Biodistribution and antitumour efficacy study of novel Her2-targeting DARPins

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Introduction

DARPins are small-sized (16 kDa) proteins derived from the naturally occurring protein ankyrin, which are engineered to exhibit high affinity for target proteins. The epithelial growth factor receptor 2 (Her2/neu) is a target of interest for targeted therapies as it is overexpressed in epithelial tumours such as breast and gastric cancers. Antitumour activity and biodistribution of five novel biparatopic Her2-targeting DARPins (CME114, CME115, CME118, CME119 and SPA28) were evaluated in mice bearing Her2-positive human breast tumours. Biparatopic DARPins bind simultaneously to two different epitopes in Her2 and trigger apoptosis in Her2-expressing tumour cells. The tested molecules are five biparatopic DARPin variants combining different amino acid sequences, relative domain orientations, and PK-extension technologies.

Material and Methods

Antitumour activity of DARPins in BT-474 tumour bearing mice

Breast cancer cells

The BT-474 human cells were cultured in DMEM medium containing 4mM L-glutamine supplemented with 10% fetal bovine serum.

Animals

Healthy female Balb/c Nude mice were obtained from Charles River. Animal experiments were performed according to ethical guidelines of animal experimentation(1-3) and were approved by Oncodesign's internal ethical committee (OncoMet).

Tumour induction

BT-474 tumour cells were subcutaneously injected (with matrigel) in the right flank of Balb/c Nude mice on D0. Randomization was performed on D25 (mean tumour volume at randomization: 264 ± 57 mm^3).

Treatments

All treatment groups were composed of 8 mice. The four CME compounds had a similar PK in mice and were dosed at 35 mg/kg. The DARPin SPA28 had a faster clearance and was dosed at 45 mg/kg to ensure a comparable AUC to the other constructs. The vehicle and all DARPins were dosed via IV injections with a Q3Dx11 scheme (indicated by blue arrows in the graphs).

Histological analysis

Liver, kidneys and heart were collected and embedded in paraffin. The histology of organs was investigated after hematoxylin and eosin staining.

Evaluation of antitumour activity

Tumour growth inhibition (T/C%) was calculated as the ratio of the median tumour volumes of treated versus control groups.

Radiolabelling of DARPins with technetium-99m (^{99m}Tc)

The DARPins were radiolabelled with ^{99m}Tc using the tricarbonyl technique (Isolink® kit). The radiochemical purity (RCP) of the ^{99m}Tc-radiolabelled DARPins was assessed by thin layer chromatography (TLC). Quality control of ^{99m}Tc-radiolabelled DARPins was also performed by exclusion chromatography. The ability of the ^{99m}Tc-radiolabelled DARPins to bind albumin and Her2 was confirmed by both ELISA and BT-474 tumour cell binding assays.

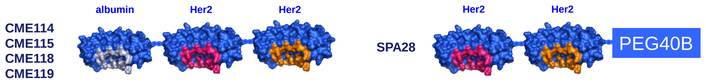
Biodistribution of ^{99m}Tc-radiolabelled DARPins in BT-474 tumour bearing mice

BT-474 tumour bearing mice received a single IV injection of ^{99m}Tc-radiolabelled DARPins (25-63 µg/mouse, n=3 per timepoint). Mice were terminated 4 and 24 hours post-injection (p.i.). Tumour, blood and organs were collected and radioactivity in these samples was determined using a γ-scintillation counter. The penetration of ^{99m}Tc-radiolabelled DARPins in tumour was evaluated by planar imaging on 7 consecutive 0.3 mm thick frozen sections.

(1) Principe d'éthique de l'expérimentation animale, Directive n°2010/63 CEE du 22 septembre 2010, Décrêt n°2013-118 du 01 février 2013.
(2) NRC Guide for the Care and Use of Laboratory Animals.
(3) United Kingdom co-coordinating committee on cancer research guidelines for welfare of animals in experimental neoplasia, Br. J. Cancer 2010, 102: 1555-1577.

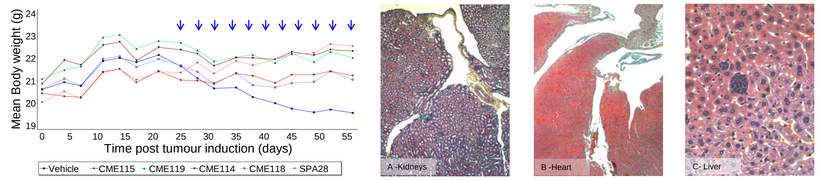
Results

DARPins



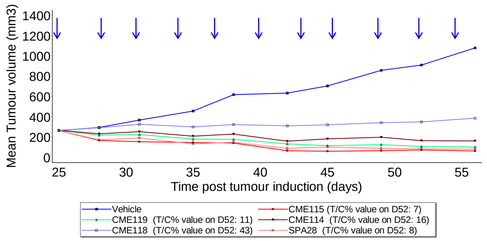
All tested DARPins were composed of a core anti-Her2 biparatopic DARPin (red and orange) and a PK extension moiety. The latter consisted of either a genetic fusion to an albumin-binding DARPin (grey) or a conjugation to PEG40B.

Safety profile



No body weight loss was observed during the course of treatments. No lesion was observed by histology in kidneys, heart and liver (A – B- C).

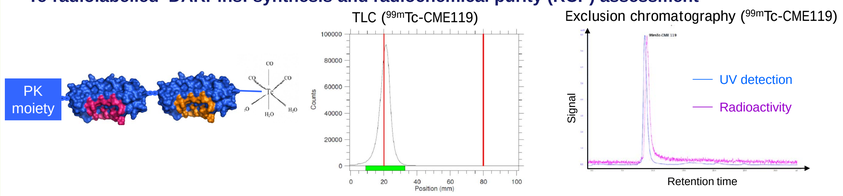
Antitumour activity



CME114, CME115, CME119 and SPA28 induced tumour regression after the first treatment which was sustained during the course of treatments whereas CME118 only induced tumour stasis.

Treated-to-control ratios (T/C%) were lower than 42% except for CME118.

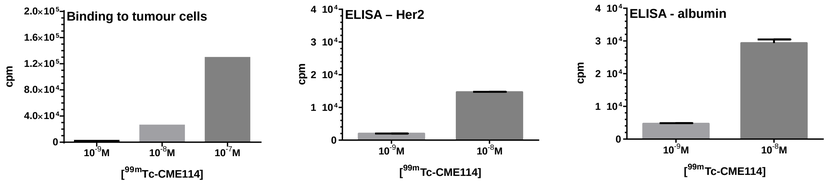
^{99m}Tc-radiolabelled DARPins: synthesis and radiochemical purity (RCP) assessment



RCP (assessed by TLC) was >90% for ^{99m}Tc-CME115 and ^{99m}Tc-CME119. RCP was 65% for ^{99m}Tc-CME114 and 49% for ^{99m}Tc-CME118. For all radiolabelled DARPins, exclusion chromatography showed that DARPins and radioactivity were co-eluted, indicating that ^{99m}Tc was bound to DARPins.

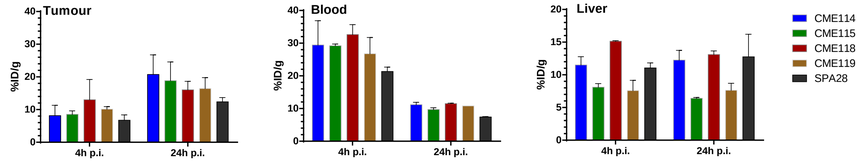
Results

Quality control of ^{99m}Tc-radiolabelled DARPins (target binding assessment)



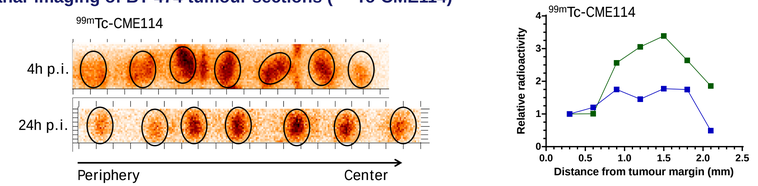
All ^{99m}Tc-radiolabelled DARPins maintained their binding capabilities as they were able to bind to BT-474 tumour cells and to the targets in ELISA assay; SPA28 binds only Her2 and not albumin. Binding of the five ^{99m}Tc-radiolabelled DARPins increased with concentration.

Biodistribution of ^{99m}Tc-radiolabelled DARPins



Uptakes of ^{99m}Tc-radiolabelled DARPins in BT-474 tumours increased between 4 and 24 hours post injection up to >15%ID/g. The four radiolabelled DARPins were slowly cleared from blood while uptakes remained quite stable in liver.

Planar imaging of BT-474 tumour sections (^{99m}Tc-CME114)



All ^{99m}Tc-radiolabelled DARPins showed a similar distribution pattern in tumours as ^{99m}Tc-CME114. They rapidly (after 4 h) penetrated 2.1 mm into the tumors, which was further enhanced with time (after 24 h) for ^{99m}Tc-CME114 and ^{99m}Tc-SPA28.

Conclusions

- DARPins are a flexible platform allowing easy conjugation to payloads, including radiolabeling.
- Tumour models allow ranking of CME candidates according to their in vivo antitumour properties.
- All tested DARPins, except CME118, induced tumour regression.
- DARPins showed good tumor targeting and penetration. CME115 showed the best tumour to blood/liver ratios.
- No side-effects related to treatment with DARPins were evidenced.
- PK extension via albumin provided longer PK and easier DARPin production than via PEG40B conjugation.

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