Integrative surfaceome profiling identifies immunotherapeutic targets in osteosarcoma and preclinical testing of BT1769, an MT1-MMP-targeted Bicycle® toxin conjugate, in osteosarcoma by the Pediatric Preclinical Testing Consortium (PPTC)

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Introduction

- Current developments in the immunotherapeutic strategies show potential in the treatment of solid tumors.
- In osteosarcoma, surface proteins such as GD2 and HER2 have been identified as cell surface antigens of interest, but therapeutic mAb such as dinutuximab and trastuzumab were inadequate to generate robust clinical responses.
- Immunotherapies such as immunoconjugates or CAR-T cell therapy have not been sufficiently studied in OS.
- *Bicycle* Toxin Conjugates have low molecular weight (~4 kDa) compared to antibody drug conjugates (~150 kDa) - rapid tumor penetration and short half-life.
Identifying New Targets in Osteosarcoma

- Hypothesis: surface proteins such as those related to bone differentiation or other consistent osteosarcoma features may be targeted therapeutically.
Identification of candidate OS cell surface antigens: approach

**Osteosarcoma surface protein profile**
Surface protein extraction and mass spectrometry identification in a panel of osteosarcoma cell lines and PDX models. 825 surface proteins were identified.

**Surface proteins overexpressed in osteosarcoma vs normal tissues**
ProteomicsDB database: surface proteins overexpressed in osteosarcoma vs normal human tissue. 141 overexpressed surface proteins were identified.

**Surface proteins overexpressed in OS vs normal tissues**
64 surface proteins were confirmed to be expressed in the OS cell lines and PDX models, and overexpressed in OS compared to normal tissues.

**Select surface genes overexpressed in OS vs normal tissues**
209 overexpressed surface genes were identified by RNA-seq data for osteosarcomas (TARGET) compared with normal human tissues (GTEX).

**Protein targets of ADCs and/or CAR T cells**
126 surface protein targets were selected by literature review.

**Candidate surface proteins**
11 surface targets were enriched at both the protein and mRNA levels.

**Candidate therapeutic targets**
4 candidate targets (MT1-MMP, MRC2, CD276, and LRRC15) were identified.

**Validation of candidate targets**
WB, FCM, and IHC with OS cell lines, PDX tissues and patient samples validated the overexpression of MT1-MMP, MRC2, and CD276 in osteosarcomas.

**Preclinical test of novel therapeutic agents**
MT1-MMP targeted bicycle toxin conjugate (BTC) BT1769 were tested in preclinical OS PDX models.
825 surface proteins are identified in OS cell lines/PDX models by mass spectrometry.
Integrative proteomic and transcriptomic analysis identifies overexpressed cell-surface proteins in OS

- Mass spectrometry: 825 surface proteins that were highly expressed in OS cell lines/PDX were identified by quantitative proteomics mass spectrometry.

- RNAseq: 209 OS specific plasma membrane-associated genes were identified.

- MS public database: 141 proteins overexpressed in OS were identified.

- The overlapped targets among these 3 datasets were filtered by current 126 ADC/CAR-T targets

- 4 surface proteins (MT1-MMP, MRC2, CD276, and LRRC15) were found to be enriched in OS by all RNA-seq and mass spectrometry datasets. There are immunoconjugates and/or Bicycle toxin conjugates targeting these 4 surface proteins.
The expression of CD276, MRC2 and MT1-MMP in OS vs Normal tissues
Validation of the expression of MT1-MMP, MRC2 and CD276 in OS cell lines and PDXs

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>PDX models</th>
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<tbody>
<tr>
<td>MT1-MMP</td>
<td>MT1-MMP</td>
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<tr>
<td>MRC2</td>
<td>MRC2</td>
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<tr>
<td>Cofilin</td>
<td>Cofilin</td>
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<tr>
<td>CD276</td>
<td>CD276</td>
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**Graphs**

- MT1-MMP expression in HOS, OS252, OS17, OS31, OS33, OS39, OS43.
- MRC2 expression in HOS, OS252, OS17, OS31, OS33, OS39, OS43.
- CD276 expression in HOS, OS252, OS17, OS31, OS33, OS39, OS43.
Validation of the expression of the candidate surface proteins - IHC with patient and PDX TMA

Patient sample | PDX tissue | Control

MT1-MMP

MRC2

CD276

Patient sample

PDX

Preclinical test of MT1-MMP targeted BT1769

RNAseq - MT1-MMP

FPKM

OS-33
OS-17
OS-2
OS-9
OS-31

Control
BT1769

Days Post Treatment Initiation
Tumor Volume (cm^3)

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Post-treatment recurrent tumor showed a lower MT1-MMP expression level.
Preclinical test of MT1-MMP targeted BT1769

Osteosarcoma PDX models

- OS33
- OS17
- OS2

Ewing sarcoma PDX models

- ES1
- OS1
- OS31
- OS9
- TC-71

**DO NOT POST**
Summary

- Integrated proteomic and transcriptomic osteosarcoma surfaceome profiling identified high-confidence osteosarcoma cell-surface antigens (MT1-MMP, CD276, MRC2, and LRRC15) as therapeutic targets.
  - ADC targeting LRRC15 is active against osteosarcoma PDXs (Hingorani, et al. Mol Cancer Ther 2021)
  - ADC targeting CD276 is active against osteosarcoma PDXs (Kendsersky, et al. Clinical Cancer Research, 2021)

- MT1-MMP’s high expression and BT1769’s preclinical activity support the potential of MT1-MMP-targeted Bicycles in osteosarcoma.
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