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Research Paper

Urinary biomarkers in metastatic bone pain: Results from a multicentre randomized trial of ibandronate compared to single dose radiotherapy for localized metastatic bone pain in prostate cancer (RIB)

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HIGHLIGHTS

- Bone marker data paired with pain scores from a large prospective randomised trial.
- No correlation seen between NTx or Cystatin C and pain response.
- Reduction in NTx concentrations seen 4 weeks after ibandronate.
- No change NTx after radiotherapy suggesting a different mechanism of action to ibandronate.

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ABSTRACT

Background: The Radiotherapy IBandronate (RIB) trial compared single dose radiotherapy and a single infusion of ibandronate in 470 bisphosphonate naïve patients with metastatic bone pain from prostate cancer randomised into a non-inferiority two arm study. Results for the primary endpoint of pain score response at 4 weeks showed that the ibandronate arm was non-inferior to single dose radiotherapy.

Patients and method: In addition to pain assessments including analgesic use made at baseline, 4, 8, 12, 26 and 52 weeks, urine was collected at baseline, 4 and 12 weeks. It was subsequently analysed for urinary N-telopeptide (NTx) and cystatin C. Linear regression models were used to compare the continuous outcome measures for urinary markers within treatment arms and baseline measurements were included as covariates. Interaction terms were fitted to allow for cross-treatment group comparisons.

Results: The primary endpoint of the RIB trial was worst pain response at 4 weeks and there was no treatment difference seen. Urine samples and paired pain scores at 4 weeks were available for 273 patients (radiotherapy 168; ibandronate 159)

The baseline samples measured for the RIB trial had an average concentration of 193 nM BCE/mM creatinine (range of 7.3–1871) compared to the quoted normal range of 33 nM BCE/mM creatinine (3 to 63). In contrast the average value of Cystatin C was 66 ng/ml (ranges ND – 1120 ng/ml) compared to the quoted normal range of 62.9 ng/ml (ranges 12.6–188 ng/ml). A statistically significant reduction in NTx concentrations between baseline and 4 weeks was seen in the ibandronate arm but not in the radiotherapy arm. No correlation between pain response and urinary marker concentration was seen in either the ibandronate or radiotherapy cohort at any time point.

Conclusion: NTx was significantly raised compared to the normal range consistent with a role as a biomarker for bone metastases from prostate cancer. A significant reduction in NTx 4 weeks after ibandronate is consistent with its action in osteoclast inhibition which was not seen after radiotherapy implying a different mode of action for radiation. There was no correlation between bone biomarker levels and pain response.

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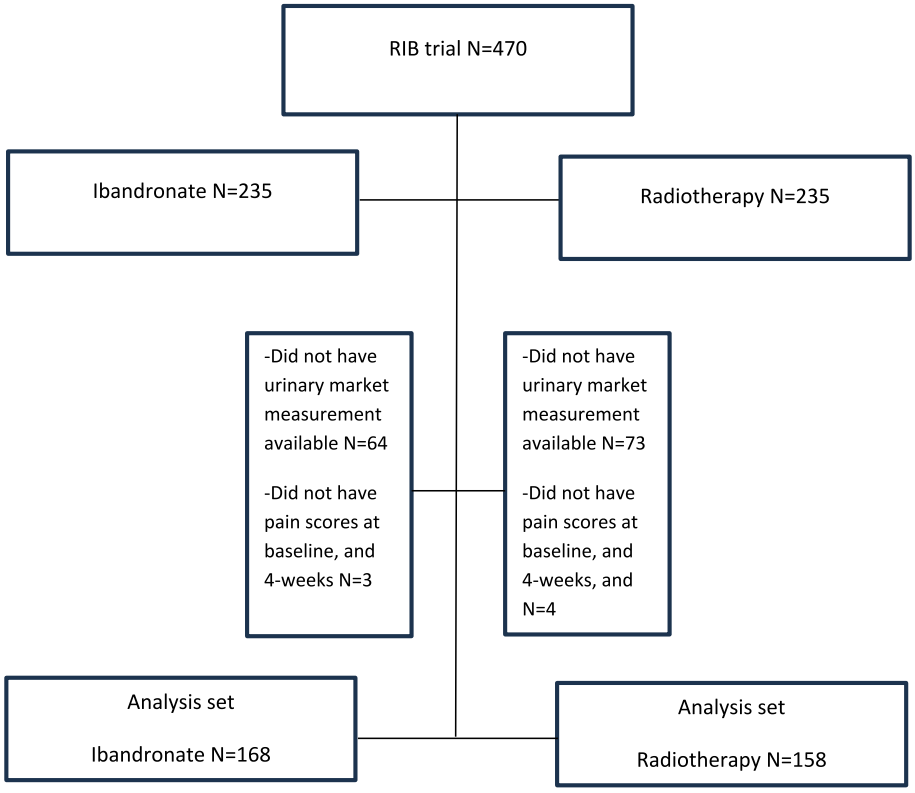

Fig. 1. Consort diagram of subset.

1. Introduction

The Radiotherapy IBandronate (RIB) trial compared single dose radiotherapy and a single infusion of ibandronate in 470 bisphosphonate naïve patients with prostate cancer who had metastatic bone pain [1] with accrual between April 2003 and November 2009. This was a non-inferiority two arm study with the primary endpoint of pain response at 4 weeks. There was little difference in worst pain response at 4 weeks (ibandronate 49.5 % vs. RT 53.1 %; $p = 0.49$), or 12 weeks (ibandronate 56.1 % vs. RT 49.4 %; $p = 0.24$). RIB is the only randomised trial to directly compare radiotherapy with bisphosphonates for pain relief from metastatic bone pain in prostate cancer where, unlike in breast cancer [2] and myeloma [3] there is very little data on their efficacy.

At present, there are no good predictive biomarkers for response of metastatic bone pain to current therapies. In the RIB trial, pain assessments and urine samples were collected to explore a potential role for bone biomarkers in this setting. The purpose was to examine the correlation between urinary N-telopeptide (NTx) and cystatin C [4,5] with pain response score. These two biomarkers were chosen as NTx is a marker of osteoclastic activity and cystatin C is a potential biomarker with osteoclastogenic activity [6].

2. Patients and method

Details of the RIB trial are described elsewhere. [1] Following baseline measurements pain assessments including analgesic use were made at 4, 8, 12, 26 and 52 weeks post-baseline with quality of life and urine sample collections at 4 and 12 weeks.

Pain response was measured by the Brief Pain Inventory (BPI) incorporating analgesic use which was scored between 1 and 3, based on the strongest pain medication taken at each time point and also using the Effective Analgesic Score (EAS), as described by Mercadante et al [7]. This also takes into account patient pain score and the type and dose of pain medication used, expressed as a morphine equivalent (mg). [1] This endpoint (on a continuous scale 0 to 150), was considered more sensitive to changes over time for a given patient than the BPI. Response was defined by a score of zero at the time point of interest or a reduction of 20 % or more from baseline.

Urine samples were collected at the treating centre and sent by post to the trial co-ordinator at Mount Vernon Cancer Centre. They were centrifuged to remove any cellular and precipitated material and were aliquoted into ~5 ml samples and stored at <-60 °C. Samples were initially stored at the Gray Laboratories, Mount Vernon Hospital Northwood, and with the laboratory transfer moved to the Oxford Institute for Radiation Oncology, Oxford University where the marker analyses were undertaken.

The laboratory analysts were blind to treatment arm, pain score and response. Urinary N-telopeptide was measured using Osteomark NTx with an ELISA kit from Alere (cat. no. X9006) [8] which included standards and QC samples. Urinary Cystatin C was measured using an ELISA kit from R & D Systems (cat. no. DSCTC0) and QC samples prepared from Quantikine® immunoassay control groups (cat. no. QC23) [9]. Creatinine measurements were used as a normalisation factor for the measurement of urinary Cystatin C and Osteomark NTx.

Linear regression models were fitted with baseline measures included as covariates to compare the continuous outcome measures for urinary markers within treatment arms from baseline to 4 weeks. Cross-treatment arm comparisons were made using linear regression models with interaction terms between the treatment group (Radiotherapy or Ibandronate) and each of the urinary markers and, where applicable, pain measurements. Where it was deemed appropriate, the scales of the continuous urinary markers were changed to aid interpretation and presented alongside 95 % confidence intervals and P-values using a threshold of 0.05 for statistical significance. Analyses of treatment groups were conducted according to intention-to-treat and included participants with at least one follow-up time point. Where appropriate, crossovers between treatment arms that occurred during the RIB trial were considered to further investigate treatment effects. Controls were

Table 1
Mean change in urinary markers from baseline to 4 weeks, by treatment arm.

		Ibandronate N=168	Radiotherapy N=158
NTx [BCE]	Baseline	1995.7	3040.8
	4 weeks	1238.3	2822.7
	Mean change (95 % CI)	−757.3 (−443.6 – −1071.1)	−218.1 (−493.2 – 929.6)
		Ibandronate N=40	Radiotherapy N=142
Cystatin C	Baseline	0.06	0.05
	4 weeks	0.07	0.06
	Mean change (95 % CI)	0.01 (0.004 – 0.03)	0.003 (0.01 – −0.02)

based on normal ranges which were provided by the manufacturer of the assay kits; urinary N-telopeptide quoted in men (mean age 56 years, range 31–87) [8] and Cystatin C from 'apparently healthy volunteers' [9]. All analyses were conducted in Stata 16 [10].

3. Results

A total of 470 patients with metastatic bone pain were prospectively randomised in the RIB trial to receive either 8 Gy single dose radiotherapy (235) or a single dose intravenous infusion of 6 mg ibandronate (235).

Urine samples for both time points and paired pain scores at 4 and 12 weeks were available for 273 patients (radiotherapy 168; ibandronate 159) as shown in Fig. 1, reflecting the considerable attrition rate in this population of patients.

There was greater inter-patient variability in the urine

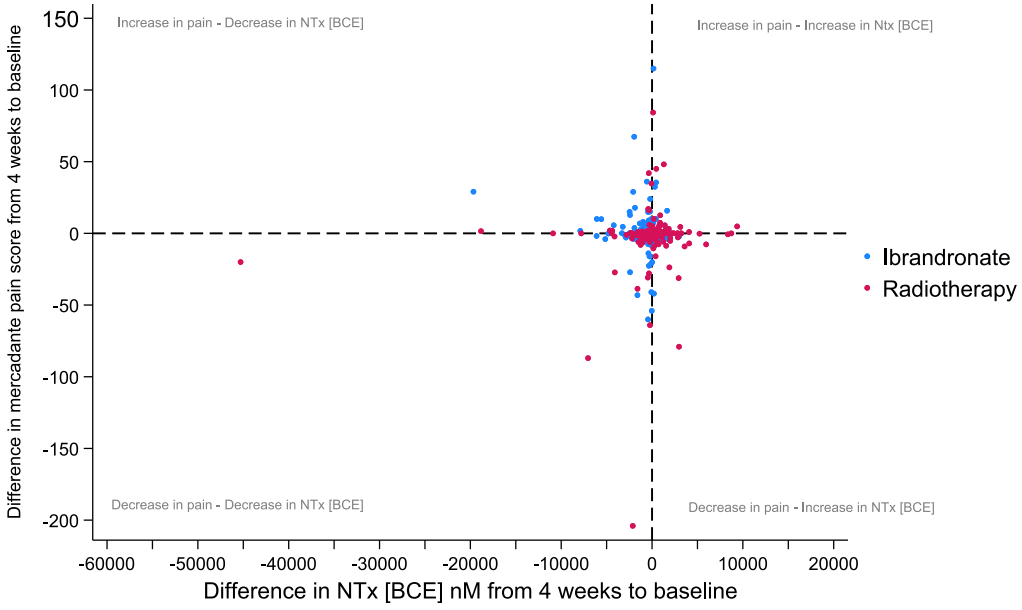

Fig. 2. Scatter plot of NTx [BCE] by Mercadante pain score change from 4 weeks to baseline, by treatment arm.

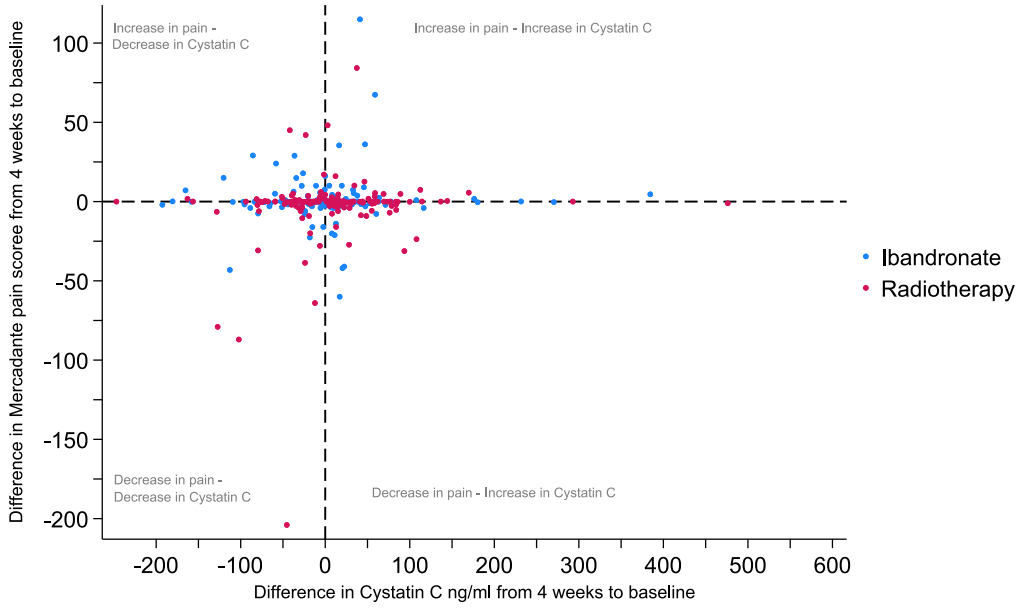

Fig. 3. Scatter plot of Cystatin C by Mercadante pain score change from 4 weeks to baseline, by treatment arm.

Table 2
Mean change in the EAS pain score at 4 weeks per change in urinary markers at 4 weeks from baseline, by treatment arm.

	Coefficient for change in Mercadante pain at 4 weeks	(95 % CI)	P-Value
Ibandronate N = 168			
NTx [BCE] per 100 units – change from baseline at 4 weeks	−0.10	−0.21 – 0.01	0.08
Radiotherapy N = 158			
NTx [BCE] per 100 units – change from baseline at 4 weeks	−0.02	−0.02 – 0.06	0.34
Ibandronate N = 142			
Cystatin C per 1 unit change-change from baseline at 4 weeks	2.0	−23.6 – 27.6	0.88
Radiotherapy N = 142			
Cystatin C per 1 unit change-change from baseline at 4 weeks	5.4	−18.2 – 29.1	0.65

concentrations of N-telopeptides of type-1 collagen (NTx) than there was for Cystatin C, with no correlation between concentration of NTx and Cystatin C measured in the urine samples. The average concentrations of NTx measured in healthy male volunteers by the supplier of the kits is 33 nM BCE/mM creatinine with a range of 3 to 63 nM/Mm creatinine [8] . The baseline samples measured for the RIB trial had an average concentration of 193 nM BCE/mM creatinine with an evaluable range of 7.3–1871 nM BCE/mM creatinine which extended far beyond the range of healthy volunteers. In contrast the quoted average concentrations of Cystatin C measured in apparently healthy volunteers is 62.9 ng/ml with ranges of 12.6–188 ng/ml [9] and the baseline samples from this study had average values of 66 ng/ml with ranges from ND to 1120 ng/ml and the vast majority of samples falling within the range measured in apparently healthy volunteers, see Supplementary Figs. 1 and 2.

The mean change in urinary markers from baseline to 4 weeks, by treatment arm is shown in Table 1. A statistically significant reduction in NTx concentrations between baseline and 4 weeks was seen in the ibandronate arm (−0.5 nM BCE/mM, 95 % CI 0.36–0.61, $P < 0.001$) but was not seen in the radiotherapy arm (−0.05 nM BCE/nM, 95 % CI −0.19 – 0.09, $P = 0.47$). No significant change was seen in Cystatin C concentrations between baseline and 4 weeks for either arm.

Overall, there was no strong relation between pain response and urinary marker concentration in either the ibandronate or radiotherapy cohort at any time point, see Figs. 2 and 3. There was evidence of an interaction between NTx[BCE] per 100 units and treatment arm for Mercadante pain score at 4 weeks ($P = 0.03$), but this was no longer seen when the NTx concentrations were normalised to serum creatinine as shown in Table 2. No effect was seen when the same analysis was undertaken with the Effective Analgesia Score (EAS) pain score. Further subgroup analysis showed a marginal association between NTx [BCE] and Mercadante pain score at 4 weeks among patients treated with Ibandronate, with there being a decrease in pain per 100 unit decrease in NTx [BCE] at 4 weeks from baseline (−0.10, 95 % CI −0.21 – 0.01, $P = 0.08$).

4. Discussion

Our study hypothesis was that pain relief after treatment for painful bone metastases with either radiotherapy or ibandronate is related to baseline and subsequent changes in osteoclast activity as measured by urinary levels of N-telopeptide and Cystatin C. The baseline levels of NTx were significantly raised in all patients consistent with a role in identifying those patients with bone breakdown caused by metastases. However no subsequent correlation with bone pain response was seen at baseline or any subsequent time point.

One sub-study from a previous radiotherapy study suggested a relationship between baseline and changes in the osteoclast marker deoxypyridinoline and pain response [11], whilst another failed to confirm this [12]. Analysis of the NCIC trial of reirradiation for painful bone metastases however showed a clear correlation between urinary markers pyridinoline and deoxypyridinoline at baseline and response, those patients having higher levels predicting non-response to re-irradiation [13]. The markers of bone resorption used in these older studies, pyridinoline, deoxypyridinoline and hydroxyproline have been criticized as relatively non-specific. Telopeptides which are direct breakdown products of type I collagen have been shown to have greater specificity as markers of bone resorption [3].

Cystatin C has also been shown to reflect osteoblastic activity in prostate cancer patients with bone metastasis receiving bisphosphonates [14]. It may also prevent tumour progression in prostate cancer by inhibition of lysosomal cysteine proteases and reduce cancer cell invasiveness through inhibition of extracellular matrix proteins [15]. Low levels of cystatin C are associated with metastases and a worse prognosis. In this population of patients with metastatic prostate cancer low levels of Cystatin C might have been expected but in fact the average was within the normal range at baseline and at 4 weeks no significant changes seen with either ibandronate or radiotherapy. This would imply no compensatory osteoblastic activity to either treatment intervention at 4 weeks or later time points to 12 weeks. The mechanism of action of ibandronate is osteoclast inhibition. This was seen with a statistically significant fall in NTx levels. The mechanism of action of radiotherapy in pain relief when used for painful bone metastases is not well characterised [16] and an effect on osteoclast activity has been proposed. The data from this analysis would suggest this is not the case with unlike the ibandronate arm no significant change seen in NTx levels from baseline to 4 weeks. This is an important observation in eliciting the mechanism of radiotherapy in pain relief from bone metastases and points to a direct effect on tumour cells and possibly neurogenic mechanisms of pain.

The strength of this study is the correlation with data from a large randomised trial with prospective pain measures at fixed time points and paired urinary samples. An important observation is the lack of osteoclast inhibition as identified by NTx levels in the radiotherapy arm and it is unfortunate that PSA levels were not also measured at the relevant time points to evaluate tumour cell activity. Radiotherapy was delivered to sites of local pain in patients with multiple metastases which may be an alternative explanation for the lack of reduction in NTx levels compared to ibandronate which is a systemic treatment. A further criticism would be the absence of a measure of tumour burden which might be expected to correlate with bone marker levels.

In conclusion urinary markers NTx and Cystatin C failed to predict for pain relief from local radiotherapy or systemic ibandronate. Whilst a fall in NTx levels with ibandronate was seen consistent with osteoclast inhibition no change occurred after radiotherapy implying a different mechanism for pain relief after radiotherapy.

CRediT authorship contribution statement

P.J. Hoskin: Writing – review & editing, Writing – original draft, Validation, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Aman Malhi:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis. **Krystyna Reczko:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Data curation. **Allan Hackshaw:** Conceptualisation, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft and final draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbo.2024.100624.

References

[1] P. Hoskin, S. Sundar, K. Reczko, S. Forsyth, N. Mithal, B. Sizer, D. Bloomfield, S. Upadhyay, P. Wilson, A. Kirkwood, M. Stratford, M. Jitlal, A. Hackshaw, A multicenter randomized trial of ibandronate compared with single-dose radiotherapy for localized metastatic bone pain in prostate cancer, J. Natl. Cancer Inst. 107 (2015) 10.

[2] B. O'Carrigan, M.H.F. Wong, M.L. Willson, et al., Bisphosphonates and other bone agents for breast cancer, Cochrane Database Syst. Rev. 10 (2017) 1465–1858.

[3] R. Mhaskar, A. Kumar, B. Miladinovic, et al., Bisphosphonates in multiple myeloma: an updated network meta-analysis, Cochrane Database Syst. Rev. 12 (2017) 1465–1858.

[4] P. Peichl, A. Griesmacherb, R. Marteau, et al., Serum crosslaps in comparison to serum osteocalcin and urinary bone resorption markers, Clin. Biochem. 34 (2001) 131–139.

[5] E. Kulcsar-Jakab, Z. Petho, Z. Pap, et al., Cystatin C as a potential predictor of osteoprotegerin levels in healthy men, a cross-sectional, observational study, BMC Musculoskelet. Disord. 16 (2015) 227.

[6] G. Leto, M. Crescimanno, C. Flandina, On the role of cystatin C in cancer progression, Life Sci. 202 (2018) 152–160.

[7] S. Mercadante, Scoring the effect of radiotherapy for painful bone metastases, Support. Care Cancer 14 (2006) 967–969.

[8] Osteomark NTx data sheet, Alere Scarborough, Inc. Scarborough, ME 04074 USA.

[9] Human Cystatin C Quantikine data sheet https://resources.rndsystems.com/pdfs/datasheets/dsctc0.pdf?v=20240217&_ga=2.32934334.1244816779.1708187416-1402475106.1708187416; accessed 17th February 2024.

[10] StataCorp, Stata Statistical Software: Release 16, StataCorp LLC, College Station, TX, 2019.

[11] P.J. Hoskin, M.R.L. Stratford, L.K. Folkes, J. Regan, J.R. Yarnold, Effect of local radiotherapy for bone pain on urinary markers of osteoclast activity, Lancet 355 (2000) 1428–1429.

[12] E. Chow, A. Hird, L. Zhang, E. Sinclair, C. Danjoux, E. Barnes, M. Tsao, L. Barbera, S. Wong, R. Vieth, Change in urinary markers of osteoclast activity following palliative radiotherapy for bone metastases, Clin. Oncol. (R. Coll. Radiol.) 21 (4) (2009) 336–342.

[13] E. Chow, C. DeAngelis, B.E. Chen, A. Azad, R.M. Meyer, C. Wilson, M. Kerba, A. Bezjak, P. Wilson, A. Nabid, J. Greenland, G. Rees, R. Vieth, R.K. Wong, P. Hoskin, Effect of re-irradiation for painful bone metastases on urinary markers of osteoclast activity (NCIC CTG SC.20U), Radiother. Oncol. 14 (2015), https://doi.org/10.1016/j.radonc.2015.02.025. S0167-8140(15)00121-8.

[14] F.M. Tumminello, G. Badalamenti, L. Incorvaia, F. Fulfaro, C. D'Amico, et al., Serum interleukin-6 in patients with metastatic bone disease: correlation with cystatin C, Med. Oncol. 26 (2009) 10–15.

[15] B. Wegiel, T. Jiborn, M. Abrahamson, L. Helczynski, L. Otterbein, J.L. Persson, A. Bjartell, Cystatin C is downregulated in prostate cancer and modulates invasion of prostate cancer cells via MAPK/Erk and androgen receptor pathways, PLoS ONE 4 (11) (2009) e7953.

[16] P.J. Hoskin, Scientific and clinical aspects of radiotherapy in the relief of bone pain, Cancer Surv. 7 (1) (1988) 69–86.