Keynotes

[Monday 23 April 2001, 09.00–09.40]

Immunological mechanisms underlying anti-tumor efficacy of humanized monoclonal antibodies

Zdenka L. Jonak, Stephen Trulli, Francis L. McCabe, Alexander Taylor, Louis Elefante, Yi-Jiun Chen, Jacqueline Fishman-Lobell, Kyung Johanson, Michael R. Mattern, Michael T. Lotze and Randall Johnson
SmithKline Beecham Pharmaceuticals, 709 Swedeland Rd., King of Prussia, PA 19406, USA

In clinical use, cancer immunotherapy with humanized monoclonal antibodies (mAbs) has showed success. It is clear that the choice of the target antigen, the characteristics of the mAb and their biology have to be carefully chosen in order to show in vivo efficacy. The target antigen should be commonly expressed on tumor cells (anti-tumor approach) or on the tumor neovasculature (anti-angiogenic). In the present study we will focus on two examples: (1) drug targeted delivery via mAb to the tumor cells, and (2) the criteria and issues for the development of the anti-angiogenic mAbs.

SB-408075, an antibody-directed small-molecule cytotoxin. SB-408075 is a conjugate of a humanized mAb, huC242, with a potent semi-synthetic maytansinoid, DM1. The mAb recognizes the CanAg antigen that has been reported as being expressed on 70+% of colon cancer. Immunohistochemistry confirmed high level of expression of this antigen in colon cancer biopsies. It was also observed that the antigen was expressed in a significant proportion of non-small cell lung carcinoma. Selectivity for tumor vs. normal tissue for this agent appears to be due to expression of the antigen only on the lumenal surface of normal gastrointestinal tissue and secretory epithelium of the pancreas and salivary glands. We confirmed the results obtained with SB-408075 by ImmunoGen, Inc. in advanced human colon cancer xenografts. In athymic mice bearing established antigen-positive HT-29 and Colo-201 human colon tumors, treatment with SB-408075 at doses well below an MTD resulted in complete and long-term regressions. Colon carcinoma SW-620 appeared to be negative for expression of CanAg by Flow cytometry but showed ca. 5% expression in tumor by immunohistochemistry. Treatment of mice bearing this tumor with SB-408075 resulted in partial regressions and tumor growth delay at high doses of SB-408075, indicating that even minimal antigen expression can generate some sensitivity to this immunotoxin. In many tumor specimens, including HT-29 and Colo-201, expression of antigen was heterogeneous, with only 20–50% of cells demonstrating reactivity with the C242 mAb. An important question is why tumors with heterogeneous antigen expression respond to SB-408075 with complete regression as opposed to brief tumor growth delay before outgrowth of antigen-negative cells. This was addressed in vitro comparing the homogeneous high expressing Colo-205 with HT-29. In a colony-forming assay, SB-408075 had a subnanomolar IC_{50} in both cell lines. The IC_{50} for SB-408075 in Colo-205 cells was identical when evaluad in a monolayer XTT assay, but, in this assay format, the IC_{50} was shifted 100-fold higher for HT-29 cells. This suggests that there is likely a bystander effect in which SB-408075 binds to and is internalized by antigen-positive cells with intracellular release and activation of DM1. The activated drug is then passively or actively effluxed and kills neighboring antigen-negative cells (MDR cell lines are resistant to maytansine). SB-408075 is currently in Phase I clinical trial in patients with colorectal, pancreatic and non-small cell lung cancers.

SB 392423, an anti-$\alpha V\beta 3$ integrin mAb (anti-angiogenic target). The formation of new blood vessels (angiogenesis) plays an important role in a variety of pathologic processes (e.g., cancer, rheumatoid arthritis, restenosis). Adhesion molecules contribute to these processes. Among these are several members of the integrin family. The heterodimeric $\alpha V\beta 3$ integrin is upregulated on the sprouting endothelial tumor neovasculature and on vascular smooth muscle cells after restenosis. We pursued two hypotheses (a) that inhibition of $\alpha V\beta 3$-mediated angiogenesis may provide a therapeutic benefit in the treatment of cancer, and (b) inhibition of $\alpha V\beta 3$-mediated smooth muscle cell migration following balloon angioplasty prevents restenosis. Murine and humanized mAbs with anti-$\alpha V\beta 3$ activity were generated. The high affinity (1.3 nM) $\alpha V\beta 3$-specific mAb, D12 was humanized and evaluated in in vitro and in in vivo systems. Since the mAbs are specific/selective for given inte-

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grins, their cross-reactivity with other species, especially mice are limited. Marginal cross-reactivity of D12 with rabbit $\alpha V\beta 3$ led to its evaluation in the rabbit restenosis model, showing that D12 mAb facilitated compensatory remodeling of the restenosis vessel and inhibited outgrowth of rabbit capillary tubes (anti-angiogenic activity). The lack of cross-reactivity of the D12 mAbs with murine integrins prevented progression of the mAb for cancer indications. To validate the target for cancer, we have shown by immunohistology that the expression of $\alpha V\beta 3$ is associated with tumor neovasculature in all human solid tumors and is expressed on surface of a few tumor specimens (e.g., breast and ovarian carcinomas). We plan to radio-iodinate the mAb and evaluate anti-tumor activity against human tumor xenografts. We are also generating mAbs against murine $\alpha V\beta 3$ to test the role of this integrin as an angiogenic target in tumor models. In summary, more data are needed to determine whether the anti-$\alpha V\beta 3$ D12 mAb (SB 392423) will prove useful in the treatment of cancer.

[Monday 23 April 2001, 17.15–18.00]
Human mature lymphocyte subsets: Receptor revision plays a major role in antibody diversity
J. Donald Capra
Oklahoma Medical Research Foundation, USA

Contrary to the general precepts of the clonal selection theory, several recent studies have provided evidence for the secondary rearrangement of immunoglobulin (Ig) genes in peripheral lymphoid tissues. These analyses typically used transgenic mouse models and have only detected secondary recombination of Ig light chain genes. Although Ig heavy chain variable region (V_H) genes encode a substantial element of antibody combining site specificity, there is scant evidence for V_H gene rearrangement in the periphery, leaving the physiological importance of peripheral recombination questionable. The extensive somatic mutations and clonality of the IgD^+Strictly-IgM^-CD38^+ human tonsilar B cell subpopulation have now allowed detection of the first clear examples of receptor revision of human V_H genes. The revised VDJ genes contain "hybrid" V_H gene segments consisting of portions from two separate germline V_H genes, a phenomenon previously only detected due to the pressures of a transgenic system.

Further study shows that the likely mechanism for these events is RAG mediated recombination. This conclusion is supported by the following: (1) There are cryptic RSS near the site of every donor/acceptor recombination. (2) RAG is known to be expressed in germinal centers (either newly arriving cells contain RAG or it is re-expressed-not clear at present). (3) Preliminary data suggests binding of RAG to human VH genes that are often seen in receptor revision events, but not human VH genes not involved in these events. (4) Very preliminary data suggest that RAG cleaves VH genes at or near these cryptic RSS.

Collectively, our results suggest that receptor revision is a common mechanism for antibody diversification. Current studies are directed toward understanding the underlying "cause": diversity, rescue or tolerance.

[Tuesday 24 April, 08.30–09.00]
Clinical effectiveness of human antibodies derived by conventional and non-conventional methods
James W. Larrick, Lloyd Yu, Clarissa Naftzger, Sudhir Jaiswal and Keith Wycoff
Planet Biotechnology, Inc., 2438 Wyandotte St., and Palo Alto Institute of Molecular Medicine, 2462 Wyandotte St., Mountain View, CA 94043, USA
Tel.: +1 650 694 4996; Fax: +1 650 694 7717; E-mail: jwlarrick@aol.com

Genetically engineered agricultural plants with improved traits (e.g. herbicide and pest resistance) have yielded significant agricultural revenues over the past 15 years. In addition, numerous immunotherapeutic proteins, antibodies and vaccines have been successfully produced using plant "bioreactors" (J.W. Larrick, L. Yu, J. Chen, S. Jaiswal and K. Wycoff, Production of antibodies in transgenic plants, *Research Immunology* **149**, 1998, 603–608); however, only a limited number have made their way into clinical trials. The most advanced product in human clinical trials is a secretory IgA antibody comprised of four polypeptide chains that inhibits the binding to teeth of Streptococcus mutans, the major causal agent of tooth decay. Numerous other plantibodies are in development. This talk will summarize recent work demonstrating the potential of plants to synthesize and assemble complex antibodies suitable for human therapeutic use.

[Wednesday 25 April 2001, 08.30–09.00]
n-CoDeR^{TM}: a unique source of highly specific and functional antibody fragments
E. Soderlind
Bioinvent SA, Lund, Sweden

The n-CoDeR^{TM} recombinant antibody gene libraries are build on a single master framework into which diverse in vivo formed complementarity deter-

mining regions (CDR:s) are allowed to recombine. These CDR:s are sampled from in vivo processed and proofread gene sequences, thus ensuring an optimal level of correctly folded and functional molecules. By the modularized assembly process, up to six CDR:s can be varied at the same time providing a possibility for the creation of a hitherto undescribed genetic and functional variation. The n-CoDeR^{TM} antibody gene libraries can be used to select highly specific, human antibody fragments with specificities to virtually any antigen, including carbohydrates and human selfproteins and with affinities down in the subnanomolar range. Furthermore, combining CDRs sampled from in vivo processed into a single framework result in molecules exhibiting a lower immunogenicity compared to normal human immunoglobulins, as determined by computer analyses. In conclusion, the libraries encode highly diverse antibody specificities, to be used in a range of applications, e.g. target discovery, antigen function modulation, and therapy and in the proteomic field.