O-191 Assessing the use of tumour-specific DARPin-toxin fusion proteins for ex vivo purging of cancer metastases from human ovarian cortex tissue fragments before autotransplantation

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Study question: Is it possible to eradicate cancer cells from ovarian cortex by using tumour-specific designed ankyrin repeat protein (DARPin)-toxin fusion proteins, without compromising the ovarian tissue?

Summary answer: Purging ovarian cortex *ex vivo* from experimentally induced breast cancer tumour foci is possible by tumour-targeted DARPin-toxin fusion proteins trough inhibition of protein synthesis.

What is known already: Ovarian tissue cryopreservation and autotransplantation is a successful technique for fertility restoration in cancer patients. The procedure is not without risk since malignant cells may still be present in the graft. Procedures to detect cancer cells render the tissue fragment useless for autotransplantation. Strategies to circumvent this problem such as *in vitro* maturation of follicles or the construction of artificial ovaries are pursued but are still experimental. Alternatively, we have shown *ex vivo* purging of ovarian cortex is possible by elimination of rhabdomyosarcoma after treatment with verteporfin. This allows treatment of cortex fragments before autotransplantation without compromising ovarian tissue integrity.

Study design, size, duration: Human ovarian cortex fragments harbouring breast cancer tumour foci were exposed for 24 h to DARPins fused to the translocation and catalytic domain of *Pseudomonas aeruginosa* exotoxin A (DARPin-toxin fusion proteins) targeting EpCAM or HER2. After treatment with the DARPin-toxin fusion proteins the tissue was cultured for an additional 6 days to allow any remaining tumour cells to form foci. In addition, the functional integrity of the ovarian tissue was analysed after purging.

Participants/materials, setting, methods: Breast cancer cell lines expressing different levels of EpCAM and HER2 were introduced in human ovarian tissue to form tumour foci. After purging with DARPin-toxin fusion proteins, the presence of any remaining cancer cells in the tissue was analysed with (immuno)histochemistry and RT-qPCR. Possible detrimental effects on the viability of ovarian cortex and follicles were determined by (immuno)histology, a follicular viability assay and an assay to determine the *in vitro* growth capacity of small follicles.

Main results and the role of chance: Ovarian cortex harbouring EpCAM-positive breast cancer cells showed a significant decrease in the number of tumour foci after treatment with the EpCAM-targeted DARPin-toxin fusion proteins. Although exposure to the EpCAM-specific DARPin had no effect on morphology or viability of follicles, a decrease in oocyte viability after *in vitro* growth experiments was observed, presumably due to low level expression of EpCAM on oocytes. In contrast to the EpCAM-specific DARPin-toxin fusion protein, the DARPin-toxin fusion protein targeting HER2 had no detrimental effects on morphology, viability or *in vitro* growth of follicles while foci of HER2-positive breast cancer cells were severely affected as indicated by the presence of apoptotic bodies, tumour cell remnants and the absence of viable tumour cells. The histological results after purging with the HER2-specific DARPin-toxin fusions proteins were confirmed by RT-qPCR, showing a decrease to basal levels of HER2 mRNA in the ovarian cortex tissue.

Limitations, reasons for caution: The effect of DARPin-toxin fusion proteins depends heavily on the expression of their target on the cancer cell. The target protein should not be expressed by ovarian cortex as this may lead to tissue damage. The functional integrity of ovarian cortex after the treatment requires further investigation *in vivo*.

Wider implications of the findings: Purging metastases from ovarian cortex without harming ovarian tissue is possible by targeting tumour specific surface expressed antigens with DARPin-toxin fusion proteins. Purging ovarian cortex tissue with DARPin-toxin fusion proteins provides a feasible therapeutic strategy to prevent reintroduction of cancer by autotransplantation in case of malignancies expressing tumour-specific surface markers.

Trial registration number: not applicable