A Peptidase-Potentiated Alkylating Agent Melflufen Is an Effective Anti-Neoplastic Agent in Osteosarcoma

Poster 1726P

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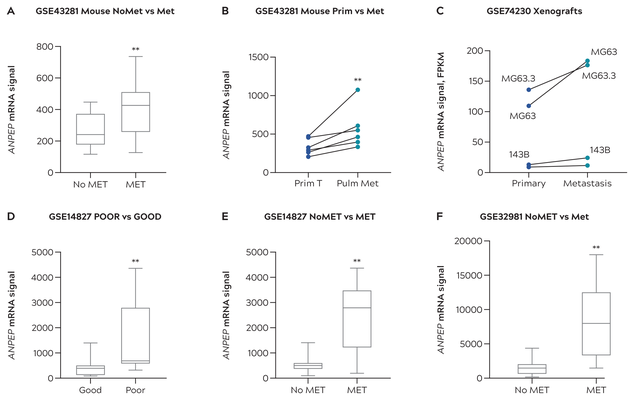
BACKGROUND

- High-grade osteosarcoma (HGOS) is the most common primary malignant bone tumor and has a high invasive and metastatic potential[1-3]
- Survival rates in metastatic patients are poor and remained virtually unchanged over the past 30 years; recent randomized trials have failed to improve the efficacy of current chemotherapy[4-6]
- Melflufen is a lipophilic peptide-conjugated alkylator; due to its high lipophilicity, melflufen rapidly enters tumor cells where it is immediately cleaved by peptidases leading to entrapment and enrichment of alkylating payload[7]
- Melflufen has demonstrated high anti-neoplastic efficacy in numerous pre-clinical models as well as clinical trials[7]
- Overexpression of peptidases is often seen in tumor cells and can thus lead to increased melflufen sensitivity[7]
- Aminopeptidase N (ANPEP/CD13) has been identified as a driver of osteosarcoma cell migration and invasion,[8-10] and overexpression of ANPEP/CD13 in osteosarcoma has been associated with poor survival and metastasis[11-13]
- Melflufen may therefore represent a valid new therapeutic option for osteosarcoma
- The objective of this study was to test the anti-neoplastic activity of the novel peptide-conjugated alkylator melflufen in the preclinical osteosarcoma setting

METHODS AND RESULTS

Figure 1. Expression of *ANPEP/CD13* mRNA in a Murine p53 Osteosarcoma Model, Xenografts, and HGOS Patient Samples



(A) *ANPEP* mRNA is up-regulated in murine osteosarcoma tumors with metastatic (MET) potential when compared to the tumors that did not produce pulmonary metastasis (No MET), GSE43281, ** *P*<0.001 Mann-Whitney U test. (B) The tumors capable of producing pulmonary metastasis in p53 murine model display higher *ANPEP* mRNA expression in the metastasis (Pulm Met) when compared to the primary bone tumor (Prim T), GSE43281, ** *P*<0.001 Wilcoxon test. (C) MG63, MG63.3 and 143B osteosarcoma express higher *ANPEP* mRNA when isolated from pulmonary metastasis in xenograft when compared to the primary culture, GSE74320, no statistical test (not enough data points). (D) *ANPEP* mRNA is higher expressed in tumor samples of osteosarcoma patients with poor response to chemotherapy, GSE14827, ** *P*<0.001 Mann Whitney U test. (E) *ANPEP* mRNA is upregulated in osteosarcoma tumors which produced pulmonary metastasis (MET) within 5 years upon initial diagnosis when compared to the samples from patients where no metastasis was detected 5 years upon initial diagnosis (No MET), GSE14827 ** *P*<0.001. (F) *ANPEP* mRNA is upregulated in the primary tumor samples of patients with pulmonary metastasis presented at diagnosis (MET) compared to the patients where no pulmonary metastasis was detected at 5 years upon diagnosis, GSE32981, ** *P*<0.001.

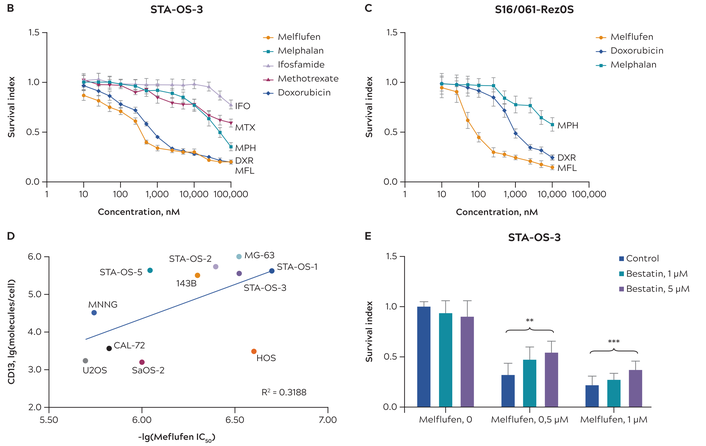
METHODS AND RESULTS

Figure 2. Drug Sensitivity Profiling of HGOS Cell Lines

A

	HOS	143B	MNNG	U2OS	SaOS-2	CAL-72	MG-63	STA-OS-1	STA-OS-2	STA-OS-3	STA-OS-5
Melflufen	6.60	6.30	5.74	5.70	6.00	5.82	6.52	6.80	6.40	6.52	6.05
Melphalan	4.60	4.60	4.30	4.00	4.60	4.70	4.30	4.80	5.00	4.30	4.60
Busulfan	4.00	4.00	4.30	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Bendamustine	5.00	4.82	4.60	4.30	4.92	4.85	5.00	5.20	5.00	4.60	4.87
Ifosfamide	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Cyclophosph	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Busulfan	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Doxorubicin	7.30	6.30	6.00	5.74	6.30	6.00	7.00	6.30	7.00	6.30	6.30
Methotrexate	7.60	7.49	8.00	7.60	6.30	6.64	7.30	7.30	6.60	4.00	7.00
Etoposide	6.30	6.12	5.60	5.00	5.30	6.00	6.00	4.00	6.00	4.00	5.00
Talazoparib	5.60	6.25	4.60	4.00	4.60	5.30	6.30	4.20	6.60	4.30	5.00
Olaparib	4.30	4.00	4.00	4.00	4.30	4.00	5.00	4.00	5.00	4.00	4.00
Niraparib	5.30	5.57	5.00	4.57	5.00	4.60	6.00	4.60	5.60	5.00	4.60
Pamiparib	4.30	4.30	4.30	4.22	4.30	4.30	4.60	4.30	4.30	4.00	4.00
AZD2461	4.00	4.00	4.00	4.00	4.00	4.00	4.60	4.00	4.30	4.00	4.00

4.00 $-\log_{10}(IC_{50})$ 8.00
100 µM 10 nM



(A) Drug sensitivity profiling of a panel of osteosarcoma cell lines against alkylating agents, chemotherapy drugs, and PARP inhibitors. *NOTE*: the depicted values are -lg(IC50 *M*) meaning that lower values (in green) correspond to higher IC50 hence resistance whereas higher values correspond to lower IC50 indicating drug sensitivity. (B) STA-OS-3 cell line established from a patient with an aggressive, multifocal, chemo-resistant disease shows sensitivity to melflufen and doxorubicin and resistance to methotrexate, ifosfamide, etoposide, and melphalan, N=3. (C) Primary cells from a refractory osteosarcoma patient show resistance to doxorubicin and melphalan but succumb to melflufen. (D) Melflufen sensitivity of osteosarcoma cells correlates with the surface expression of CD13, Spearman correlation R=0.63. (E) Aminopeptidase inhibitor bestatin hinders cytotoxic effect of melflufen in osteosarcoma cell lines.

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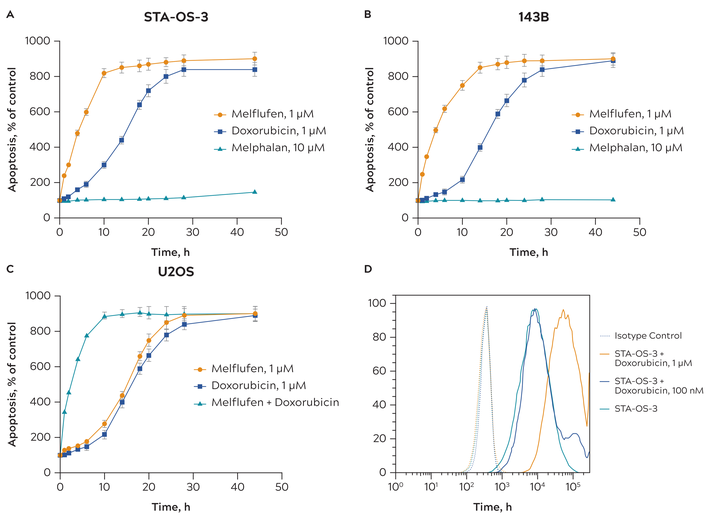
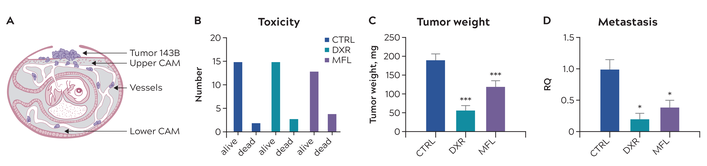
Figure 3. Apoptosis



Melflufen is rapidly inducing apoptosis in multidrug-resistant STA-OS-3 (A) and 143B (B) osteosarcoma cell lines. (C) CD13-low cell line U2OS slowly reacts to the action of melflufen, but the apoptosis is accelerated by the addition of doxorubicin. (D) Doxorubicin upregulates CD13 expression.

Figure 4. In Vivo Studies



(A) Schematic representation of the chick embryo system used in the study. (B) Toxicity profile showing the number of dead chick embryos upon addition of the drugs (100 µM doxorubicin (DXR), 500 µM melflufen (MFL)). (C) Average tumor weight isolated from the xenografts, ANOVA test. (D) Metastasis rate measured by the presence of tumor cells in the lower CAM. * *P*<0.05; *** *P*<0.001 (ANOVA test).

CONCLUSIONS

- Melflufen is the first-in-class alkylating agent showing anti-neoplastic activity in osteosarcoma models in vitro and in osteosarcoma xenograft models
  - Melflufen's cytotoxic activity positively correlates with ANPEP/CD13 surface expression and is reduced by bestatin, an aminopeptidase inhibitor, showing the direct contribution of aminopeptidase activity into melflufen's anti-osteosarcoma effect
  - Melflufen rapidly induces apoptosis in osteosarcoma cells, especially in CD13-high cells, thus eradicating the aggressive clones
  - Melflufen synergizes with doxorubicin, a long-term first-line agent for osteosarcoma, in CD13-low cells due to the positive effect of doxorubicin treatment on CD13 expression
  - Melflufen can insert cytotoxic activity within the malignant cells where methotrexate and etoposide failed
- Melflufen did not show any significant toxicities at the concentration at which anti-neoplastic and anti-metastatic activity was demonstrated in osteosarcoma cells
- Melflufen represents a novel anti-osteosarcoma drug with remarkable anti-neoplastic and anti-metastatic activities; to our knowledge, melflufen is the only alkylating agent to show high efficacy in preclinical osteosarcoma models
- Combined with a favorable toxicity profile,[6] melflufen may be an effective adjunct treatment for high-risk osteosarcoma patients

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DISCLOSURES

KB, **AS**, **FL**: employment and equity ownership with Oncopeptides; **TL**, **LK**, **STM**: no relevant relationships to disclose