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2nd PEGS Annual Symposium on Antibodies for Cancer Therapy

April 30–May 1, 2012, Boston, MA USA

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Abbreviations: CEA, carcinoembryonic antigen; CD, cluster of differentiation; CHO, Chinese hamster ovary; CTLA-4, cytotoxic T lymphocyte antigen 4; CXCR, C-X-C chemokine receptor type 4; DLL, delta-like ligand; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; Fab, antigen-binding fragment; HER, human epidermal growth factor receptor; HGF, hepatocyte growth factor; JAM-A, junctional adhesion molecule A; ICAM, intercellular adhesion molecule; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor-1 receptor; IL, interleukin; PSMA, prostate-specific membrane antigen; Trail-R, tumor necrosis factor-related apoptosis-inducing ligand receptor; TNF, tumor necrosis factor; TSN-1, transfer syntax notation 1; VEGF, vascular endothelial growth factor; VEGF-R, vascular endothelial growth factor receptor

The 2nd Annual Antibodies for Cancer Therapy symposium, organized again by Cambridge Healthtech Institute as part of the Protein Engineering Summit, was held in Boston, USA from April 30th to May 1st, 2012. Since the approval of the first cancer antibody therapeutic, rituximab, 15 y ago, 11 have been approved for cancer therapy, although one, gemtuzumab ozogamicin, was withdrawn from the market. The first day of the symposium started with a historical review of early work for lymphomas and leukemias and the evolution from murine to human antibodies. The symposium discussed the current status and future perspectives of therapeutic antibodies in the biology of immunoglobulin, emerging research on biosimilars and biobetters, and engineering bispecific antibodies and antibody-drug conjugates. The tumor penetration session was focused on the understanding of antibody therapy using ex vivo tumor spheroids and the development of novel agents targeting epithelial junctions in solid tumors. The second day of the symposium discussed the development of new generation recombinant immunotoxins with low immunogenicity, construction of chimeric antigen receptors, and the proof-of-concept of 'photoimmunotherapy'. The preclinical and clinical session presented antibodies targeting Notch signaling and chemokine receptors. Finally, the symposium discussed emerging technologies and platforms for therapeutic antibody discovery.

Day 1: April 30, 2012

The 2nd Annual Antibodies for Cancer Therapy symposium was opened by the Chairman, **Mitchell Ho** (National Cancer Institute). He welcomed the attendees and thanked the Cambridge Healthtech Institute, Inc. for organizing this symposium. He also thanked his fellow Scientific Advisory Board members, **Soldano Ferrone** (University of Pittsburgh) and **Horacio G. Nastri** (Pfizer, Inc.) for nominating and selecting this year's speakers. He dedicated the opening Keynote session to the celebration of the 15th anniversary of rituximab, the first approved cancer antibody drug, and then he introduced two keynote speakers on Day One, **Ivor Royston** (Forward Ventures) and **Alain Beck** (Pierre Fabre).

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Keynote Session

Ivor Royston gave the first keynote speech, "Monoclonal antibodies: the origins of cancer targeted therapy." He remarked that monoclonal antibody (mAb)-based therapeutics have a 30 y history. They are major components of the oncologist's armament in treating cancer and the forerunners of today's targeted therapy of cancer. His talk focused on the historic views of cancer antibody therapy by discussing the evolution and ups and downs of these "magic bullets."

Dr. Royston generated mAbs to T cell leukemia lines, but not the Epstein-Barr virus- transformed lymphoblastoid B cell lines from the same patient, when he was on the oncology faculty of the University of California at San Diego in 1977, two years after the Nobel Prize winning work was published in Nature by Kohler and Milstein.[1] In 1986, he co-founded IDEC Corporation. Rituximab (Rituxan®), which is a chimeric anti-CD20 mAb, was originally developed by IDEC under the name IDEC-C2B8.

With his post-doctoral fellow and later colleague Bob Dillman, Dr. Royston began pilot clinical studies in patients with T cell lymphoma and chronic lymphocytic leukemia (CLL) in the early 1980s.[2,3] One of their patients with cutaneous T cell

lymphoma (CTCL) showed a great response to the murine mAb, but after several cycles of treatment, the response was abrogated by the production of human anti-murine antibody (HAMA). At the same time, Drs. Ronald Levy and Richard Miller at Stanford were developing anti-idiotypic mAbs against CTCL and B cell lymphoma.[4] Drs. Lee Nadler, Jerome Ritz and Stuart Schlossman at the Dana-Farber Cancer Institute were running clinical trials on lymphoma and acute myeloid leukemia (AML).[5] Dr. Bismarck Lozzio at the University of Tennessee was also working on AML, and Dr. Stefan Thierfelder at the University of Munich in Germany was evaluating mAb therapy on acute lymphoblastic leukemia (ALL). Given that murine mAbs have immunogenicity, induce HAMA in humans, and have a short half life, it became clear that human mAbs were necessary for cancer therapy.

In 1983, Dr. Royston published a paper on the creation of a drug-resistant human lymphoblastoid B cell line (UC 729–6) that could be used to make human-human hybridomas, which secrete antibodies.[6] In this paper, Dr. Royston and colleagues described the human-human hybridomas derived from lymphocytes isolated from regional draining lymph nodes (LN) of cancer patients. UC 729–6 can be fused with human lymphocytes to generate stable human-human hybridomas, some of which secrete antibodies reactive to human cell surface antigens. Dr. Royston discussed how 30 y ago, the first cancer patient in the world was treated with a fully human mAb. Dr. Hideaki Hagawara, Dr. Royston's post-doctoral fellow from Japan, learned of a Japanese patient with advanced cervical cancer and asked if he could bring the patient's tumor draining LN to the lab to make hybridomas. With Dr. Royston's approval, Dr. Hagawara personally transported the LN to the lab from Japan. They made a number of hybridomas and found a few that made antibodies that reacted with cervical cancer cell lines, as well as the LN Dr. Hagawara brought from Japan. Dr. Hagawara abruptly returned to Japan with hybridoma cells. The Japanese patient was eventually treated with human mAbs derived from this human-human hybridoma, and the event was featured in 1983 on a "CBS evening news with Dan Rather" episode. Dr. Royston showed a video clip of the broadcast to the audience. The video also showed a CLL patient being treated with an early murine mAb; this patient had a good clinical response and no HAMA response. Dr. Royston and others also noted this effect later in lymphoma patients, and they quickly realized that B cell lymphoma patients or CLL patients only infrequently had an immune response to murine mAbs, which is why rituximab was so successful in B cell lymphomas. The human mAbs to the Japanese patient's tumor were later described in 1987.[7]

In 1981, Drs. Richard Miller and Ronald Levy were able to put a patient with follicular B cell lymphoma into complete remission for a number of years with a murine anti-idiotype antibody.[4,8] Such an antibody was truly tumor specific since it only reacted with the tumor B cell idiotype. In light of this, Dr. Ivor Royston, as a co-founder of IDEC, tried to commercialize the idea of producing a customized anti-idiotype mAb for lymphoma patients. However, the idea was not economically feasible, so the company turned its attention to a pan-B cell mAb and subsequently produced the chimeric anti-CD20 rituximab. In 1997, this product became the first antibody approved by the US Food and Drug Administration (FDA) to treat cancer. The differential expression of CD20 on lymphoma cells provides the rationale for targeted agents against the antigen. Additionally, CD20 is an ideal target for therapy because it is neither shed nor modulated. Rituximab is a genetically engineered antibody that consists of the variable light- and heavy-chain regions from the murine anti-CD20 antibody IDEC-C2B8 grafted on human immunoglobulin (Ig) G1 and κ constant regions.

Rituximab is indicated for the treatment of CD20+, relapsed or refractory, low-grade or follicular non-Hodgkin lymphoma (NHL), but the exact mechanism of CD20 mAb–induced antitumor or anti–B cell effect remains unknown. It is generally accepted that host immune effector mechanisms, which include antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CDC), are involved.[9,10] This notion has been supported by several observations. First, while rituximab (IgG1) effectively depleted CD20+ B cells, an equivalent IgG4 version of rituximab was unable to deplete B cells in vivo in primates.[11] This is consistent with the role played by complement or ADCC effector cells, given that they mediate cytotoxic effects best via the IgG1 constant region. Second, rituximab showed a significant decrease in its antitumor effect in FcγRIII receptor–deficient mice.[12] This argues that an Fc receptor–dependent mechanism (i.e., ADCC) contributes substantially to rituximab's antitumor effect. Third, in vitro studies showed that lymphoma cell lines and freshly isolated follicular lymphoma cells are targets for ADCC and CDC in the presence of rituximab.[9,10] In addition to immune-mediated mechanisms via ADCC and CDC, a recent study has shown that rituximab can induce apoptosis of some lymphoma cell lines via the caspase pathway.[13] This suggests a possible role of CD20-mediated signal transduction in rituximab's antitumor effect. It is likely that each of these mechanisms plays a role in the clinical activity of rituximab, although the relative contribution of each, and whether certain mechanisms are more important in selected histologies, is not yet clear.

Since the landmark approval of rituximab in 1997, there have been seven additional unconjugated mAbs, including trastuzumab (Herceptin®), bevacizumab (Avastin®), alemtuzumab (Campath®), cetuximab (Erbitux®), panitumumab (Vectibix®), ofatumumab (Arzerra®), and ipilimumab (Yervoy®), that have been approved by the FDA for cancer therapy. Four conjugated mAbs, ibritumomab tiuxetan (Zevalin®), tositumomab-I131 (Bexxar®), brentuximab vedotin (Adcetris®), and gemtuzumab ozogamicin (Mylotarg®), have also been approved by the FDA. Gemtuzumab ozogamicin, an IgG4 conjugated to the drug calicheamicin, has been removed from the market because further studies failed to show efficacy. The unconjugated antibodies are quite effective, have long half-lives in the blood stream, and can interact with human complement and effector cells in the patients's immune system. The two radiolabeled anti-CD20 mAbs (ibritumomab tiuxetan and tositumomab-I131) are murine, which expedites clearance from the circulation. The latest approval was Seattle Genetics' brentuximab vedotin (SGN-35) for refractory Hodgkin disease and anaplastic large

cell lymphoma (ALCL). It is an IgG1 conjugated to auristatin, a microtubule inhibitor. Overall, there are 11 approved mAbs (plus one approval withdrawn) for cancer therapy.

Dr. Royston concluded by stating that modern recombinant techniques have made it possible to rapidly produce chimeric, humanized and totally human antibodies. These mAbs are changing the face of cancer therapy and have ushered in the era of targeted therapy. There are now more than 300 antibody drugs being evaluated in clinical trials. Along with the targeted small molecule drugs that can act on mutated or aberrant gene products, the anti-cancer mAbs are resulting in more remissions and better outcomes with far less toxicity.

As the second keynote speaker, **Alain Beck** focused on the strategies and challenges for the next generation of therapeutic antibodies.[14] In the first part of his talk, Dr. Beck discussed selection of targets for mAb therapy (clinical relevance vs. biological functionality), optimization of mAb structures (pharmaceutical properties, functions, antibody-drug conjugation or antibody-drug conjugates (ADC), bispecifics, oligoclonals, and different scaffolds), and emerging research on biosimilars and biobetters. He reviewed the regulatory approvals of antibodies and related structures and selection technologies (chimerization, humanization, phage display and transgenic mice). Among 165 anti-cancer antibodies currently in clinical study, 84 (51%) are unmodified IgG, 25 (15%) are ADC, 10 (6%) are bispecific antibodies, 17 (10%) are engineered, and 16 (10%) are fragments.[15] Dr. Beck discussed how to select targets based on competition, safety, proof of concept and intellectual property. He also analyzed targets in three categories: (a) clinically validated targets (e.g., CD20, HER2, EGFR, VEGFA, EpCAM, and CTLA-4), (b) pre-validated targets (e.g., IGF-1R, IGF1/2, HGF, c-Met, Her3, VEGF/VEGF-R, Trail-R, IL6/IL6R, IL4/IL13, CD19, CD22, CD30, CD33, CD44, CD80 min CXCR4, and ICAM-1), and (c) new functional targets (e.g., RAAG12, CD9, JAM-A, CD151, TSN-1). Dr. Beck first reviewed the crystal structures of HER2/EGFR mAbs and explained how functional mAbs against HER2 or EGFR can be achieved by targeting different sub-domains on the receptors. For example, functional mAbs to the HER2 extracellular domain target three different sub-domains (I, II, IV). Functional mAbs to EGFR target sub-domain (III) except for mAb 806, while small tyrosine kinase inhibitors are directed at enzyme catalytic intracellular domains. Cetuximab Fab interacts exclusively with sub-domain III of soluble EGFR. It partially occludes the ligand binding region on this domain and sterically prevents the receptor from adopting the extended conformation required for dimerization.[16] These effects contribute to inhibition of EGFR activation by cetuximab. Necitumumab (IMC-11F8) recognizes an epitope in sub-domain III like cetuximab.[17] Pertuzumab binds with the HER2 sub-domain II overlapping with the heterodimer interface.[18] Interestingly, when used as first-line treatment for HER2-positive metastatic breast cancer in a study with 808 randomly assigned patients, the combination of pertuzumab, trastuzumab, and docetaxel (pertuzumab group) significantly prolonged progression-free survival with no increase in cardiac toxic effects, compared with the placebo, trastuzumab, and docetaxel (control group).[19] The median progression-free survival was 12.4 mo in the control group, as compared with 18.5 mo in the pertuzumab group. Dr. Beck also briefly described the ongoing development of next generation mAbs targeting IGF1-R, HGF/SF-Met, CXCR4 and a novel human junctional adhesion molecule A (hJAM-A).

Dr. Beck then discussed the optimization of IgG structure for antibody therapy with an emphasis on physicochemical and functional analysis of glycosylation.[14,20] He compared main glycoforms found in human and recombinant Fc produced in CHO, NS0, and PERC.6 mammalian cell lines, analyzed immunogenicity and half-life related to glycoforms produced in NS0, SP2/0, and plants, and discussed glyco-engineering to increase cytotoxicity or anti-inflammatory properties of mAbs. For example, he gave one example showing that genetically removing the C-terminal lysine residues in the constant region of IgG yielded more homogeneous IgG molecules. He also compared IgG1 and IgG4, and he showed that the single amino acid mutation of the IgG hinge region (S225P) could avoid half antibody of IgG4 in vivo.

Dr. Beck then discussed the current status of ADC development.[21] He started with two ADC drugs, inotuzumab ozogamicin (Pfizer/Wyeth), a calicheamycin conjugated antibody targeting CD22 being evaluated in NHL patients and trastuzumab emtansine (Genentech), a maytansinoid (DM) 1 conjugated antibody targeting HER2 for breast cancer. Both ADC drugs are currently being evaluated in Phase 3 clinical trials. Brentuximab vedotin (Seattle Genetics), consisting of bretuximab and auristatin E (4 cytotoxic drugs per IgG molecule), was developed to treat relapse CD30-positive lymphomas. In a Phase 2 trial for Hodgkin Lymphoma, 94% (96 of 102) of patients achieved tumor reduction.[22] Trastuzumab-DM1 contains ~3.5 cytotoxic drugs per IgG molecule. It combines both biologic activity by blocking downstream HER2 signaling to inhibit proliferation of cells and intracellular delivery of the DM1 cytotoxic agent.[23,24] Tratuzumab-DM1 is currently being evaluated in Phase 3 clinical trials for metastatic breast cancer.

The reactive thiol in cysteine is used for coupling maleimide linkers in the generation of ADCs. To make 2nd generation "ThiomAbs," efforts have been made to engineer site-specific thio-trastuzumab variants for coupling to thiol-reactive linkers without perturbing antibody structure and function.[25] Based on antibody structural modeling, cysteines were engineered into the IgG molecule at three sites differing in solvent accessibility and local charge. The higher stability and superior in vivo efficacy of the LC-V205C variant conjugate may be due to faster maleimide ring hydrolysis, which prevents drug loss through the maleimide exchange from antibody to thiol-reactive constituents in the plasma.

In the final part of his talk, Dr. Beck discussed biosimilars, biobetters (or biosuperiors), and next-generation antibody derivatives. He started with the definitions of biosimilars and biobetters because there is confusion in the field. He defined biosimilars as copies of an approved mAb with the same amino acid sequence. Biosimilar antibodies can be produced from a different clone, and the manufacturing process can result in differences in glycosylation and other microvariations. Biobetters are mAbs with

a very close amino acid sequence, but optimized glycosylation profile (e.g., low fucose to enhance ADCC, less immunogenic). Biobetters may have engineered Fc domains to increase the serum half-life (usually two or three amino acid mutations). Next-generation antibodies are follow-up mAbs with different amino acid sequences and improved variable domains (e.g., better affinity) or improved Fc domains (glyco- or amino acid-engineered to increase effector functions or half-life). The first review in the European Union of the marketing application of a biosimilar mAb is in progress, and a draft of regulatory guidance is in the works.

Dr. Beck then noted that there is an urgent need to analyze the structures of biosimilar and biobetter antibodies qualitatively and quantitatively. He and colleagues recently used a mass spectrometry method to characterize structures of trastuzumab and cetuximab.[26] Cetuximab produced in SP2/0 (a murine myeloma cell line) contains > 30% of mouse glycoforms such as Gal-α-1,3 gal. The Gal-α-1,3 gal epitope in cetuximab induces anaphylaxis in patients and their IgEs were specific to the Gal-α-1,3 gal epitope. However, IgEs are not able to bind other chimeric mAbs (rituximab and infliximab) produced in CHO cells. A recent Phase 1 study of anti-EGFR mAb in patients with solid tumors was completed in China.[27] A recombinant anti-EGFR mAb (CMAB009) that has the same amino acid sequence as cetuximab was made but produced in CHO cells. Therefore, unlike cetuximab, the new mAb does not have the Gal-α-1,3 gal epitope.

Dr. Beck ended his talk by discussing the challenges to be faced in the next 10 y, such as identification and validation of new targets, addressing the resistance to current drug treatments, understanding target cross talk and regulation. In the meantime, efforts must be made to decrease the costs of industrial production by increasing the productivity of the current cell lines, developing alternative production systems and purification processes, and optimizing the design of more homogeneous and stable IgGs. Dr. Beck also pointed out that the availability of regulatory pathways to register biosimilar antibodies (European Medicines Agency, FDA) might provide further motivation to achieve these goals.

In the next section of the symposium, a featured panel presentation chaired by Mitchell Ho, Ivor Royston and Alain Beck discussed the future of mAbs for cancer therapy. The panel focused on three topics: (a) strategies to identify or select good targets (clinical relevance vs. biological effects), (b) strategies to develop good antibodies (monoclonal, polyclonal, bispecific, alternative scaffolds) and (c) biosimilar antibodies and emerging markets (e.g., China, India). Dr. Ho reiterated Dr. Beck's points about how some targets are clinically relevant (for example, overexpression of the target in a large percentage of patients with the same tumor type) and how some have biological effects (for example, inhibition of cell proliferation or migration, induction of apoptosis). Dr. Ho then asked Drs. Royston and Beck how the selection of therapeutic targets in cancers has evolved since the 1970s and whether major advances in genomics or proteomics may play a role in target identification. The panel believes that functional genomics has already played a role in antibody therapy and will play a more important role in target identification in the future. For example, tumor specific mutations have already provided opportunities for novel cancer therapies. On the other hand, quick analysis of the clinical relevance of novel functional targets remains challenging. The panel then discussed strategies to develop better antibodies (monoclonal, polyclonal, bispecific, alternative scaffolds). Dr. Royston emphasized the engagement of effector cells. Dr. Beck emphasized the target (such as IGF and EGF) crosstalk. In conclusion, the panel discussed drug resistance and how the current trend in bispecific or multifunctional antibodies could potentially work better than single antibodies in cancers that are more resistant to single agents.

Tumor Penetration

In the session focused on tumor penetration. **Mitchell Ho** (NCI) started by discussing the discovery of human mAbs targeting mesothelin in mesothelioma, ovarian cancer, cholangiocarcinoma, and other cancers. Dr. Ho described the new human mAb HN1 specific to cell surface mesothelin in various tumor types and showed that the new antibody and recombinant immunotoxin based on the HN1 Fv exhibited potent anti-tumor activity.[28] He then discussed how tumor microenvironments may present significant barriers to anti-tumor agents including mAbs. Molecules involved in multicellular tumor microenvironments are difficult to study ex vivo. In collaboration with V. Courtney Broaddus (University of California, San Francisco) and Shuichi Takayama (University of Michigan), Dr. Ho uses in vitro tumor spheroids in the field of antibody therapy.[29-31] He generated a tumor spheroid model using the NCI-H226 mesothelioma cell line and patient cells.[31] He recently compared the gene expression profiles of spheroids and monolayers using microarray analysis. Microarray analysis revealed that 142 probe sets were differentially expressed between tumor spheroids and monolayers.[32] Gene ontology analysis revealed that upregulated genes were primarily related to immune response, wound response, lymphocyte stimulation and response to cytokine stimulation, whereas downregulated genes were primarily associated with apoptosis. These genes, including MMP2, BAFF/BLyS/TNFSF13B, RANTES/CCL5 and TNFAIP6/TSG-6, which are specific to the three-dimensional biological structure of tumors, may also have potential as targets for cancer therapy and diagnostics. Both tumor penetration and acquired apoptotic resistance cause the major barriers for antibodies, including antibody-drug/toxin conjugates in solid tumors. Dr. Ho identified a key cell contact protein, E-Cadherin, related to tight junctions in tumor penetration using mesothelioma spheroids. He showed that the downregulation of E-Cadherin by siRNA and an E-Cadherin-specific functional blocking antibody could sensitize cancer cells to anti-mesothelin immunotoxin killing.[31]

Andre Lieber (University of Washington) discussed junction opener 1 (JO-1), which is a small, recombinant adenovirus serotype 3-derived protein specific for the epithelial junction protein desmoglein 2 (DSG2).[33] In mouse xenograft models employing HER2/neu- and EGFR-positive human cancer cell lines, JO-1 mediated cleavage of DSG2 dimers and reduced E-Cadherin expression in tight junctions. He found that JO-1-triggered

changes that allowed for increased intratumoral penetration of the anti-HER2/neu mAb trastuzumab and increased therapeutic efficacy of trastuzumab in mouse xenograft models using breast, gastric, and ovarian cancer cells that were HER2/neu-positive. His group also combined JO-1 with the EGFR-targeting mAb cetuximab and found improved therapeutic outcomes in a metastatic model of EGFR-positive lung cancer.

Novel Formats against Cancer: Antibody-Drug Conjugates and Bispecifics

The afternoon session on Day One entitled "Novel formats against cancer: antibody-drug conjugates and bispecifics" was chaired by **Horacio G. Nastri** (Pifzer).

Syd Johnson (MacroGenics) gave an update of bispecific antibodies. Dr. Johnson described the pre-clinical development of several DART proteins that recruited effector cells to tumor targets.

Berengere Vire (National Heart, Lung, and Blood Institute) discussed the development of a novel antibody-drug conjugate (ADC) derived from the IgM-Fc portion for CLL therapy. She noted that the Fc receptor for IgM (also known as FcμR) was recently identified,[34] and that Adrian Wiestner's group at the NIH showed that FcμR was overexpressed in CLL cells. Aggregation of FcμR on CLL cells by IgM prompted rapid internalization of both IgM and FcμR, reaching half-maximal internalization of cell-bound IgM within one minute.[35] Upon internalization, FcμR transported IgM through the endocytic pathway to the lysosome, where it was degraded, indicating a potential pathway for the delivery of therapeutic mAb-drug conjugates into CLL cells. In collaboration with Christoph Rader (National Cancer Institute), Dr. Vire generated an ADC derived from the Fc portion of IgM to selectively target CLL cells using a selenocysteine-based site specific conjugation with cemadotin and monomethylauristatin F.[36]

Jin-San Yoo (PharmAbcine) started with tanibirumab (TTAC-0001), a human mAb specific for both human and murine VEGFR-2. VEGF binds to its receptor (VEGFR) and stimulates angiogenesis, an important step in tumor growth and metastasis. They found that tanibirumab inhibited the binding of VEGF to VEGFR-2 (also known as KDR) and, therefore, inhibited angiogenesis. They compared tanibirumab with bevacizumab in a human malignant glioblastoma (U-87 MG GBM) orthotopic model, as well as lung cancer, breast cancer, colon cancer, and liver cancer models in mice and showed that tanibirumab had better efficacy in all of the models. Dr. Yoo then discussed a bispecific antibody format called "DIG-body" and a specific example: DIG-KT, a DIG-body inhibiting the binding of VEGF and angiopoietin to their receptors VEGFR-2 and Tie-2. DIG-KT can inhibit both VEGF- and angiopoietin- mediated cancer cell migration in vitro. DIG-KT has better efficacy than tanibirumab in U87-MG GBM and Hep3B (hepatocellular carcinoma) orthotopic models. DIG-KT also inhibits the growth of pancreatic tumors and murine colon tumors. Finally, Dr. Yoo discussed PIG-KM, a bispecific antibody for KDR (VEGFR-2) and cMet and showed that PIG-KM had better efficacy than tanibirumab in a U-87 MG GBM orthotopic model.

Christoph Spiess (Genentech) presented a knobs-into-holes technology to generate bispecific antibodies with non-common light chains.[37] The solution eliminates the need of linkers to prevent light chain mispairing. Each heavy chain is expressed with its corresponding light chain in separate *Escherichia coli* cultures. Individual half-antibodies are purified and combined. Finally, the bispecific antibody is purified by conventional means.

The first day ended with four concurrent problem-solving breakout discussions. The first forum entitled "Effective Penetration of Tumor Targets" was moderated by **Mitchell Ho** (NCI). It focused on: (a) penetration of solid tumors and the blood-brain barrier: challenges and opportunities, (b) role of cell junction proteins in tumor microenvironments and the identification of novel targets, and (c) 3D tumor culture technologies and applications. The second forum entitled "Clinical Potential of Immunotherapy against Advanced Cancers" was moderated by **Richard A. Morgan** (NCI). It discussed immunotherapy categories (antibody-based therapy, cell-based therapy, vaccines/gene therapy, what cancers to target and clinical trial design/endpoints). The third forum entitled "Analyzing Trends for Success of mAbs" chaired by **Alain Beck** (Pierre Fabre) discussed (a) target selection and validation, (b) antibody structure optimization, (c) alternative formats, (d) synergistic mechanisms of action, (e) biomarker identification and patient selection, (g) biosimilar and biobetter mAbs. The fourth forum entitled "Anticalins: Diagnostic and Therapeutic Applications" was moderated by **Laurent Audoly** (Pieris Ag).

Day 2: May 1, 2012, Opening Remarks

The second day symposium was chaired by **Soldano Ferrone** (University of Pittsburgh), who reviewed the origin of hybridomas by Kohler and Milstein and the substantial challenges that faced the field of therapeutic antibodies in the 1990s. Dr. Ferrone suggested that a lesson from that time is that it is critical to discuss important problems in the field so that solutions can be found.

Immunotherapies in the Fight against Cancer

Ira H. Pastan (NCI) gave a keynote presentation entitled "Immunotoxin with low immunogenicity for cancer treatment." Recombinant immunotoxins are hybrid proteins containing an Fv that reacts with a cancer cell and a bacterial or plant toxin that can induce antibody responses and limit the number of treatment cycles.[38] Dr. Pastan and colleagues have developed approaches to identify human B cell and T cell epitopes and produced active immunotoxins in which both types of epitopes have been removed.[39]

Examples of the recombinant immunotoxins currently being developed include HA22 (CAT-8015; moxetumomab pasudotox), which targets CD22, and SS1P, which targets mesothelin. Each of these molecules contains PE38, a truncated form of Pseudomonas exotoxin A (PE) containing amino acids 253–364 and 381–613. CD22 is a cell surface protein only expressed on B cells and B cell malignancies. It is not present on stem cells;

thus normal B cells can be regenerated after treatment stops. Phase 1 studies of moxetumomab pasudotox in patients with hairy cell leukemia (HCL) are completed.[40] Among the patients who failed standard chemotherapies, the overall response rate for moxetumomab pasudotox was 86%, and 46% achieved complete remission. Therefore, moxetumomab pasudotox at doses up to 50 μg/kg every other day (QOD) X 3 has activity in relapsed/refractory HCL and has a safety profile that supports further clinical development for treatment of this disease. Mesothelin is a cell surface glycoprotein overexpressed in mesothelioma, ovarian cancer, pancreatic cancer, lung cancer and many other cancers.[41] Phase 1 clinical studies of SS1P in patients with advanced mesothelin-positive cancers are completed.[42] SS1P, given as an intravenous infusion QOD for six or three doses, was administered to 34 patients with advanced mesothelioma (n = 20), ovarian (n = 12), and pancreatic (n = 2) cancer. Seventeen patients were treated on the QOD x 3 schedule, and the maximum tolerated dose (MTD) was 45 microg/kg/dose. Four patients had minor responses. Recent studies indicate that chemotherapy plus SS1P are well tolerated. However, immunogenicity, i.e., the formation of neutralizing antibodies, prevents retreatment and better responses in patients.

BL22, an early version of anti-CD22 immunotoxin, has been reported to induce complete remissions in 47–61% of patients with chemoresistant hairy cell leukemia.[43,44] Unfortunately, in the Phase 2 trial of BL22, four (11%) patients produced levels of neutralizing antibodies sufficiently high to prevent retreatment, and others made lower levels of antibodies. In solid tumors, immunogenicity is much higher; nearly all patients treated with SS1P produced significant levels of neutralizing antibodies.[42,45] Therefore, it is critical to reduce the immunogenicity of immunotoxins for cancer therapy.

To reduce immunogenicity, Dr. Pastan and colleagues identified B and T cell epitopes on Pseudomonas exotoxin and removed them while retaining toxin activity. To determine B cell epitopes on PE38, Dr. Pastan and colleagues hypothesized that mice and humans may have similar B cell epitopes. Therefore, initially they made 60 murine mAbs against PE38 and identified seven major epitope groups. The locations of many of these epitopes were validated by mutating large surface-exposed residues to alanine.[46] A mutant of moxetumomab pasudotox containing eight epitope-eliminating mutations (HA22–8X) was prepared, and this greatly reduced immunogenicity in mice.[47] In parallel, two large sections of PE38 containing lysosomal protease cleavage sites were removed, leaving only amino acids 274–284 and 394–613 of the toxin.[48] The resulting molecule (named HA22-LR) with the deletion of domain II of Pseudomonas exotoxin, retained cytotoxicity toward CD22+ cell lines, killed primary CLL cells more potently than moxetumomab pasudotox, was much less toxic to mice, and had significantly improved antitumor activity toward murine xenografts. They also made a new molecule called HA22-LR-8M to destroy the epitopes on domain III of Pseudomonas exotoxin by mutating eight bulky amino acids to alanine, glycine or serine.[49]

HA22-LR-8M is fully cytotoxic against malignant B-cell lines, has high cytotoxic activity against cells directly isolated from patients with CLL, and has strong antitumor activity in mice. The immunotoxin does not induce antibody formation in mice when given repeatedly by intravenous injection, and it does not induce a secondary antibody response when given to mice previously exposed to HA22. HA22-LR-8M also has greatly reduced antigenicity when exposed to sera from patients who have produced antibodies to HA22. The properties of HA22-LR-8M make it an excellent candidate for further clinical development.

In addition to mouse B cell epitopes, Dr. Pastan and colleagues have also investigated human B cell epitopes on Pseudomonas exotoxin. They recently generated several phage display libraries from the B cells in the patients treated with immunotoxins and identified major human B cell epitopes in order to remove them in immunotoxins. In the last part of his talk, Dr. Pastan discussed their ongoing research in T cell epitope mapping of Pseudomonas exotoxin and mutants that induced less T cell activity.

Richard A. Morgan (NCI) discussed the development of a chimeric antigen receptor (CAR), a hybrid protein composed of the antigen recognition domain from a Fv fused to an intracellular T cell activation domain(s). Gross et al. first reported the generation of CARs as functional receptors with antibody-type specificity.[50] Since then, three generations of CARs have been developed by adding additional T cell signaling domains.[51,52] Dr. Morgan described different strategies to make CAR gene transfer vectors. Several clinical trials using CAR-transduced T cells have been reported.[52] The molecules targeted antigens such as CD19 (B cell lymphoma and leukemia), CD20 (mantle cell lymphoma and relapsed/refractory B-cell lymphoma/leukemia), GD_2 (neuroblastoma), CEA (adenocarcinoma, stomach carcinoma, breast cancer, colorectal carcinoma), PSMA (prostate cancer), HER2/neu (lung malignancy, metastatic cancer, advanced osteosarcoma) and IL-13R ‹2 (glioblastoma). Dr. Morgan discussed results obtained from ongoing anti-CD19 CAR gene therapy protocol to treat follicular lymphoma, CLL, splenic marginal zone lymphoma, and large B cell lymphoma.[53] The protocol consisted of chemotherapy followed by an infusion of anti-CD19-CAR-transduced T cells and a course of IL-2 (clinicaltrials.gov, NCT00924326). Six of eight patients treated on their protocol obtained remissions of their advanced, progressive B cell malignancies. Four out of eight patients treated on the protocol had long-term depletion of normal polyclonal CD19(+) B-lineage cells. He believes that anti-CD19-CAR-transduced T cells have great promise to improve the treatment of B cell malignancies because of a potent ability to eliminate CD19(+) cells in patients.

Dr. Morgan then discussed the development of CARs to treat other cancers. An anti-EGFR variant III (EGFRvIII) CAR vector based on human mAb 139 is specific for EGFRvIII and has no reactivity against wild-type EGFR. EGFRvIII-CAR transduced glioblastoma multiforme (GBM) patient T cells recognize EGFRvIII-expressing targets, including glioma stem cell lines. A Phase 1/2 study led by Steven Rosenberg (NCI) of the safety and feasibility of administering T cells expressing anti-EGFRvIII CAR to patients with malignant gliomas expressing EGFRvIII is currently ongoing (clinicaltrials.gov, NCT01454596). Dr. Morgan also discussed the development by his NCI colleagues

of anti-mesothelin CAR targeting pancreatic cancer. In his collaboration with Ira Pastan, Steven Rosenberg is leading a Phase 1/2 study of metastatic cancer using anti-mesothelin CAR engineered peripheral blood lymphocytes in patients (clinicaltrials.gov, NCT01583686).

Hisataka Kobayashi (NCI) described a new antibody conjugate with a photosensitive near infrared (NIR) phthalocyanine dye, IR700, which can be used as an optical imaging agent at low doses of light, but becomes a photoimmunotherapeutic (PIT) at higher doses of light.[54]

Dr. Kobayashi previously developed numbers of targeted 'activatable' fluorescent imaging probes using rational chemical designs.[55] These agents are activated after cellular internalization by sensing the pH change in the lysosome or catabolizing by acidic enzymes using clinically feasible materials.[56,57] Among them, a novel acidic pH-activatable probe based on the boron-dipyrromethene fluorophore was synthesized. It was then conjugated to a cancer-targeting mAb and tested ex vivo and in vivo imaging of human EGFR type 2-positive lung cancer cells in mice. The probe was highly specific for tumors with minimal background signal.

In a recent study, Dr. Kobayashi used IR700 conjugated to mAbs targeting EGFRs. Massive necrotic cell death was induced immediately after irradiating mAb-IR700-bound target cells with NIR light. The mAb-IR700 conjugates were most effective when bound to the cell membrane, and they produced no phototoxicity when not bound.

Pre-Clinical and Clinical Data

Timothy Hoey (OncoMed) discussed antibodies targeting DLL4 signaling. Cancer stem cells (CSCs; also known as tumor initiating cells) were first demonstrated in leukemia.[58] Clarke et al. first identified CSCs in solid tumors.[59] The Notch pathway mediates intercellular signaling in stem cell self-renewal, proliferation, and differentiation; the ligand DLL4 for the Notch receptor is required for embryonic vascular development, and it is upregulated in tumor angiogenesis. Dr. Hoey and colleagues generated demcizumab, which has subnanomolar affinity for human DLL4 but not murine DLL4, and found that the antibody was a potent and complete blocker of the interaction of DLL4 and its Notch receptor.

Treatment with an anti-human DLL4 mAb inhibited the expression of Notch target genes and reduced proliferation of tumor cells. They found that specifically inhibiting human DLL4 in the tumor, either alone or in combination with the chemotherapeutic agent irinotecan, reduced CSC frequency.[60] The antibody also reduced expression of CSC markers in colon tumors. They also found that an anti-murine DLL4 antibody reduces tumor growth by de-regulating angiogenesis. Furthermore, they showed that anti-DLL4, not anti-EGFR, is active in K-Ras mutant colon tumors in mice.[61] Anti-DLL4 as a single agent inhibits pancreatic tumor growth in mice. The combination of anti-DLL4 and gemcitabine exhibits more potent tumor growth inhibition. DLL4 is active in gemcitabine-resistant pancreatic tumors by reducing CSC frequency. They believe that mechanisms of action for anti-DLL4 antibody are based on: (a) reduction of CSC frequency and (b) disruption of tumor angiogenesis.[62] A Phase 1 clinical study using anti-DLL4 antibody is ongoing. Finally, Dr. Hoey summarized the antibodies currently being developed at OncoMed that target: (1) Notch Pathway [DLL4 (demcizumab, Phase 1 as a single agent and combination therapy), DLL4/VEGF (bispecific), Notch2/3 (Phase 1), Notch 1]; (2) Wnt pathway [Fzd7 (Phase 1), Fzd8Fc, SMOLs]; and (3) RSPO-LGR pathway.

Laurent Audoly (Pieris Ag) gave the presentation entitled "Path to Clinical Development and Completion of a Phase I Clinical Trial for a New Class of Therapeutic Protein—Anticalins." Dr. Audoly described the safety and efficacy of Anticalins in humans, the identification and characterization of a highly specific and purely antagonistic cMet-targeting Anticalin, and the manufacturing of bispecific Anticalins. He described PRS-050, an anti-VEGF PEGylated Anticalin. The results of the Phase 1 trial demonstrated PRS-050 was well-tolerated, and no MTD was reached.[63] The data supported the advancement of PRS-050 into Phase 2 studies in patients with solid tumors.

Wayne A. Marasco (Harvard Medical School) gave the presentation entitled "Human Anti-CCR4 mAb Immunotherapy for the Treatment of Cutaneous T Cell Lymphoma." CTCL is a heterogeneous group of neoplastic disorders characterized by clonally derived and skin-homing malignant T cells. These cells express a high level of chemokine receptor CCR4 that is associated with their skin-homing capacity. CCR4 is also expressed as high levels on regulatory T cells (Tregs). Dr. Marasco described a humanized anti-CCR4 mAb and showed its potent anti-tumor effects in a mouse CTCL tumor model. In vitro studies were used to elucidate the mechanism(s) of tumor cell killing. The anti-CCR4 mAb was also able to reverse Treg-induced suppression on effector T cell (Teff) proliferation. This latter property may allow this anti-CCR4 mAb to be used to augment natural anti-cancer immunity against other solid tumors and hematologic malignancies where a contribution of Treg to immune evasion by the tumor cells has been established.

Emerging Companies and Trends in Oncology

David M. Hilbert (Zyngenia) discussed a new format of multi-specific, multi-valent mAbs, called Zybodies. This technology recombinantly fuses short target-binding peptides to the N- or C-termini of the Ig heavy and light chains. The resulting proteins can be bi-, tri-, tetra, or penta-specific, with each specificity represented bivalently. He discussed the production, stability, binding attributes, and biological function of several lead therapeutic candidates for the treatment of breast cancer. Zyngenia made a trastuzumab IgG-based penta-specific Zybody simultaneously bound to five targets (Ang2, $\alpha v\beta 3$, HER2, IGF-1R, and EGFR) and showed that the Zybody was a more potent inhibitor of cell proliferation than trastuzumab alone. Dr. Hillbert showed that a bi-specific Zybody (HER-ang2) targeting both HER2 and Ang2 showed more efficacy than trastuzumab alone in a SK-OV-3 xenograft model. They also developed an adalimumab-based bispecific antibody called HUM-ang2, which targets TNF-α and Ang2.[64] Interestingly, treatment with the bi-specific HUM-ang2

Zybody inhibits both NF-κB and Akt pathways. Treatment with adalimumab inhibited only the NF-κB pathway, and treatment with a control Ang2 antibody inhibited only the Akt pathway. They found that HUM-ang2 is more potent than adalimumab in an hTNF-α transgenic mouse model of autoimmune polyarthritis and colitis.

Hans de Haard (arGEN-X) gave the presentation entitled "New Insights into Modulation of c-Met Function in Cancer through a Novel Human Antibody Platform." Dr. Haard discussed arGEN-X's SIMPLE Antibody™ platform with a focus on the c-Met antibody program.

Marc A. van Dijk (4-Antibody AG) discussed a B cell based antibody display technology called Retrocyte Display. This technology allows the stable expression of full-length human IgG antibodies on the cell surface of B lymphocytes with monoclonal diversity > 1 x 10^9 for flow cytometry sorting of antigen-binding cells. Using the library, they found human antibodies for a cell surface protein with affinity less than 2 nM and cross-reactivity with non-human primate and rodent homologs. They can also screen multiple targets (e.g., TNF, EGFR) simultaneously by flow cytometry.

Note

Summaries were prepared from PDFs of the presentations provided by speakers after the meeting. In cases where a speaker was not able to share his or her presentation, detailed summaries are not included, although the speaker's name, affiliation, topic and abstract appear in the report.

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