

MODIFIED ABSTRACT

Purpose: Bcl-2 is anti-apoptotic. Overexpression has been associated with the development of resistance to androgen deprivation (AD) and poor outcome in relatively favorable risk patients treated with radiotherapy (RT). Bax is pro-apoptotic, regulating bcl-2 through heterodimer formation. Our prior results indicate that abnormal expression (both overexpression and underexpression) of bax has also been associated with prostate cancer recurrence after RT in men with relatively favorable features. However, bcl-2 and bax were not related to outcome in more locally-advanced patients treated in RTOG 86-10 with RT alone or RT+ short term AD (STAD). In this report, these markers are investigated in more contemporary high risk men treated with RT+ STAD or RT + long term AD (LTAD).

Methods: Of the 1518 assessable men in RTOG 92-02, there was sufficient paraffin-embedded formalin-fixed pretreatment diagnostic tissue for bcl-2 and bax analyses by immunohistochemical methods in 502 and 343 patients, respectively. Bcl-2 expression was classified as overexpressed if any cluster >20 tumor cells was positive (n=229; 45.6%). Bax expression was underexpressed (n=26; 7.6%) or overexpressed (n=159; 46.4%) relative to normal prostatic epithelial cells in the specimen. Median follow-up was about 9 years for both the bcl-2 and bax cohorts.

Univariate and multivariate analyses (MVA's) by Cox proportional hazards models were applied using the endpoints overall mortality (OM), cause-specific mortality (CSM), distant metastasis (DM), local failure (LF), biochemical failure (BF) by both the ASTRO consensus (three consecutive rises in PSA or salvage hormones) and Phoenix (PSA> nadir + 2 ng/ml after treatment or salvage hormones) definitions, and any failure (AF; clinical and biochemical failure). The MVA's included dichotomized covariates: Age (<70 vs. ≥ 70 years), iPSA (≤30 vs >30 ng/mL, cutpoint based on protocol stratification), Gleason score (2-6 vs. 7-10), T-stage (T2 vs T3/T4) and Assigned Treatment (STAD + RT vs LTAD + RT).

Results: Those in whom bcl-2 was assessed had a greater proportion of Gleason 7-10 (66% vs 56%; p=0.0005) and fewer were treated with STAD+RT (46% vs 52%; p=0.04) compared to those in whom bcl-2 was not available (n=1016); there was no difference in the distribution of other factors. There was no statistical difference in outcome by BF, LF, DM, AF), CSM or OM between the bcl-2 assessed and unavailable groups. The same trends were seen for the bax assessable versus unavailable groups.

Bcl-2 overexpression was significantly related to Gleason 7-10 disease (71% vs 61%; p=0.03; **Table I**), while abnormal bax expression was not related to any pretreatment factor (age, Gleason score, iPSA, T-stage) or assigned treatment (**Table II**). **Table III** displays the distribution of bcl-2 and bax staining, using the presence of any detectable bcl-2 staining and any abnormal (more or greater than internal epithelial cell controls) bax staining as the criteria for positive bcl-2 and abnormal bax, as described previously (1, 2).

In multivariate analyses, bcl-2 was not independently related to any of the endpoints tested, although the relative risks for failure were higher (**Table IV**). Bax was significantly associated with AF and marginally with BF using the ASTRO definition (**Table V**). The combination of negative (0 intensity) bcl-2 and normal bax expression was significantly related to both BF and AF in multivariate (**Table VI**) and univariate (**Figure 1**) analyses; these correlations appeared to be more robust than for bax expression alone. **Table VII** shows that the difference between negative bcl-2/normal bax and the others was most pronounced when STAD+RT was used.

1. Pollack A, Cowen D, Troncso P, Zagars GK, Von Eschenbach AC, Meistrich ML, McDonnell T. Molecular markers of outcome after radiotherapy in patients with prostate carcinoma. *Cancer* 2003;97:1630-1638.
2. Khor LY, Desilvio M, Li R, McDonnell TJ, Hammond ME, Sause WT, Pilepich MV, Okunieff P, Sandler HM, Pollack A. Bcl-2 and bax expression and prostate cancer outcome in men treated with radiotherapy in Radiation Therapy Oncology Group protocol 86-10. *Int J Radiat Oncol Biol Phys* 2006;66:25-30.

RESULTS

Table I. Distribution of patients by Bcl-2 staining intensity (N=502)

Characteristics	Group	Bcl-2 Negative (n=273)		Bcl-2 Positive (n=229)		p-value
		n	%	n	%	
Age (yr)	<70	129	47	100	44	0.422
	≥70	144	53	129	56	
Gleason Score	2-6	99	39	63	29	0.031
	7-10	156	61	152	71	
Clinical Stage	T2	123	45	96	42	0.481
	T3 or T4	150	55	133	58	
iPSA (ng/mL)	≤30	182	67	160	70	0.443
	>30	91	33	69	30	
Assigned Tx	STAD + RT	123	45	110	48	0.505
	LTAD + RT	150	55	119	52	

Abbreviations: STAD, short-term androgen deprivation; Tx, treatment; RT, radiotherapy; LTAD, long-term androgen deprivation.
*P-value is from Chi-square statistics.

Table III. Distribution of Bcl-2 and Bax by Staining

Bcl-2	n (%)	Bax	n (%)
Negative	273 (54%)	Normal	158 (46%)
Weakly Positive	148 (30%)	Overexpressed	159 (46%)
Mod Positive	63 (13%)	Underexpressed	26 (8%)
Strongly positive	18 (4%)		

Table IV. Multivariate analysis of Bcl-2 intensity

Endpoint	Covariate	Group	RR	95% CI	p-value*
OM	Bcl-2 intensity	>0	1.24	(0.96, 1.60)	0.102
CSM	Bcl-2 intensity	>0	1.24	(0.79, 1.94)	0.345
DM	Bcl-2 intensity	>0	1.37	(0.90, 2.08)	0.148
LF	Bcl-2 intensity	>0	1.27	(0.78, 2.07)	0.341
BF Phoenix	Bcl-2 intensity	>0	1.12	(0.87, 1.45)	0.374
BF ASTRO	Bcl-2 intensity	>0	1.13	(0.85, 1.51)	0.386
	Assigned Tx	STAD+RT	3.01	(2.24, 4.04)	<0.0001
	iPSA	>30	1.53	(1.14, 2.05)	0.004
	GLSC	7-10	1.09	(0.80, 1.48)	0.587
	T-stage	T3/T4	1.03	(0.77, 1.37)	0.844
Any Failure	Bcl-2	>0	1.16	(0.91, 1.48)	0.225
	Assigned Tx	STAD+RT	2.19	(1.72, 2.79)	<0.0001
	iPSA	>30	1.30	(1.01, 1.68)	0.044
	GLSC	7-10	0.97	(0.75, 1.25)	0.796
	T-stage	T3/T4	1.01	(0.79, 1.29)	0.939

Abbreviations: RR, relative risk; CI, confidence interval; OM, overall mortality; CSM, cause-specific mortality; DM, distant metastasis; Tx, treatment; STAD, short-term androgen deprivation; RT, radiotherapy; BF, biochemical failure; iPSA, pretreatment initial PSA; GLSC, Gleason score.
*P-values from Chi-square test using Cox proportional hazards model.

Table II. Distribution of patients by Bax staining intensity (N=343)

Characteristics	Group	Bax Normal (n=158)		Bax Abnormal (n=185)		p-value
		n	%	n	%	
Age (yr)	<70	71	45	73	40	0.306
	≥70	87	55	112	60	
Gleason Score	2-6	55	38	59	34	0.502
	7-10	90	62	113	66	
Clinical Stage	T2	76	48	88	48	0.921
	T3 or T4	82	52	97	52	
iPSA (ng/mL)	≤30	101	64	124	67	0.547
	>30	57	36	61	33	
Assigned Tx	STAD + RT	72	46	81	44	0.740
	LTAD + RT	86	54	104	56	

Table V. Multivariate analysis of Bax staining

Endpoint	Covariate	Group	RR	95% CI	p-value*
OM	Bax	Abnormal	0.99	(0.73, 1.34)	0.937
CSM	Bax	Abnormal	1.05	(0.60, 1.84)	0.856
DM	Bax	Abnormal	1.06	(0.63, 1.80)	0.814
LF	Bax	Abnormal	0.95	(0.54, 1.67)	0.852
BF Phoenix	Bax	Abnormal	1.15	(0.84, 1.58)	0.394
BF ASTRO	Bax	Abnormal	1.37	(0.96, 1.97)	0.085
	Assigned Tx	STAD + RT	3.28	(2.26, 4.76)	<0.0001
	iPSA	>30	1.64	(1.14, 2.36)	0.007
	GLSC	7-10	1.21	(0.82, 1.79)	0.334
	T-stage	T3/T4	1.29	(0.89, 1.85)	0.175
Any Failure	Bax	Abnormal	1.43	(1.05, 1.95)	0.023
	Assigned Tx	STAD+RT	2.33	(1.71, 3.16)	<0.0001
	iPSA	>30	1.30	(0.95, 1.78)	0.103
	GLSC	7-10	0.96	(0.70, 1.33)	0.827
	T-stage	T3/T4	1.18	(0.87, 1.61)	0.279

Table VI. Multivariate analysis of Combined Bcl2/Bax Staining

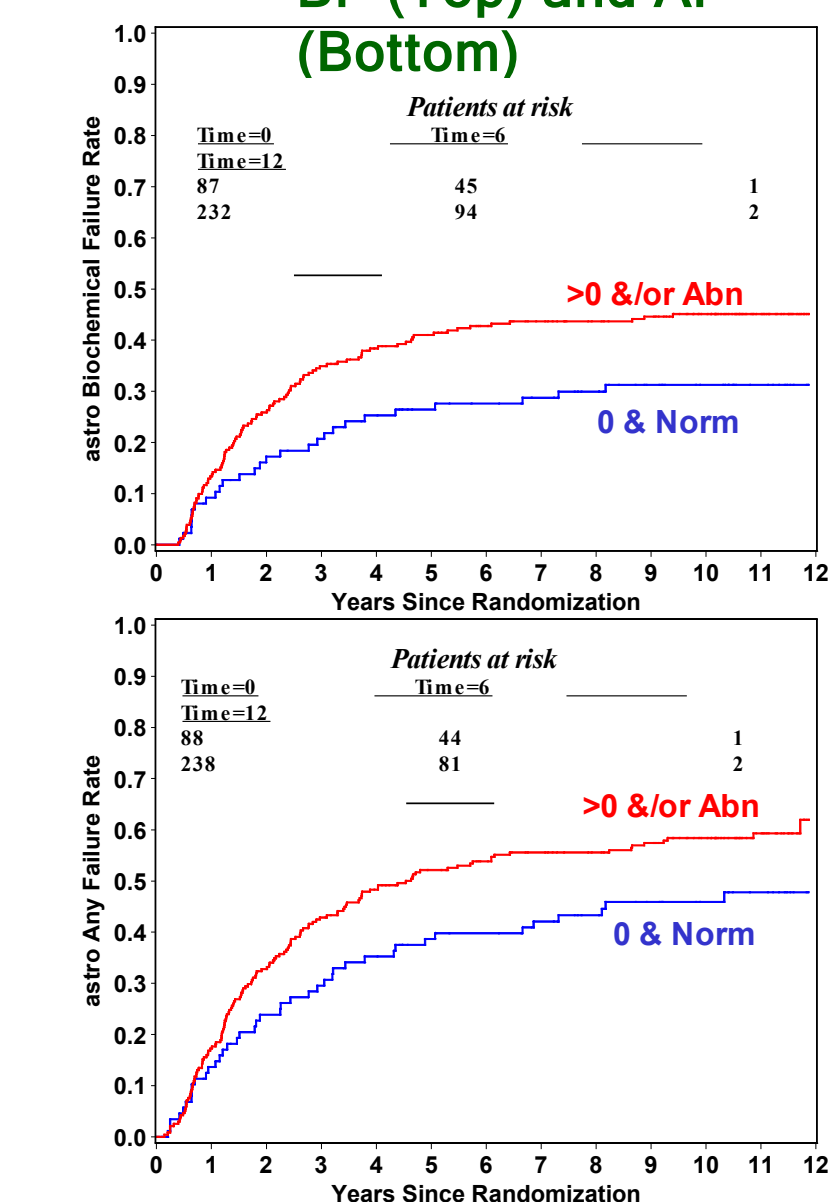
Endpoint	Covariate	Group	RR	95% CI	p-value*
BF ASTRO	Bcl-2/Bax	>0 &/or Abn	1.60	(1.03, 2.49)	0.036
	Assigned Tx	STAD + RT	3.14	(2.16, 4.56)	<0.0001
	iPSA	>30	1.71	(1.19, 2.47)	0.004
	GLSC	7-10	1.13	(0.76, 1.67)	0.555
	T-stage	T3/T4	1.23	(0.85, 1.77)	0.276
Any Failure	Bcl-2/Bax	>0 &/or Abn	1.45	(1.01, 2.10)	0.046
	Assigned Tx	STAD+RT	2.26	(1.66, 3.08)	<0.0001
	iPSA	>30	1.34	(0.97, 1.84)	0.074
	GLSC	7-10	0.91	(0.66, 1.26)	0.559
	T-stage	T3/T4	1.16	(0.85, 1.59)	0.333

Abbreviations: RR, relative risk; CI, confidence interval; OM, overall mortality; CSM, cause-specific mortality; DM, distant metastasis; Tx, treatment; STAD, short-term androgen deprivation; RT, radiotherapy; BF, biochemical failure; iPSA, pretreatment initial PSA; GLSC, Gleason score; Abn, abnormal bax expression.
*P-values from Chi-square test using Cox proportional hazards model.

Table VII.

Group	Tx	n	% Fail	p-value
BF ASTRO				
0 & norm	STAD+RT	36	33.3	0.116
	LTAD+RT	51	21.6	
>0 &/or Abn	STAD+RT	106	61.3	<0.0001
	LTAD+RT	126	23.9	
Any Failure				
0 & norm	STAD+RT	27	48.7	0.056
	LTAD+RT	51	31.4	
>0 &/or Abn	STAD+RT	107	69.2	<0.0001
	LTAD+RT	131	38.2	

Figure 1. Bcl-2/Bax by ASTRO BF (Top) and AF (Bottom)



CONCLUSIONS

- Abnormal bax expression was more predictive of outcome (AF) than bcl-2 when tested individually.
- There appeared to be an advantage when bcl-2 and bax were combined, with negative bcl-2 and normal bax associated with the best prognosis (BF and AF).
- The greatest difference between negative bcl-2/normal bax versus the others was in those who received STAD+RT
 - ❖ LTAD+RT was most effective at reducing failure in those tumors with positive bcl-2 and/or abnormal bax expression.