Human Antibodies 15 (2006) 1–2
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Session 1: Cancer – I

Wednesday 10 May, 2006. Moderators: Mark C. Glassy and Peter Vollmers

[09.00-10.00]
[Opening Keynote Lecture]
Antibodies: Key determinants of normal and malignant B cells'
Klaus Rajewsky
CBR Institute for Biomedical Research, Boston, USA

Abstract not received.

[10.00-10.30]
Feasibility of human antibody cocktails in oncology
Mark C. Glassy
Shantha West, San Diego, USA

Abstract not received.

[10.30-10.50]
Antibody therapeutic approaches in cancer
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Fully human antibodies are rapidly becoming the norm in antibody therapy. Genmab has a number of clinical programs in cancer including antibodies against CD4, CD20 and CD38. This presentation will address the selection of highly potent therapeutic antibodies against lymphoid tumors. Insights into the mechanisms of action and recent data from clinical development will be discussed.

HuMax-CD4 is in phase III clinical development for cutaneous T cell lymphoma (CTCL). Our studies suggest that the clinical effects of HuMax-CD4 are mediated by three distinct mechanisms of action, all of which have the effect of down-regulating activated T cells: first, an effective ADCC-mediated deletion of memory-effector T cells; second, an inhibitory action on the initiation of TCR signal transduction coupling brought about by sequestration of $p56^{lck}$; and, third, direct inhibitory signals mediated via Dok-1 and SHIP-1 that inhibit AKT activation and may perturb normal cytoskeletal protein regulation.

HuMax-CD20 is in phase I/II and phase II clinical development for follicular lymphoma, B-CLL and rheumatoid arthritis. Using human Ig transgenic mice, we have generated a panel of fully human CD20 mAb directed against the CD20 molecule expressed on B cells, which is considered the best validated antibody therapeutic target for cancer. *In vitro* experiments of our lead candidate, HuMax-CD20, showed that it recognized a novel epitope on CD20, displayed an unusually slow off-rate, and rapidly induced translocation of CD20 into lipid rafts. Importantly, HuMax-CD20 was exceptionally active in complement-dependent cytotoxicity (CDC) in the presence of human plasma or whole blood, being able to lyse a range of rituximab-resistant targets expressing low levels of CD20, such as CLL.

In vivo experiments showed HuMax-CD20 to decrease tumor load and increase survival in a SCID xenograft model. Intravenous infusion of HuMax-CD20 in cynomolgus monkeys led to a profound, long lasting B cell depletion.

HuMax-CD20's superiority in inducing complement-mediated lysis of CD20-positive cells compared to rituximab therefore translates to a more efficient reduction of tumor growth *in vivo*.

[10.50-11.10]
Proteomic mapping and immunotargeting of endothelial caveolae for transcytosis and penetration into solid tumors and lungs
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The overwhelming molecular complexity of each tissue and the *in vivo* inaccessibility of most cells within a tissue greatly limit the abilities of global genomic and proteomic analysis to discover and

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validate key targets for directing tissue-specific de livery of many therapies and imaging agents *in vivo*. A novel hypothesis-driven systems biology approach is described that reduces data complexity to a small subset of proteins induced at the critical tissue-blood interface inherently accessible to antibodies injected intravenously. We use subcellular fractionation, monoclonal antibody generation, phage library screening, mass spectrometry, *in silico* subtraction, and bioinformatics to unmask, from >100,000 of tumor proteins, <50 proteins apparently induced in solid tumors at the endothelial cell surface and its caveolae. Expression profiling and γ-scintigraphic imaging with antibodies validates several of these proteins as specifically exposed *in vivo* to permit selective immunotargeting and imaging of solid tumors in 1 hour. Targeted radio-immunotherapy destroys various solid tumors to increase animal survival and induce complete remissions. These accessible targets are expressed on the blood vessels of not only multiple rodent tumor models but also human solid tumors including primary and metastatic lesions of breast, kidney, liver, prostate, lung, and brain. Many of our new targets were discovered to be concentrated endothelial caveolae which are specialized plasmalemmal invaginations that can transcytose their molecular cargo to theoretically provide a means for transporting imaging agents, drugs, and gene vectors across the normally restrictive endothelial cell barriers to reach underlying tissue cells, the usual desired targets of pharmacotherapies. Compared to antibodies to endothelial cell surface proteins not found in caveolae, antibodies targeting caveolae permit not only more rapid and specific targeting and imaging but also selective transendothelial transport in both normal tissues and solid tumors within minutes of intravenous injection. Live dynamic imaging using both fluorescence intravital microscopy and γ-scintigraphy (SPECT/CT) showed extensive penetration into and accumulation throughout solid tumors. This unexpected speed of vascular targeting and caveolae-mediated transcytosis *in vivo* further encourages utility in tissue-specific molecular imaging as well as drug and gene delivery *in vivo*. This new integrated, multi-step analytical strategy can map tissue- and disease-modulated expression of proteins at the endothelial cell surface and its caveolae to reveal promising novel intravenously accessible cancer targets useful for imaging and therapy.