An Automated High-throughput Platform for the Design and Production of Recombinant Immunotoxins with Anticancer Activity in Plants

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Introduction

Complex biopharmaceuticals

Complex biopharmaceuticals like antibody drug conjugates (ADCs) and recombinant immunotoxins (RITs) enable the targeted delivery of toxic protein drugs to cancer cells. While ADCs have high production costs because the monoclonal antibody and the toxic drug require separate production and then chemical coupling, RITs can be produced as one fusion protein, which might reduce costs from >150.000 €/g to <30.000 €/g. Plants proved to be most efficient for their production since RITs can be targeted to different compartments where they do not interfere with the host metabolism.[2, 3, 4]

Plant Cell Pack (PCP) technology for high-throughput screening of RITs

PCPs, derived from plant suspension cells, provide a high-throughput screening platform for the production of recombinant protein candidates in plants and has already been used to develop the RIT VisA-H22 for the treatment of acute myeloid leukemia (AML), but the accumulation of 40 mg/kg was low compared to 1-6 g/kg that are required for an economic production.[5,6] The latter might be due to the manual PCP protocol, which suffers from low throughput (320 samples per week) and high variation between biological replicates (<55 %).

Objectives & Methodology

1. The automation of the PCP screening platform for the development of RITs in 96-well format on an industrial robotic station. This includes the implementation of protocols for the following steps based on previously developed methods [5, 6, 7, 8]:
   - (i) Cloning of RIT expression cassettes in pTRAc vectors by a PCR amplification, restriction and ligation method using PCR plates.
   - (ii) Transformation of RIT vectors into *Agrobacterium tumefaciens* (*A. tumefaciens)* by electroporation and subsequent limited dilution to obtain monoclonal cell lines for glycerol stocks using electroporation plates.
   - (iii) Cultivation of *A. tumefaciens* and Tobacco Bright Yellow-2 (BY-2) cells on a heating shaker system.
   - (iv) Generation of PCPs from BY-2 and its infiltration with *A. tumefaciens* containing the RIT plasmids using filter plates.
   - (v) Extraction of RITs by a detergent-based chemical lysis of PCPs and subsequent centrifugation method.
   - (vi) Quantification of RITs using an enzyme-linked immunosorbent assay in combination with a plate reader for detection.
2. The proof-of-concept of the automated PCP screening platform by the development of various RIT candidates against AML and multiple myeloma (MM) combined with the production and purification of RITs in whole plants to produce sufficient quantities of RITs for pre-clinical tests.
   - (i) Cloning of RITs with antibodies against CD64 (AML) and CD38/CD47 (MM) coupled with 4 different toxins Abrin A chain, Bryodin I, Melittin and Diphtheria toxin and identification of candidates with high accumulation levels of >1 g/kg in PCP.
   - (ii) Production of selected RIT candidates in *Nicotiana benthamiana (N. benthamiana)* plants and subsequent purification of RITs by Protein A chromatography to obtain RITs with 95-98 % purity.
   - (iii) *In-vitro* tests on cancer cell lines and *in-vivo* tests on mice and identification of RIT with an bioactivity comparable to that of a reference ADC.

Expected Outcome

1. An automated PCP platform increasing the throughput from 320 to 3.200 samples per week while reducing the variance from 55 % to <5 %.
2. Identification of RIT candidates against AML and MM with high accumulation levels of >1 g/kg in PCPs (compared to 40 mg/kg) and a high bioactivity (comparable to a reference ADC).

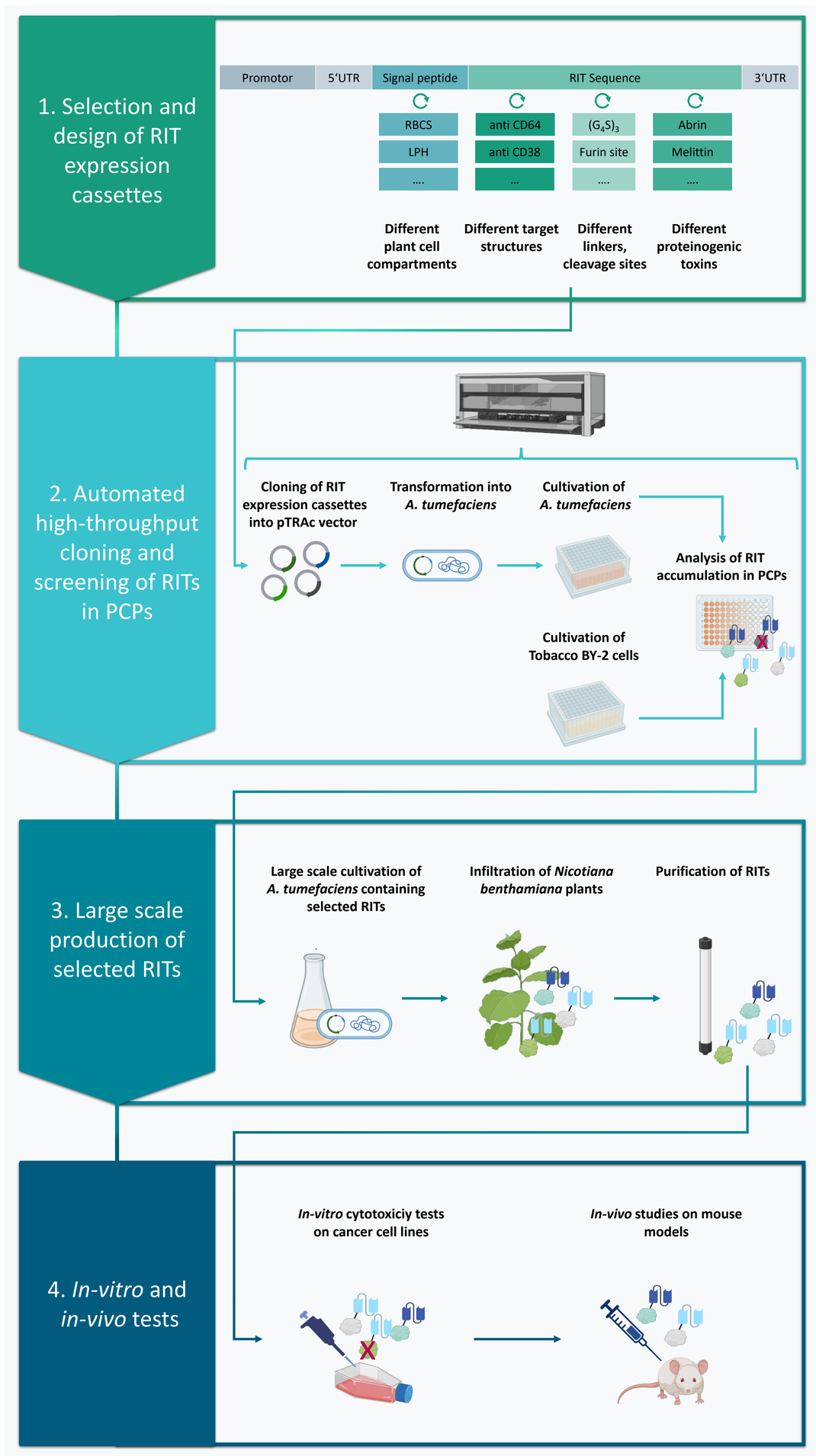

Fig1: Schematic illustration of the workflow for the design and production of recombinant immunotoxins (RITs).[9]

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9 The illustrations were created with BioRender.com by Henrik Nausch.

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