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Session 11: Applied technologies – III

Friday 8 October 2004. Moderators:Kathy Bowdish and Mark C. Glassy

[14.00–14.30]
Tailoring antibodies for human therapy
Dee Athwal
Celltech, Slough, UK

Abstract not received.

[14.30–14.50]
Anti-pseudomonal antibodies for treatment of pseudomonas aeruginosa infections: Efficacy of anti-flagellin antibodies in a murine burn wound model
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Vaccination (active or passive) against Pseudomonas aeruginosa and immunotherapy are desirable options in an era of increasing drug resistance. Because it is an important virulence factor, P. aeruginosa flagellum was targeted for vaccination and immunotherapy.

Polyclonal and monoclonal anti-pseudomonal type b flagellin antibodies (anti-bFla IgG) were produced and their efficacy was evaluated in a murine burn wound model infected by a subeschar injection of P. aeruginosa strain PA01 (2×10^6 and 5×10^6 CFU). Mice were treated with systemic anti-bFla IgG, non-specific IgG, and imipenem (0.5 mg twice daily), and with topical silver sulfadiazine (4 days, twice daily). The control groups included: burn alone, untreated infected burn, and infected non-burned.

Three separate regimens were examined: prophylaxis (pre-infection), treatment (post-infection), and combined. The mortality and morbidity (weight loss) follow-up lasted for 2 weeks and burns were examined histopathologically.

The uninfected burn group and the group infected with P. aeruginosa without burns had a 0% mortality rate. The mortality rate ranged between 58–83% in groups with P. aeruginosa-infected burns. The mortality rate in the anti-bFla IgG-treated groups ranged between 0–17% ($P < 0.05$ vs. non-treated groups), and was similar to groups treated with imipenem or silver sulfadiazine (8% mortality rate). The three tested regimens yielded similar results. Morbidity paralleled survival results. Complete re-epithelialization of the wound occurred earlier in the anti-bFla IgG-treated mice compared to untreated mice.

We conclude that anti-bFla IgG, given either as prophylaxis or treatment, effectively reduced mortality and morbidity and improved wound healing in a P. aeruginosa-infected murine burn model.

[14.50–15.20]
GPEx$^{\mathrm{TM}}$: Gene insertion technology for the rapid development of stable, high-expressing mammalian cell lines for initial product screening through large scale manufacturing
Paul Weiss
Gala Design, Inc.

Abstract: Typically, developers of biologic products use multiple methods to produce a number of different mammalian cell lines along the path from discovery to commercial production for a recombinant protein or antibody. Transient cell protein production is followed by initial stable cell line production, and after multiple gene amplification steps a final production cell line is obtained. The flexibility of the GPEx$^{\mathrm{TM}}$ technology allows for rapid production of research and clinical-scale quantities of protein early in the develop-

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ment process, as well as the ability to produce a final production cell line for commercial-scale manufacturing. The GPEx^{TM} process generates cell lines producing initial quantities of protein within two months from the start of the project, and produces a commercial production master cell bank candidate within six months. GPEx^{TM} utilizes replication defective retrovectors to insert multiple copies of a transgene, each at a unique genomic location, within any mammalian cell line. The insertions target open or active regions of the host cell genome allowing for higher, more consistent expression per copy of the gene inserted. Repeated cell transductions increase gene copy number and subsequent protein production levels. After a single GPEx^{TM} cell transduction, a pool of stable clonal lines is generated that expresses the target protein. This pool of cells normally produces levels of recombinant protein in the range of 25–60 mg/L after 14 days in non-optimized terminal T-flask culture without feeding. Depending on the desired cell production level, as well as client timing requirements, either clonal selection or additional cell transductions are performed on the pool of cells. Upon clonal selection from the cell pool, clones in the range of 3–7 fold higher expression than the pool are identified. Analysis of results from the GPEx^{TM} process will be discussed, in conjunction with an overview of the technology and its utility.

Biography: Dr. Paul Weiss has been President of Gala Design, Inc. since February 2002. He served as a director on Gala's Board from 1998 to 2001, when he joined the management team as Senior Vice President of Business Development. While serving on Gala's Board, Dr. Weiss also held the position of Vice President of Technology and Product Licensing at 3-Dimensional Pharmaceuticals, a company he helped take public in 2001 and which now is a division of J&J. Prior to joining 3-Dimensional Pharmaceuticals, Dr. Weiss was Director of Licensing for Wyeth-Ayerst Laboratories, now Wyeth Pharmaceuticals. In October 2002, Cardinal Health acquired Gala Design and it is now a wholly owned subsidiary of Cardinal Health. Currently, Dr. Weiss also serves on the Board of Directors for Alfacell Corporation, a company focusing on ribonuclease therapy for the treatment of cancer. Dr. Weiss holds a Ph.D. in Biochemistry and an MBA from the University of Wisconsin-Madison and a B.Sc. in Biochemisty from Carleton University Institute of Biochemistry in Ottawa, Ontario.

[15.20–15.50]
[Keynote Lecture]

Human monoclonal antibodies as vectors for cancer vaccines

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Small antigen/antibody complexes are taken up by the CD64 receptor on dendritic cells. This results in up regulation of costimulatory molecules and processing and presentation of the antigen on both class I and class II MHC molecules. Targeting CD64 therefore results in efficient stimulation of both helper and cytotoxic T cell responses. We have shown that a monomeric human IgG1 monoclonal antibody, 105AD7, can also stimulate helper and cyotoxic T cell responses in over 300 cancer patients with no associated toxicity. If the Fc region of this antibody is removed it is 1,000 fold less efficient at stimulating T cells. Similarly an anti-idiotypic antibody mimicking CEA was very inefficient at stimulating human T cells as a mouse IgG2b but when it was chimerised to a human IgG1 antibody it stimulated CEA specific helper and CTL responses. These results suggest that not only antigen/antibody complexes but monomeric human IgG1 that express T cell epitopes within their CDR regions can also target CD64. We have therefore engineered a humanised monoclonal antibody to express a TRP-2 CTL epitope and demonstrated in both C57Bl and HLA-A2,1 transgenic mice that it stimulates CTLs that kill melanoma cells more efficiently than peptide. We have also shown that it is possible to splice a functional domain of a protein to human IgG1 Fc and stimulate CTL, helper and antibody responses. We have spliced the first 196 amino acids of Tie-2 to FcγI and shown in HLA-A2.1 transgenic mice that it stimulates helper and CTL responses that recognise endothelial cells over-expressing Tie-2. Similarly the SCR1-3 domains of CD55 have been spliced to FcγI and this vaccine has been used to stimulate neutralising antibodies in mice. This provides a platform for the development of a wide range of cancer vaccines.