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New Experimental Therapies Targetting Breast Cancer Cell

Di Benedetto Melanie
*INSERM U940/CSPBAT, Université P13,
France*

1. Introduction

Proliferation/survival, migration and invasion are processes common to both primary tumor angiogenesis and metastases formation. Among the treatment approaches being investigated, the most developed is the use of genomics and proteomics research to assist the identification of unique targets involved in tumor angiogenesis or invasiveness. In addition, metastasis in breast cancer patients accounts for over 90% of the deaths. Preclinical studies reveal that many drugs used in the management of primary tumors are not or little effective against metastasis (Perret & Crepin, 2008). Although the mechanism by which metastases develop is still not fully understood, it is generally believed that tumor cells acquire features that affect their metastatic potential during the progression of the tumor; these features include increased survival, invasive and migratory abilities of breast cancer cells. Breast cancer progression is a complex cascade of sequential steps, none of which being fully understood. Many studies implicated stroma in the development of metastases. Stroma and cancer cell interactions were found to contribute to cell detachment from primary tumors, intravasation into the blood stream, and extravasation at distant sites where tumor cells can seed and form tumor metastases (Shekhar et al., 2003). Previously, fibroblasts, endothelial cells and macrophages and other stroma cells were reported to be implicated in the occurrence of metastases (Cunha et al., 1992; Haslam et al., 2001; Shekhar et al., 2003). In these conditions, new strategies as well as the identification of novel therapeutic targets will be needed to effectively target the interactions between stroma and tumor cells via growth factor. Thus, it is important to take into account not also the context near tumor cells (like growth factors as well as chemokines) but also the distribution of the tumor cells in the metastatic sites.

As of today, the poorly predictive preclinical models, lack of tumor target specificity, lack of effective cellular and intracellular delivery, development of resistance have slowed down the progress in anti-cancer therapy. Although the pharmaceutical industry prefers to develop small molecule therapy that can be orally administered to patients, it is now admitted that development of new drug delivery system strategy is essential to increase therapeutic index. Synthetic and natural polymers have an established role as in several biomedical applications, including their use as prosthesis or implants (Anderson, 2001). During the past decade, polymer implants have been used in cancer therapy to treat locally hormone-dependent tumors (Zoladex, Lupron depot) or brain tumors by implanting chemotherapy delivering polymer post-surgically. Over than 10 water-soluble polymer

drugs conjugates have entered in phase I/II clinical trials as I.V. administrated anticancer agents. These include six conjugates based on methacrylamide polymers (HPMA). These polymers have been developed in the basis of the achieved tumor-specific targeting by the enhanced permeability and retention effect (Ducan et al., 2005; Matsumura et al., 1986). This increasing tumor retention has been observed and attributed to the better extravasation in the blood vessel of macromolecules and the absence of their drainage release (Noguchi Y et al., 1998; Seymour et al., 1995). HPMA copolymer conjugate with chemotherapeutic agents as doxorubicin has shown high retention and efficacy toward tumors without side effects (Ducan et al., 2005). The specific tumor cell targeting can also be attributed to the interaction of copolymers and heparin growth factors which are highly expressed in the tumor cell environment. Better retention is also attributed to the endocytic internalisation of conjugates which allows also to bypassing MDR efflux responsible for drug resistance. Also, another type of natural polymer interesting to be evaluated is the glycoaminoglycans analogs since it is well-known that they importantly interact with growth factors and receptors on the cell membranes. In another hand, the different type of glycosaminoglycans on the cell surface support in relation with normal or tumoral statues supported their involvement. We have recently developed the two kinds of copolymers that show interesting results in basis of the possibility to functionalize them with active biomolecules. Among active biomolecules, the interesting ones are those which inhibited the ancrage of rho/ras signaling molecules to tumor cells since this pathway plays a key role in invasion, migration and proliferation of tumor cells. The inhibition of the prenylation of ras and rho leads to the blocking of ancrage to the membrane. We have focused our attention on two types of such small molecules. The first one, phenylacetate (NaPa), comes from the metabolism (Fig. 1). NaPa, which has been originally used for urea children disorders (Samid et al., 1992, 1993) has since been demonstrated to efficiently inhibits cancer cell lines proliferation *in vitro*. The second small molecules are bisphosphonates (PBs, fig 1), which are mostly known for their efficacy in the treatment of bone disorders and are also efficient *in vitro* in inhibiting cancer cell proliferation. For both molecules, the main obstacle with their use in cancer therapy stems

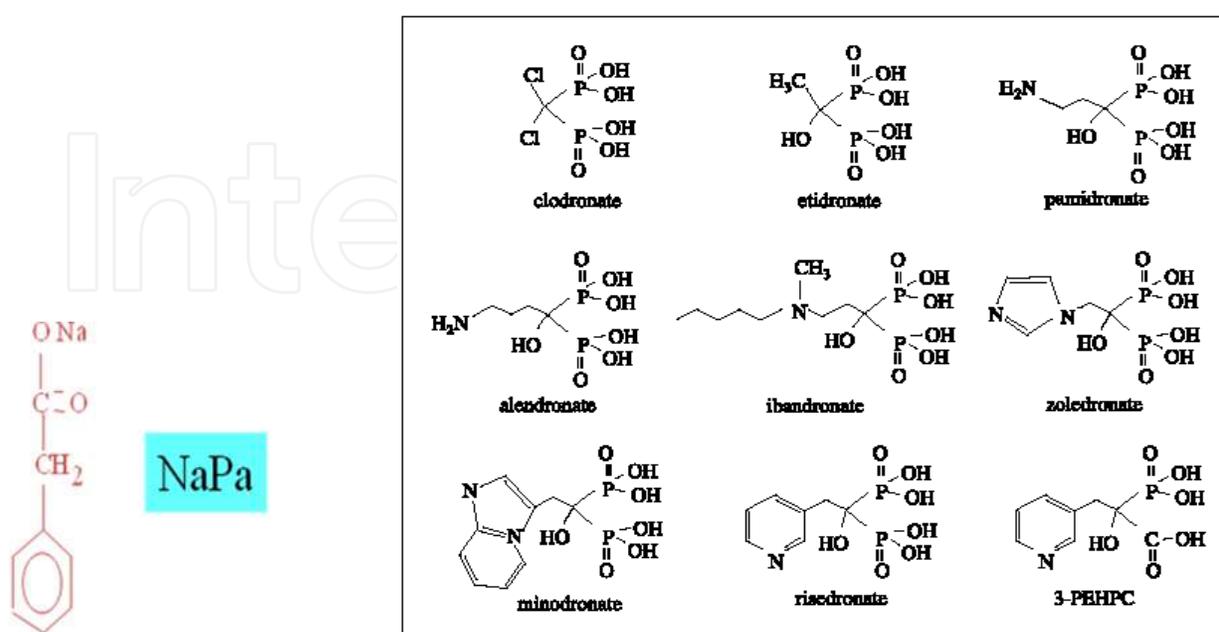


Fig. 1. Phenylacetate (NaPa) and bisphosphonates (BPs) molecules.

from the high concentrations (up to micromolars) that are needed to achieve efficacy *in vivo*. Since we think that these molecules remain of potential use in cancer because they target specific step involved in both primary tumor growth and metastasis formation, we are developing new strategies that aim to increase their efficacy using drug delivery systems in specific cancer cells localized in specific sites. Herein, we will present all these strategies, first by using polymers for NaPa, and secondly using chemical transformation of the compounds, in particular esterification for the bisphosphonates. Also we will present possible future directions, such as the use of new polymers as well as new delivery systems like nanotechnologies.

2. Glycoaminoglycan polymer strategy

2.1 Carboxybenzylamide dextran (CMDB) and NaPa combination

Carboxymethyl benzylamide dextran derivative (in particular CMDB7) inhibits breast cancer cell proliferation *in vitro* and in nude mice (Bagheri-Yarmand et al, 1992, 1997, 1998a, b, 1999). This *in vitro* effect is associated with a decrease in the S-phase cell population and with an accumulation of cells in G1 phase of cell cycle (Bagheri-Yarmand et al, 1992). CMDB7 disrupts the mitogenic effect of growth factors by preventing their binding to specific receptors as reported for Fibroblast Growth Factor-2 and -4 (FGF2, FGF4, Bagheri-Yarmand et al, 1998a), Platelet-Derived Growth Factor-BB and Transforming Growth Factor- β 1 (PDGF-BB, TGF β Bagheri-Yarmand et al, 1998b). *In vivo*, CMDB7 treatment reduces the growth of MCF-7ras (Bagheri-Yarmand et al, 1998b) and FGF4-transfected HBL100 xenografts and decreases the tumor angiogenesis (Bagheri-Yarmand et al, 1998a). Sodium phenylacetate (NaPa), a physiological metabolite of phenylalanine, is normally found in human plasma at micromolar concentrations. At higher concentrations, NaPa is reported to induce the cytostasis and the reversion of malignant phenotype of different cancer cells *in vitro* (Samid et al, 1993, 1994, 1997, 2000; Adam et al, 1995). Furthermore, NaPa is described to modulate the synthesis and/or the release of some growth factors (Ferrandina et al, 1997; Thibout et al, 1998) and to increase, in synergistic manner, the effect of some molecules affecting the growth factor pathways (Prasanna et al, 1996; Samid et al, 1993). For example, NaPa potentiates the antitumor activity of tamoxifen by increasing apoptosis in breast cancer xenografts in nude mice. Finally, NaPa has been used in phase I and II clinical trials on patients with malignant tumors (Chang et al., 1999; Thibault et al, 1994). In basis of this data, we have evaluated *in vitro* and *in vivo* the efficacy of combined treatment with NaPa and an industrial dextran derivative LS4 (Sterilyo Laboratories) whose composition is similar to CMDB7 one, on breast cancer cell growth. We have used the MCF-7ras cell line obtained by transfection of MCF-7 cells, isolated from pleural metastasis of breast adenocarcinoma, with v-Ha-ras oncogene. The MCF-7ras cells secrete high quantities of TGF α , TGF β , epithelial growth factor (EGF) and insulin growth factor (IGF) (Albini et al, 1986). This cell line represents an oestrogen-independent cellular model corresponding to some malignant breast tumors (Spandidos and Agnantis, 1984) and does not require oestrogen supplementation to induce a high incidence of tumors in nude mice (Sommers et al, 1990). The analysis of CMDBLS4-NaPa combination effect is performed by the isobole method.

NaPa enhances the dextran derivative CMDBLS4 antiproliferative effect on breast cancer MCF-7ras cells both *in vitro* and *in vivo*. Indeed, NaPa or CMDBLS4, delivered alone for 7 weeks, inhibits MCF-7ras tumor growth by 60% and 40%, respectively, while the CMDBLS4-NaPa combination decreases MCF-7ras tumor growth by 83% without any toxicity. The

effectiveness of the NaPa and CMDBLS4 combination can be explained by their distinct mechanisms of action. MCF-7ras breast cancer cells secrete an important amount of mitogenic growth factors such as TGF β and PDGF (Bronzert et al, 1987; Dickson et al, 1987; Knabbe et al, 1987). The mitogenic effects of these growth factors can be reduced by inhibition of their synthesis or/and their action on target cells. Treatment of cells with NaPa decreases the mitogenic activity of MCF-7ras conditioned medium on BALBc/3T3. The possible mechanism is a modulation of the synthesis and the release of growth factors like TGF β in MCF-7ras breast cancer cells (Thibout et al, 1998). CMDBLS4, when added to conditioned medium, inhibits conditioned medium mitogenic effect on BALBc/3T3 fibroblasts. This finding argues for CMDBLS4 interactions with growth factors contained in CM. Indeed, previous studies have shown that dextran derivatives interact with heparin-binding growth factors like TGF β , PDGFBB or FGF-2 and inhibit their mitogenic effect (Bagheri-Yarmand et al, 1998a, b). All our and others' observations suggest that NaPa and CMDBLS4 act on distinct targets involved in the tumor development. NaPa alters the mitogenic growth factor production and renders the tumor cells quiescent in the G1 phase while CMDBLS4 interacts with MCF-7ras growth factors and inhibits their mitogenic activities.

aCMDBLS4 (Ac; mM)	NaPa (Bc; mM)	% I	CMDBLS4 (Ae; mM)	NaPa (Be; mM)	D
48 h					
3.7	0.75	33	18.5	17	0.44b
7.4	1.5	45.8	>18.5	30	0.45b
14.8	3	48.1	>18.5	32.1	0.89
18.5	4	50	>18.5	34	1.12
72 h					
3.7	0.75	51.4	>18.5	20.6	0.23b
7.4	1.5	54.8	>18.5	23	0.46b
14.8	3	53.3	>18.5	24	0.93
18.5	4	60.2	>18.5	29	1.13

Table 1. Effects of NaPa and CMDBLS4 combination with a ratio = 5 on MCF-7ras cell proliferation. ^a Ac and Bc, concentrations of agents A and B used in the combination treatment; Ae and Be, concentrations of agents A and B able to produce the same magnitude of effect if used individually. D combination index If D=1 the effect is additive, if D<1, the effect is synergistic. ^b P < 0.05. % of inhibition determined by MTT assay: $[1 - (\text{absorbance of cells in medium containing agents} / \text{absorbance of cells in control culture medium})] \times 100$. (Di Benedetto et al., 2001).

2.2 Carboxybenzylamide –phenylacetate dextran (NaPaC)

NaPa molecule enhances, in a synergistic or additive manner, the inhibitory effect of CMDB on breast cancer cell growth *in vitro* and in nude mice when administrated at a CMDB/NaPa ratio of 4 (Di Benedetto et al., 2001). To obtain a new drug with the same properties but easier to use as a future anti-cancer molecule than the combined treatment, we have performed the esterification of CMDB by NaPa respecting the synergistic CMDB/NaPa ratio. We have then investigated the *in vitro* and *in vivo* effects of this new

dextran derivative, phenylacetate carboxymethyl benzylamide dextran, named NaPaC, on breast cancer cell proliferation as well as its apoptotic and anti-angiogenic effects.

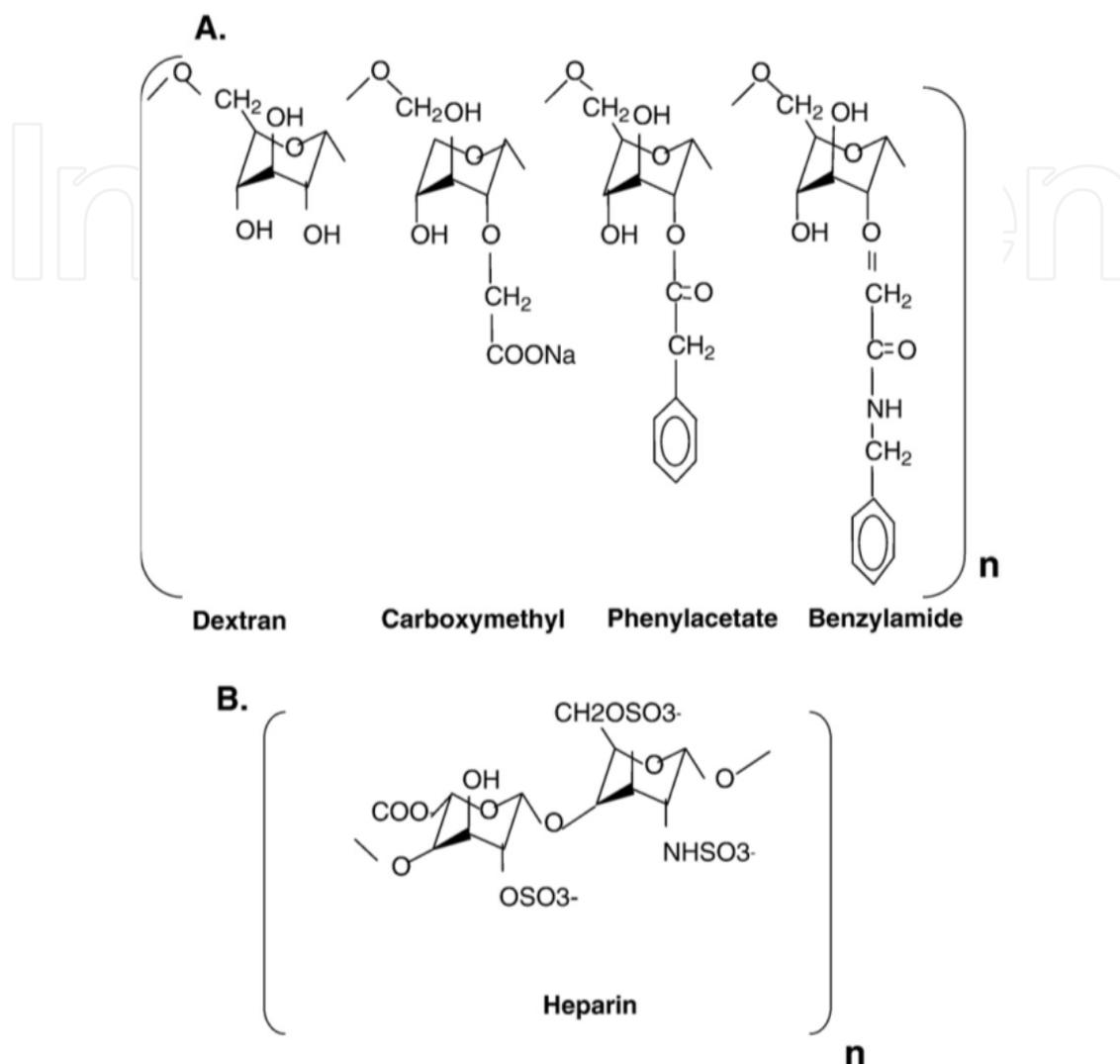


Fig. 2. Carboxymethylbenzylamide-NaPa (NaPaC) (A) and heparin (B) molecules (Di Benedetto et al., 2002).

Interestingly, NaPaC inhibits the growth of breast cancer MCF-7ras cells at a concentration lower than CMDB or NaPa. The comparison of IC₅₀ for three drugs supplies the additional evidence for the highly enhanced efficiency of NaPaC as compared to CMDB and NaPa (Table 2). Therefore, the hybrid molecule retains at least the additive effect of its two components observed previously (Di Benedetto et al., 2001). This effect is not only specific to MCF-7 ras cells as similar results are obtained for other breast cancer cell lines, including MCF-7, MDA-MB-231 and MDA-MB-435. NaPaC inhibited *in vivo* MCF-7ras tumor growth more efficiently and at lower dose than CMDB or NaPa. This can be explained by the fact that NaPaC gathers the antiproliferative, apoptotic and anti-angiogenic actions generally admitted to lead *in vivo* to concerted inhibition of tumor growth. The inhibition of the endothelium growth, causing the impaired delivery of nutrients and oxygen to tumor, leads to tumor cell death. Indeed, we observed *in vivo* that the inhibition of MCF-7ras tumor

growth by NaPaC is concomitant with a poor microvessel density (as compared to control) at short-time treatment and with multifocal necrotic areas at long-term administration. Therefore, NaPaC is more efficient than CMDB or NaPa and decreases the microvessel density at dose lower than its components.

Treatment	Tumor (volume) at Day 1 (mm ³)	Tumor (volume) at day 5(mm ³)	Tumour growth (%)
Control	221±90	2103±328	-
NaP4 40 m/ kg (0.25 mmol/ kg)	157±30	990±192	57
CMDB 150 mg/kg (1.85 µmol/kg)	199±70	1326±281	33
NaPaC 15 m/kg (0.18 mmol/ kg)	167±40	717±203	66

Table 2. Inhibition of MCF-7ras tumor growth by NaPaC and its components, CMDB and NaPa. MCF-7ras cells were inoculated s.c in nude mice near the fad pad. After tumor uptake, the animals were treated for 7 weeks with NaPa (n=10), CMDB (n=10) and NaPaC (n=10). Tumor volume (+s.e.m.) for different experimental groups were compared to control (Di Benedetto et al., 2002).

Of note, no necrotic areas were detected in tumors treated with CMDB or NaPa alone. In accord with our *in vivo* results on tumor neovascularization, NaPaC *in vitro* inhibits the growth of human endothelial cells more efficiently than CMDB or NaPa. The mechanisms involved in CMDB and NaPa actions on endothelial cell proliferation seem to be distinct. CMDB interacts directly with VEGF165 (Hamma-Kourbali et al., 2001), the most specific angiogenic factor (Plouet et al., 1989) and inhibits the VEGF165-induced HUVE-C-C growth.

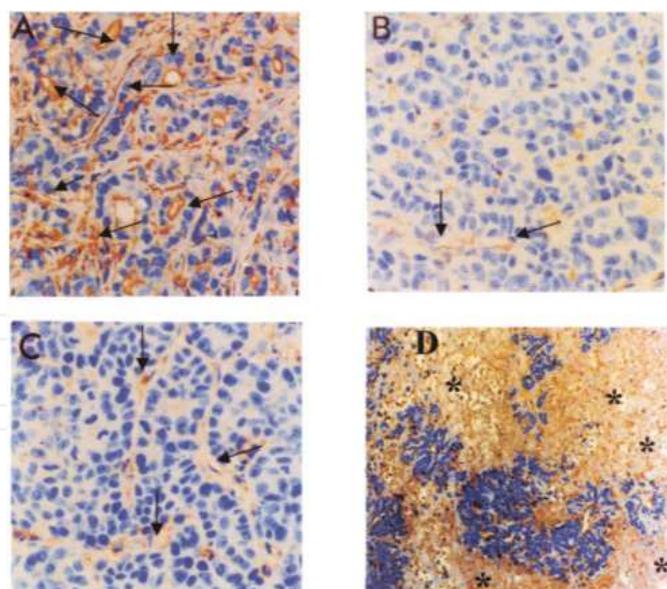


Fig. 3. Inhibition of MCF-7ras tumor angiogenesis by NaPaC, CMDB and NaPa. Microvessel staining of tumors untreated (A) and treated with 150 mg/kg CMDB (B), 40 mg/kg NaPa (C) or 15 mg/kg NaPaC (D), twice a week, for 7 weeks, was performed using GSL-1 lectin. Necrotic areas in panel d are indicated with the asterisks. Magnification X250 was used for panels A,B and C, and magnification X100 was applied for panel D. The representative microvessels in panels A±C are marked with the arrows (Di Benedetto et al., 2002).

In our laboratory, we observe that NaPa do not interact with VEGF 165 molecule, and has no effect on VEGF165-dependent cell growth. Up to date, the mechanism of NaPa action on HUVE-C-C growth is unknown. Concerning NaPaC, it inherits the CMDB ability to interact with angiogenic growth factor and blocks the VEGF165-induced endothelial cell proliferation at lower concentration than CMDB (Fig. 4). However, the involvement of the NaPa mechanism in NaPaC action on HUVE-C-C growth is still unknown.

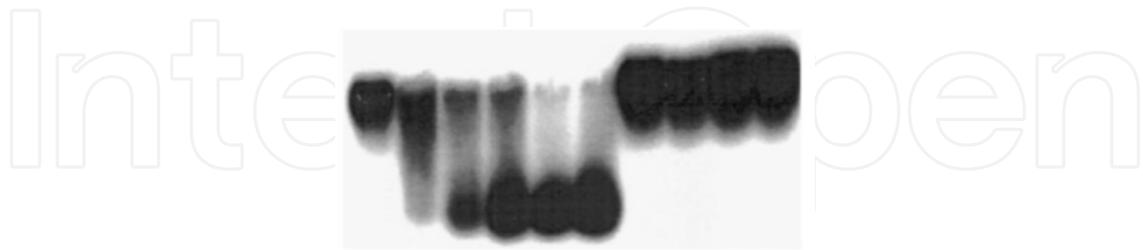


Fig. 4. NaPaC directly interacts with VEGF165. After a 1 h incubation of ^{125}I -VEGF165 (105 c.p.m.=3 ng) with NaPaC or NaPa at 48C, the mixtures (10 ml) were electrophoretically analysed in non denaturant 1% agarose gel at pH 7.0. Lane 1 represents the migration of ^{125}I -VEGF165 alone; lanes 2 \pm 6 correspond to 0.6, 1.8, 5.5, 15 or 48 mM NaPaC; lanes 7 \pm 10 represent the shifts after addition of 1.0, 5.0, 10 or 20 mM NaPa (Di Benedetto et al., 2003).

Finally, it is noteworthy that NaPa treatments at high concentrations can induce pathological effects (Thibault et al., 1994; Chang et al., 1999). The use of non-toxic new molecule, NaPaC, should limit side effects of NaPa and increase its therapeutic efficacy. In conclusion, NaPaC provides additional interesting clues in developing new anti-cancer drugs with specific triple activity: antiproliferative, aponecrotic and anti-angiogenic. The inhibition of angiogenesis is crucial for blocking tumor progression since tumor-associated high-density neovascularization is responsible for development of metastases.

3. Bisphosphonate esterifications

Bisphosphonates (BPs) have long been used in metabolic bone disease as osteoporosis, tumor-associated hypercalcaemia and metastases-induced osteolysis due to their ability to inhibit bone resorption. BPs are able to bind divalent cations like Ca^{2+} or zinc, constituting the basis of their bone-targeting property and their inhibition of the proteolytic activity of matrix metalloproteinases (MMP), respectively. The nature of their side chains gives rise to a variety of possible structures and stereochemistry determining their different potencies (Cleardin et al., 2005; Cleardin et al., 2003; Caraglia et al., 2006; Green, 2003). Non-nitrogen containing BPs (non N-BPs) act by forming non hydrolysable ATP-analogues and are less effective than nitrogen-containing BPs (N-BPs) in inhibiting bone metastasis (Roger et al., 2003). However, Zoledronate treatment of patients are reported to induce toxic side effect characterised by osteonecrosis of the jaw while non N-BP did not produce this effect (Van den Wingaert, 2006; Diel et al., 2007). N-BPs, such as zoledronate, act on the mevalonate pathway, inhibiting the farnesyl diphosphate synthase (FPP) and thereby depleting the cells of the farnesyl (FPP) or geranylgeranyl (GGPP) diphosphate isoprenoids (Roelofs et al., 2006). Isoprenoids are required for translocation and anchorage of small G proteins like Rho or Ras to the plasma membrane assuring their ultimate involvement in signal transduction during several important normal and tumor cellular pathways. However, *in vivo* efficacy of all BPs on extra-osseous sites or primary tumors is still debated. Only a small number of studies have demonstrated their *in vivo* antiproliferative activity on tumors or metastasis

present in soft tissues (Stresing et al., 2007). The reasons are the poor oral bioavailability (0.3-7% in humans) due to chelation of metal ions by phosphonic acid group inside the digestive lumen, poor membrane permeability due to poor BP lipophilicity as well as strong uptake by bone tissue (Ezra & Colomb, 2000). Previously, new strategy to overcome BP hydrophilicity is masking the phosphonic acid with organic protecting groups and introducing hydrophobic functions in the side chain (Migianu et al., 2005). An esterified BP with methyl group displays antitumor growth and antiangiogenic activities on A431 tumors, being more effective *in vivo* than *in vitro* (Ledoux et al., 2006). In order to further increase the lipophilicity of BPs (and their entering into the cells), we have synthesized new aromatic 1-hydroxymethylene-1,1-bisphosphonic acids containing phenyl or halogen phenyl ring in their side chains. Interestingly, these compounds exhibit potent antiproliferative activities *in vitro* on human epidermoid A431 cells (Guenin et al., 2005). In parallel, recently crystallographic and computational investigation reveal that the presence of phenyl ring in the side chain permitted non N-BPs to interact with farnesyl enzyme (Mao et al., 2006). Based on these data, we have synthesized a class of BPs that contains bromobenzyl in their side chains (BP7033Br, Fig. 5), as well asymmetrically esterified one of each phosphonic acids with aromatic groups (BP7033Br ALK, Fig. 5).

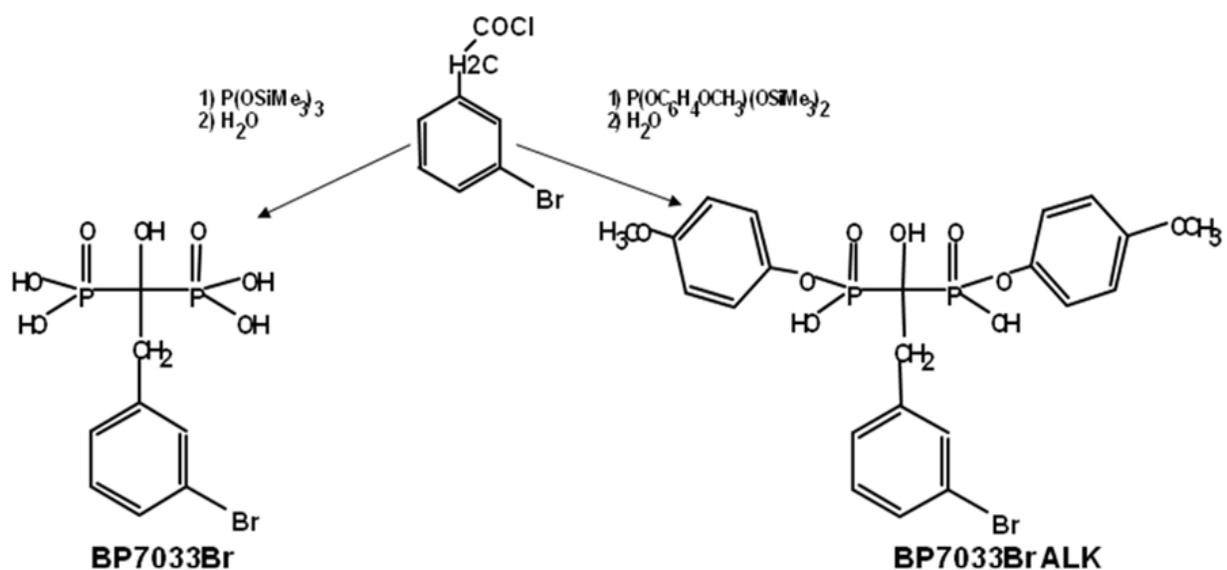


Fig. 5. Chemical structure of BP7033Br and BP7033Br ALK (Abdelkarim et al., 2009).

BPs represents an emerging class of drugs for cancer therapy and new class of non-N-BPs which exhibits higher antiproliferative activities on breast cancer cells compared to previously described non-N-BPs such as clodronate (Journe et al., 2004). Both types of m-bromobenzyl BPs inhibit the viability of several breast cancer cell lines with different estrogen-receptor statuses (Fig. 6). The esterified BP is the more effective on estrogen-responsive cells since the maximal inhibition is reached at 250 μ M in contrast to non esterified BP that does not induce maximal inhibition even at 1 mM. In addition, at 250 μ M, BP7033Br ALK is effective on cells independently from the estrogen-receptor status. Both types of our BP inhibits viability of estrogen non-responsive cells and particularly that of MDA-MB-231 and D3H2LN cells, the last cell line being the more aggressive ones. Indeed, it is worth to note that dramatic improvement of antiproliferative effect of non-N-BPs on breast cancer cells is reached since clodronate at the same concentration range (200 μ M) and

the same time-treatment (72h) do not reduce MDA-MB-231 cell viability (Fromigue et al., 2000; Monkkonene et al., 2008; Senaratne et al., 2000). In addition, clodronate demonstrates mitogenic effects via MCF7 estrogenic receptor (Journe et al., 2004) and we never observe this effect with our BPs. Based on our results, it appears also that BP7033Br ALK antiproliferative effect is estrogen-receptor-independent. The occurrence of this new effect of non-N-BPs could result from the addition of aromatic functions in the side chain. Heterocycle in the side chain is implicated in the induction of cell apoptosis by preventing the prenylation of signalling proteins such as Ras or Rho (Luckman et al., 1998). Inhibition of Ras processing using non bromo-containing BP7033 is also reached (Hamma-kourbali et al., 2003). Also, the addition of phenyl function in the side chain of BPs rendered the catalytic pocket of geranyl and/or farnesyl synthase enzymes of the mevalonate pathway more accessible (Mao et al., 2006; Steeg et al., 2006). BP7033Br ALK reduces MDA-MB-231 and D3H2LN cell viabilities about 90% with a concentration 4-fold inferior to that of BP7033Br.

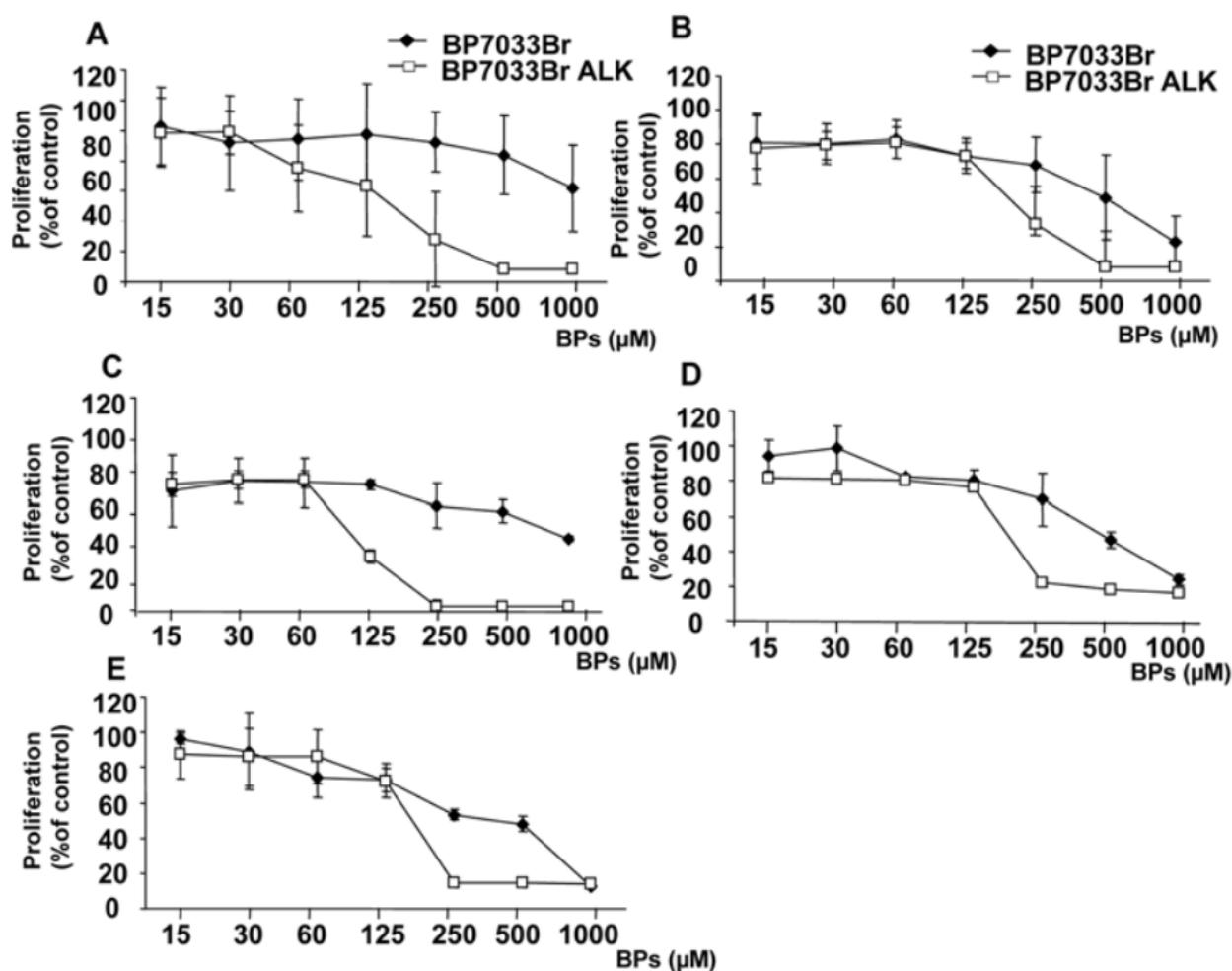


Fig. 6. BP7033Br and BP7033Br ALK inhibited viability of different breast cancer cells. T47D (A), MCF-7 (B), SKBR3 (C), MDA-MB-231 (D) or D3H2LN cells (E) (1×10^5) were treated with BP7033Br and BP7033Br ALK at increasing concentrations for 72h. Then, the cells were washed and incubated with 0.1 mL of MTT (2 mg/mL) for 4 h. Optical density was measured at 570 nm using a Labsystems Multiskan MS microplate reader. Data represents the mean value (\pm SD) of three independent experiments (Abdelkarim et al., 2009).

These data are in agreement with previous results on epidermoid A431 cell proliferation that show a beneficial effect of esterification of the phosphonic groups (Guenin et al., 2005; Ledoux et al., 2006). Our hypothesis is that such esterified compounds rendered BPs more hydrophobic increasing their cell uptake and could therefore act like prodrugs releasing active BP into the cells. In accordance, characterisation of the hydrophilicity demonstrated a shift toward lipophilicity of the BP7033Br ALK compound (Log P values of -0.75 versus -0.31, respectively). Alternatively, one could also hypothesize that esterified BPs have their own mechanisms since they block the cells into the S phase while non esterified BPs inhibits the G0/G1 cell phase transition. On the other hand, both type of BPs (esterified or not) induce cell death apoptosis of both MDA-MB-231 and D3H2LN cells. These results are interesting since D3H2LN cells have a greater metastatic potential than MDA-MB-231 and consequently could be more resistant to apoptosis, as it is described for metastatic cells (Wang et al., 2002; Winter et al., 2008). Both BPs induce strong D3H2LN metastatic cell apoptosis but the concentration of the esterified analogue used is 2-fold lower. Also, the two BPs inhibits migration of MDA-MB-231 cell lines with a more important effect obtained with BP7033Br ALK. In contrast, BP7033Br ALK is less effective in inhibiting D3H2LN cell lines invasion concomitant with a less important effect on MMP-9 and MMP-2 activities. BP7033Br strongly inhibits MMP-9 and MMP-2, the major form of metalloproteinases present in extracellular matrix. Since MMPs are zinc-dependent endopeptidases, we speculate that the reduction of BP7033Br ALK effect could be due to a reduction of available phosphonic acid groups able to chelate zinc and consequently inhibit MMPs. These results are in agreement with previous studies which show that MMPs inhibition activity by BPs is related to zinc chelation (Cleazardin et al., 2003). However, we hypothesise that release of BP7033Br ALK with free phosphonic group could be more important in *in vivo* system because of the presence of phosphodiesterases in serum.

BPs antitumor effects have been mainly observed on D3H2LN xenografts growth and metastasis (Fig. 7). D3H2LN cells have been derived from a MDA-MB-231 subclone isolated from a lymph node metastases and induce an increased xenograft tumor growth as compared to parental cells when injected *in vivo* (Jenkins et al., 2005). Both BP7033Br and BP7033Br ALK inhibit D3H2LN tumor growth after intratumoral injection of about 286 µg BPs per mouse twice a week during 21 days. We establish that this new class of BPs is the most potent among the current non N-BPs since clodronate, even used at 1600 mg twice daily during several weeks (as compared to BP7033 ALK corresponding human dose of 770 mg twice a week during only 2 weeks) fails to reduce primary tumor growth (Winter et al., 2008). Also, BPs are 10-fold more potent than the non halogenated phenyl analogues (Sebbah-Louriki et al., 2002). In addition, BP7033Br ALK better inhibits D3H2LN vessel density than BP7033Br. This point is also supported by the large necrosis area not detected in BP7033Br treated tumors. In addition, we cannot exclude that these large necrosis areas could also be due to a greater amount of esterified BP penetrating into tumor to induce cell death. As compared to N-BPs, it is noteworthy that risedronate or ibandronate failed to inhibit MDA-MB-231 tumor growth in nude mice (Higara et al., 2001, Sasaki et al., 1995). Furthermore, no pre-clinical works demonstrate an antiproliferative effect of zoledronate on primary breast tumor growth in nude mice. The only study demonstrating an inhibition effect of zoledronate on primary tumor growth uses mesothelioma tumors which involves calcification that could uptake the drug (Wakchoure et al., 2006) In addition, the efficacy of zoledronate on bone metastasis seems to be supported by its affinity for osseous tissues rather than its direct antiproliferative effect on tumor cells (Winter et al., 2008).

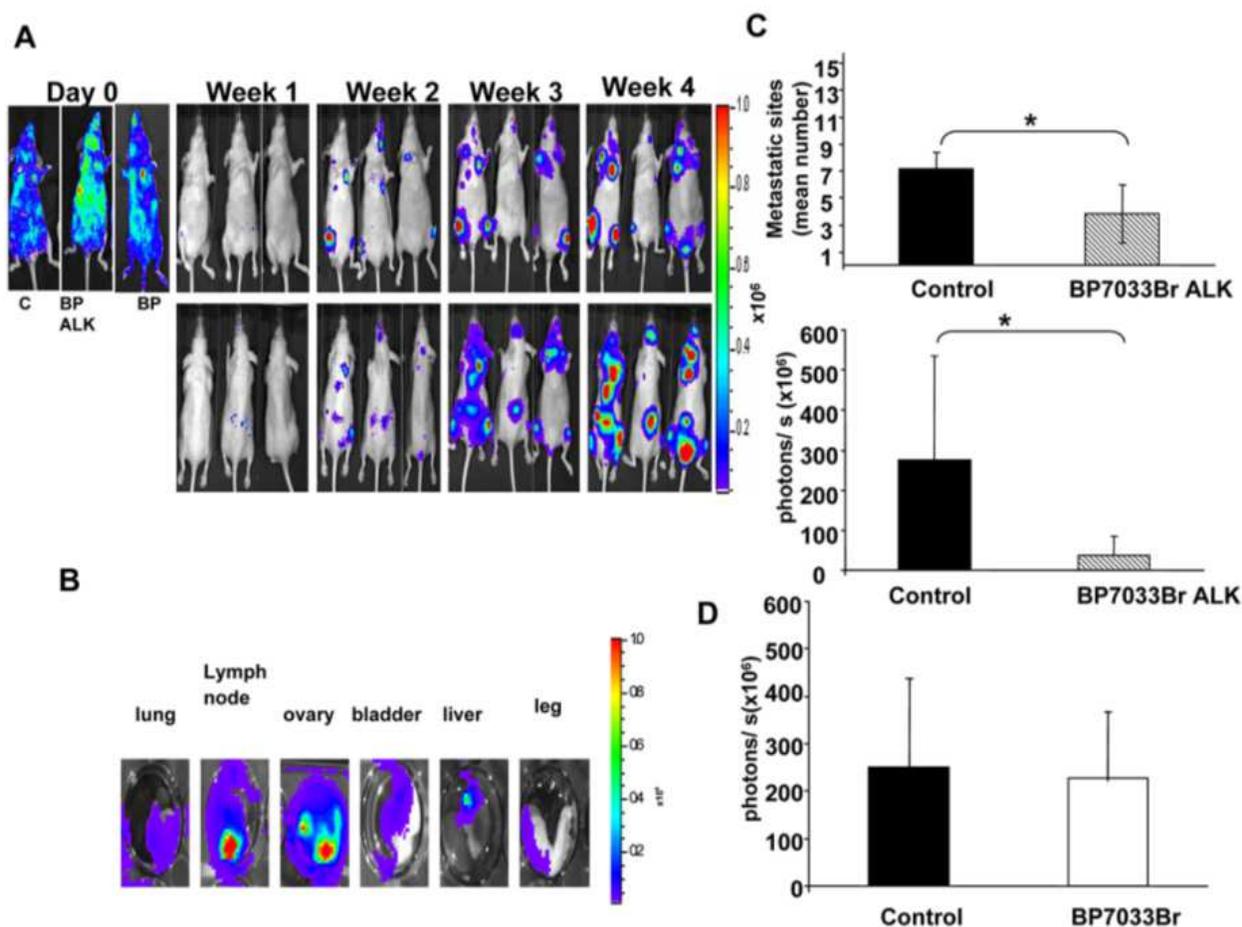


Fig. 7. Only BP7033Br ALK inhibited D3H2LN metastasis. D3H2LN cells were injected into the left ventricle of nude mice (n=7). Day 0 showed the successful intracardiac cells injection. Within 2 weeks, when metastasis were initiated, mice were treated with BP7033Br ALK or BP7033Br (A). At the indicated days, the bioluminescence images were acquired for control (c, left panel) and BPs treated mice (BP7033BrALK and BP7033Br middle and right panel, respectively). *Ex vivo* data confirm soft tissue metastasis from D3H2LN cells injection (B). Quantification of the mean metastatic sites and the photons/s after BP7033Br ALK treatment (C). Quantification the photons/s after BP7033Br treatment (D). Each column represents a mean (\pm SD) of three independent experiments. * $P_{\text{versus control}} < 0.05$ (Abdelkarim et al., 2009).

Also, zoledronate is a compound rapidly eliminated from plasma, resulting in renal excretion, rapid bone or calcified tissues uptake and accumulation partly due to its phosphonic functions. We have recently showed that the symmetrical esterification of the phosphonic groups may improved BPs soft tissue bioavailability limiting osseous or calcified tissue uptake (Ref). Also, as their chemical structures are close to the apomine BP, which presents aliphatic ester group, their half-life are expected to be close to that found for this drug (156 h with micromolar plasma concentration, (Alberts et al., 2001). Thus, esterified BP7033Br ALK abrogates angiogenesis, both soft tissue and bone metastasis whereas BP7033Br does not. Noteworthy, in BP7033Br treated mice, luminescence signalling of leg osseous metastasis is not significantly reduced because 2/7 mice do not respond to the

treatment in contrast to BP7033Br ALK treatment that induces significant reduction (Ref). Indeed, the esterified functions seem to be important for the BPs distribution within the systemic system and less for local injection (subcutaneous tumors) since the two N-BPs studied both inhibit D3H2LN xenograft growth. Of note, N-BPs induced osteonecrosis of treated patients (Diel et al., 2007) whereas no apparent side effects have been observed with non N-BPs. Therefore, esterified m-bromobenzyl non N-BPs constitute a new class of drugs with potent direct antitumor, antiangiogenic and antimetastatic efficacy on breast tumors (Abdelkarim et al., 2009).

4. Future directions Polynass/nanotechnologies

4.1 PolyNASS polymers

Polymers display antiproliferative, aponecrotic and anti-angiogenic effects without cytotoxicity on endothelial and cancer cells. Indeed, after 4 days of incubation, only 0,6 μ M of 20MA/80NaSS is required to induce 85% of cell growth inhibition of MDA-MB-231 cell growth. In contrast and interestingly, in HUVECs cells, polymers 50MA/50NaSS is more efficient with 70% inhibition achieved with 0,06 μ M (Fig. 8). The effect of 20MA/80NaSS on breast tumor cell is not specific only to MDA-MB-231 cells since it also inhibits other breast cancer cell lines MCF-7 (Skhiri *et al.*, 2008). The inhibition achieved with MA/NaSS polymers is comparable to that obtained in non invasive breast carcinoma cell MCF-7 (Skhiri *et al.*, 2008) and the inhibition effect varies in function of percentage of carboxylate and sulfonate content in polymers. Interestingly, the strongly percentage of tumor cell proliferation inhibition was observed in presence of 20MA/80NaSS polymer. At 6 μ M, this polymer induces up to 80% of inhibition of the cellular MDA-MB-231 cancer cell proliferation after 4 days of culture. On the other hand, at the same concentration, the polyNaSS effect on cellular proliferation was much lower (60%). Indeed, the presence of two groupings carboxylate and sulfonate is necessary and plays an important role in breast cancer anti-proliferation inhibition activity. Also, it seems that similar proportion (50 MA/50 NASS) of functional subunits is rather an interesting chemical composition to obtain the optimal inhibition of endothelial cells proliferation. In addition, it seems that a strong proportion of sulfonate units in polymers is necessary for a high antiproliferative activity in cancer cells while the equal proportion of the functional groups are necessary for the endothelial cells. These results suggest that the mechanism implicated in the inhibition of the two types of cells is different. The effect of these two copolymers on HUVEC and MDA-MB231 cell cycle was also different as a decrease of cells in the S-phase and an accumulation of cells in G0/G1-phase were observed. In conclusion, these new type of polymers could lead to the development of new anti-cancer drugs with specific triple activity: antiproliferative, aponecrotic and anti-angiogenic. Since the development of resistant tumor cell clones can be a serious problem, the inhibition of angiogenesis is crucial for blocking tumour progression since tumor associated high density neovascularization is responsible for development of metastases. Therefore, polymers could be good candidate by selectively inhibiting tumor cells or endothelial cells in basis of their composition.

In addition, these kinds of molecule could be conjugated with specific drugs (copolymers) that will target genes specific to endothelial or tumors cell activities enhancing in this way, the benefit of the therapies. It is to note that in contrast to glycoaminoglycan like polymers, PolyNASS polymer synthesis is better controlled in basis of their chemical composition that is determined by titration of carboxylic groups and ^1H NMR.

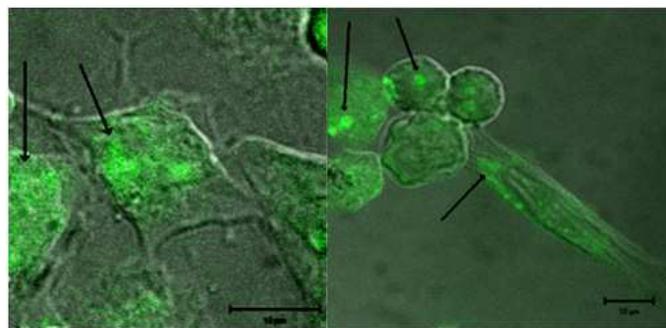
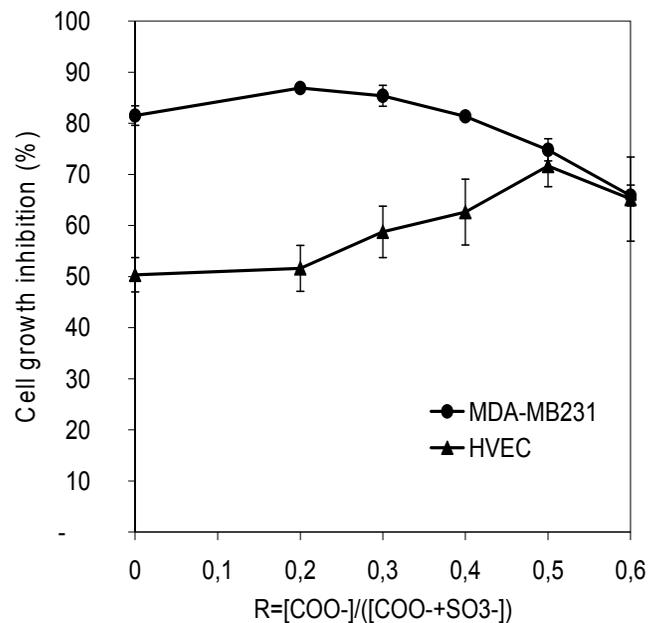


Fig. 8. Structure-function relationship of PolyNASS copolymers and penetration into MDA-MB-231 tumor (left) and HUVEC endothelial cells (right).

4.2 Nanotechnologies

An innovative multimodal system, which combines magnetic targeting of therapeutic agents with both magnetic resonance and fluorescence imaging into one system has been recently described (Bennayou et al., 2011). This new magnetic nanoplatform consists of superparamagnetic $\gamma\text{Fe}_2\text{O}_3$ nanoparticles, used clinically as an MRI contrast agent, conjugated to therapeutic molecules of the hydroxymethylene bisphosphonate family (HMBPs): alendronate with an amine function as the terminal group. *In vitro* tests with breast cancer cells show that the $\gamma\text{Fe}_2\text{O}_3$ @alendronate hybrid nanomaterial reduces cell viability and acts as a drug delivery system. The presence of both $\gamma\text{Fe}_2\text{O}_3$ @alendronate and a magnetic field significantly reduced the development of tumors. The amine functionalities can be used as precursor groups for the covalent coupling of peptides or monoclonal antibodies for specific biological targeting. The feasibility of this process is demonstrated by coupling rhodamine B, a fluorescence marker, to the $\gamma\text{Fe}_2\text{O}_3$ @alendronate nanohybrid. The system shows fluorescent properties and high affinity for cells. Therefore, magnetic and fluorescent nanoparticles are potential candidates for smart drug-delivery systems. Also, the superparamagnetic behaviour of such nanoparticles may be exploited as MRI contrast agents to improve therapeutic diagnostics.

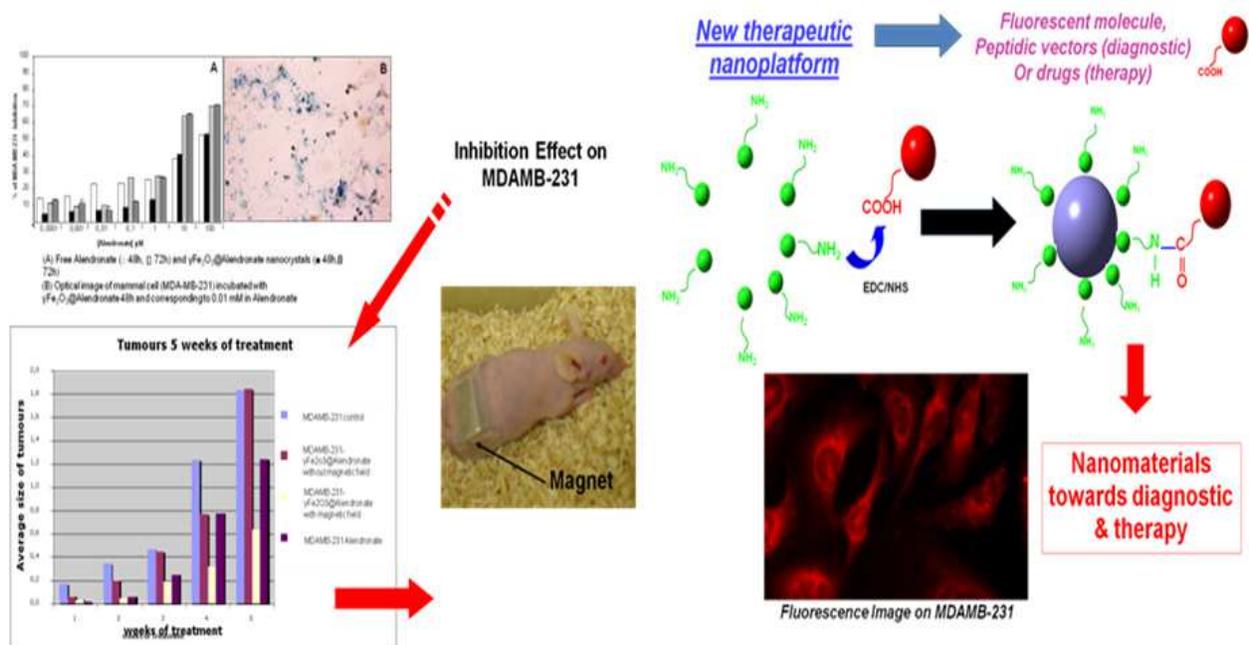


Fig. 9. Nanoparticle platform showing the possibility to graft BPs on superparamagnetic γ -Fe₂O₃ nanoparticles.

5. References

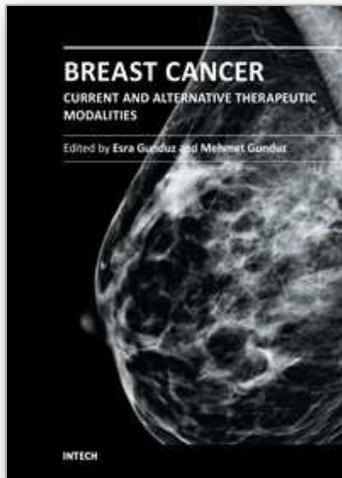
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Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various therapeutic modalities from signaling pathways through various anti-tumor compounds as well as herbal medicine for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

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Phone: +385 (51) 770 447
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InTech China

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中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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