Effective and Rapid Osteoclast Inhibition by Denosumab, is Reflected in Serum C Telopeptide (CTX), Across Different Tumor Types

S. Tuyls[1], F. Geurs[1,*] and Y. Deprest[2]

[1]Depts. of Medical Oncology; [2]Laboratory, Regional Hospital Sint Maria, Ziekenhuislaan 100, 1500 Halle, Belgium

Abstract: Denosumab, a monoclonal antibody against RANKL has superior efficacy against bone metastases. Its *in vivo* activity can be measured recently by dosing of serum C telopeptide, produced by collagen destruction by the osteoclast. We report the first patient series, across several tumor types of the efficacy of denosumab in normalising serum CTX.

Keywords: Bone metastases, denosumab, serum c telopeptide.

INTRODUCTION

Denosumab recently received reimbursement and approval by the FDA and EMEA, due to its superior efficacy in decreasing the morbidity of bone metastases of breast, prostate and other tumor types [1-3]. This was the result of the initial phase I and II trials indicating its superior efficacy compared to pamidronate in normalizing bone resorption parameters in breast cancer and myeloma patients [4-6]. These initial studies used older urinary parameters of bone resorption and alkaline phosphatise in serum to measure osteoblast activity. However the effect of denosumab on other tumor types and more new serum markers of bone resorption such as C-telopeptides (CTX) however, have only recently became the subject of investigation [7, 8].

The osteoclasts cause breakdown of type I collagen transverse cross-links, the main constituent of the bone's organic matrix, resulting in the release of degradation molecules such as pyridinoline, deoxypyridinoline, N-telopeptides (NTX) and C-telopeptides (CTX) [7]. These products are released into the blood and excreted in the urine, and constitute markers of bone formation and resorption (e.g. alkaline phosphatase and osteocalcin). The detection of the recent CTX markers in blood is associated with the presence and progression of bone metastases and is linked to the prognosis and the response to pharmacological treatment [7-9].

*Address correspondence to this author at the Depts. of Medical Oncology, Regional Hospital Sint Maria, Ziekenhuislaan 100, 1500 Halle, Belgium;
Tel: 00 32 2 363 66 10; E-mail: g@regzhsintmaria.be

MATERIALS AND METHODS

Study Design

This single centre study ran from June 2012 to September 2012. The aim of this study was to determine the efficacy of a single injection of denosumab in 20 patients with solid tumors, and to evaluate the influence on serum CTX not only in breastcancer and multiple myeloma but also in other common metastatic tumors. Patients with radiological evidence of lytic or mixed bone metastases were enrolled into the study as well as patients with a bone isotope scan confirming diffuse bone metastases. Patients were at least 18 years old and were treated for metastatic disease. Patients were excluded if they had disorders of the parathyroid or serum bilirubin >43 µmol/L, All patients provided written informed consent before enrolment, and the study was approved by the local Ethics Committee.

Study Procedures

During the morning of dosage, patients received a subcutaneous injection of 120 mg denosumab. Medications affecting bone metabolism, such as bisphosphonates, were withdrawn during 30 days before randomization and were not scheduled after randomization. Chemotherapy within 21 days following denosumab treatment, was allowed to reproduce the actual clinical situation where bisphosphonates or denosumab are given together with chemotherapy.

Patients were followed for at least 28 days. Blood samples for alkaline phosphatise and serum CTX were scheduled on days 1or 4, and then during weeks 2 or 3. If a patient could not present for blood sampling in between chemotherapy appointments, the CTX and AF

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at start of the second chemotherapy date (usually day 14 or 21) was taken in account. Radiological assessment, medical history, medication history, and scintigraphic bone involvement were recorded prestudy.

To assess the effect of denosumab administration on bone metabolism, serum CTX dosage was performed using Elecsys® ß-CrosslapsTM/Serum assay (Roche Diagnostics). Precision was evaluated on a two-level quality control. Level 1 at 285 pg/mL with a c.v. of 2.77 and level 2 at 638 pg/mL with a c.v. of 2.45.

Serum alkaline phosphatase activity was measured on a Cobas 6000 analyzer (Roche Diagnostics, Mannheim, Germany) using a colorimetric assay. Between-day imprecision was evaluated using a bilevel quality control (Liquichek, Unassayed chemistry, Bio-Rad). Level 1 at 95 U/L with a CV% of 3.6 and level 2 at 373 U/L with a CV% of 2.6.

RESULTS

Demographics and Baseline Characteristics

A total of 19 patients (6 women, 13 men); median age 72 (range 64-90), with different tumor types with bone metastasis, were enrolled. All patients received study treatment and were assessed for effect on bone metabolism variables with CTX and AF dosaging.

Results on Bone Metabolism

One patient demonstrated normal CTX (below 350 pg/ml) and AF before treatment, and these parameters remained normal 4 days after treatment. In the other 18 patients, there were 2 patients whose CTX did not normalise between 7 and 21 days after treatment

Table 1: Serum C Telopeptide (CTX) Measured at Baseline and Between Chemotherapy Courses, if the Patient Agreed. When Possible, a Blood Sample for CTX was Done at the Start of Every Chemotherapy Course (day 21). Normal Range of CTX is < 299 pg/ml

	base line value	day 4	day 7	day 14	day 21
Prostate 1	1180				215
SCLC	914				78
Prostate 2	806				96
Prostate 3	1138		63		NA
Breast 1	1004				75
Esophagus	1148		38		NA
Skin	1230			215	NA
Prostate 4	2509		907		died
NSCLC 1	2287	344	311		299
NSCLC 2	1035	339			227
Prostate 5	1972	274			269
NSCLC 3	768			42	54
Prostate 6	797	112			100
Breast 2	578		245		199
NHL	883			587	died
HCC	819	262			250
ACUP	373		220		253
Esophagus 2	590				190
RCC	81	79			NA

('prostate 4' and 'NHL'). Both patients died during observation due to progressive disease (one patient with diffuse castration resistant prostate cancer, the other patient diffuse mantle cell lymphoma). Of the remaining 16 patients, 5 had normalization of CTX on day 4 after administration; 4 had normalized CTX on day 7, and in 2 patients the CTX normalized ond day 14. Of the remaining 5, CTX was in the normal range by day 21. In 8 patients decrease in CTX was followed by a slow decrease in AF. In these patients, the AF showed only modest decrease from baseline, on day 28. This was expected because denosumab was specifically developed to inhibit osteoclast maturation, differentiation, and survival.

Table 2: Changes in Alkaline Phospatase (Normal Range < 135 U:/l), in Patients with Initially Elevated AF – in Only One Patient there was a Normalisation of AF at Day 21

	base line value	day 21
Prostate 1	817	382
Prostate 2	171	115
Skin	153	140
Prostate 4	226	206
NSCLC 1	381	283
Prostate 5	477	269
Prostate 6	226	194
NHL	257	151
HCC	297	284
ACUP	156	146

Safety and Tolerability

Denosumab injections were well tolerated; no injection site reactions were reported. The two patients with persistently high CTX died after day 8, due to progressive prostate cancer and lymphoma respectively.

DISCUSSION

The results of this study show that denosumab, a monoclonal antibody with high affinity and specificity to inhibit osteoclasts, was effective in decreasing bone resorption rapidly and for a sustained period of time in patients with all tumor types metastatic to bone. Suppression of bone resorption was based on changes from baseline in the measured biochemical markers, namely serum CTX. Normalisation of this bone resorption marker occurred within 4 to 21 days following a single subcutaneous dose of denosumab in 18 out of 19 patients. Bone-specific alkaline phosphatate levels showed only mild and delayed suppression, confirming that denosumab does not have a direct effect to inhibit osteoblasts.

These results show that the effect of denosumab on a serum parameter of osteoclast activity can be readily measured with a serum dosage of CTX, across tumor types. There is extensive literature that normalization of bone resorption rate, as evaluated by CTX determination, correlates with a decrease of skeletal complications in metastatic bone disease.

Limitations of this study include its small size, and short follow-up. However this study confirms the efficacy and tolerability of denosumab across different tumor types with bone metastasis, as well as the potential to measure its activity with a simple serum CTX dosage.

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