Abstract #384

Immunomodulatory effect of denosumab in early breast cancer: preliminary results of a randomized window-opportunity clinical trial D-Biomark (NCT03691311)

A.Vethencourt[1], EM. Trinidad[2], C. Gómez Aleza[2], S. Pernas[1] , A. Petit[3], MT. Soler-Monsó[3], A. Urruticoechea[6], A. Gumà Martinez[4], A. García-Tejedor,[5] M.J. Pla[5] , C. Capó[7], M. Gil-Gil[1], A. Stradella[1], A. Fernández[1], S. Recalde Penabad[1], M. Cejuela[1], S. Vazquez[1], A. Iserte[8], C. Falo[1], E. Gonzalez-Suarez[2,9]

1 Medical Oncology Department, Breast Cancer Unit, Institut Català d'Oncologia (ICO); 2 Institut d'Investigacio Biomédica de Bellvitge (IDIBELL-ONCOBELL) ; 3 Departments of Pathology, 4 Radiology and 5 Gynaecology. Hospital Universitari de Bellvitge, Breast Cancer Unit, Institut Català d'Oncologia. L'Hospitalet de Llobregat, Barcelona, Spain; 6 Breast Cancer Unit, Onkologikoa, Donostia, Spain; 7 Department of Gynaecology. Hospital de Viladecans, Barcelona, Spain; 8 Clinical trials unit, Institut Català d'Oncologia, l'Hospitalet De Llobregat, Barcelona, Spain; 9 Department of Molecular Oncology, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Background and rationale

RANKL mediates the proliferative and pro-tumorigenic role of progesterone in the mammary gland and inhibition of the pathway in established mouse tumours leads to tumour cell differentiation and apoptosis, reducing recurrence and metastasis.[1,2,3] These results suggest that RANK pathway inhibitors like denosumab, which are clinically used for the treatment of bone metastasis, could also prevent and/or treat breast cancer (BC). Our pre-clinical and clinical results support that RANK pathway inhibition in tumour cells leads to anti-tumour immune response in premenopausal luminal BC.[4] However, the population of BC patients who may benefit from denosumab remains to be identified.

Hypothesis

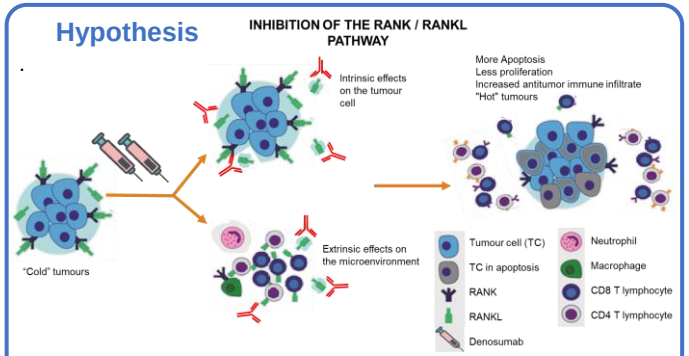


Figure. 1 Possible effect of denosumab in breast cancer. *Denosumab (monoclonal antibody against RANKL) could have an antiproliferative and / or proapoptotic effect on the tumour cell (intrinsic effect) and modulate the immune surveillance by inducing an antitumour immune response (extrinsic effect) in breast cancer.*

Methods

Patients with early-stage HER2-negative BC, candidates to tumour excision as first therapeutic approach are included. Patients are randomized 2:1 to denosumab: control (no treatment); experimental arm received 2 doses of 120 mg subcutaneous of denosumab (once per week) before surgery (2-4 weeks later). Tumour cell proliferation by Ki-67 immunohistochemistry (IHC), cell survival by cleaved caspase-3 IHC (primary endpoints) and stromal tumour infiltrating lymphocytes (TILs) were evaluated comparing baseline biopsy sample and surgical specimen. Ki-67 and TILs were quantified according to International guidelines by blinded pathologists.

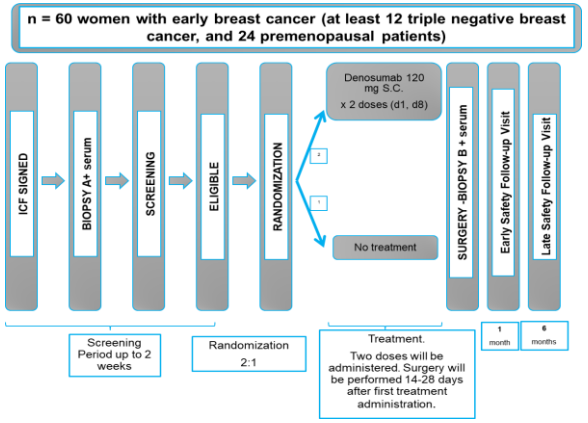


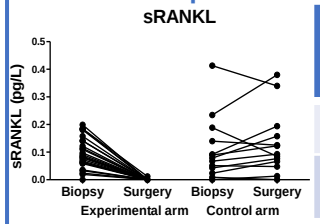
Figure 2 . D-Biomark. *The diagram shows the usual route that a patient from the clinical trial NTC03691311 goes through.*

Results

		TOTAL	ARM: DENOSUMAB	ARM: CONTROL
Patients included		36 out of 60	24	12
Age (range)		55.2 (37 - 88)	55.78 (37 - 88)	54.5 (42 - 67)
Triple negative breast cancer (TNBC)		5 out of 12	3 (12.5%)	2 (16.6%)
Pre-menopausal		21 out of 24	14 (60.9%)	7 (58.3 %)
Histological grade	G1	10 (27.8%)	8 (33.3%)	3 (25%)
	G2	21(58.3%)	13 (54.2%)	8 (66.7%)
	G3	5 (13.9%)	3 (12.5%)	1 (8.3%)
Histological subtype	Ductal	27 (75%)	18 (75%)	9 (75.0 %)
	Lobular	7 (19.4%)	6 (25%)	1 (8.3%)
	Others	3 (5.6%)	0 (0%)	2 (16.6%)
Clinical anatomical stage	IA	25 (69.4%)	16 (66.7%)	9 (75.0%)
	IB	0 (0%)	0 (0%)	0 (0%)
	IIA	10 (27.8%)	7 (29.2%)	3 (25.0%)
	IIB	1 (2.8%)	1 (4.2%)	0 (0%)

Table 1. Clinicopathological features of 36 patients. *Preliminary data from the first 36 patients enrolled out of 60. Clinical and tumour characteristics were well balanced between both groups.*

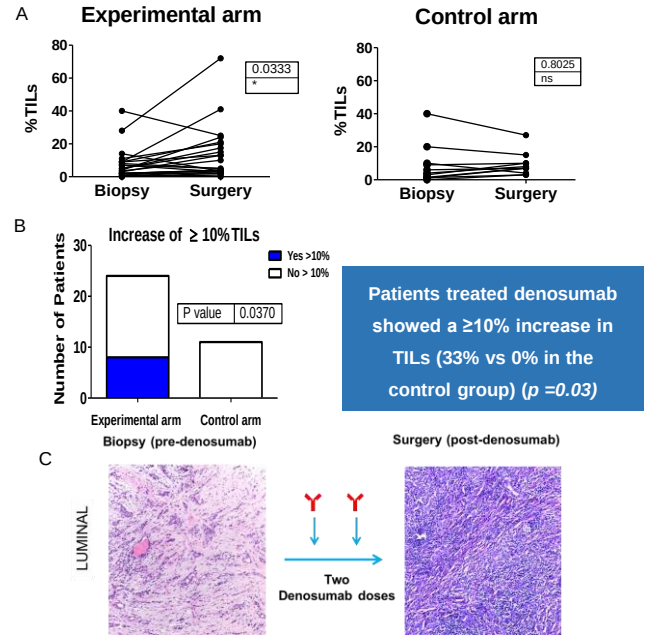
Denosumab does not reduce tumor cell proliferation or survival



	Experimental arm		Control Arm	
	Biopsy	Surgery	Biopsy	Surgery
Ki67 (mean)	23.9	29.9	24.4	28.8
Cleaved Caspase-3 (mean)	0.35	0.42	0.26	0.58

Figure 3. sRANKL (ELISA). Table 2. Means of Ki-67 (IHC) and Cleaved Caspase-3 (IHC). *Denosumab was associated with an effective systemic inhibition of RANKL but not with a reduction in proliferation (Ki67) or survival (Cleaved Caspase-3) primary end points.*

Denosumab increases TILs in early breast cancer



Patients treated denosumab showed a ≥10% increase in TILs (33% vs 0% in the control group) (*p =0.03*)

Figure 4. Denosumab increases the tumour inflammatory infiltrate. *A. An increase in TILs was observed in the denosumab treated group (p=0.03, Paired t test) but not in the control group (p=0.80). B. Patients treated denosumab showed a ≥10% increase in TILs (33% vs 0% in the control group) (p =0.03). C. Representative picture of H&E showing an increase in stromal TILs after denosumab treatment.*

Conclusions and future work

Preliminary data from the D-Biomark clinical trial show that a short treatment of neoadjuvant single agent denosumab does not reduce tumour cell proliferation or survival but increases TILs in early BC. This study will be completed with immunohistochemical characterization of immune infiltrate and gene expression data. The increase in stromal TILs will be confirmed in premenopausal and postmenopausal patients, as well as in TNBC and luminal subtypes.

Acknowledgments

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Contact: acvethencourt@iconcologia.net
egsuarez@idibell.cat