Cancer Cell International

CANCER CELL INTERNATIONAL

BioMed Central

Poster presentation

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Limiting the risk of immunogenicity by identification and removal of T-cell epitopes (DeImmunisation™)

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from Association for Immunotherapy of Cancer: Cancer Immunotherapy – 2nd Annual Meeting
Mainz, Germany, 6–7 May 2004

Published: 1 July 2004

Received: 28 April 2004

Cancer Cell International 2004, **4**(Suppl 1):S20

This article is available from: http://www.cancerci.com/content/4/S1/S20

Immunogenicity of non-human proteins such as plant derived toxin molecules and bacterial enzymes provide an almost complete barrier to their clinical implementation as novel therapeutic strategies for cancer. It is also recognised that the immunogenicity associated with therapeutic antibodies and certain human proteins can also limit their clinical effectiveness. For therapeutic antibodies a number of engineering options exist, and clearly have in part been developed to address the issue of immunogenicity. Examples include "classic" humanisation by CDR engraftment, use of human Ig libraries such as phage display systems and human Ig transgenic mice. However, in each case there should be an expectation that these can all result in antibodies still capable of eliciting an immune response in certain patients. This is connected to the process of affinity maturation during antibody generation that inevitably results in antibodies to which patients may not be naturally tolerant. Underlying this process is the presentation of T-helper peptide epitopes derived from the intracellular processing of the therapeutic antibody. Foreign T-cell epitopes are also present in plant toxins and bacterial enzymes and will cause a sustained immune response and the generation of immunological memory.

Our approach at Biovation has been the development of what we have termed DeImmunisation™ technology. This involves the identification and removal of helper T-cell epitopes from therapeutic antibody and protein candidates. We use *ex-vivo* human T-cell assays in a variety of formats to identify the T-cell epitopes, to direct the substitution of residues within epitopes and also to validate successful removal of epitopes from the protein.

Our start point is to use naïve T-cell assays to provide an outline epitope map of the protein of interest. T-cell assays are performed using healthy human PBMC preparations in MHC selected donor panels and synthetic peptide antigens. The epitope map can be further refined using the enzyme-linked immunospot (ELISPOT) assay to measure the frequencies of activated T-cells according to their particular cytokine profile. We use a software tool termed Peptide Threading to model the effect of amino acid substitutions on the ability of a T-cell epitope sequence to interact with multiple different MHC class II allotypes. This can guide the design of variant sequences and in some studies, the design phase is augmented by fine scale mapping of individual epitopes using alanine scanning mutational analysis. Antibodies and proteins engineered using this approach are tested for an improved immunogenicity profile using time course T-cell assays and selected donor panels.

DeImmunisation™ is widely applicable and we have engineered a number of therapeutic antibody and protein candidates some of which are now entering clinical trial. One such antibody in clinical development targets prostate specific membrane antigen (PSMA) and has shown no evidence of immunogenicity in >100 patients. Data will be presented on this example and other studies from our therapeutic antibody programmes and Fc-linked cytokine projects.