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The new ParaDIgm: IgM from bench to clinic

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The inaugural IgM event entitled "The new ParaDIgm: IgM from bench to clinic" brought together the increasingly active and growing IgM antibody community to discuss recent advances and challenges facing the discovery and development of IgM antibody therapies and technologies. Researchers, clinicians and biomanufacturing experts delivered 21 talks on the basic science and isolation of IgM, upstream and downstream development, and formulation and clinical development of the molecules. Participants networked around topics aimed at exploring the full potential of IgM antibodies. The meeting was held at DECHEMA Gesellschaft für Chemische Technik und Biotechnologie e.V. (Society for Chemical Engineering and Biotechnology), a non-profit scientific and technical society based in Frankfurt am Main, Germany. The meeting was sponsored by Patrys, Laureate Biopharma, Bio-Rad Laboratories, BIA Separations, Percivia and the Bio Affinity Company (BAC). The second New ParaDIgm: IgM from bench to clinic meeting, will be held on April 23–24, 2013 in Frankfurt, Germany.

Day 1: November 15, 2011

The first day of the meeting opened with two presentations on the basic science of IgM and isolation of the IgM antibody molecules, and then focused on upstream/downstream development. **Goeff Howlett** (University of Melbourne) discussed the characterization of IgM-antigen interactions using fluorescence detection in the analytical ultracentrifuge. The focus of Dr. Howlett's work, conducted in collaboration with Patrys Ltd, was the characterization PAT-SM6. This IgM antibody has been shown to effectively induce the death of tumor cells using "lipoptosis," which occurs when intracellular lipids accumulate to toxic levels within diseased cells when PAT-SM6 binds to a glycosylated form of GRP78, the target of PAT-SM6. PAT-SM6 also binds to oxidized low-density lipoprotein, which suggests that the antibody can transport this lipid in excess amounts into tumors.

Work on the generation of different IgM glycoforms in plants was discussed by **Dr. Andreas Loos** (University of Natural Resources and Life Sciences), who began his talk by positing that plants are still seen as a somewhat "exotic" expression system, although plants have been shown to express complex molecules with post-translational modifications similar to those expressed in mammalian cells. Dr. Loos in particularly highlighted the N-glycosylation patterns, which are an essential prerequisite for therapeutic efficacy. He noted the general benefits of plant expression systems, emphasizing the economic benefit and the fact that plants can express these complex molecules in a homogenous way, and mentioned also that some vaccines are currently being produced in plants in commercial settings.

Dr. Loos then described the capabilities of the MagnICON® Plant expression system, which is based on an organism, Nicotiana Benthamiana, capable of expressing an acceptable amount of multimerized IgM antibody after a 3–4 d period.[1] After briefly showing several examples of expression and purification of antibodies in plants, Dr. Loos gave a thorough overview of the glycosylation pathway in plant and mammalian cells, noting that mannose and GlcNAc structures are conserved in both plant and mammalian pathways. Dr. Loos contrasted and compared the quality of expressed antibody in terms of glycoforms, between plant-derived IgM antibodies and human serum IgM, highlighting the areas of similarity in glycosylation patterns using data from several liquid chromatography-mass spectrometry (LC-MS) experiments to support his claims, while pointing out areas of difference between expression systems, specifically sialylation and fucosylation patterns. He concluded his talk by restating that plant expression systems are becoming viable and attractive alternatives to mammalian systems for the production of biotherapeutics,[2] although more work is needed to produce sialylated structures with bisected GlcNAc and the correct α1,6-fucosylation pattern.

Dr. Christoph Binder began his presentation with a brief overview of the current theory of the pathogenesis of atherosclerosis, describing it as a chronic inflammatory condition of the inner vascular walls, in which oxidized LDL cholesterol (OxLDL) and apoptotic cells accumulate thus triggering inflammation and propagating the disease process.[3] Dr. Binder then went on to describe oxidation-specific epitopes on OxLDL and apoptotic cells as prominent target of natural antibodies (mostly IgM antibodies). One example includes phosphocholine (PC) of oxidized phospholipids, which is present in OxLDL and on the surface of apoptotic cells. PC is recognized by the prototypic germline encoded natural IgM T15/EO6. He cited findings from his team that have shown that T15/EO6 IgM protect mice from atherosclerosis. Dr. Binder further referenced research that demonstrated an inverse correlation between the concentration

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of IgM antibodies to OxLDL and incidence of cardiovascular disease in humans; such is not the case for IgG where there seems to be direct correlation. Dr. Binder suggested that these findings support the idea that IgM antibodies play an atheroprotective role in humans. He then raised the question of whether the T15/EO6-PC interaction was an exception or one of many examples of IgM produced by the same cell type that target related oxidation-specific epitopes. He went on to discuss research that has shown that other IgM antibodies do in fact exist that recognize various oxidation-specific epitopes; with approximately 30% of all natural IgM antibodies demonstrating specificity for such epitopes in mice and humans.[4] Dr. Binder described evidence that oxidation-specific epitopes can be found in a variety of condition, not just atherosclerosis, and that antibodies targeting these epitopes recognize circulating microparticles, which are membrane blebs that are shed from activated or dying cells, and are involved in many other conditions. Interestingly, circulating microparticles were found to contain a high amount of malondialdehyd-adducts, a common lipid peroxidation-derived epitope, in their membranes. Dr. Binder commented that these findings shed a new light on the potentially important role played by IgM antibodies in maintaining homeostasis. In that the immune system recognizes oxidation-specific structures as harmful species, triggering natural antibodies to clear dying cells and cell debris, or to limit their pro-inflammatory potential. Thus, a reduced level of IgM antibodies may well increase the incidence of diseases mediated by inflammation. In closing, Dr. Binder noted that the concentration and types of IgM antibodies present, can perhaps play a predictive role in chronic inflammatory diseases, and may help clinicians assess predisposition for various conditions.

Renate Kunert (University of Natural Resources and Life Sciences) provided an overview on the purification and characterization of human recombinant IgMs, and the use of layered beads as a new tool for IgM purification was then discussed by **Tobias Söderman** (GE Healthcare). Dr. Söderman first described traditional chromatography resins as homogenous, porous polymeric structures that are functionalized with a variety of chemical groups to produce various modes of interaction, e.g., cation exchange, anion exchange, hydrophobic interaction and affinity chromatography. He then introduced the layered beads concept which is an innovative approach to particle design currently in development at GE Healthcare. As implied by their name, layered beads are functionalized in different layers, allowing for new particle design options such as shell beads and core beads. Shell beads have a functionalized outer layer and an inert inner core, while core beads are designed in an opposite manner where the functionalized layer is the inner (core) portion and the outer layer is the portion that is inert, yet porous.

Dr. Söderman discussed bead properties and provided two examples of shell beads that each have a different thickness of the outer functionalized layer, noting that a thinner outer layer does produce a significant loss of binding capacity for the model protein bovine serum albumin (BSA) and also, that the loss in benzoylated diethylaminoethyl cellulose chromatography is not linear (11 micron layer gives 81% of the BDC and a 6 micron layer gives 60% of BDC) because, in chromatography, the entire bead is actually never involved in the separation. He then noted that the loss of dynamic binding capacity (DBC) is compensated for by the higher resolution afforded by the short diffusion path of these shell beads, thus providing high resolution at a high flow-rate owing to the large particle size of the beads. Dr. Söderman also described core beads, mentioning that these particles are designed for purification of viruses or other large bio-molecules. Here, the design principle is to create pores that allow impurities to enter the bead to interact with the functionalized core while letting larger molecules flow through the resin bed. Large molecules are prevented from entering by the smaller pores designed into the shell structure, i.e., pore size determines the size cutoff range. Dr. Söderman added that the inner portion of these core beads is functionalized with a multi-modal ligand that tightly binds impurities such as host cell proteins or DNA, trapping them in the core. He concluded his talk by highlighting an example of a purification process contrasting size exclusion chromatography (SEC) with the core bead technique. He showed that the core beads can be saturated to higher level with impurities (up to 100 times more that SEC), at a variety of salt concentrations and flow rates, he also showed the versatility of these resins under various chromatographic conditions.

Xuemei He (Bio-Rad Laboratories) presented a two-step chromatography workflow for the purification of monoclonal IgM. She highlighted the difficulty in obtaining biologically active IgM antibodies at acceptable yields and high purity with current purification processes. IgM antibodies are challenging to work with because they have large structures, are quite sensitive to pH and salt condition, lack an affinity handle and have a propensity to form complexes with DNA. Dr. He described the issues associated with standard purification techniques when applied to an IgM molecule.

Dr. He then discussed an alternative process that avoids the use of cation exchange or HIC purification schemes because these modes of separation are often not suitable for such sensitive molecules. The first step of the proposed process is pre-treatment of the supernatant to reduce host cell DNA and other species in the solution that can potentially compete for the biding sites on the first chromatography resin, reducing capacity. The pre-treatment is performed using AG 1-X8 resin, an anion exchange resin. The eluate from the pre-treatment step is then directly loaded onto a hydrophobic cation exchange mixed-mode column at relatively high salt concentration (without any prior dilution) and eluted using a simple pH adjustment from pH 6.5 to 7.8 along with a moderate increase in conductivity. This step cleared much of the BSA and prepared the IgM for the second chromatography step, which was performed by direct loading without any sample handling onto a ceramic hydroxyapatite (CHT™) column at pH 7 and eluted using a phosphate gradient from 20 mM sodium phosphate to 125 mM sodium phosphate to yield biologically active IgM with 80% recovery. Dr. He noted that the method presented is highly effective and productive due to the minimal sample handling involved (only pH adjustment without any dilution steps) and the mild conditions used throughout the entire purification process. She concluded by commenting on the

versatility and robustness of using sequential mixed-mode purification techniques to purify IgM antibodies with high yield.

Pete Gagnon (Bioprocessing Technology Institute) discussed the industry's quest for a broadly applicable IgM purification platform. **Jeffrey Jorgensen** provided details of an automated platform for purification of autologous human IgM. He first gave a brief overview of Biovest's 30-y history, which began with pioneering the development of disposable hollow-fiber bioreactors. He described clinical studies that Biovest is conducting with the National Cancer Institute, and Phase 2 and Phase 3 studies of patient-specific cancer vaccines, BiovaxID®, for the treatment of follicular non-Hodgkin lymphoma and mantle cell lymphoma.[5] Dr. Jorgensen also briefly touched on work being conducted with the US Department of Defense, which has interest in the rapid deployment benefits of Biovest's platform expression and purification platform in cases of sudden disease outbreak.

The focus of Dr. Jorgensen's talk was the prototype purification module of the BiovaxID® system, which has an integrated tangential flow filtration filter, several chromatography columns and a virus filter that are all part of a fully disposable unit. He noted that deep process understanding is critical to success. Expression rates are quite variable, making purification difficult. The capture step is done on ceramic hydroxyapatite (CHT™) using a step change in phosphate concentration, which helps normalize the concentration and volume. Viral inactivation for the IgM is done by diafiltration/buffer hold, which allows the process to remain closed using a pH probe. The next step in the process is an anion exchange chromatography step in flow-through mode to bind impurities. The robustness of the instrument is verified in triplicate, using crude supernatant of three tumor-specific IgM antibody expressions. The entire operation takes less than 10 h and the yield produced had a mean of 41.1% (SD 11.8%) and host cell protein (HCP) reduction was between 1.8–2.1 logs. Product quality was verified for consistency using SDS-PAGE (reduced Coomassie blue). Overall, these consistency studies demonstrated that the Biovest automated system can deliver yields of 28.5–51.1%, with HCP reduction of approximately between 2.0–2.4 logs, which are acceptable results for such a process.

Dr. Jorgensen noted that these consistency and robustness studies provide Biovest with confidence that such a small, automated instrument is effective in delivering patient-specific IgM-Id vaccines in a commercial setting. Future improvement to the instrument will include an in-process sampling method and a more effective way to control CHT load volumes at the capture step. Other improvement work will focus on better impurity clearance, perhaps by using a third chromatography step, and a more robust viral safety scheme.

The screening and quantification of human IgMs using biolayer interferometry (BLI) was discussed by **Alexander Mader** (University of Natural Resources and Life Sciences). He began by mentioning the importance of IgM antibodies in the body's defense against bacterial infections and cancer. He then described the work being done by his team in Professor Kunert's group whereby a streamlined modular approach was developed for the expression of IgMs in serum and protein-free environments. Despite such advances, large scale production remains a challenge. Dr. Mader highlighted the current use of ELISA as the screening method of choice for such processes. More importantly, more effective and productive screening and quantitation processes for IgM antibodies are needed.

Dr. Mader provided a quick overview of BLI, which is a label-free technique for measuring biomolecular interactions where the interference pattern of white light reflected from two surfaces is measured. Functionally, as the number of molecules bound cause an increase in thickness to one surface (the biosensor tip), this change causes a shift in the interference that is then measured in real-time. Dr. Mader described a novel surface used on the forteBio Octet QK system that includes Protein L, which recognizes kappa light chains and effectively binds to representatives of all antibody classes, including IgM. The sensor can be regenerated after each experiment, testing 10 times has shown that the binding rate remains constant. Using several standards, this novel Protein L sensor provides results comparable to ELISA, but at high throughput rates.

Petra Kramberger (Patrys GmbH) presented results showing monolith column technology to be an effective separation medium for IgM antibodies and other large bio-molecules. She also discussed the use of monolith columns in analytical techniques and bio-production settings. Monoliths are made from one single and uniform porous structure with uninterrupted and interconnected channels that run throughout the mass of column. These channels are optimized for size and can be modified with various functional groups to address specific applications. Mass transfer in monoliths is achieved through convection, which leads to very fast mass transfer of molecules between the mobile phase and monolith structure. These fast transfer dynamics make the technique more efficient in terms of resolution and speed, compared with traditional packed-bed resin columns that separate molecules using a diffusion mechanism, which requires more residence time for effective separation.

Dr. Kramberger also mentioned several techniques being explored for membrane-based separations. For IgM specific separation, she highlighted the need for robust and reproducible methods to support production. She discussed work done at BIA Separations using IgM antibodies from cell culture and CIM® Protein A monoliths, where initial experiment were shown to be irreproducible and displayed a slight increase in backpressure. Further investigation lead to the realization that DNA-IgM complexes could have affected the column performance after several injections. A change in buffer systems at pH 3 was attempted and results were positive. Subsequent to this change, purification was repeated 200 times without regeneration; overlays of the chromatograms displayed comparable results with no increase in pressure. Evidently, the dissociation of DNA-IgM complexes allowed for the full realization of the chromatographic performance of the monolith column technology for this application.

Day 2: November 16, 2011

Discussion of upstream/downstream development continued during the morning of the second day of the meeting, and then

turned to formulation and clinical development. **Frank Detmers** (BAC B.V.) provided an overview of a ligand development platform based on llama proteins that was used to create BAC's CaptureSelect IgM affinity matrix. He detailed the production and screening process that starts with llama immunization to generate a VHH library, from which mRNA is isolated. From the RNA, cDNA is produced. Using PCR, a VHH library is then identified. The binding properties of individual proteins are then assessed through colony picking. If insufficient binders are present in the VHH library, yeast display can be used to increase the amount. The selected proteins are screened for resistance against common process operating conditions such as pH, buffers, cleaning agents and other process parameters to produce a short list of candidate ligands. The best binders are then stabilized on polymer matrices and tested in a chromatography process. Once a suitable ligand is selected for a given application, it can be produced at large scale in baker's yeast, then purified by filtration and ion exchange chromatography. Dr. Detmers then introduced a new specialized affinity ligand, which is a 14 kDa llama antibody fragment that specifically recognizes IgM. This ligand is directed toward a unique domain on the Fc part of IgM molecule thus enabling specific purification of human and mouse IgMs without cross reactivity with human and mouse IgG and IgA antibodies.

John Schreffler (Morphotek, Inc.) discussed purification applications using CaptureSelect IgM described by Dr. Detmers. He noted that variability of IgM expression motivated the downstream purification team to seek a more specific purification approach using a specialized ligand to replace a Protein A method previously in use. He showed data displaying high purity and high binding capacity (albeit at a longer residence time) using the BAC CaptureSelect IgM; however, the BAC IgM affinity matrix was not available at the needed scale at the time of the scale-up, it was nonetheless effective and easy to use. The Morphotek team also tested GE Healthcare's CaptureSelect IgM resin, a material based on NHS-activated Sepharose 4 Fast Flow, which displayed a lower dynamic binding capacity (DBC) and required a low elution pH. Dr. Scheffler noted that Morphotek has in place clearly defined performance criteria, e.g., DBC, purity, recovery, impurity clearance and process time, that meet their processing needs and guide all experimental resin screening. Dr. Schreffler also discussed several data sets generated from multiple rounds of testing using several IgM antibodies and multiple conditions that lead the team to conclude that an acceptable level of quality is certainly within reach using CaptureSelect IgM resins, although more work is needed to reduce process time.

Michiel Ultee (Laureate Biopharmaceutical Services) began his presentation on upstream and downstream challenges of clinical production of a human IgM antibody therapeutic with a brief introduction of Laureate Biopharmaceutical Services, a well-established CMO with extensive manufacturing experience of a wide variety of molecules, such as IgG and IgM antibodies, enzymes, Fc-fusion proteins and other complex molecules. Dr. Ultee also mentioned that his firm has experience with many well-known expression cell lines, they can handle large projects from early concept to fill/finish operations, and they use many new disposable technologies to optimize process performance and overall project costs.

The majority of his talk was on the manufacturing of PAT-SM6 at the Laureate Biopharmaceutical Services facility in New Jersey.[6] PAT-SM6 is expressed in the PER.C6 human cell line, which produces antibodies with fully human glycosylation and other post-translational modification patterns. Dr. Ultee and his team transferred the process and worked to significantly increase titer and improve other aspects of the manufacturing scheme. The antibody was expressed at the 250 L scale in a disposable reactor. PAT-SM6 titer increased greatly after the process was changed from a batch to a fed-batch mode; the cells responded well and cell viability remained high, reaching 30 million vc/ml. Nutrient consumption was studied in great detail during the expression and changes were made to the feeding regiment to significantly increase productivity.

Dr. Ultee then described the downstream process used to purify PAT-SM6 for clinical use, saying that the process had been adapted for large-scale manufacturing from a purification process designed by Validated Biosystems Inc. The first step of the multi-step process used ceramic hydroxyapatite (CHT™) for initial capture, followed by an inline strong anion-exchange membrane step used in a flow-through mode. Viral inactivation was performed using a Triton X-100 wash in static on-column mode. This was followed by other washes with phosphate buffer. After the washes, the material was loaded onto Sartobind® Q membrane to further reduce host cell DNA levels. Dr. Ultee noted that high recovery rates were obtained from the CHT/Q elution scheme, and mentioned that urea and polyethylene glycol (PEG) were used to stabilize the antibody during processing. Lastly, successive ion-exchange steps using convective interaction media (CIM) monolithic columns were also used to complete the purification process. Viral filtration was performed on a nanopore filter followed by ultrafiltration and diafiltration. The downstream purification process yielded clinical grade PAT-SM6 protein with residual HCP at 44.4 ng/mg, residual DNA < 0.03 ng/mg, residual Triton < 0.4 μg/mL and endotoxin at 0.10 EU/mg. Dr. Ultee concluded his talk by mentioning that final formulation of PAT-SM6 was conducted according to standard industry practices, and that clinical grade IgM antibody can be effectively expressed, purified and formulated at a large scale.

The final presentation of the morning session on downstream development of IgM was given by **Aleš Štrancar** (BIA Separations), who discussed lowering the production costs of IgM by using fast in-process HPLC and multiuse disposable columns. The meeting program then turned to consideration of antibody patents, and the formulation and clinical development of IgM antibodies.

Gerda Redl (REDL Life Science Patent Attorneys) discussed what room exists for antibody patents in the crowded antibody R&D field. She began her talk with a quick overview of well-known patents in the field of monoclonal antibodies, but noted that there is still room for innovation and new intellectual property (IP) asset ownership, which are essential elements for securing continued investment from private funders. Further, she mentioned that IP assets can be related to specific discovery

platforms, composition of matter or combinations thereof. She stated that patents can also protect production processes, formulations or clinical regimens. Ms. Redl stressed the importance of a thorough IP review, advising the audience that a clear IP strategy must be put in place early-on to inform any work that may lead to possible innovation and to ensure that a defensible IP portfolio can be secured by inventors.

Ms. Redl then briefly reviewed what constitutes the basis of "patentability," discussing trends in the field, as well as the guidance and philosophy of the various patent authorities on what is considered "innovation" and the required level of detail regarding the description and scope of invention. She noted that in the case of antibodies, claims can be related to antibody function or to antibody structure, i.e., sequence, or variations thereof, stressing that a more robust approach would claim both structure and function to define the invention. She highlighted an example where publication of a given antibody function may reduce the chances of patentability even if the structure is not revealed, and even if it is considered innovative by the inventors, because a known function may make the structure seem obvious to a skilled person in the field. She concluded her overview with a summary of the evolution of inventive standards, which has changed, just as the field on monoclonal antibodies has, over the last few decades. Ms. Redl's overview touched upon the important aspects of IP and gave the audience a good perspective on this critical topic in the field of antibody research and therapeutics development.

Monika Mueller (Singapore, Agency for Science, Technology and Research) discussed the stabilization of IgM from cell culture through formulation. She stated that the objective of her work is to explore the stability and viability of IgM antibodies (post-purification) under various conditions to derive suitable formulations that allowed stable long-term storage and maintained activity of the molecules. She enumerated and briefly described the techniques that are employed in this work, e.g., size exclusion chromatography (SEC) to measure aggregation, degradation and fragmentation, differential scanning calorimetry (DSC) to monitor the various IgM molecules for aggregation, denaturation and other conformational changes that can affect the activity of the protein. Design of Experiment (DOE) principles are also employed to determine the most effective ranges of formulation parameters for optimal stability.

Dr. Mueller then highlighted the various transition states and resulting species for model antibodies (IgM and IgG) as determined by DSC and SEC to describe key concepts. Dr. Mueller briefly summarized the effect of solution pH (between pH 5 and pH 8) on several IgM antibodies before summarizing her findings that a solution pH near or at the isoelectric point of the antibody increased the conformational stability of the protein, preserving the highest levels of monomer in solution (as observed for six of the eight IgM antibodies tested). Regarding excipients, Dr. Mueller mentioned that the effects of various concentrations of commonly used formulation additives, e.g., NaCl, glycine, sucrose, arginine, glycine, PEG 6000, sorbitol, were explored using DOE. She stated that the experimental results clearly showed that only three excipients tested had a significant positive impact on IgM stability in solution—sucrose, sorbitol and glycine—with the highest concentration in the ranges tested, producing the best results. This effect was observed for all eight IgM antibodies evaluated. Dr. Mueller then noted that exchanging sucrose for sorbitol further increased stability, but cautioned that one must be mindful of viscosity increases with the addition of additives.

Dr. Mueller summarized the results of thermal stress testing (37°C), shear testing (24 h stirring) and long-term storage (4°C and -20°C) on all eight IgM molecules in their optimal formulations as previously determined. In all cases, stability was confirmed over time; in some cases, however, a pH decrease for some formulations was required for best long-term stability results. Lastly, Dr. Mueller shared bioactivity test results (human embryonic stem cell binding assays) after six months storage at -20°C and 4°C that demonstrated that binding affinity is maintained without changes in cytotoxicity levels. Dr. Mueller concluded by saying that, demonstrably, IgM antibodies can be formulated for long-term stability and activity.

An overview of the long-term storage of IgM as a liquid formulation was provided by **Marcus Tarköy** (Kenta Biotech Ltd.), and then **Deanne Greenwood** (Patrys Ltd.) discussed preliminary results from a first-in-human study of PAT-SM6 IgM in patients with melanoma. Dr. Greenwood began her talk with a brief description of the technology used by Patrys Ltd. to generate IgM antibodies with specificity toward cancer cells. The company's unique approach begins with the isolation from donor patients of B1 cells that produce antibodies that specifically bind to cancer tissue yet ignore unaffected normal tissue. In the second step of the Patrys process, the most effective antibodies are selected based on their anti-cancer activity for further development. Dr. Greenwood noted that this selection is determined predominately by immunohistochemistry.

PAT-SM6, one of the company's lead drug candidates, is a natural human IgM antibody derived from the two-stage technique. PAT-SM6 was isolated from a patient with gastric cancer and binds to GRP78, an endoplasmic reticulum chaperone protein that is overexpressed in many types of cancer cells. Dr. Greenwood described an on-going, open-label Phase 1 study being conducted in patients with in-transit melanoma, a form of recurrent melanoma that is confined to the skin and where progress can be directly assessed by tumor biopsy. The protocol for the study is designed to assess the safety profile of PAT-SM6, and determine the pharmacokinetics and immunogenicity profile of the antibody. PAT-SM6 is administered as a single dose given in a "three plus three" study format, commonly used in cancer clinical trials, where three groups of three patients are given three different doses of the drug. The first cohort received 0.15 mg/kg of PAT-SM6; the dose was then doubled to 0.3 mg/kg in the next cohort. Recruiting was underway for the third cohort, and this group was set to receive a dose of 0.6 mg/kg of PAT-SM6 given intravenously over a 60 min period. As of mid-November 2011, six patients had been treated and Dr. Greenwood noted that no adverse effects or adverse immune responses to PAT-SM6 had been reported and an increased level of cancer cell apoptosis was observed to be widespread in the post-treatment samples of one

of the patients. Dr. Greenwood concluded her talk by saying that results were very encouraging and that the safety and specificity of IgM antibodies had been verified, thus prompting Patrys to move forward with other IgM antibodies and clinical studies for other cancer indications for PAT-SM6.

Andre Choo (Bioprocessing Technology Institute) presented an interesting application of IgM antibody technology to the elimination of residual pluripotent stem cells. He began by saying that perhaps we did not choose IgM antibodies, but rather IgM antibodies have chosen us. IgM antibodies are natural molecules for which new and exciting applications are being discovered in variety of fields, including stem cells. Dr. Choo briefly discussed recent developments in the area of human embryonic stem cells (hESC), noting that these cells have great potential in regenerative medicine because of their capacity to differentiate into cells of any type of tissue in the human body using a variety of differentiation techniques. Irrespective of the technique employed, however, cell differentiation is always incomplete (i.e., the process is inefficient), which poses substantial risks if stem cells are to be transplanted into tissue in vivo because undifferentiated cells can cause teratoma.

Dr. Choo cited work by his group demonstrating that mAb 84 can effectively eliminate teratoma formation by undifferentiated hESC in a SCID mouse model. mAb 84, a cytotoxic IgM antibody of 960 kDa, was found to specifically bind and kill undifferentiated stem cells within 30 min of incubation. Importantly, a similar effect was observed for human embryonic carcinoma NCCIT cells and induced pluripotent stem (IPS) cells.[7] Dr. Choo noted that mAb 84's target seems to be conserved across pluripotent cells lines as the cells are reprogrammed to pluripotency. Western blot and mass spectrometry studies have revealed that the antigen is a podocalyxin-like protein-1 (PODXL-1) and that this protein is heavily glycosylated. More importantly, it has also been shown that the expression of PODXL-1 is downregulated as cells are differentiated, thus explaining the reduced killing effect of mAb 84 of differentiated stem cells. Dr. Choo mentioned as a side note that the PODXL-1 antigen is cited in literature as a cell marker in aggressive types of breast and prostate cancer, which may suggest that stem cells and cancer cells do share some conservation of antigens.

To further understand the mechanism responsible for stem cell killing by mAb 84 after binding to PODXL-1, the team conducted a literature search that indicated the responsible mechanism may be oncosis, which is a form of cell aggregation and increased cell permeability that occurs when a pro-oncosis receptor is targeted by an IgM mAb. Interestingly, the PODXL-1 protein found on stem cells and the pro-ocosis receptor share many structural similarities. Further work by Dr. Choo's group confirmed that similar cytosekeleton changes are observed on both incidences of oncosis. Dr. Choo showed a time-lapse video that displayed this oncosis effect on a sample of ES cells treated with mAb 84 after 10 min of incubation. Dr. Choo also shared several data sets showing that mAb 84 binds to sugar residues on the PODXL-1 protein.

In concluding, Dr. Choo briefly discussed mAb 85, an IgM antibody that effectively binds but does not kill undifferentiated stem cells. He stated that such a non-cytotoxic antibody can be employed for stem cell separation techniques in a research setting or, perhaps one day, a manufacturing setting. Overall, Dr. Choo's work demonstrated unique and innovative uses of IgM antibodies in the fast-moving field of stem cell research and regenerative medicine.

Michael Rudolf (Kenta Biotech AG) presented data on the comparability of effector function of fully human IgM derived from hybridoma or recombinant CHO expression. **Leo Rasche** (University Hospital Wuerzburg) then discussed how the natural IgM antibody PAT-SM6 specifically targets and kills primary multiple myeloma cells by induction of apoptosis and complement activation. Dr. Rasche first noted that, ironically, the scientific community had somewhat ignored a main component of innate immunity, B1 cells secreting IgM antibodies, for a long time. He provided a brief overview of the process for the isolation and identification of IgM antibodies previously described by speakers at the workshop. Dr. Rasche highlighted the special nature of PAT-SM6, most notably the cytotoxicity due to its capacity to induce apoptosis upon accumulation in diseased tissue, which was verified in vivo in animal models. He also noted that PAT-SM6 has a broad spectrum of activity against many types of cancer cells.

Dr. Rasche shifted his focus to multiple myeloma (MM), and described how myeloma cells can be effectively isolated from diseased tissue. He presented data showing that PAT-SM6 does indeed bind specifically to MM cells, with data from 11 newly diagnosed patients showing significant binding in all cases as evidenced by staining patterns. A similar result for binding was also observed in 9 patients whose diseased had relapsed, with 100% binding observed. In all cases, 90 - 100% of cells displayed homogeneous and pervasive binding by PAT-SM6. Notably, PAT-SM6 does not bind to healthy primary hematopoietic tissue. In addition to the immunohistochemistry work presented, Dr. Rasche shared data obtained using flow cytometry techniques on primary myeloma samples, which also showed that PAT-SM-6 had positively induced apoptosis of the cancer cells.

Dr. Rasche reminded the audience that PAT-SM6 specifically binds GRP78, then posed the questions: what is the role of GRP78 in myeloma, is there a function or link that we need understand? He stated that the current thinking is that GRP78 is active in the endoplasmic reticulum (ER) where it plays a chaperoning role. It is therefore quite possible that myeloma cells accumulate large quantities of unfolded proteins due to stress brought on by the cancer and thus require a very large amount of GRP78 chaperons (overexpression), which is also expressed on the surface of the cells. Regarding apoptosis, Dr. Rasche showed that MM cells incubated with PAT-SM6 demonstrate significantly higher levels of cell death and that the effect was observed independent of myeloma subtype and stage of disease. In his concluding remarks, Dr. Rasche posited that the demonstrated capability of PAT-SM6 to induce apoptosis presents a potentially powerful immunotherapeutic approach to treat multiple myeloma, as well as other types of cancer.

Nelson Teng (Stanford Cancer Center) discussed a Phase 1 trial of mAb 216, a novel human monoclonal IgM antibody, in

patients with relapsed or refractory B cell acute lymphoblastic leukemia (ALL). He first provided a brief history of how mAb 216 (a natural human monoclonal IgM antibody) was generated and noted that mAb 216's ability to bind normal B lymphocytes, B cell lymphomas and B-progenitor lymphoblasts was shown early on, which led researchers to explore mAb 216 as a possible treatment for ALL. Dr. Teng showed data demonstrating the cytotoxicity of mAb 261 at levels as low 1 ug/ml, and he mentioned that there is a range of binding affinities for the various B cell types and that the binding patterns of mAb 216 are distinctly different from those of anti-CD20 rituximab (Rituxan®; a monoclonal antibody used in the treatment of ALL). He briefly presented preclinical data demonstrating that lymphoma cell suspensions that bound mAb 216 showed significant uptake of the staining agent propidium iodide (PI) compared with a control mAb, and that two lymphoma cell suspensions that did not bind mAb 216 did not take up PI. These immunostaining studies showed that mAb 216 caused significant disruption of the plasma membrane and formation of large pores resulting in cell lysis. Dr. Teng noted that, because mAb 216 binds to a different epitope than rituximab, it is quite likely that mAb 216 can eventually be used in combination or as a second-line treatment of refractory lymphoma patients. Importantly, when administered in combination with vincristine (a chemotherapeutic agent commonly used to treat non-Hodgkin lymphoma) mAb 216 has proven to be significantly more potent in inducing cell lysis. Dr. Teng also quickly reviewed the production process of mAb 216, highlighting the fact that, once lyophilized, mAb 216 is extremely stable.

Dr. Teng briefly reviewed the typical symptomatology, methods used to diagnose ALL, and the current strategies used to treat leukemia in children and adults, then discussed results from the trial that had for a primary objective, to determine the maximum-tolerated dose and toxicity of mAb 216 administered alone (starting dose of 1.25 mg/Kg; standard 3+3 dose escalation design) and in combination with the chemotherapeutic agent vincristine, in children and adults While the secondary objective of the study was to examine the pharmacokinetic behavior of mAb 216 and assess tumor targeting capacity and clinical efficacy in relapsed or refractory B cell ALL patients. Study subjects received two treatment courses of mAb 216, with the same dose of antibody administered on day one and day seven. Good responders (defined as those with less than 25% leukemic blasts in bone marrow or a more than 75% reduction in peripheral blood blast count) received a second dose of mAb 216, while poor responders (defined as those with a rise in peripheral blood blast count) received the second dose of mAb 216 in combination with vincristine. Importantly, vincristine was chosen because it has a well-understood toxicity profile.

Dr. Teng presented results that are partially summarized here: All 13 patients showed a poor response to the first dose of mAb 216 (as per defined criteria) with a decrease in peripheral blasts from 6–65% in 9 patients (measured at 24 h and at 7 d after administration).[8] In eight of the patients, the addition of vincristine to mAb 216 produced an average reduction of the peripheral blasts of 81%. mAb 216 was detected on peripheral blasts in all study patients. In conclusion, this Phase 1 study showed that early responses are favorable and that mAb 216 in combination with vincristine is a promising treatment option for leukemia patients. Also, that the therapy is tolerated in patients with relapsed or refractory B-cell ALL and that mAb 216 does effectively bind to peripheral blasts. Dr. Teng then shared two poignant and painful stories of disease progression in two very ill patients who had failed many treatment options before being admitted in the study. He noted that severe symptoms had been alleviated while on the mAb 216 study regiment.

Closing Comments on the Meeting

Dr. Teng's excellent presentation was a befitting close to this first "New ParaDIgm: IgM from bench to clinic" meeting. As a treating oncologist, Dr. Teng conveyed the human side of disease and the impact of treatment on patients. His talk was a stark, yet hopeful, reminder to all attendees that we must continue to explore the therapeutic potential of IgM antibodies. Attendees came to the meeting with different motivations from a broad spectrum of backgrounds and professional expertise. However, in my estimation, everyone left the meeting with a great sense of responsibility and renewed energy to pursue work in the field of IgM antibodies. I am confident that the 2013 meeting will be even bigger, as more effective techniques and promising results are achieved and shared by this increasingly active community.

Note

Summaries were prepared from the author's notes or from PDFs of the presentations provided by speakers after the meeting. When the notes were insufficient and speakers were not able to share their presentations, detailed summaries were not included, although the names, affiliations and presentation topics of all speakers appear in the report.

References

1. Nagels B, Van Damme EJ, Pabst M, Callewaert N, Weterings K. Production of complex multiantennary N-glycans in Nicotiana benthamiana plants. Plant Physiol 2011; 155:1103-12; PMID:21233332; http://dx.doi.org/10.1104/pp.110.168773.
2. Klimyuk V, Pogue G, Herz S, Butler J, Haydon H. Production of recombinant antigens and antibodies in Nicotiana benthamiana Using 'Magnifection' technology: GMP-compliant facilities for small- and large-scale manufacturing. Curr Top Microbiol Immunol 2012; In press; PMID:22527176; http://dx.doi.org/10.1007/82_2012_212.
3. Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. Biochim Biophys Acta 2012; In press; PMID:22305963.
4. Bieghs V, van Gorp PJ, Walenbergh S, Gijbels MJ, Verheyen F, Buurman WA, et al. Specific immunization strategies against oxidized LDL: A novel way to reduce non-alcoholic steatohepatitis in mice. Hepatology 2012; In press; PMID:22334337; http://dx.doi.org/10.1002/hep.25660.
5. Schuster SJ, Neelapu SS, Gause BL, Janik JE, Muggia FM, Gockerman JP, et al. Vaccination with patient-specific tumor-derived antigen in first remission improves disease-free survival in follicular lymphoma. J Clin Oncol 2011; 29:2787-94; PMID:21632504; http://dx.doi.org/10.1200/JCO.2010.33.3005.
6. Valasek C, Cole J, Hensel F, Ye P, Conner M, Ultee ME. Production and purification of a PER.C6-expressed IgM antibody therapeutic. BioProcess International 2011; 9:29-37.
7. Choo AB, Tan HL, Ang SN, Fong WJ, Chin A, Lo J, et al. Selection against undifferentiated human embryonic stem cells by a cytotoxic antibody recognizing podocalyxin-like protein-1. Stem Cells 2008; 26:1454-63; PMID:18356574; http://dx.doi.org/10.1634/stemcells.2007-0576.
8. Liedtke M, Twist CJ, Medeiros BC, Gotlib JR, Berube C, Bieber MM, et al. Phase I trial of a novel human monoclonal antibody mAb216 in patients with relapsed or refractory B-cell acute lymphoblastic leukemia. Haematologica 2012; 97:30-7; PMID:21993685; http://dx.doi.org/10.3324/haematol.2011.045997.