Novel bone-targeting bisphosphonate-chemotherapeutic conjugates: from clinical proof-of-concept to second generation compounds

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ABSTRACT

MBC-11, a conjugate of etidronate and cytarabine, has recently demonstrated significant reduction of cancer cell activity in the majority of bone lesions in patients with prostate, breast and cervical cancer metastases [1]. In an effort to extend this success, a second-generation conjugate of ibandronate and gemcitabine (GEM-IB) is designed to increase (i) antiresorptive activity with the use of ibandronate, and (ii) anticancer activity directed at solid tumors with the use of the self-potentiating antimetabolite gemcitabine. The development of synthetic methods and purification of GEM-IB have enabled *in vivo* testing. In a mouse model of osteosarcoma, GEM-IB reduced tumor growth and in combination with docetaxel demonstrated preservation of bone architecture and antitumor efficacy synergistically. These results warrant further clinical development of this promising approach.

INTRODUCTION

Bisphosphonate (BP)-drug conjugates are a very promising approach to treating Cancer-Induced Bone Disease (CIBD). Desired properties of ideal bone-targeting drug conjugates have been suggested:

(i) specific targeting of bone mineral or bone localized cells,
(ii) stable to systemic exposure prior to bone binding,
(iii) labile enough to release its drug payloads after bone localization,
(iv) the kinetics of drug release promote efficacy and enable synergistic benefit with other drugs,
(v) the linker enabling conjugation is nontoxic ,
(vi) efficacy at the bone lesion is achieved with very limited systemic exposure,
(vi) healthy tissue including bone is not adversely affected.

Many BP-drug conjugates have been reported [2]; however, none have reported a complete characterization of all the desired properties and few have provided the critical parameters needed to optimize a therapeutic for human use, e.g. tissue distribution, maximum-tolerated-dose, toxicity PK/PD correlations. Such information would be of great benefit to the rational design of future conjugates.

MBC is pursuing complete data sets with an approach using an unconventional linear conjugation of drug to the phosphate moiety of BPs (other reports of BP-drug conjugates have the drug attached via a linker to the geminal carbon). MBC-11 (figure 1) safety and efficacy was demonstrated in a Phase I clinical trial on CIBD patients. MBC-11 reports described its synthesis, bone affinity [3], in vitro [4] and in vivo activity [5-7], pharmacokinetics and recently human efficacy. These results have suggested the ability to generate synergistic drug ratios in the bone compartment. GEM-IB (figure 2) is being developed with the aim of bone localizing the known synergy of gemcitabine (GEM) and docetaxel (DTX) as a treatment for osteosarcoma.

RESULTS AND DISCUSSION

MBC-11 Pharmacokinetics and Pharmacodynamics: Prostate, breast and cervical cancer patients with established bone metastases were treated with MBC-11 as described [1]. Figure 1 illustrates the chemical structure of MBC-11 and its products of metabolism and a representative single patient's plasma profile. Despite a large fraction of MBC-11 hydrolysis observed in the blood, Figure 2 demonstrates the majority of bone lesions are significantly reduced.

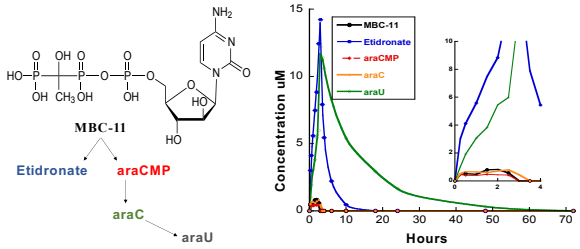

Figure 1: **Plasma pharmacokinetics**, araCMP is aracytidine-monophosphate, araC is aracytidiine, araU is arauridine (the deamination product of araC)

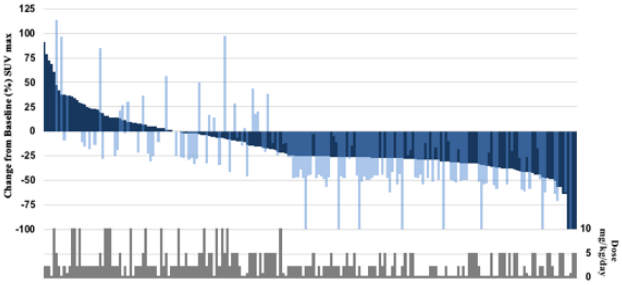

Figure 2: Bone Lesion Activity: Of 14 patients, 211 bone lesions were detected at baseline using ^{18}F-FDG-PET/CT imaging. The change in SUV$_{max}$ after 2 months (2 cycles of MBC-11 therapy) are shown with dark-blue bars; -100% indicates reduction to below the limit of detection. Five patients continued on therapy for an additional 2 months (4 cycles total); further changes from baseline are shown by light-blue bars overlapping their respective bone lesion at the 2-cycle time point. Of the 211 lesions, 110 (52%) showed a reduction in SUV$_{max}$ of ≥ 25% after 2 cycles of MBC-11. Of the 133 bone lesions present at baseline in the five patients that received 4 cycles, 85 (64%) showed a reduction. The underlying grey bars correspond to blue bars and indicate the dose administered - scale on right.

Bone-Targeted Combination Concept - GEM-IB Preliminary in vivo studies: Human proof of concept with MBC-11 and prior *in vivo* studies suggest the creation of a gradient of cytarabine from the bone surface into the marrow. The ability to use such a gradient to improve treatment with combination therapy is illustrated in Figure 3. As shown, a bone targeted drug (Drug 1) can drive bone localized concentrations above the systemic toxic limit and in combination with an additional non-targeted drug (Drug 2) can achieve drug ratios not otherwise possible. We applied this concept using GEM and DTX due to reports of GEM and DTX synergy in osteosarcoma patients [8]. GEM-IB (structure in Figure 4) was tested alone and in combination with Docetaxel in a mouse model of osteosarcoma. Figure 4 indicates free or conjugated ibandronate moiety preserved bone architecture, bone volume density and trabecular thickness and number. Figure 5 demonstrates GEM-IB and DTX additive or synergistic activity with regard to tumor volume reduction and improved survival.

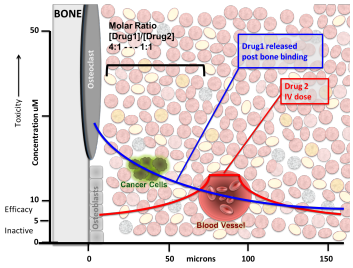

Figure 3. Synergistic drug ratios near bone-localized cancer lesions: scale incorporates reported blood vessel and cell sizes as well as average distances of micrometastases and blood vessels from bone surface. Y-axis Inactive, Efficacy, Toxicity matches plasma levels reported for araC and GEM.

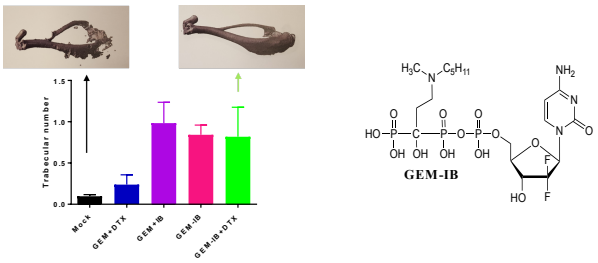

Figure 4: **GEM-IB chemical structure and effects on bone lysis**, GEM-IB, IB, DTX and GEM were administered to mice once weekly (DTX) or twice weekly (GEM, GEM-IB, IB) alone or in combination. DTX was administered at 15 mg/kg while GEM was administered at 6 mg/kg, GEM-IB at molar equivalent to the GEM dose (14 mg/kg) and IB at molar equivalent to the conjugate dose (6 mg/kg). A control group of mice (mock) were administered isotonic saline. Tumor-laden tibias of three mice from each group were imaged using Micro CT on day 22 after treatment initiation. Representative images are shown.

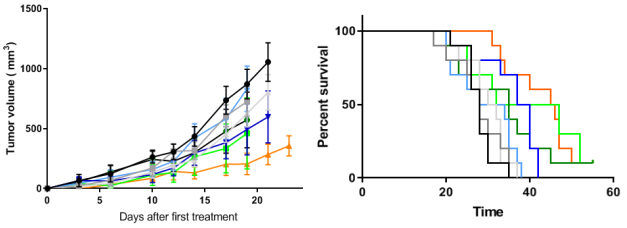

Figure 5: Tumor Volume and Survival: Nude mice were treated once-weekly 4 days after intratibial implant of 143B human osteosarcoma cells with the following drugs: 20 mg/kg GEM-IB, or equimolar amounts of GEM, IB, or both GEM + IB, plus or minus 5 mg/kg DTX. Median survival time of GEM-IB + DTX = 45.5 days; untargeted combination = 35 days.

CONCLUSIONS

MBC-11 significantly reduces bone-localized cancer cell activity in human patients.

MBC-11 PK indicates a small fraction of dose binds bone and drives efficacy.

Bone-to-blood drug gradients enable synergistic drug combos in the bone marrow.

GEM-IB has prevented cancer-induced bone lysis in an OS mouse model.

GEM-IB in combination with DTX reduces tumor volume and improves survival in an OS model.

Further development of this bone targeted platform is warranted.

ACKNOWLEDGEMENT

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