Activatable Antibody-Zinc-Finger Conjugates (AZFCs): a novel concept of gene therapy.

Proposers
Joao Goncalves (iMed-Research Institute of Medicines, Faculdade de Farmacia da Universidade de Lisboa), Raquel Aires-Barros (iBB- Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisbo=

Zinc-finger (ZF) proteins have the intrinsic capability of penetrating the cell (protein transduction), allowing it to be directly delivered and used in therapeutics in a new concept of gene therapy without direct genetic intervention. We propose to use this property to create a new protease-activated drug improving the existent antibody-based (trastuzumab) therapy against HER2+ breast cancer, by adding a second mechanism of action able to act even on trastuzumab-resistant cells. A ZF artificial transcription factor, designed to down-regulate Bcl-2 and induce apoptosis, will be fused to an antibody against HER2 with a linker containing a target sequence for matrix metalloproteinase-9 (MMP-9). The antibody ensures the delivery to HER2+ tissues and MMP-9 overexpression in tumorous tissue will improve efficiency by selectively releasing the ZF-ATF and inducing apoptosis of cancer cells through an HER2-independent mechanism. This project aims to develop a new concept of activatable antibody-drug conjugate delivery for cancer gene therapy by fusing a zinc-finger artificial transcription factor (ZF-ATF) to an antibody with a linker containing a protease-target sequence that will allow the release of the ZF-ATF for efficient transduction. The primary application, and model for the development of the technology, is highly specific and effective therapy in breast cancer, including trastuzumab-resistant tumours.

Due to its complexity, the production of this chimeric protein will be optimized. A battery of 4 reactors available at IST-IBB with control of dissolved oxygen, pH, temperature and agitation rate will be used. The concentration levels of nutrients will be followed throughout the culture using a multi-parameter metabolite analyser and consumption/production rates will be calculated. Operational parameters such as agitation speed, initial cell densities, feeding regimen and dissolved oxygen will be optimized to maximize titer and volumetric productivity of protein production. In order to optimise and intensify the purification of the chimeric protein, different unit operations will be evaluated within each purification stage (clarification, capture and polishing) with respect to yields, purity, time, energy, raw materials, etc. Particular focus will be given to aqueous two phase systems and chromatography.