

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Gnant M, Mlineritsch B, Schippinger W, et al. Endocrine therapy plus zoledronic acid in premenopausal breast cancer. *N Engl J Med* 2009;360:679-91.

Supplemental Data

Supplemental Table 1: Test of the Proportionality Assumption

Model		HR	95% CI	P Value
1	ANA vs TAM†	1.148	0.23-5.73	0.87
	Interaction: ANA/TAM * Log-Time	0.987	0.62-1.57	0.95
2	ZOL vs No ZOL†	0.704	0.14-3.62	0.67
	Interaction: ZOL/No ZOL * Log-Time	0.974	0.61-1.56	0.91

HR denotes hazard ratio; CI denotes confidence interval; ANA denotes anastrozole; TAM denotes tamoxifen; ZOL denotes zoledronic acid.

†Reference category.

Supplemental Table 2: Sensitivity Analysis*

Analysis	HR	95% CI	P Value
Excluding patients in the BMD substudy (n=404)			
ANA vs TAM†	1.39	0.92-2.10	0.12
ZOL vs No ZOL†	0.70	0.46-1.06	0.09
Excluding patients who received any 8-mg zoledronic acid dose (n=98)‡			
ANA vs TAM†	1.18	0.83-1.66	0.36
ZOL vs No ZOL†	0.73	0.50-1.05	0.09

HR denotes hazard ratio; CI denotes confidence interval.

*Because patients in these two subgroups were enrolled early in the trial, the median follow-up for the sensitivity analyses is only 45 months. Subgroups were evaluated for statistical heterogeneity and no conclusions regarding subgroup efficacy can be drawn from these analyses.

†Reference category.

‡Median duration of 8-mg dose was 5.5 months.

Supplemental Table 3: Full Multivariate Cox Regression Analysis of DFS*

	HR	95% CI	P Value
Therapy (ANA vs TAM†)	1.08	0.77-1.52	0.07
Therapy (No ZOL† vs ZOL)	0.65	0.46-0.91	0.01
Age Group ($\leq 40^{\dagger}$ vs > 40 years)	0.95	0.62-1.44	0.79
T-Stage (T1† vs $\geq T2$)	1.52	1.06-2.18	0.03
Grading (G1/2/X† vs G3)	2.22	1.57-3.14	<0.001
LN (negative† vs positive)	2.13	1.51-3.02	<0.001
ER (-, +, ++† vs +++‡)	1.08	0.76-1.54	0.65
PgR (-, +, ++† vs +++‡)	0.51	0.34-0.76	0.001

DFS denotes disease-free survival; HR denotes hazard ratio; CI denotes confidence interval; ANA denotes anastrozole; TAM denotes tamoxifen; ZOL denotes zoledronic acid; LN denotes lymph node; ER denotes estrogen receptor; PgR denotes progesterone receptor.

*Includes all potential risk factors used for randomization.

†Reference category.

‡Reiner Score for staining: +, 10 to 50%; ++, 51 to 80%; and +++, 81 to 100%.

Supplemental Table 4: Complete List of All Adverse Events in All Treatment Groups

Adverse Event — no. (%)	TAM (n=451)	TAM + ZOL (n=449)	ANA (n=453)	ANA + ZOL (n=450)	P Value†
Other/minor (not categorized)	108 (24.0)	112 (24.9)	107 (23.6)	119 (26.4)	0.76
Arthralgia	52 (11.5)	65 (14.5)	112 (24.7)	150 (33.3)	<0.001
Hot flushes	28 (6.2)	27 (6.0)	25 (5.5)	25 (5.6)	0.96
Myalgia	5 (1.1)	7 (1.6)	6 (1.3)	6 (1.3)	0.93
Depression, sleep disturbances	70 (15.5)	74 (16.5)	97 (21.4)	80 (17.8)	0.11
Vaginal inflammation	8 (1.8)	4 (0.9)	4 (0.9)	2 (0.4)	0.26
Breast inflammation	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.2)	0.87
Hair loss	6 (1.3)	8 (1.8)	6 (1.3)	9 (2.0)	0.81
Hypertonia	14 (3.1)	20 (4.5)	20 (4.4)	25 (5.6)	0.35
Peripheral nerve disease	17 (3.8)	22 (4.9)	14 (3.1)	29 (6.4)	0.09
Weight gain	7 (1.6)	7 (1.6)	8 (1.8)	4 (0.9)	0.71
Vaginal discharge	0 (0.0)	3 (0.7)	2 (0.4)	1 (0.2)	0.32
Vaginal dryness	2 (0.4)	2 (0.5)	4 (0.9)	2 (0.4)	0.85
Muscle cramp	9 (2.0)	8 (1.8)	2 (0.4)	4 (0.9)	0.10
Obstipation	5 (1.1)	5 (1.1)	1 (0.2)	0 (0.0)	0.04
Leg edema	9 (2.0)	10 (2.2)	2 (0.4)	2 (0.4)	0.02
Tachycardia	2 (0.4)	9 (2.0)	5 (1.1)	10 (2.2)	0.07
Tinnitus/hearing loss	2 (0.4)	1 (0.2)	3 (0.7)	4 (0.9)	0.63
Stomach pain	8 (1.8)	8 (1.8)	13 (2.9)	17 (3.8)	0.18
Infection of ear, nose, or throat	7 (1.6)	8 (1.8)	5 (1.1)	5 (1.1)	0.78
Torn ligament/Meniscus lesion	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Sensory disturbance	2 (0.4)	0 (0.0)	1 (0.2)	3 (0.7)	0.38
Dizziness	13 (2.9)	9 (2.0)	7 (1.6)	18 (4.0)	0.11
Lung inflammation	13 (2.9)	9 (2.0)	12 (2.7)	22 (4.9)	0.09
Hypercalcemia	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.2)	0.87
Vaginal bleeding	5 (1.1)	4 (0.9)	2 (0.4)	5 (1.1)	0.63
Thrombosis	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0.50
Uterine polyp	5 (1.1)	0 (0.0)	1 (0.2)	1 (0.2)	0.07
Skin disease	23 (5.1)	32 (7.1)	16 (3.5)	26 (5.8)	0.11
Lymphedema (arm, hand)	29 (6.4)	31 (6.9)	28 (6.2)	26 (5.8)	0.92
Hemorrhoids	0 (0.0)	2 (0.5)	1 (0.2)	0 (0.0)	0.25
Leukopenia	0 (0.0)	1 (0.2)	1 (0.2)	0 (0.0)	0.75
Fracture	1 (0.2)	1 (0.2)	1 (0.2)	0 (0.0)	0.91
Cognitive disorder	0 (0.0)	4 (0.9)	3 (0.7)	9 (2.0)	0.01
Irregular vaginal discharge	3 (0.7)	2 (0.5)	2 (0.4)	2 (0.4)	0.97
Thyroid disease	2 (0.4)	0 (0.0)	2 (0.4)	5 (1.1)	0.14
Incontinence	0 (0.0)	2 (0.5)	0 (0.0)	0 (0.0)	0.06
Uro-genital tract infection	3 (0.7)	7 (1.6)	0 (0.0)	1 (0.2)	0.01
Tendonitis	3 (0.7)	1 (0.2)	4 (0.9)	4 (0.9)	0.58
Ovarian changes	1 (0.2)	0 (0.0)	1 (0.2)	2 (0.4)	0.72
Spinal troubles/pain	13 (2.9)	15 (3.3)	15 (3.3)	16 (3.6)	0.95
Periodontal disease*	5 (1.1)	3 (0.7)	0 (0.0)	6 (1.3)	0.05
Diarrhea	10 (2.2)	17 (3.8)	11 (2.4)	12 (2.7)	0.51
Fever	9 (2.0)	34 (7.6)	11 (2.4)	46 (10.2)	<0.001
Nausea and vomiting	23 (5.1)	29 (6.5)	32 (7.1)	48 (10.7)	0.01
Morning stiffness	11 (2.4)	14 (3.1)	33 (7.3)	35 (7.8)	<0.001
Fatigue	70 (15.5)	82 (18.3)	93 (20.5)	98 (21.8)	0.08
Headache	59 (13.1)	59 (13.1)	63 (13.9)	85 (18.9)	0.05
Impaired vision	36 (8.0)	27 (6.0)	22 (4.9)	29 (6.4)	0.29
Hypocalcemia	0 (0.0)	3 (0.7)	0 (0.0)	1 (0.2)	0.03
Cutaneous reaction	19 (4.2)	5 (1.1)	18 (4.0)	15 (3.3)	0.02
Bone pain	94 (20.8)	132 (29.4)	128 (28.3)	185 (41.1)	<0.001

TAM denotes tamoxifen; ANA denotes anastrozole; ZOL denotes zoledronic acid.

*No confirmed cases of osteonecrosis of the jaw.

†P values are using Fisher's exact test.

Supplemental Table 5: Complete List of All Serious Adverse Events in All Treatment Groups

Serious Adverse Event — no. (%)	TAM (n=451)	TAM + ZOL (n=449)	ANA (n=453)	ANA + ZOL (n=450)	P Value†
Arthralgia	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.2)‡	0.37
Depression, sleep disturbances	1 (0.2)	3 (0.7)	0 (0.0)	1 (0.2)	0.20
Vaginal inflammation	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.2)	0.37
Breast inflammation	3 (0.7)	0 (0.0)	1 (0.2)	1 (0.2)	0.35
Hypertonia	2 (0.4)	0 (0.0)	1 (0.2)	3 (0.7)	0.38
Peripheral nerve disease	4 (0.9)	1 (0.2)	4 (0.9)	10 (2.2)	0.04
Obstipation	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Tachycardia	1 (0.2)	0 (0.0)	1 (0.2)	1 (0.2)	1.00
Tinnitus/hearing loss	1 (0.2)	1 (0.2)	0 (0.0)	1 (0.2)	0.72
Stomach pain	1 (0.2)	3 (0.7)	2 (0.4)	2 (0.4)	0.73
Torn ligament/Meniscus lesion	3 (0.7)	1 (0.2)	2 (0.4)	2 (0.4)	0.91
Sensory disturbance	0 (0.0)	2 (0.5)	2 (0.4)	2 (0.4)	0.57
Dizziness	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)	0.62
Lung inflammation	6 (1.3)	5 (1.1)	5 (1.1)	2 (0.4)	0.56
Vaginal bleeding	6 (1.3)	13 (2.9)	7 (1.6)	3 (0.7)	0.07
Thrombosis	3 (0.7)	5 (1.1)	0 (0.0)	0 (0.0)	0.01
Uterine polyp	40 (8.9)	51 (11.4)	7 (1.6)	5 (1.1)	<0.001
Skin disease	8 (1.8)	8 (1.8)	3 (0.7)	5 (1.1)	0.36
Lymphedema (arm, hand)	1 (0.2)	1 (0.2)	2 (0.4)	1 (0.2)	1.00
Hemorrhoids	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Leukopenia	1 (0.2)	0 (0.0)	0 (0.0)	0 (0.0)	0.75
Fracture	6 (1.3)	4 (0.9)	4 (0.9)	7 (1.6)	0.75
Cognitive disorder	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)	0.50
Melanoma	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)	0.62
Irregular vaginal discharge	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Thyroid disease	3 (0.7)	0 (0.0)	3 (0.7)	2 (0.4)	0.40
Incontinence	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Uro-genital tract infection	6 (1.3)	7 (1.6)	4 (0.9)	8 (1.8)	0.67
Tendonitis	2 (0.4)	5 (1.1)	3 (0.7)	3 (0.7)	0.66
Ovarian changes	2 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0.19
Spinal troubles/pain	0 (0.0)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Periodontal disease*	0 (0.0)	1 (0.2)	0 (0.0)	1 (0.2)	0.37
Other tumors	3 (0.7)	2 (0.5)	2 (0.4)	3 (0.7)	0.93
Disease of liver / gallbladder	5 (1.1)	3 (0.7)	2 (0.4)	2 (0.4)	0.64
Insult / TIA	1 (0.2)	0 (0.0)	1 (0.2)	0 (0.0)	1.00
Diarrhea	1 (0.2)	0 (0.0)	1 (0.2)	1 (0.2)	1.00
Fever	1 (0.2)	1 (0.2)	1 (0.2)	2 (0.4)	0.88
Headache	1 (0.2)	0 (0.0)	0 (0.0)	1 (0.2)	0.62
Cutaneous reaction	3 (0.7)	5 (1.1)	1 (0.2)	3 (0.7)	0.41
Bone pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)‡	0.50

TAM denotes tamoxifen; ANA denotes anastrozole; ZOL denotes zoledronic acid.

*No confirmed cases of osteonecrosis of the jaw.

†P values are using Fisher's exact test.

‡One case each of arthralgia and bone pain in the ANA + ZOL group were associated with prior hip replacement.

Supplemental Table 6: Complete List of All Adverse Events With and Without Zoledronic Acid

Adverse Event — no. (%)	ZOL (n=899)	No ZOL (n=904)	P Value†
Other/minor (not categorized)	231 (25.7)	215 (23.9)	0.35
Arthralgia	215 (23.9)	164 (18.2)	0.003
Hot flushes	52 (5.8)	53 (5.9)	1.00
Myalgia	13 (1.4)	11 (1.2)	0.69
Depression, sleep disturbances	154 (17.1)	167 (18.6)	0.46
Vaginal inflammation	6 (0.7)	12 (1.3)	0.24
Breast inflammation	1 (0.1)	1 (0.1)	1.00
Hair loss	17 (1.9)	12 (1.3)	0.36
Hypertonia	45 (5.0)	34 (3.8)	0.21
Peripheral nerve disease	51 (5.7)	31 (3.4)	0.02
Weight gain	11 (1.2)	15 (1.7)	0.55
Vaginal discharge	4 (0.4)	2 (0.2)	0.45
Vaginal dryness	4 (0.4)	6 (0.7)	0.75
Muscle cramp	12 (1.3)	11 (1.2)	0.84
Obstipation	5 (0.6)	6 (0.7)	1.00
Leg edema	12 (1.3)	11 (1.2)	0.84
Tachycardia	19 (2.1)	7 (0.8)	0.02
Tinnitus/hearing loss	5 (0.6)	5 (0.6)	1.00
Stomach pain	25 (2.8)	21 (2.3)	0.55
Infection of ear, nose, or throat	13 (1.4)	12 (1.3)	0.84
Torn ligament/Meniscus lesion	0 (0.0)	2 (0.2)	0.50
Sensory disturbance	3 (0.3)	3 (0.3)	1.00
Dizziness	27 (3.0)	20 (2.2)	0.30
Lung inflammation	31 (3.4)	25 (2.8)	0.42
Hypercalcemia	1 (0.1)	1 (0.1)	1.00
Vaginal bleeding	9 (1.0)	7 (0.8)	0.63
Thrombosis	1 (0.1)	0 (0.0)	0.50
Uterine polyp	1 (0.1)	6 (0.7)	0.12
Skin disease	58 (6.5)	39 (4.3)	0.05
Lymphedema (arm, hand)	57 (6.3)	57 (6.3)	1.00
Hemorrhoids	2 (0.2)	1 (0.1)	0.62
Leukopenia	1 (0.1)	1 (0.1)	1.00
Fracture	1 (0.1)	2 (0.2)	1.00
Cognitive disorder	13 (1.4)	3 (0.3)	0.01
Irregular vaginal discharge	4 (0.4)	5 (0.6)	1.00
Thyroid disease	5 (0.6)	4 (0.4)	0.75
Incontinence	2 (0.2)	0 (0.0)	0.25
Uro-genital tract infection	8 (0.9)	3 (0.3)	0.14
Tendonitis	5 (0.6)	7 (0.8)	0.77
Ovarian changes	2 (0.2)	2 (0.2)	1.00
Spinal troubles/pain	31 (3.4)	28 (3.1)	0.69
Periodontal disease*	9 (1.0)	5 (0.6)	0.30
Diarrhea	29 (3.2)	21 (2.3)	0.25
Fever	80 (8.9)	20 (2.2)	<0.001
Nausea and vomiting	77 (8.6)	55 (6.1)	0.05
Morning stiffness	49 (5.5)	44 (4.9)	0.60
Fatigue	180 (20.0)	163 (18.1)	0.31
Headache	144 (16.0)	122 (13.6)	0.14
Impaired vision	56 (6.2)	58 (6.5)	0.92
Hypocalcemia	4 (0.4)	0 (0.0)	0.06
Cutaneous reaction	20 (2.2)	37 (4.1)	0.03
Bone pain	317 (35.3)	222 (24.7)	<0.001

ZOL denotes zoledronic acid.

*No confirmed cases of osteonecrosis of the jaw.

†P values are using Fisher's exact test.

Supplemental Table 7: Complete List of All Serious Adverse Events With and Without Zoledronic Acid

Serious Adverse Event — no. (%)	ZOL (n=899)	No ZOL (n=904)	P Value†
Arthralgia	2 (0.2)‡	0 (0.0)	0.25
Depression, sleep disturbances	4 (0.4)	1 (0.1)	0.22
Vaginal inflammation	2 (0.2)	0 (0.0)	0.25
Breast inflammation	1 (0.1)	4 (0.4)	0.37
Hypertonia	3 (0.3)	3 (0.3)	1.00
Peripheral nerve disease	11 (1.2)	8 (0.9)	0.50
Obstipation	0 (0.0)	1 (0.1)	1.00
Tachycardia	1 (0.1)	2 (0.2)	1.00
Tinnitus/hearing loss	2 (0.2)	1 (0.1)	0.62
Stomach pain	5 (0.6)	3 (0.3)	0.51
Torn ligament/Meniscus lesion	3 (0.3)	5 (0.6)	0.73
Sensory disturbance	4 (0.4)	2 (0.2)	0.45
Dizziness	1 (0.1)	1 (0.1)	1.00
Lung inflammation	7 (0.8)	11 (1.2)	0.48
Vaginal bleeding	16 (1.8)	13 (1.4)	0.58
Thrombosis	5 (0.6)	3 (0.3)	0.51
Uterine polyp	56 (6.2)	47 (5.2)	0.36
Skin disease	13 (1.4)	11 (1.2)	0.69
Lymphedema (arm, hand)	2 (0.2)	3 (0.3)	1.00
Hemorrhoids	0 (0.0)	2 (0.2)	0.50
Leukopenia	0 (0.0)	1 (0.1)	1.00
Fracture	11 (1.2)	10 (1.1)	0.83
Cognitive disorder	1 (0.1)	0 (0.0)	0.50
Melanoma	1 (0.1)	1 (0.1)	1.00
Irregular vaginal discharge	0 (0.0)	1 (0.1)	1.00
Thyroid disease	2 (0.2)	6 (0.7)	0.29
Incontinence	0 (0.0)	1 (0.1)	1.00
Uro-genital tract infection	15 (1.7)	10 (1.1)	0.32
Tendonitis	8 (0.9)	5 (0.6)	0.42
Ovarian changes	0 (0.0)	2 (0.2)	0.50
Spinal troubles/pain	0 (0.0)	1 (0.1)	1.00
Periodontal disease*	2 (0.2)	0 (0.0)	0.25
Other tumors	5 (0.6)	5 (0.6)	1.00
Disease of liver / gallbladder	5 (0.6)	7 (0.8)	0.77
Insult / TIA	0 (0.0)	2 (0.2)	0.50
Diarrhea	1 (0.1)	2 (0.2)	1.00
Fever	3 (0.3)	2 (0.2)	0.69
Headache	1 (0.1)	1 (0.1)	1.00
Cutaneous reaction	8 (0.9)	4 (0.4)	0.26
Bone pain	1 (0.1)‡	0 (0.0)	0.50

ZOL denotes zoledronic acid.

*No confirmed cases of osteonecrosis of the jaw.

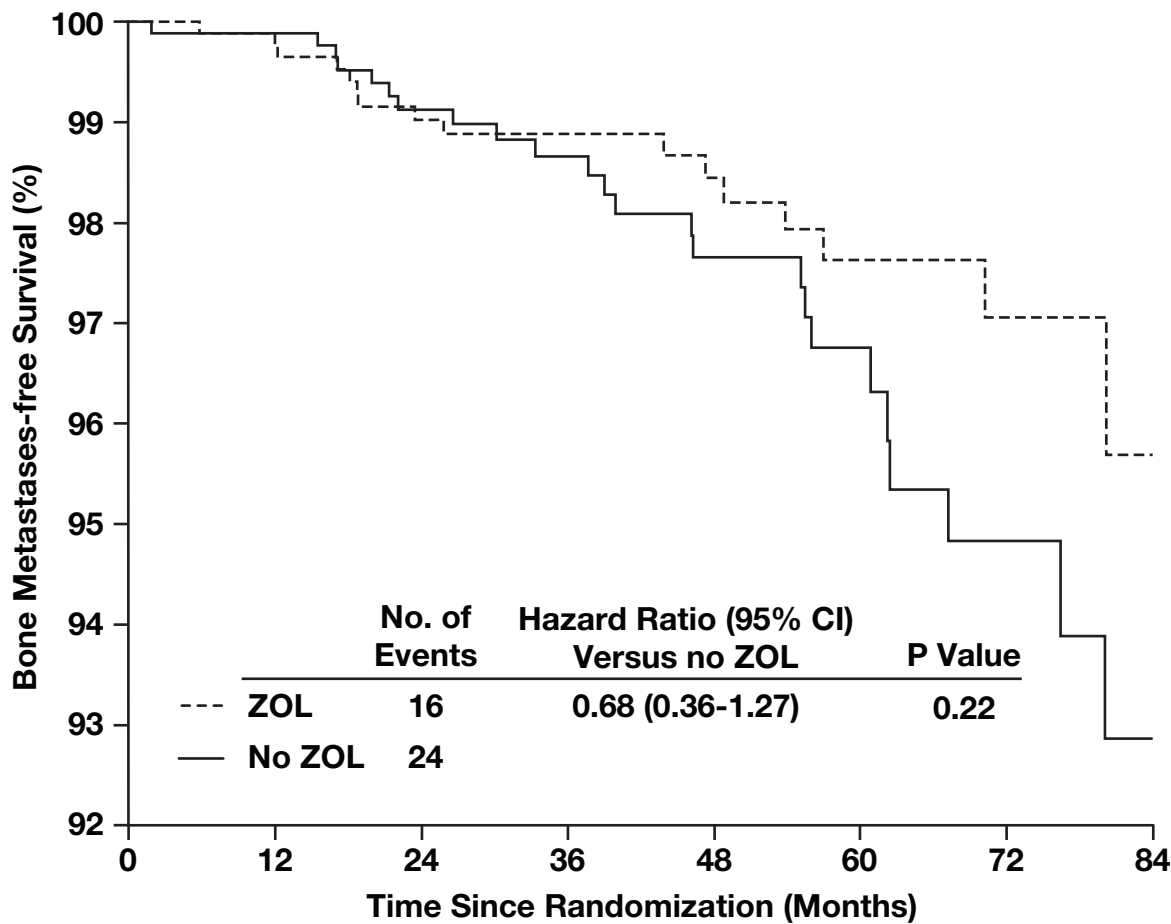
†P values are using Fisher's exact test.

‡One case each of arthralgia and bone pain in the ANA + ZOL group were associated with prior hip replacement.

Supplemental Figure 1. Kaplan-Meier Plots of Bone-metastases-free Survival.

Women with breast cancer received adjuvant endocrine therapy plus zoledronic acid (ZOL) compared with adjuvant endocrine therapy without zoledronic acid (No ZOL). ZOL denotes anastrozole/goserelin plus zoledronic acid and tamoxifen/goserelin plus zoledronic acid; No ZOL denotes anastrozole/goserelin and tamoxifen/goserelin.

Supplemental figure 1





Statistical Analysis Plan

Drug Substances: Anastrozole, Tamoxifen, Zoledronate
Study Code: ABCESG-12
Version No.: 1.0
Date: March 12, 2008

An Austrian Breast Cancer Study Group Phase III Trial to Evaluate the Activity of Tamoxifen in Comparison with Anastrozole, either alone or in Combination with Zoledronate, in Premenopausal Stage I and II Breast Cancer Patients with Hormone-Responsive Tumors

**Austrian Breast and Colorectal Cancer Study Group
ABCESG Protocol number 12**

Coordinating Investigator _____

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List of Abbreviations

Abbreviation or special term	Explanation
Ana	Anastrozole
ABCSG	Austrian Breast and Colorectal Cancer Study Group
AE	Adverse event (see definition in Section 4.7.1.1. in the study protocol).
AI	Aromatase inhibitor
AC	Anastrozole combined with Zometa control group
AZ	Anastrozole combined with Zometa group
BMD	Bone mineral density
Con	Control group
CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
CTIBL	cancer treatment-induced bone loss
DFS	Disease-free survival
Endpoint	A status of the patient that constitutes the 'endpoint' of a patient's participation in a clinical study and that is used as the final outcome.
ER-ICA	Oestrogen receptor
GCP	Good Clinical Practice
IAB	International advisory board
ICH	International Conference on Harmonisation
i. v.	intravenous
Measurement	An observation made on a variable using a measurement device.
MedDRA	Medical dictionary for Regulatory Activities
Nol	Nolvadex
OS	Overall survival
PgR-ICA	Progesterone receptor
RFS	Recurrence free survival
SAE	Serious adverse event
SAP	Statistical analysis plan
STD	Standard deviation
SOP	Standard operational procedure
Tam	Tamoxifen
TC	Tamoxifen combined with Zometa control group
TZ	Tamoxifen combined with Zometa group
Zol	Zoledronic acid

Introduction

The Austrian Breast & Colorectal Cancer Study Group (ABCSCG) is a cooperative institution that was set up to conduct controlled clinical trials in breast and colorectal cancer and to facilitate communication and the dissemination of knowledge among scientists and others dedicated to the cancer problem. The ultimate goal of the ABCSCG is to enhance the standard of cancer treatment in this country and abroad by developing innovative approaches and testing increasingly more effective therapeutic strategies.

Prospectively randomized clinical investigations have come to be seen as the only instrument to generate valid and reliable clinical data, to gain insights into significant prognostic and predictive factors, and thus to enhance all aspects of evidence-based medicine. As a target of oncological research and practice, the ABCSCG has selected two of the most common malignancies affecting women and men in the present-day western world: carcinoma of the breast and bowel.

As a whole, the ABCSCG collaborates toward the common goal of controlling, effectively treating, and ultimately curing cancer by means of large, multi-center cancer trials in the (neo-)adjuvant setting. Research results are provided to the medical community through scientific publications and professional meetings.

This Statistical Analysis Plan (SAP) is based on the Amended Clinical Study Protocol for ABCSCG study 12 and gives a detailed description of all statistical analyses to be conducted within this trial at predefined time points.

Study Details

Study Design

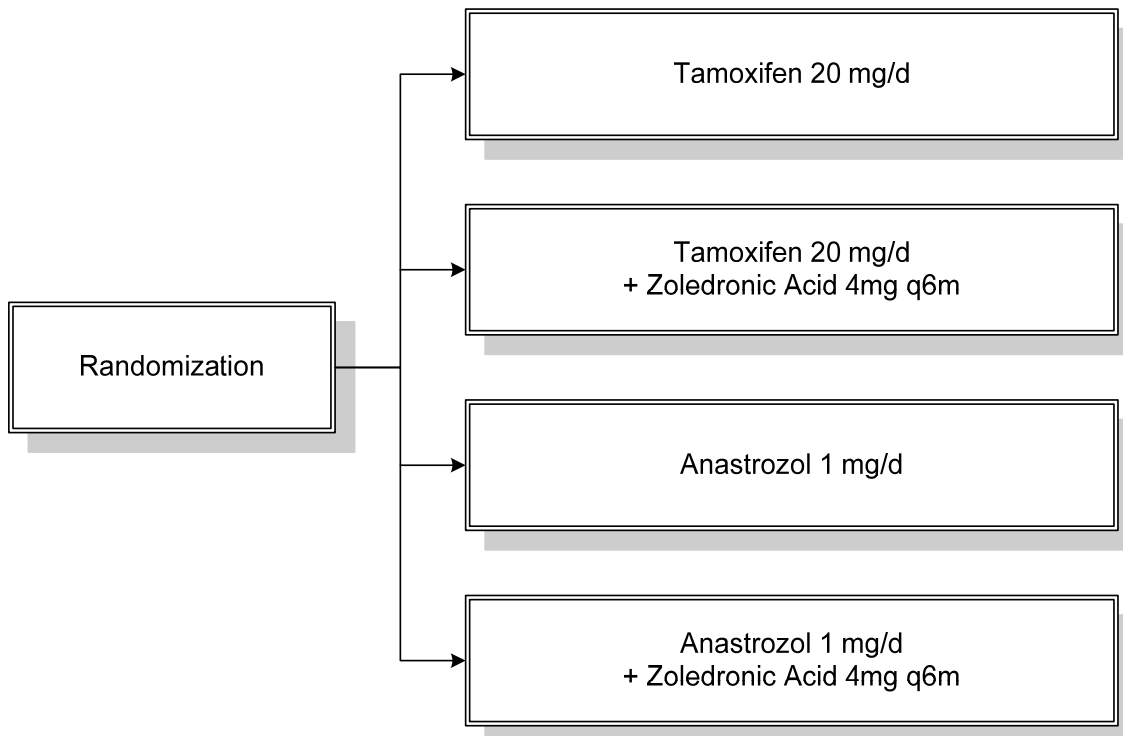
Overall Study Design

The trial is conducted as an open multi-center phase III study, in a two-factorial study design and according to GCP guidelines. Patients will be randomly assigned to a total of 4 study groups in a 1:1:1:1 assignment ratio. Several stratification criteria will be used in order to ensure balanced distribution of known risk factors.

Patients will either be treated with anastrozole (1mg daily for 3 years) or tamoxifen (20mg daily for 3 years), and will additionally receive either zoledronate (8mg q4 weeks for 3 years) or no zoledronate.

- arm A: Nolvadex alone
- arm B: Nolvadex plus zoledronate
- arm C: Arimidex alone
- arm D: Arimidex plus zoledronate

Figure 1: Therapy arms of ABCSG-12



Zoledronate will be administered by i.v. injection at a dose of 8 mg/month (4 mg/month after amendment 3) for the treatment period of 3 years. For the primary objective in the zoledronate group, 50 patients in each treatment arm will be necessary to obtain an acceptable number of BMD measurements to show a difference in the two treatment groups (with bisphosphonate vs. without bisphosphonate). Five BMD measurements are planned to be performed per patient. The randomization group will be generated by the trial statistician.

The study medication should start to be administered to eligible patients no later than 8 weeks post surgery. The treatment period will last 3 years, and the observation period another 5 years.

FPFV: May 1999

LPFV: May 2004

LPLV: May 2007

A Data Safety Monitoring Board will be installed to monitor the safety parameters of this study upon a regular basis. This committee will consist of the following members: one statistician and two oncologists experienced in this indication.

Randomisation

The actual treatment given to individual subjects will be randomized according to the adaptive randomization method of Pocock and Simon (1975), using a computer program.

The detailed randomization process is as follows:

Patient will be identified and all necessary investigations for the entry visit will be done. Patient will receive all the information (verbal and written) to understand the purpose and the course of the trial and consents (written consent). Eligibility of the patient will be checked using the randomization check list (cf. separate SOP). Investigator will call the Randomization Center, University of Vienna Surgical Department (contact Mrs. Eichwalder, Mrs. Gustavson or Mrs. Chalupa, tel: (01) 40400 ext. 2238, or (01) 4081416; Mo-Fr 08:00-15:30). The caller should identify himself, state the trial number (12) and provide patient information together with all data necessary for randomization, including the stratification criteria. The investigator will receive the continuous patient numbering and the randomized treatment arm. Note: Investigator will have the option to alternatively fax

the randomization check list, containing the patient identification, to the Study Center (fax: (01) 4090990) and in turn will receive randomization results by fax.

In summary, the patient identification received by the Randomization Center consists of the following 2 elements:

A four-digit number for the sequential numbering of the patient.

A letter code identifying the treatment arm: A for Nolvadex
 B for Nolvadex and zoledronate
 C for Arimidex
 D for Arimidex and zoledronate

Number of Subjects

Determination of Sample Size

Disease-free Survival

A difference in 5-year disease-free survival of 10% (70% and 80% survival) between the therapy groups (tamoxifen compared to anastrozole and zoledronate compared to no zoledronate) can be detected - with a significance level of 5% and a power of 90% - with a two-sided test, if 250 patients per year are recruited over 5 years and a follow-up period of 0.6 years subsequently (George and Desu 1974, STPLAN 1987). An exponential distribution of survival times is assumed. Summarizing, 1,250 patients will be recruited to the study over 5 years (roughly half of the patients being affected in Austria), and a final analysis will be carried out 5.6 years after start of the study.

Bone Density

Bone density examinations will be carried out pre-study, at 6 months, 12 months, 36 months and 60 months.

A difference of 1.5 percent points in measurements of bone density after 1 year of treatment between therapy without zoledronate (tamoxifen and anastrozole alone) and therapy with zoledronate (tamoxifen + zoledronate and anastrozole + zoledronate) can be detected - with a power of 85%, a two-sided significance level of 5%, and a common standard deviation in all 4 groups of 3.5 - with a two-factorial analysis of variance, if 50

patients are recruited in each group. This leads to an overall sample size of 200 patients. All measurements are related to a baseline measurement before start of treatment (= 100% bone density).

Enhancement of Case Number for BMD (Protocol Amendment 4)

The enhancement of the case number is based on an interaction between therapy and age (not grouped). The estimation of the case number is based on the assumption, that bone density of older patients within the control group, measured as percent of the baseline value, is lower than bone density of younger patients (0.4% each year of life). Within the therapy group the bone density of older patients, measured as percent of the baseline value, increases in comparison to younger patients (0.3% each year of life). Those assumptions result in a case number of 360 analyzable patients. If a drop-out rate of 10% is assumed the additional protocol suggests the inclusion of 400 patients into the trial.

Statistical rationale for planning of the case number to assess the interaction between BMD measurements and age:

To assess the interaction between therapy and age a sample size of 90 patients in each group (summarized 180 with and 180 without zoledronate) reveals different slopes of the regression line of -0.4 in the control group and 0.3 in the zoledronate group with a power of 80% assuming an alpha error of 5%, STD of the age of 5.5 years and a STD of the residuals of 13 years. A power of 80% is sufficient for the occurrence of an interaction.

Increase of Case Number for primary Outcome (Protocol Amendment 5)

The IAB reviewed a report of trial data on cancer treatment-induced bone loss (CTIBL) in the lumbar spine and trochanter in patients treated with zoledronate (vs. controls).

The IAB recommended the study be continued as planned, on the basis of a hazard rate of 1.8 for the sample size calculation in order to answer the underlying scientific question. Furthermore, precautionary measures are to imperatively include bisphosphonate administration to patients (i) who exceed a T-Score of -2.5 and (ii) who lose a total of 10% of BMD within 12 months of treatment. Along with continuous treatment (and yearly BMD measurements), adherence is to be maintained in accordance with the American Society of Clinical Oncology 2003 Update on the role of bisphosphonates. The IAB also reviewed the proposed accrual increase, and concurred with the decision to recommend increase in the patient recruitment to a sample size of 1,800 patients. Finally, the IAB determined that

it would be appropriate to rapidly share data on the incidence and potential prevention of CTIBL with the wider scientific community.

To reveal a hazard ratio of 1.8 between the two therapy arms with a 2-sided alpha error of 0.05 and a power of 90%, 124 events has to be observed the final analysis. Assuming a constant hazard one can expect, that the 5-year survival is 92.6% for the standard therapy can be improved to 95.9% by a hazard ratio of 1.8. An average recruiting rate of 278 patients a year, a recruiting duration of 6.5 years and subsequent observation of 2.8 additional years an observation of 124 events can be expected. A total of 1800 patients will be recruited. The expected duration of the trial will be 9.3 years.

Study Objectives

The study protocol is not completely precise with regard to the definition of the primary goal. In 3.2 it is claimed that the “2x2-factorial design allows answering two equally important questions” (comparison of goserelin with tamoxifen and zeldronate with control). The study was powered for the primary outcome variable disease free survival (DFS) for the comparison between anastrozole vs tamoxifen. In an amendment (see below) due to a recommendation of the International Advisory Board (IAB) an increase of sample size was performed so that the final analysis should be performed after 124 events have been observed. The comparison between zoledronate vs control was planned to be primarily based on bone density (BMD) measurements, although in the protocol a potential positive effect of zoledronate on survival (as a possible consequence of the positive effect on bone density) has been mentioned within the two important goals of the design. In the following we give an exact definition of the primary objectives, a strategy to address the problem of multiplicity, the secondary and explorative objectives, a precise definition of the outcome variables and a sensitivity analysis.

Primary Objectives

Comparison of Tamoxifen and Anastrozole Therapy

The objective is the comparison between tamoxifen (nolvadex) and anastrozole (arimidex) treatment of disease-free survival (DFS) in patients with non-metastatic breast cancer.

Comparison of Zoledronate and Control Group

The objective is to assess whether zoledronate (zometa) added to standard adjuvant treatment can increase DFS in patients with non-metastatic breast cancer as compared to those not receiving zoledronate.

Adjusting for Multiplicity

To adjust for multiple testing a two-sided significance level of $\alpha=0.025$ is applied for each of these primary comparisons, applying a stepwise Bonferroni-Holm procedure if one the two comparisons rejects.

Secondary Objectives

Recurrence free Survival

For the assessment of the recurrence free survival (RFS) patients receiving tamoxifen will be compared to patients receiving anastrozole and patients receiving zoledronate will be compared to controls, respectively.

Overall Survival

For the assessment of the overall survival (OS) patients receiving tamoxifen will be compared to patients receiving anastrozole and patients receiving zoledronate will be compared to controls, respectively.

Bone Loss

The objective is to assess whether zoledronate added to standard adjuvant therapy can decrease or even prevent bone loss in patients treated with hormonal blockade combined with an anti-estrogen or aromatase inhibitor (AI).

Safety

Adverse and severe adverse events will be listed for all therapy subgroups “anastrozole / zoledronate” (AZ), “anastrozole / zoledronate control” (AC), “tamoxifen / zoledronate” (TZ), “tamoxifen / zoledronate control” (TC).

Exploratory Objectives

Bone Metastases

The objective is to explore the effect of zoledronate on the development of bone metastases in patients with primary breast cancer as compared to those patients not treated with zoledronate.

Treatment Interaction

The objective is the assessment of differences between the impact of zoledronate on DFS within the tamoxifen and within the anastrozole group in comparison to controls.

Sensitivity Analysis

Additionally, a sensitivity analysis is planned in order to control a potential bias caused by the BMD measurements in about 400 patients during recruitment period which have been analyzed, showed a clear positive effect of zoledronate and have been published early (following a recommendation of the IAB). To investigate the impact of the release of early results of 400 patients on the further course of the trial the primary comparisons between zoledronate and control in the total sample will be also performed in the (stochastically independent) sub-sample of patients without BMD measurements. If major deviations in the results will appear this will be a concern.

Definition of primary, secondary and exploratory Outcome

Primary Endpoint

Disease free Survival

Generally a disease event is defined as local recurrence, contra-lateral carcinoma, distant metastasis, secondary carcinoma and/or death. Time-to-disease starts at the randomization date and ends at the occurrence of a disease event. If the observation period of a patient ends before any disease event occurs the patient will be censored.

Secondary Endpoints

Recurrence free Survival

A recurrence is defined as occurrence of local relapse, contra-lateral carcinoma, distant metastasis and/or secondary carcinoma. Time-to-recurrence starts at randomization date and ends at the time of the first recurrence. If the observation period of a patient ends before any recurrence the patient will be censored.

Overall Survival

Death is defined as occurrence of breast cancer related and other death. Time-to-death starts at the randomization date and ends at death of the patient. If the patient is alive at the of the observation period the patient will be censored. This period is called censoring time.

Bone Loss

Bone loss is defined as chronological decrease of bone mineral density measured in the lumbar spine and trochanter (pre-study, 6 months, 12 months 36 and 60 months). The interaction of zoledronate with the age of patients (addressed in amendment 4) is interpreted as difference between the impact of zoledronate on BMD between young and old people.

Safety

An adverse event is defined in the International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice as “any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment” (ICH E6:1.2).

Exploratory Endpoints

Bone Metastases

Time-to-occurrence of bone metastases starts at randomization date and ends at the time of the first occurrence of bone metastases. If the observation period of a patient ends before any bone metastases have been diagnosed the patient will be censored.

Interaction of Therapies

Interaction of therapy is defined as the difference of the impact of zoledronate on DFS between patients receiving anastrozole and tamoxifen, respectively.

Analysis Set

Definition of Analysis Sets

The efficacy evaluation of investigated treatments will be based on all randomized patients and an intention-to-treat analysis will be performed. All patients will remain in their randomized treatment group for the purpose of statistical analysis independent of their actual treatment received.

Safety data for this study will be summarized using treatment actually received.

Violations

Any data of patients collected relating to protocol violations will be listed and summarised.

Analysis Methods

The analyses will be carried out using statistical analysis system (SAS) software, by members of the biostatistics department at ABCSG. All analyses are two-sided and follow the intention-to-treat principle.

Analysis Methods for primary Endpoint

Two tests will be performed for primary endpoint DFS referring to the following two pairs of hypotheses (H0 vs H1), respectively.

- H0: There is no difference between patients receiving tamoxifen and patients receiving anastrozole in respect of DFS.
H1: There is a difference between patients receiving tamoxifen and patients receiving anastrozole in respect of DFS.
- H0: The addition of zoledronate does not have an impact on DFS in comparison to controls not receiving zoledronate.
H1: The addition of zoledronate has an impact on DFS in comparison to controls not receiving zoledronate.

Analysis Methods for Disease free Survival

The two tests of the primary endpoint will be calculated starting with the 2-sided significance level of 2.5% applying the adjustment for multiplicity according to Bonferroni-Holm. Treatment comparisons of the 2x2-factorial design will be conducted with the proportional hazards regression model of Cox (1972) considering the stratification criteria of randomization. The primary predictors are: therapy 1 [0: Tamoxifen vs 1: Anastrozole] and therapy 2 [0: Controls vs 1: Zoledronate]. Potential confounding effects will be assessed with the variables age [0: ≤40 vs 1: >40], T-stage [0: T1 vs 1: T2], grading [0: G1/2 vs 1: G3], number of affected lymph nodes [0: negative vs 1: positive], ER-ICA [0: negative, 1: positive], PgR-ICA [0: negative, 1: positive].

The proportionality assumption of the Cox model will be investigated with a time-dependent exploratory variable, which is defined as treatment multiplied by the logarithm (base e) of the time-to-event. If there is evidence of a departure from the adjusted model assumptions the reason will be explored and reported.

All effects are quantified with hazard ratios. Therapy effects are also graphically shown with estimated survival functions according to Kaplan and Meier (1958). The results of the

analysis will be presented in terms of hazard ratios together with associated 95 % confidence intervals and 2-sided p-values.

Table 1: Baseline Prognostic Variables

Baseline Covariate	Values
oestrogen receptor expression	negative
	+
	++
	+++
progesteron receptor expression	negative
	+
	++
	+++
axillary lymph-node status	N0
	N1
	N2
	N3
tumour stage	T1
	T2
	T3
differentiation grade	G1
	G2
	G3
	GX

SAS Code Fragments

The following statements are code fragments used for the main statistical analyses. They are based on SAS® version 9.1. Italic words will be replaced by correct names of the datasets and variables (according to the database). In survival time data events are coded with 1 and 0 otherwise.

Kaplan-Meier estimation

```
proc lifetest data=dataset plot=(s) notable;
  time time*status(0);
  strata covariate;
run;
```

Cox regression model

a) Univariate:

```
proc phreg data=dataset;  
  model time*status(0)=covariate / rl;  
  strata stratification_factors;  
run;
```

b) Stratified:

```
proc phreg data=dataset;  
  model time*status(0)=covariate / rl;  
  strata stratification_factors;  
run;
```

c) Multiple:

```
proc phreg data=dataset;  
  model time*status(0)=list_of_covariates / rl;  
run;
```

Analysis Methods for secondary Endpoints

Analyses of secondary endpoints will be calculated at the 2-sided significance level of 5%.

Recurrence free Survival

The same model for statistical analysis will be applied as for disease free survival.

Overall Survival

The same model for statistical analysis will be applied as for disease free survival.

Bone Loss

Differences in bone density after one year between patients with vs. those without zoledronate therapy will be analyzed using an analysis of variance. As bone density for each patient will be measured several times following the begin of therapy, a repeated measures analysis of variance will be also used to evaluate the time effect, effect of zoledronate, and a possible interaction between zoledronate therapy and time. Appropriate transformations (e.g. logarithmic transformation) are applied in cases of skew distributions or heteroscedasticity. BMD measurements will be analyzed with a mixture model accounting for interactions with age of patients.

SAS Code Fragments

Repeated Measures Mixed Model

```
proc mixed data=dataset;  
  class treatment;
```

```

model BMD = time*treatment time2*treatment;
random int / type=un subject=subject;
repeated / subject=subject type=ar(1);
contrast 'chronological sequence Zol vs Con (quadratic)'
      time*treatment 0.5 -0.5 0.5 -0.5,
      time2*treatment 0.5 -0.5 0.5 -0.5 ;
contrast 'chronological sequence Ana vs Nol (quadratic)'
      time*treatment 0.5 0.5 -0.5 -0.5,
      time2*treatment 0.5 0.5 -0.5 -0.5 ;
contrast 'chronological sequence interaction Zol/Con and Nol/Ana
(quadratic)'
      time*treatment 0.5 -0.5 -0.5 0.5,
      time2*treatment 0.5 -0.5 -0.5 0.5 ;
contrast 'chronological sequence Ana vs Nol (without Zol)'
      time*treatment 0.5 0 -0.5 0,
      time2*treatment 0.5 0 -0.5 0;
contrast 'chronological sequence Ana vs Nol (with Zol)'
      time*treatment 0 0.5 0 -0.5,
      time2*treatment 0 0.5 0 -0.5;
contrast 'chronological sequence Zol/Ana'
      time*treatment 0 1 0 0,
      time2*treatment 0 1 0 0;
contrast 'chronological sequence Zol/Nol'
      time*treatment 0 0 0 1,
      time2*treatment 0 0 0 1;
contrast 'chronological sequence Con/Ana'
      time*treatment 1 0 0 0,
      time2*treatment 1 0 0 0;
contrast 'chronological sequence Con/Nol'
      time*treatment 0 0 1 0,
      time2*treatment 0 0 1 0;
contrast 'chronological sequence Con'
      time*treatment 1 0 1 0,
      time2*treatment 1 0 1 0;
contrast 'chronological sequence Zol'
      time*treatment 0 1 0 1,
      time2*treatment 0 1 0 1;

run;

```

Safety

Safety and tolerability data will be presented by treatment received. Appropriate summaries of these data will be presented with descriptive statistics. Safety and tolerability will be assessed in terms of AEs, SAEs and laboratory data outside reference intervals, which will be collected for all patients. Data from all treatment periods will be combined in the presentation of safety data. AEs (both in terms of MedDRA preferred terms and CTCAE grade), and laboratory data will be listed individually by patient and summarised by treatment received. Time-to-serious-adverse-events will be used for treatment comparisons applying a Cox proportional hazard model with the two treatment factors and their interaction.

For patients who have a treatment modification, all AE data (due to toxicity or otherwise) will be assigned to the initial treatment received.

Analysis Methods for exploratory Endpoints

Analyses of exploratory endpoints will be calculated at the 2-sided significance level of 5%.

Bone Metastases

The same model for statistical analysis will be applied as for disease free survival.

Interaction of Therapies

The distribution of disease free survival time within the 4 subgroups (TC, TZ, AC, AZ) will be visualized by Kaplan-Meier curves in the four treatment sub-groups. The treatment interaction will be tested using the same model as in the primary analysis including an additional multiplicative interaction term.

SAS Code Fragments

Cox regression model

```
proc phreg data=dataset;
  model time*status(0)=therapy1 therapy2 therapy1_therapy2
        / r1 include=number_of_covariates selection=f;
  therapy1_therapy2= therapy1*therapy2
run;
```

Other Calculations

Additionally paired and unpaired Student's t-tests will compare baseline BMD and T-score with 36 month and 60 month values within the treatment groups. Descriptive statistics will be given as mean and standard deviations, optionally as median, quartiles, minimum and maximum. Frequencies will be given as case numbers and percent values.

Sensitivity Analysis

To assess sensitivity the analyses conducted for the primary outcome will be repeated on the sub-sample of patients who had no BMD measurements analyzed and reported in the interim analysis of bone density.

Tabular Summaries

Description of tabular summaries:

The following demographic information will be summarised by randomised treatment group:

- Age: this summary will include number of observations, mean, STD, minimum, maximum and number of subjects with missing values in case of an approximately normal distribution. Otherwise, mean and STD are replaced by the median.

The following breast cancer history information will be summarised by randomised treatment group:

- Any prior or current cytotoxic chemotherapy, except preoperative chemotherapy, oestrogen and progesterone receptor expression, axillary lymph-node status, tumour diameter and differentiation grade the number and percentage of subjects will be summarised.

The following withdrawal information and protocol violence will be summarised by randomised treatment group:

- Subject disposition status (randomised, treated, withdrawn, completed); the number and percentage of subjects will be summarised.
- Reason for withdrawal (adverse event, recurrence of breast cancer, patient request, death, other reasons): the number and percentage of subjects will be summarised.

Serious adverse event data, whilst patients are receiving study treatment, will be summarised by study treatment group.

Interim Analyses

The interim analysis which was planned in the protocol was skipped because of the low overall number of events.

A potential bias which may be caused by the enhancement of the case number (recommended early by the IAB, Amendment 5) should not arise in the sensitivity analysis.

Changes of Analysis from Protocol

Since the study protocol has not been precise enough on some statistical issues these have clarified in the SAP.

References

- Cox D.R. (1972) Regression models and life-tables (with discussion). Journal of the Royal Statistical Society, Series B 34, 187-220.
- Kaplan E.L. & Meier,P. (1958) Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53, 457-481.
- Pocock S J., & Simon R. (1975) Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 31, 103-115.