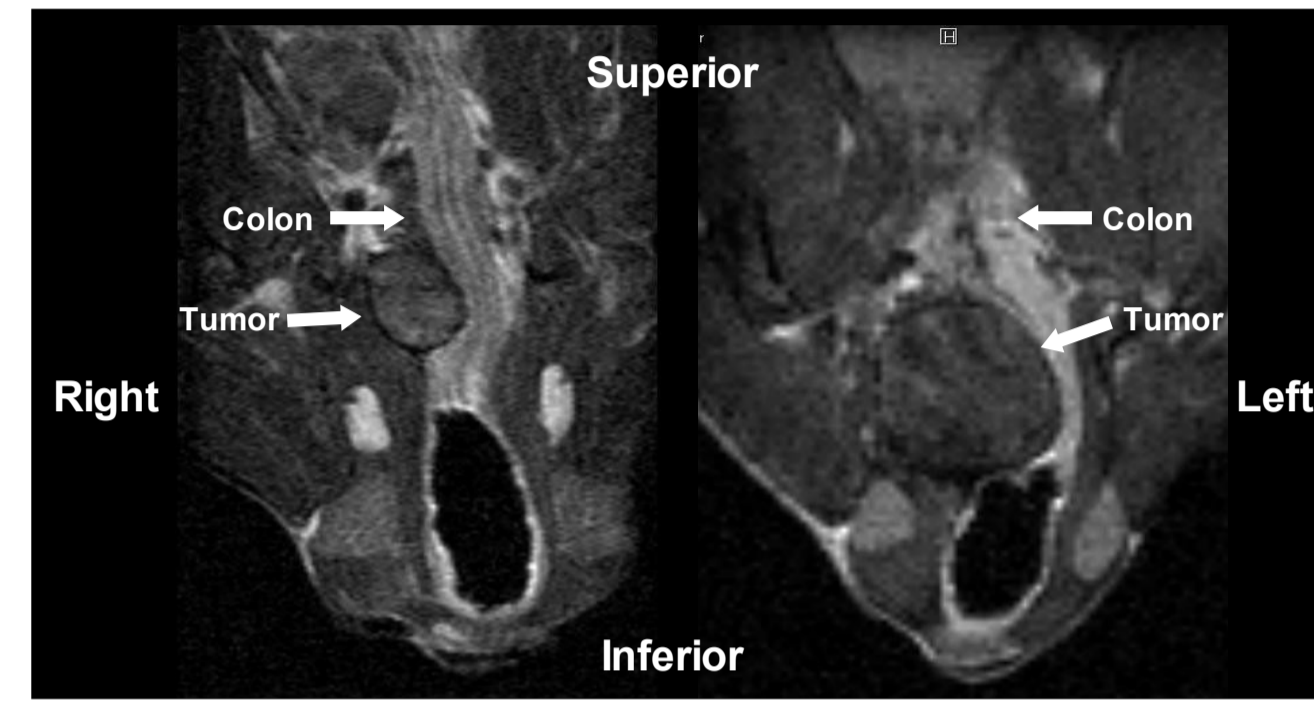
Material/Methods: LNCaP cells were grown in the prostates of nude mice. Beginning at three weeks after orthotopic implantation of cells, weekly tail vein bleedings to obtain 70 ml of blood for serum PSA measurements were initiated. Once the PSA level exceeded 2.0 ng/ml, animals were separated in two main categories, mismatch (MM) and AS, and further within each category were assigned at random to one of the following four treatments: oligonucleotide, oligonucleotide +RT, oligonucleotide+AD and oligonucleotide+RT+AD. Tumor volumes by MRI and serum PSA were obtained weekly after the beginning of treatment. Imaging was done at field strength of 7 Tesla in a vertical wide bore (10 cm) Bruker DRX magnet. The efficacy of the treatment was assessed by estimating failure using definitions modeled after freedom from failure assessment in patients with prostate cancer. Two parameters were used; freedom from biochemical failure (FFBF, defined as PSA \leq 1.5 ng/ml 6 weeks after treatment) and freedom from tumor volume failure (FFTVF, defined as an MRI-based tumor volume \leq 20 mm³ 10 weeks after treatment). The combined freedom from failure endpoint was defined as both FFBF and FFTVF.

Results: *In vivo* pilot studies were carried out to optimize the experimental conditions. The rate of tumor formation from 5 x 10⁵ LNCaP-MST and LNCaP (wild-type) cells was examined. Only 15.7% of the mice formed detectable tumors when LNCaP-MST cells were injected orthotopically. In contrast, 74% of mice injected with LNCaP cells formed tumors. Consequently, the experiments focused on LNCaP cells.

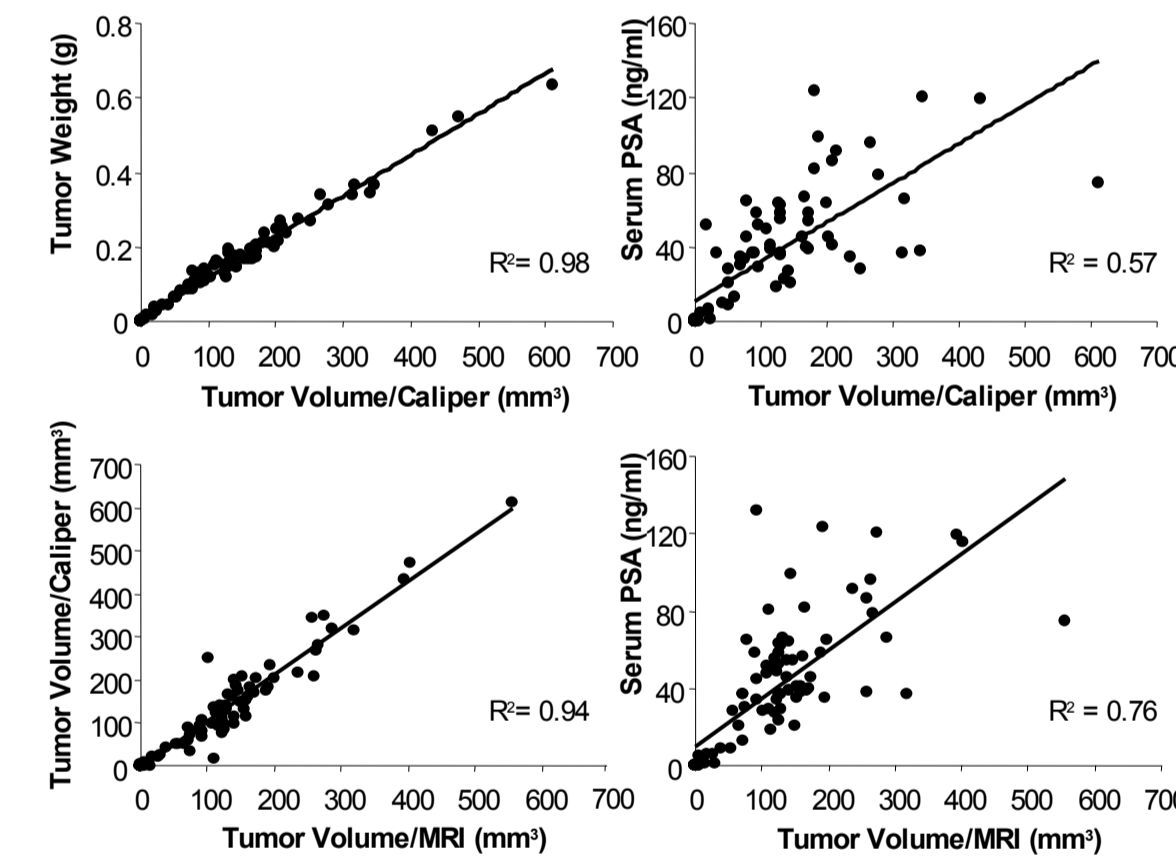
We also carried out pilot experiments to determine the optimal RT dose and AS concentration and the results are summarized in Table 1. These data suggest that 5.0 Gy causes a borderline undetectable response, which is desirable in testing for radiosensitization. The animals in Group 2 were treated with 12.5 mg/kg/day AS for 10 days. This treatment resulted in a minimal effect on FFBF. For this reason, a higher dose of AS-MDM2 (25 mg/kg/day) for a longer time period (15 days) was used.

Table 1. Summary of pilot experiment results for determining optimal RT dose (Group 1) and AS-MDM2 treatment conditions (Group 2) in LNCaP tumors grown orthotopically.

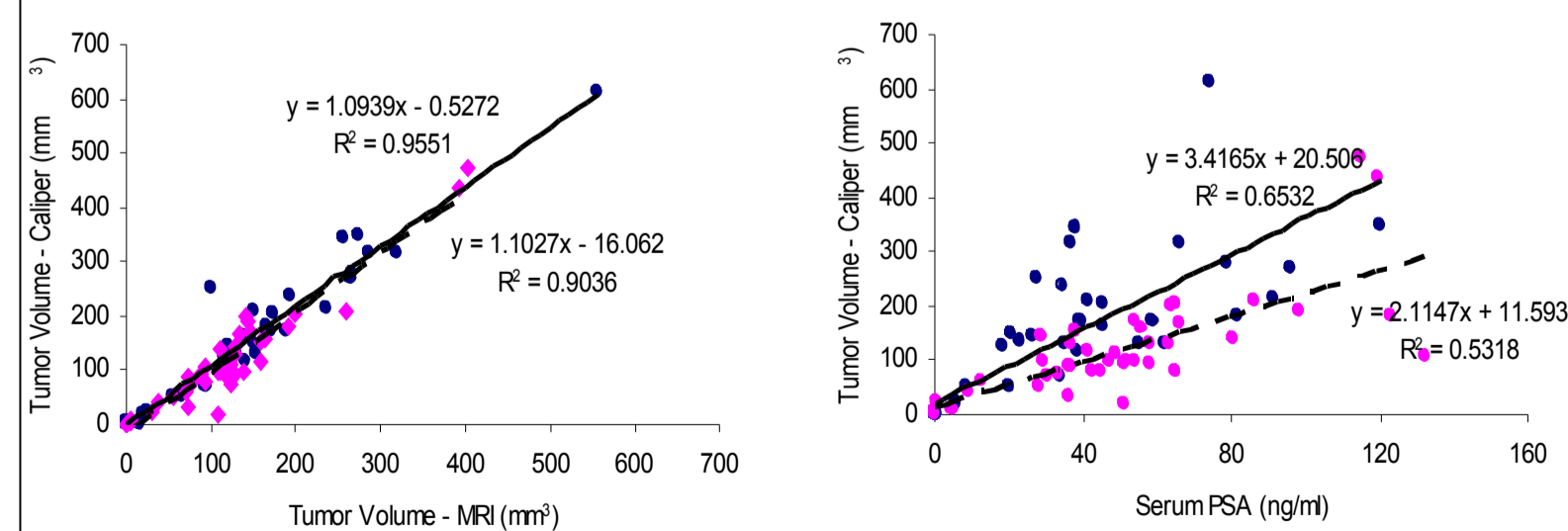
Group	Androgen Deprivation	AS-MDM2 12.5 mg/kg 10 days	RT Dose	FFBF (tumor < 20 mm ³ at 10 wks)
1	----	----	2.5 Gy	0% (0/4)
1	----	----	5.0 Gy	0% (0/4)
1	----	----	7.5 Gy	25% (1/4)
1	----	----	10 Gy	50% (2/4)
2	----	Yes	----	0% (0/13)
2	----	Yes	5 Gy	18% (4/22)
2	Yes	Yes	----	33% (5/15)
2	Yes	Yes	5 Gy	32% (6/19)



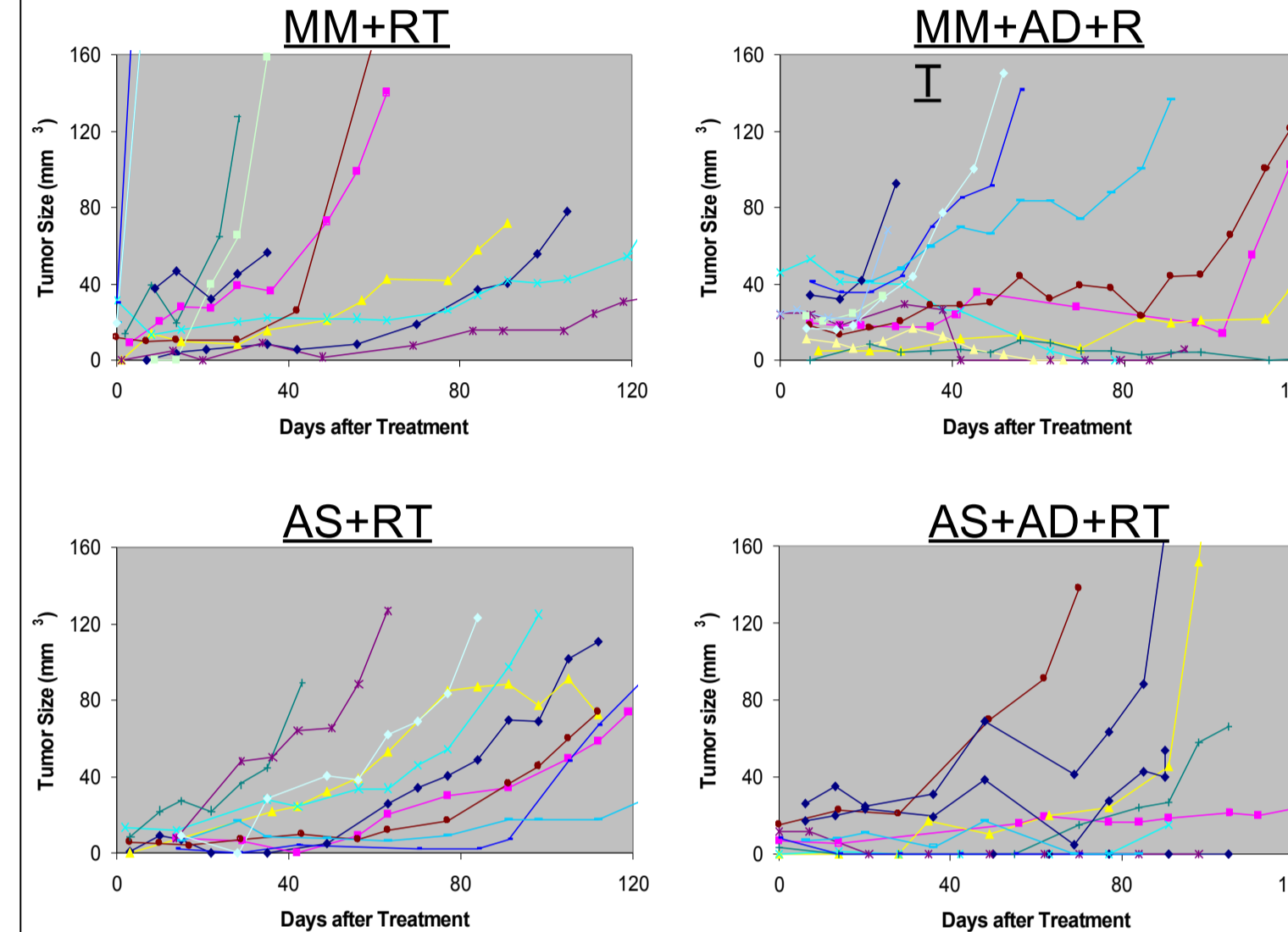
MRI resulted in excellent images of tumors, allowing for accurate determination of tumor volumes. Clear depiction of the tumor borders and anatomic structures are apparent. The images above were taken 6 weeks apart from the same animal with the earlier smaller tumor (10.1 mm³) associated with the PSA of 4.3 ng/ml and the larger tumor (127.5 mm³) is associated with a PSA of 21.9 ng/ml.



There is a strong correlation between caliper measurements of the excised tumor and tumor weight and tumor volume, measured with MRI. The relationships of serum PSA with caliper and MRI-based tumor volume, although weaker, were also significant.



We explored the source of the variability of PSAs relationship to tumor volume by separating the data into two groups – tumors with and without AD treatment. Androgen deprivation regulates PSA expression and may, like other therapeutic methods, alter the relationship of PSA to tumor volume. The graphs above show that AD does not affect the association between MRI and caliper-measured TV (panel A); however, the slope of the caliper-based tumor volume to PSA plot is significantly lower ($p < 0.05$) in the setting of AD (panel B, pink series).



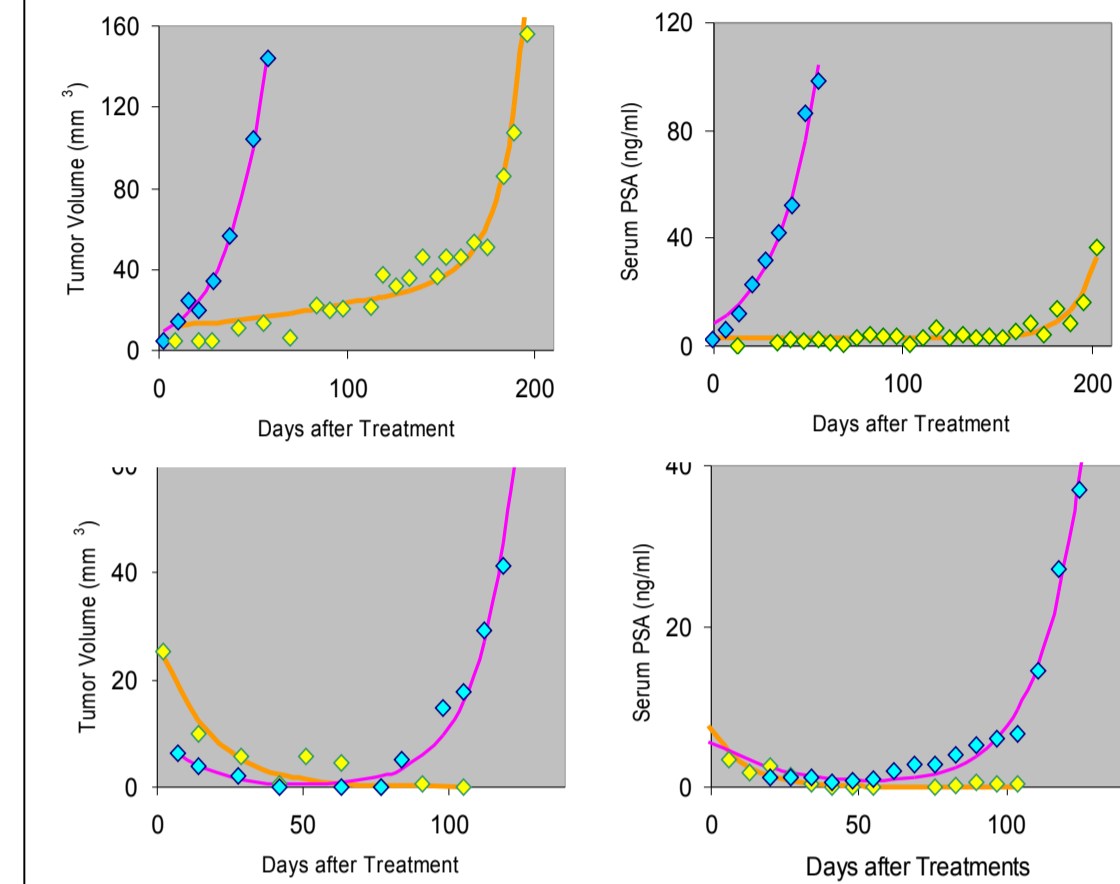
Differential response to RT and RT+AD of the AS and MM groups. The experiment consisted of 94 mice, divided into 8 experimental groups. The time-related data collection involved a total of 812 MRIs and 1013 PSA measurements.

Table 2. Freedom From Biochemical Failure (FFBF) (PSA < 1.5 ng/ml at 6 weeks) and Freedom from MRI-Based Tumor Volume Failure (FFTVF) (tumor volume less than 20 mm³ at 10 weeks) and combined endpoint in LNCaP cells grown orthotopically in the prostates of nude mice

Group	FFBF	FFTVF	Combined
MM (pooled)	23% (9/48)	23% (11/48)	17% (8/48)
AS (pooled)	43% (20/46) [†]	50% (23/46) [†]	43% (20/46) [†]
MM	8% (1/12)	16% (2/12)	8% (1/12)
AS	20% (2/10)	30% (3/10)	20% (2/10)
MM+RT	9% (1/11)	9% (1/11)	9% (1/11)
AS+RT	10% (1/10)	30% (3/10)	10% (1/10)
MM+AD	16% (2/12)	25% (3/12)	16% (2/12)
AS+AD	54% (7/13) [†]	54% (7/13)	54% (7/13) [†]
MM+AD+RT	38% (5/13)	38% (5/13)	31% (4/13)
AS+AD+RT	77% (10/13) [†]	77% (10/13) [†]	77% (10/13) [†]

[†]Statistically significant difference ($p < 0.05$), compared to the group above

The results of the *in vivo* experiment examining the effect of AS are depicted in Table 2. The findings of two endpoints (FFBF and FFTVF) are displayed together with the combined endpoint. The pooled data from the two main oligonucleotide treatment categories (MM and AS) are displayed in the first two rows. The increases in freedom from failure for all of the endpoints were significant ($p < 0.05$). The outcomes of the individual treatments were also compared. The proportion of failure free mice by all three criteria (FFBF, FFTVF and combined) was higher for AS relative to MM in all 4 pairs of comparisons – alone, AD, RT, AD+RT. For all of the endpoints, AS+AD+RT was significantly different from the corresponding MM control. Likewise, AS+AD was significantly different from MM+AD for FFBF and the combined endpoint.



Longitudinal tumor volume and PSA data was modeled analytically in order to characterize tumor growth kinetics. Examples of different tumor volume (left panels) and corresponding PSA (right panels) kinetics with overlaid exponential fits. (upper). Rapid growth without delay from treatment and delayed growth; (below) Decline and initial decline and then rapid growth (solid triangles).

Table 3. Average values (\pm SEM) of tumor parameters, calculated by modeling of longitudinal data from MRI-based tumor volume and serum PSA measurements.

Group	Tumor Doubling time (days)	PSA Doubling time (days)	Tumor Volume at 6 wks (mm ³)	PSA at 6 wks (ng/ml)	Tumor Volume at 10 wks (mm ³)	PSA at 10 wks (ng/ml)
MM	25 \pm 9	27 \pm 10	208 \pm 61	64 \pm 17	276 \pm 68	77 \pm 17
AS-MDM2	39 \pm 13	34 \pm 11	75 \pm 27	37 \pm 13	250 \pm 72	80 \pm 27
MM+RT	18 \pm 5	23 \pm 10	173 \pm 65	41 \pm 14	250 \pm 68	70 \pm 16
AS+RT	22 \pm 3	22 \pm 3	26 \pm 8 ^{†*}	15 \pm 6 ^{†*}	79 \pm 36 ^{†*}	34 \pm 13 ^{†*}
MM+AD	34 \pm 14	22 \pm 9	183 \pm 54	52 \pm 15	308 \pm 66	77 \pm 17
AS+AD	57 \pm 14	58 \pm 13 [†]	103 \pm 51	25 \pm 13	180 \pm 66 [‡]	48 \pm 17 [‡]
MM+AD+RT	43 \pm 11	39 \pm 12	61 \pm 20 [†]	28 \pm 14 [†]	146 \pm 54 [†]	41 \pm 15 [†]
AS+AD+RT	70 \pm 11 ^{†*}	71 \pm 11 ^{†*}	10 \pm 5 ^{†*}	2 \pm 1 ^{†*}	22 \pm 11 ^{†*}	6 \pm 4 ^{†*}

[†] Statistical significant difference ($p < 0.05$), compared to the group above
[‡] $p < 0.1$, compared to the group above
^{*} Statistical significant difference ($p < 0.05$), compared to MM group
[#] Statistical significant difference ($p < 0.05$), compared to AS-MDM2 group

The data in Table 3 summarize the fitted tumor growth kinetic results, showing that the most dramatic effects were seen with AS+AD+RT. When these three treatments were administered together, MRI-based tumor volume and PSA doubling times were significantly longer, calculated MRI-based TVs at six and 10 weeks were significantly lower and calculated PSAs at six and 10 weeks were significantly lower than for all of the other groups tested.

Conclusions:

- AS has wide-ranging action in inhibiting orthotopically grown prostate cancer (LNCaP) cells;
- AS significantly sensitizes androgen sensitive prostate cancer cells to AD;
- further inhibition is seen when all three agents, AS+AD+RT, are given together.

These are the first results to demonstrate that AS significantly sensitizes androgen sensitive prostate cancer cells to AD and AD+RT *in vivo*. AS should substantially enhance the response of prostate tumors locally to AD+RT and has the potential to promote cell killing distantly by potentiating the apoptotic action of AD. The results with AD underscore the potential to affect micrometastatic disease, which is probably responsible for treatment failure in 30-40% of men with high risk disease.