Antibody engineering to evaluate binding, internalisation, and intracellular routing of tumour-targeting fusionproteins.

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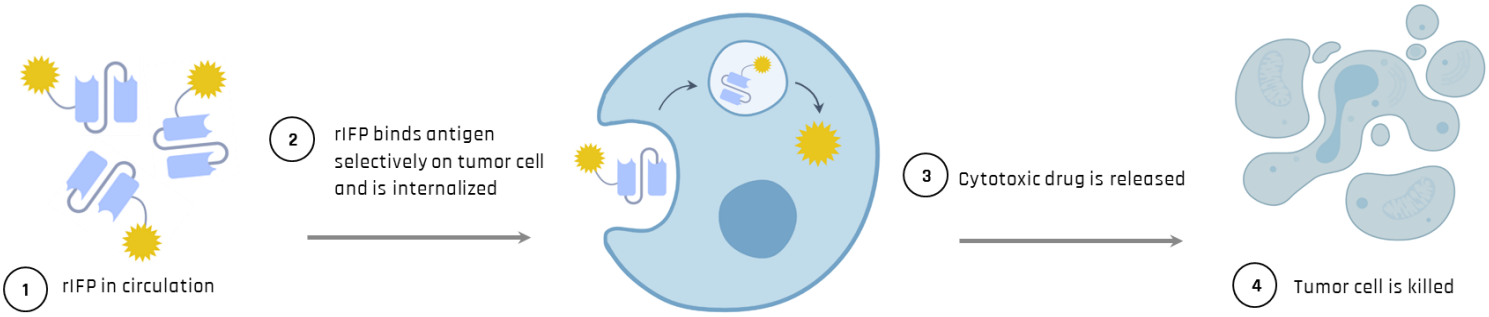

Figure 1. Recombinant immunotherapeutic fusion protein (rIFP) mechanism of action.

Introduction: Recombinant immunotherapeutic fusion proteins (rIFPs) were designed to target triple-negative breast cancer (TNBC). This is a heterogeneous and aggressive subset of breast cancer accounting for 15-20% of all diagnosed breast cancer cases, with women of premenopausal age and African descent inordinately predisposed [1]. Based on past findings, we hypothesised that the ability to bind to two identical antigens through a bivalent antibody increases the total strength of the reaction and that increasing the affinity and valency of tumour-targeting antibodies would result in improved tumour uptake [2]. Furthermore, the rate of internalisation and intracellular routing of rIFPs may be significant for their therapeutic efficacy [3]. Understanding these factors could impact the use of such biopharmaceuticals for targeted treatment with relevant cell surface biomarkers.

Methodology: The rIFPs were transiently expressed in mammalian cell culture and purified using immobilised metal- ion affinity chromatography (IMAC). Following SDS-PAGE and Western Blot protein analysis, the proteins were fluorescently labelled and the differences between the mono- and bivalent rIFP formats were evaluated *in vitro* using confocal imaging. The efficacy in binding to targeted cell surface receptors, rate of internalisation, and intracellular routing of internalised rIFPs were evaluated to determine such differences. Differences in rIFP-mediated cytotoxicity were evaluated *in vitro* using XTT-based cell viability assays.

Results: The rIFPs were successfully expressed, purified, and characterised. Imaging results indicate that the bivalent rIFPs display increased binding affinity and faster internalisation rate compared to the monovalent counterpart when applied to a confirmed antigen-positive TNBC cell line. This correlated with an enhanced cytotoxic effect of the bivalentrIFP.

Discussion: Protein characterisation, confocal imaging, and cytotoxicity assay data of mono- and bivalent rIFPs will be discussed. These results may have implications for the future designs of rIFPs, however, *in vivo* pharmacokinetic studies are needed to further elucidate the effect of valency on the efficacy of recombinant immunotherapeutic fusion proteins.

References:

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