Antibody Therapeutics, 2019, Vol. 2, No. 4 ***88–96***
doi:10.1093/abt/tbz012
Advance Access Publication on 10 December 2019

Meeting reports and interviews

Next-Generation Antibody Therapeutics: Discovery, Development and Beyond: highlights of the third annual conference of the Chinese Antibody Society

Hongyu Zhang[1,†], Mi Deng[2,†], Fen Pei[3,†], andShouye Wang[4,*] and Mitchell Ho[5,*]

[1]Department of Biochemical Engineering, University College London, London, WC1E 6BT, UK, [2]Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA, [3]BioLegend, 8999 BioLegend Way, San Diego, CA 92121, USA, [4]Chinese Antibody Society, 955 Massachusetts Ave #276, Cambridge, MA 02139, USA and [5]NCI Antibody Engineering Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

Received: September 19, 2019; Revised: November 7, 2019; Accepted: November 21, 2019

ABSTRACT

The Chinese Antibody Society (CAS) convened the third annual conference in Cambridge, Massachusetts, USA on April 7, 2019. More than 600 global members attended the meeting. The theme of this conference was Next-Generation Antibody Therapeutics: Discovery, Development and Beyond. The meeting covered a vast variety of topics including cancer immunotherapy, single-domain antibodies as well as bispecific antibodies, immunotoxins, transgenic mouse platforms for next-generation monoclonal antibody discovery and antibody chemistry, manufacturing and controls (CMCs). Two hot topics were comprehensively discussed by the prestigious panelists and hosts at the panel discussions during the conferences, i.e., bispecific antibodies and antibody CMC.

Statement of Significance: The Chinese Antibody Society convened the third annual conference in Cambridge, Massachusetts, USA on 7 April 2019. The meeting covered a variety of topics, including cancer immunotherapy, single-domain antibody, bispecific antibody, immunotoxin, transgenic mouse platforms for next-generation monoclonal antibody discovery and antibody CMC.

KEYWORDS: antibody therapeutics; bispecific antibodies; chemistry manufacturing and Controls (CMC); immuno-oncology; single-domain antibody; immunotoxin; transgenic mouse platforms; Chinese Antibody Society;

OPENING REMARKS

The third annual conference organized by the Chinese Antibody Society (CAS) was held on 7 April 2019 in Cambridge, MA. Dr. Fubao Wang, vice president of Sarepta Therapeutics, and a board director of CAS delivered the opening remark. He welcomed all attendees of the conference and thanked the speakers, moderators, panelists, session chairs, the conference organizing committee, CAS volunteers (Fig. 1) and the partners and sponsors of the conference. Dr. Wang summarized the major achievements of global antibody therapeutics in 2018. At least 13 antibody drugs were approved by the regulatory authorities for commercialization, which include three calcitonin gene-related peptide (CGRP)/CGRP receptor antibodies, the first camelid single-domain antibody and the first anti-HIV therapeutic antibody (Ibalizumab). Ibalizumab, approved by the FDA and manufactured by WuXi Biologics, is the first commercial antibody product produced in China for the US market. In China, 10 antibody drugs were approved in 2018 including two anti-PD-1 antibodies, Toripalimab and Sintilimab,

*To whom correspondence should be addressed. Shouye Wang or Mitchell Ho. Email: shouye.wang@gmail.com or homi@mail.nih.gov.
†These authors made equal contributions to this article.

Figure 1. A group portrait of the conference speakers, panelists, CAS advisors and volunteers taken during the third annual conference.

from Shanghai Junshi Biosciences (Shanghai, China) and Innovent Biologics (Suzhou, China), respectively. In addition, nine new drug applications (NDAs, equivalent to Biologics License Application [BLA] in the USA) were filed for marketing in China. Importantly, four Chinese companies with pipelines that mainly focused on therapeutic antibodies were listed on the Hong Kong Stock Exchanges (HKEX) last year. Dr. Wang highlighted the theme of this year's conference—Next-Generation Antibody Therapeutics: Discovery, Development and Beyond—with an agenda that included hot topics of therapeutic antibodies: cancer immunotherapy, single-domain antibodies as well as bispecific antibodies, immunotoxins, transgenic mouse platforms for next-generation monoclonal antibody discovery and antibody CMC.

YEARLY REVIEW AND ANNOUNCEMENTS OF THE CAS

At the beginning of the conference, Dr. Shouye Wang, the founder and the first president, handed over the symbolic cup of the society to Dr. Zhidan Tu, the current president of CAS. As the new president and on behalf of the society, Tu later shared the CAS yearly review with the audience. Founded on 27 April 2016, the CAS is an international nonprofit professional organization focusing on therapeutic antibodies and relevant therapeutic modalities. The mission of the society is to build a platform that facilitates the communication and collaboration of the global community in the discovery, development, manufacturing and commercialization of antibody-based products. Professionals interested in therapeutic antibodies, regardless of race, citizenship or location, are welcome to join the CAS through the website (https://chineseantibody.org/membership-register/). CAS has been in collaborations with other organizations including the Antibody Society. The two organizations will have a joint special section on December 13, 2019 at the coming Antibody Engineering & Therapeutics event, which is to be held in San Diego on December 9–13 2019.

During the CAS talk, Tu introduced the Board of Directors, Advisory Board and Executive Committee members. The featured events and activities, so called as 'Five Ones', were also introduced. The Five Ones plan includes the most featured platforms and events through which the society has been contributing to the community and members. These include one newly launched Experts' Perspectives, one journal—*Antibody Therapeutics* that was launched in June 2018 and published by Oxford University Press (https://academic.oup.com/abt), one monthly webinar series, one PharmaConnect event series, which brings the society members a deep dive into selected biopharmaceutical and biotechnology companies and one annual conference. The major goal of these efforts is to achieve the mission of the CAS, as well as to serve all members of the society and beyond. On behalf of CAS, Tu expressed the appreciation to all parties and individuals who have supported the growth and development of the society and community. In the end, Tu announced that the Fourth Annual Conference will be held on 3 May 2020 in Cambridge, Massachusetts.

COMBINING DRUGS FOR CANCER IMMUNOTHERAPY

Dr. Nils Lonberg, former senior vice president of Bristol-Myers Squibb, gave a presentation on the history and development of combining drugs for immuno-oncology (IO) therapy. It has been decades since scientists identified the T-cell co-stimulator CD28 and co-inhibitory CTLA-4 and PD-1. In late 1990s and early 2000s, CTLA-4 inhibitor ipilimumab (Yervoy) and PD-1 inhibitor Nivolumab (Opdivo) was discovered respectively. In 2009, the co-administration of these two antibodies was conducted to clinical studies for the synergy use of both checkpoint blockade (CPB) molecules, which was finally approved in the USA on 10 Nov 2015.

The combination therapy of nivolumab and ipilimumab had made tremendous success in the survival rate of metastatic melanoma patients. The overall survival is 64%, 58% and 53% in 24, 36 and 48 months after the treatment, respectively, which is a significant improvement from chemotherapy that is merely 6% at the end of 36 months. Beyond melanoma, anti-CTLA-4 and anti-PD-1 antibody combination has been proved effective in a variety of tumors such as non–small-cell lung cancer (NSCLC), small-cell lung cancer, kidney cancer, hepatocellular cancer, gastric cancer, microsatellite unstable colorectal cancer, triple negative breast cancer and head & neck cancer. NSCLC patients with highly mutated tumors receiving nivolumab-ipilimumab combination have a progression-free survival of 43% compared to 13% in chemotherapy.

Resistance to IO therapy in some patients affected clinical trial results. Tumors usually have complex intrinsic antigenicity such that some cancer cells with low antigenicity could cause resistance. Therefore, PD-1 attenuation to the T cells that are shown to be effective in preclinical studies does not necessarily translate into success in clinical trials. Can the combination of IO therapy lead to less resistance in the tumor? In the nivolumab-ipilimumab CM-067 trial for the first-line treatment of metastatic melanoma, patients that exhibited less than 1% PD-L1 expression level had significantly higher overall survival when treated with combination therapy compared to monotherapy. However, when PD-L1 expression level is greater than 1%, the combination therapy did not show significant advantage compared to nivolumab monotherapy. Interestingly, when PD-L1 expression is greater than 10%, the advantage of combination therapy over monotherapy is observed again. This suggests the IO resistance of the tumor is a multidimensional phenomenon that requires combination therapies to target distinct and orthogonal mechanisms. This also indicates our understanding of the IO mechanism is still insufficient to support the rational design, especially the effects of combination therapy on different cancer types. The lack of understanding of the mechanism gives rise to the question whether mouse models used to study the IO therapy could provide firm evidence on the mechanism. Dr. Lonberg suggested mouse experiments should be used as *in vivo* assay rather than a disease model and highlighted the need to follow specific immunologic endpoints rather than just tumor control. For instance, although PD-1 and CTLA-4 in mouse models could give similar reduction in the tumor volume, tumor-specific CD8 antigen concentration showed that CTLA-4 provides higher memory response as compared to PD-1. This indicates PD-1 and CTLA-4 produces different immunologic endpoints.

At the end of the talk, Dr. Lonberg marked our current position in the IO R&D landscape: we know certain IO combination can work, but we have not fully explored the mechanism. Moreover, it is still not clear whether those identified immune attenuation pathways are relevant to human cancer immunology. It will be too costly to validate such relevance *via* human clinical trials. New thoughts need to be introduced to guide the future development of IO therapy.

ANTIBODY-BASED THERAPEUTICS: PAST, PRESENT AND FUTURE

Prof. Kerry Chester, University College London and president of the Antibody Society, highlighted pivotal milestones contributing to the success of antibodies, which are expected to comprise almost half of the USD $22.7 billion biologics discovery market by 2025. Since the initial discovery of murine monoclonal antibody technology, the clinical restriction of mouse monoclonals has urged researchers to develop chimeric, humanized and eventually fully human monoclonal antibodies over decades of progress. Meanwhile, the monoclonal antibody repertoire has achieved extremely diverse specificity against almost any target. If we consider antibodies entering Phase-I trials, those for cancer treatments have been rapidly increasing. Thus in 2010, equal number of antibodies against cancer and non-cancer targets (29 vs. 28) entered Phase I whereas in 2018, only 23 antibodies were developed against non-cancer targets compared to 97 antibodies against cancer targets.

Antibody formats entering clinic as approved drugs have also been evolving. From 1997 to 2013, monoclonal antibodies of IgG1, IgG2 and IgG4 subclasses have been developed against CD20, HER2, EGFR, VEGF, VEGFR2, GD2, PDGFRα, SLAMF7, etc. in cancer treatment. Some of these antibodies were conjugated to drug molecules (ADCs), radioisotopes or glycoengineered to improve performance. Since 2014, the immune-checkpoint inhibitors have seen significant success in cancer treatment. This is achieved through the fine-tuning of T-cell activation in the tumor microenvironment via the blockade of CTLA-4 and PD-1. Although most monoclonal antibodies have been of IgG1, IgG2 and IgG4 subclasses, humanized or fully human antibodies have largely replaced murine antibodies along with newer formats such as single-chain Fv (scFv) in bispecific T-cell engagers (BiTE) were also approved during 2014–2018 against CD19 and CD22. In general, the landscape of approved antibodies trend toward more humanized, more checkpoint inhibitors and more diverse structures and conjugations.

For Phase-I studies, contributions from China have been particularly marked. Although there were only four antibodies produced from Chinese companies in Phase I before 2014, this number doubled in 2015 and the annual number has steadily increased to 21 by 2018. This contributes to about a quarter of the global market. These antibody drugs also show diversity as 13 are immune-checkpoint inhibitors, four ADCs and five bispecifics.

Prof. Chester also commented that the scFv has made particularly valuable contributions to the field. This simplified format of VH tethered to VL by a flexible linker retains the binding specificity of a Fab antibody arm and can be readily expressed on the surface of filamentous bacteriophage, leading to the potential for rapid selection of desired binders from vast diverse phage-display libraries of many millions. This technology can be used for antibody discovery and humanization and has been the foundation of many successful commercial ventures. The scFv format itself forms the basis of T-cell recruiting agents, BiTEs and chimeric antigen receptors (CAR), the antigen binding moiety of CAR-T cells. ScFvs also form the basis for

fragment–drug conjugates (FDC), a new drug format currently in development by Antikor Biopharma Ltd (UK) and Essex Bio-Technology (China).

SINGLE-DOMAIN ANTIBODIES AND ADVANCES IN CANCER IMMUNOTHERAPY

Prof. Mitchell Ho, a Senior Investigator in the Laboratory of Molecular Biology and the Director of Antibody Engineering Program (AEP) at the National Cancer Institute (NCI), NIH, presented his latest progress on the research of single-domain antibodies (also called nanobodies). Single-domain antibodies have small molecular weight (13–15 kDa) and simple structure, equivalent to the size of the V_H domain of a full IgG antibody (150 kDa). These properties make single-domain antibodies easy to produce, are highly stable and capable of binding to buried sites on the antigen that are inaccessible to conventional IgG antibodies.

Prof Ho's group investigates how to block cell-surface glypicans (GPCs) with single-domain antibodies in various cancers. GPC3 is one of the six glypicans identified in mammals (GPC1–GPC6) that recruit Wnt on the cell surface to initiate and modulate Wnt/β-catenin signaling pathway [1–3]. The upregulation of Wnt signaling by GPC3 has been reported in various cancer types particularly in liver cancer [4]. Using computational modeling, the Ho lab has recently showed that Wnt binds to a very obscure hydrophobic groove on the N-lobe of GPC3 as predicted and experimentally validated the Wnt binding site by mutagenesis of a panel of key hydrophobic residues on the Wnt binding site (Phe41, Trp260, Tyr264 and Met269) [4]. Furthermore, HN3, a human nanobody isolated by phage display [5], binds to GPC3 on the same hydrophobic groove that Wnt binds and thereby blocks the interaction between GPC3 and Wnt [4, 6]. Moreover, the Ho lab reported another human nanobody (called LH7) highly specific to GPC2, another member of the GPC family they identified as a new target in the treatment of neuroblastoma [7]. LH7 also inhibits Wnt/β-catenin signaling pathway. The blockage of Wnt/β-catenin signaling downregulates the expression of N-Myc, the oncogenic driver of neuroblastoma pathogenesis, inside the cell that leads to tumor cell death.

In the second part of his talk, Prof Ho introduced the construction of a new phage library for shark single-domain antibodies from nurse sharks and the establishment of the new NCI AEP in which he serves as the founding director. Sharks have multimillion years of history and are one of the first animal species evolved with adaptive immunity. Remarkably, the B-cell receptor (BCR) and T-cell receptor (TCR) of sharks use the same antigen recognition domain, which is highly related to its single-domain antibody (V_{NAR}). Prof Ho's lab constructed a large phage-displayed V_{NAR} library from six naïve nurse sharks (*Ginglymostoma cirratum*), which was published earlier in *Antibody Therapeutics* [8]. The V_{NAR} genes of this library come from the B- and T-cell repertoire of the naïve sharks that reaches a diversity of 1.2×10^{10}. The next-generation sequencing analysis shows 85% sequence of this library only appeared once. The sequences of this shark library present a few characteristic categories (type I–IV), in which type I and type II are two major types. Type I V_{NAR} sequences have two non-canonical cysteine residues in the complementarity-determining region-3 (CDR3) region, which can have a hallmark of the bicycle structure. Sequences in type II have one non-canonical cysteine in the CDR3. The newly established AEP at the NCI Center for Cancer Research has developed a protocol to express and purify shark nanobodies from type I and II because the number of cysteines in the CDR3 is very different from human antibodies. The expression level of these single-domain antibodies could achieve 150 mg per liter of *Escherichia coli* culture and can be purified using a single-step affinity chromatography. The affinity of the selected V_{NAR} molecules from the naïve library is typically 1–10 nM as shown in the Octet results. Apart from the shark single-domain antibodies, Prof Ho's group also established several camel single-domain antibody libraries from the Arabian camels (*Camelus dromedarius*) and isolated camel VHH binders with high affinity for both human and mouse mesothelin (MSLN) on tumor cells. Such cross-species binding capability of the single-domain antibodies is rarely found in conventional antibodies.

Prof Ho and his colleague continued to develop CAR-T cells derived from the single-domain antibodies discovered in his lab. Anti-GPC2 LH7 is implemented on CAR-T cells that suppress the growth of tumor in mice models [7]. The cross-species camel single-domain antibodies for human and mouse MSLN are currently being implemented to CAR-T to kill MSLN-positive tumor cells whereas the normal tissues were left unaffected. The data may indicate that single-domain antibodies have advantage to recognize the subtle difference in the antigen between normal and tumor cells, as Prof Ho stated, single-domain antibodies are able to recognize a unique conformation other than the primary sequence of the antigen.

MORNING PANEL DISCUSSION: STRATEGIES AND CHALLENGES FOR THE NEXT-GENERATION BISPECIFIC ANTIBODIES

Bispecific antibody (BsAb) technology is considered as the next generation of immunotherapy. A topic focusing on the current strategies and future challenges of BsAb was discussed. The panel moderator Dr. Chengbin Wu, the founder and CEO of EpimAb Biotherapeutics, briefly introduced diverse technologies and global drug development status of BsAbs. After two decades of technological exploration, a diverse panel of BsAb formats has been developed for various applications [9]. To achieve the best therapeutic efficacy and desired safety, the design of bispecific format should meet the mechanisms of action (MOA) of different targets, including cell retargeting, increasing targeting selectivity or diversity, delivery through biological barriers and immune complex formation [10]. As of the end of 2018, more than 100 BsAbs targeting over 70 molecules are currently in clinical trials. Among them, 82% of BsAbs are for cancer treatment and 22% targeting CD3 for T-cell engagement.

Four panelists included Dr. Patrick A. Baeuerle, Executive Partner from MPM Capital, Dr. Yan Wu, Direc-

tor from Genentech, Dr. Tariq Ghayur (Distinguished Research Fellow from AbbVie) and Dr. Changshou Gao (Senior Director from MedImmune) shared their experiences and insights in technology, biological mechanism and clinic application of BsAb.

According to the panelists, BsAbs can provide novel MOA that cannot be achieved by monoclonal antibodies or combinations, and one such mechanism is engaging T-cells with tumor cells. The major challenges of BsAb include 1) to identify the unique biology which differ from combinations of monoclonal antibodies, 2) to establish appropriate animal models, 3) to apply high throughput screening in the BsAb format, 4) to select specific BsAb format appropriate for each mechanism, 5) to optimize the manufacture processes and 6) to improve the safety profile. Understanding the target biology is the most important for BsAb development. The above mentioned ongoing clinical trials for 100+ BsAbs will help us better understand the biology of BsAbs and the relevant targets.

BiTE molecules showed effective T-cell engagement by recruiting T-cells to the tumor microenvironment, resulting in tumor growth inhibition. According to the panelists, the target on T-cells, CD3, is critical and sufficient for T-cell recruitment and activation.

CAR-T cell has similar mechanism as BiTE, in which they both engage the T-cells to the tumor. In contrast of the fast adaption of CAR-T technology in academia, technical issues delayed the progression of the BsAb field until anti-CD19/CD3 BiTE was shown to treat non-Hodgkin's B-cell lymphoma patients in 2008 with promising efficacy [11]. In addition, although CAR-T and BiTE were approved for B-cell acute lymphoblastic leukemia (B-ALL) treatment, CAR-T is used for pediatric patients, whereas BiTE is used for adults with improved tolerance. Moreover, current BiTE, such as EGFR-CD3 and BCMA-CD3 BsAb, have very limited toxicity.

The selection of appropriate affinity against targets is highly dependent on the target biology of BsAbs. For example, anti-CD3 may need lower affinity while higher affinity of anti-HER2 may be desired; and anti-CTLA-4 with high affinity may lead to high toxicity. In addition, high-affinity BsAbs targeting the cell-surface receptor can cause non-linear pharmacokinetics (PK) of BsAbs. The BsAb with regular Fc portion may have a good PK as monoclonal antibodies in patients. Moreover, fusion to an anti-human serum albumin single-domain antibody improves the PK *in vivo* as well [12].

Although the field has witnessed the fast progress of BsAbs within the past years, some underlying mechanisms are still unclear such as T-cell expansion by BiTE etc., and questions still remain in areas such as the proper selection of target pairs for BsAb, resistant mechanisms, prodrug design and the engagement of NK cells.

IMMUNOTOXINS: FROM CONCEPTION TO FDA APPROVAL

Dr. Ira Pastan from NIH gave a presentation on his recombinant immunotoxin (RIT) research as a follow-up to the recent approval of Lumoxiti (moxetumomab pasudotox-tdfk) by the FDA for the treatment of refractory hairy cell leukemia (HCL). Dr. Pastan's lab focuses on Pseudomonas exotoxin A—a toxin secreted by *Pseudomonas aeruginosa* that enters the cell by receptor-mediated endocytosis. In late 1980s, Dr. Pastan and his colleagues identified the function of the various domains of Pseudomonas exotoxin A (PE) and used this information to make a RIT in which the binding domain of PE is replaced with a scFv and a 38 kDa portion containing the ADP-ribosylating activity is fused to the Fv. As the Fv domain in the single chain format is often unstable, it was stabilized by the introduction of disulfide bonds between the heavy and light chains. This chimeric construct is recognized by a cell-surface receptor on a cancer cell and enters the cell via receptor-mediated endocytosis. The PE domain is subsequently cleaved from the Fv, reaches the cytosol and inactivates elongation factor 2 (EF2) leading to the arrest of protein synthesis and cell death. This property makes this immunotoxin very effective in killing chemotherapy-resistant cancer cells. Furthermore, although Pseudomonas exotoxin A causes immunogenicity and anti-drug antibody (ADA) formation in patients with normal immune systems, it does not severely impact patients with HCL and other B-cell malignancies, making it a potential treatment for blood cancers.

Dr. Pastan currently works on the development of two anti-B-cell maturation antigen immunotoxins (LMB-70, a Fab-PE24 chimeric protein and LMB-75, a Fv-PE24 chimeric protein) for the treatment of multiple myeloma expressing B-cell maturation antigen (BCMA) that develops resistance to chemotherapy. BCMA is expressed by almost all myeloma cells making it a good target for antibody-based therapy. The half-life of LMB-75 (7 min) is much shorter than that of LMB-70 (27–151 min) in mice. LMB-70 is highly toxic to BCMA expressing cell lines (IC_{50} ranges from 1.1 to 7.2 ng/mL) and to myeloma cells extracted from the marrow of chemotherapy-resistant patients (IC_{50} ranges from 0.4 to 17.9 ng/mL). In a H929 bone marrow model, LMB-70 and LMB-75 make the mice disease-free over 90 days of treatment, whereas the untreated mice died at day 41 [13]. The negative control groups were given LMB-12 (anti-MSLN RIT) or LMB-258 (RIT with inactive toxin payload). The survival rates were the same as the untreated group indicating that the inhibition of tumors require both targeting to the BCMA-myeloma cells and an active toxin [13]. It is known that the bone marrow microenvironment can protect myeloma cells from chemotherapy. LMB-70 and LMB-75 can bypass the microenvironment niche of the tumor that leads to drug resistance, making it a potential alternative therapy for multiple myeloma.

In his talk, Dr. Pastan discussed the question of how to find a good target for immunotoxin and other antibody-based therapies. His group has focused on lineage-restricted antigens as targets, such as CD22 and CD25, and has also discovered MSLN, a lineage-restricted protein that is widely found in solid tumors such as mesothelioma, ovarian cancer, pancreatic cancer and lung cancer. For solid tumor therapy, Dr. Pastan focuses on the development of immunotoxins (SS1P and LMB-100). They are composed of an anti-MSLN Fv or Fab fused to a toxin

domain. The phase I trial of SS1P shows little anti-tumor activity in 33 MSLN-positive cancer patients in which 29 patients possess stable or progressive disease at the end of the treatment. The poor response is in part due to ADA formation. The immune systems of solid tumor patients are more active than those with blood tumors, so that patients with mesothelioma developed high titers of neutralizing antibodies, but HCL and pediatric ALL patients did not. To overcome this issue, SS1P was co-administrated with pentostatin and cytoxan to advanced chemorefractory mesothelioma patients. The result shows the production of anti-SS1P antibodies is delayed. Additionally, several patients with refractory mesothelioma had major and long-lasting responses to the treatment. The responses were accompanied by evidence that the tumors were infiltrated with inflammatory cells, which implied that the drug combination was inducing anti-tumor immunity.

The possibility that SS1P treatment was inducing anti-tumor immunity was further explored in a mouse model using mice that express and tolerate human MSLN so that the mouse tumors expressing human MSLN could grow in the mice. They found that when 66C14-M mouse tumors growing subcutaneously were directly injected with SS1P and also given an anti-CTLA-4 antibody with intraperitoneal injection, there was a strong synergistic effect resulting in the complete regressions of injected and un-injected second tumors and anti-tumor immunity. This discovery has been translated into clinical trials by combining an anti-MSLN immunotoxin with a checkpoint inhibitor [14]. Moreover, Dr. Pastan and his colleagues have recently developed an anti-CD25 immunotoxin that kills mouse cells expressing CD25. It contains an anti-mouse CD25 Fv. They reported that injection of this agent into mouse tumors depleted Tregs and caused regression of injected as well as distant un-injected tumors. It also induced anti-tumor immunity. Their data also showed this anti-CD25 immunotoxin worked in three different cancer models (colon, breast and mesothelioma) to induce anti-tumor immunity. These findings will be translated into a clinical trial.

DEVELOPMENT OF NEW-GENERATION TECHNOLOGIES FOR MANUFACTURING OF ANTIBODIES

Dr. Scott Liu from Henlius gave a talk on the development of new-generation technologies for manufacturing antibodies. First, Liu shared his vision and mission on making antibody therapeutics affordable by developing new-generation manufacturing technologies and lowering the manufacturing cost of biosimilars and innovative antibodies. Manufacturing of biologics involves complex biological and biochemical engineering technologies. A consistent and well-controlled process determines the production of drugs that have consistent batch-to-batch and year-to-year high quality. However, current biologic manufacturing still relies on individual unit operation, i.e. batch bioprocess, instead of continuous bioprocess, i.e. one-piece flow. Therefore, there is a great need to develop the continuous manufacturing process to increase manufacturing robustness and reliability, reduce manufacturing cost and capital expenditure, reduce new facility building time and reduce product changeover time [15].

Next, Liu shared the progress on development of continuous processing of Helius. The company integrated the application of alternating tangential flow (ATF) into the upstream process development. Specifically, they ran the bioreactor at a fixed volume with a constant flow of media, combined with a cell retention device, such as ATF, to keep cell in a constant growth state. Liu's data demonstrate that the amount of antibody produced with continuous perfusion was significantly higher than that with traditional fed-batch approach. In addition, concentrated perfusion was even more effective to increase titer and reduce the production scale and cost. Additionally, Liu shared their efforts in developing continuous chromatography for downstream purification in order to achieve higher capacity usage, higher efficiency, lower buffer consumption and reduced capital investment. Instead of single column, they used multiple parallel columns in continuous mode. Their data have shown that the continuous chromatography not only has stable performance, shorter cycle time and higher loading capacity, it also led to increased productivity and improved usage of resins.

At last, Liu summarized the opportunities and challenges of the continuous process. The continuous bioprocess reduced hold steps, improved facility utilization and reduced capital investment with less contamination risk, less deviation and high integrity. However, there are still several challenges ahead, such as high upfront investment, core tech and experiences barrier in China, high startup costs, new control and validation strategy and regulatory uncertainties.

NEXT-GENERATION ANTIBODY THERAPEUTICS BASED ON NOVEL TRANSGENIC MOUSE PLATFORMS

Dr. Atul Deshpande from Harbour BioMed gave an introduction on the next-generation antibody therapeutics based on novel transgenic mouse platforms. Dr. Deshpande started his talk by discussing the importance of next-generation therapeutics in the drug discovery and commercialization. Despite the success in the current drug development, challenges remain in the form of unmet medical needs across the world, such as limited response in many disease and patient populations or good efficacy but significant side effects. To address these challenges, there are three emerging trends to develop next-generation therapeutics: development of human antibody generation platform in species other than mouse; genetic manipulation of the immune system to yield unconventional antibody repertoires; generation of novel transgenic mouse systems that produce non-canonical antibodies [16]. Among them, the transgenic mouse platform has created significant amount of values to the market. In 2018 sales report, all the different drugs which were discovered with transgenic mouse platform have reached over $20 billion. Therefore, if these platforms are used properly, they could add significant value to the biopharmaceutical industry and more importantly to the patients.

Dr. Deshpande also shared the heavy-chain-only antibody (HCAb) transgenic mouse platform in Harbour BioMed with the audience. The HCAb mice present a versatile platform for generation of specific human heavy-chain-only antibodies. It can be used to generate fully human HCAbs, building blocks for bispecific antibodies and generate fully human single-domain antibodies. Furthermore, Dr. Deshpande discussed how HCAb greatly expanded the landscape of bispecific antibodies with innovative formats. Their research data shared by Dr. Deshpande showed that symmetric bispecific antibodies generated by HCAb had significantly higher solubility, higher yields, higher thermostability, similar purity to monoclonal IgG and similar binding affinity to their monoclonal parent antibodies. In addition, HCAbs also have added advantages in generating asymmetric formats, such as no light chain, no scFv introduced, increased avidity or epitope accessibility by tandem VH and smaller size than IgG.

In the last piece of his presentation Dr. Deshpande shared HBM's own data on HCAb based anti-CTLA-4 monoclonal antibody, HBM4003–2, as a proof of concept story in IND enabling studies. HBM4003–2 is an HCAb-based next-generation anti-CTLA4 antibody. In preclinical studies, its potency appears to be better than ipilimumab analog in binding to CTLA-4, thereby blocking its binding to its B7–1 and B7–2 ligands. It also showed excellent SEC profiles in high concentration formulation and in heat challenge test. Overall, HBM4003–2 HCAb shows a superior Treg depletion and T-cell activation *in vitro* and *in vivo* with a very promising safety profile in monkey. Therefore, HCAb platform can be used to generate antibodies with higher efficacy and safety profiles.

In summary, HCAb platform is potentially an essential tool for next-generation drug discovery. It possesses several advantages over traditional antibody discovery platform, such as versatile formats to adapt with different applications and MOAs, targeting challenging epitopes, compatibility with regular antibody for bispecifics, flexibility to be designed as asymmetric to facilitate downstream separation and homogenous or symmetric format to avoid heavy-chain mispairing. Thus, the HCAb platform presents an exciting opportunity to help design and produce multi-specific and multiformat antibodies that could potentially lead to the discovery and development of the next generation of antibody therapeutics to better address unmet patients' needs.

TRANSLATING HUMAN IMMUNOLOGY TO MEDICINE

Dr. Yong-Jun Liu from Sanofi gave a talk on translating human immunology to medicine by sharing several examples of his own research on how our discovery in human immunology leads to medicine development in the clinics.

The first example he shared with the audience was the research discovery of CD40/CD40L, one of the hot targets for immunotherapy against autoimmune diseases. In 1991, two research groups published their sequencing data and found that antibody affinity maturation occurred within the germinal center through somatic mutations during the B-cell proliferation [17, 18]. However, it was still unknown how the immune system selected the high-affinity mutants. Liu and his colleagues found that B cells in the germinal center were programmed to die if they were not selected. Centrocytes can be prevented from entering apoptosis if they are activated both through their receptors for antigen and a surface glycoprotein recognized by CD40 antibodies [19]. CD4 follicular T helper cells express CD40L, which provides survival signal for germinal center B cells. Knock-outs of CD40L result in the lack of the germinal center. In addition, CD40 signaling co-stimulates human dendritic cells (DCs) to mature and produce IL-12 [20, 21]. Therefore, many companies are developing antibodies against CD40/CD40L to treat autoimmune disease.

During that study, Liu also discovered and isolated the plasmacytoid dendritic cells (pDC), which is a very important cell type of innate immune system. pDC can sense viral DNA and RNA through toll-like receptor 7 and 9 and produce interferon upon viral infection. In addition, they can be activated by self DNA or RNA released by dead cells [22]. ILT7, one of its receptors, downregulates the receptor-mediated innate immune response, serving as a safe guard [23]. Liu *et al*. generated antibodies against immunoglobulin-like transcript 7 (ILT7). This antibody (MEDI7734), which was licensed to Medimmune (now AstraZeneca), is now under clinical trial.

Another example Liu shared with the audience was how the early discovery research of thymus stromal cell-derived lymphopoietin (TSLP) was translated into promising immunotherapies. Although early studies on TSLP in the mouse model were not encouraging, Liu's research group discovered that TSLP links epithelial cells to DCs by allergic inflammation [24]. TLSP is now revealed to be an important regulator of DC-mediated control of Th2-based human allergic responses [24, 25]. TSLP triggers DCs to produce OX40L, which is very critical for polarizing T-cells to produce all the inflammatory Th2 and meanwhile shutting down the IL10 production, implying that both TLSP and OX40 are very important targets for allergic inflammation. Ten years after the first discovery research, the clinical trial results from phase 2b studies published by Amgen and Medimmune have shown that anti-TLSP antibodies reduce the exacerbation of severe asthma by 70% [26].

The final example Liu shared with the audience was OX40/OX40L. Since OX40L shuts down IL-10-producing regulatory T-cells [27], Liu has been focusing on developing anti-OX40L immunotherapy. Liu et al. produced multiple monoclonal antibodies to target OX40 and found that the combination of anti-OX40 and anti-CG-enriched oligodeoxynucleotide worked more efficiently in mouse model and human patients [28]. GSK and Merck initiated phase I trial of this antibody (GSK3174998) and it is under phase 2 clinical trial now.

At the end of his talk, Liu summarized how his early discovery research on CD40/CD40L, TLSP/TLSPR and OX40 has been translated into promising immunotherapies. He believed that our early discovery of human immunology would continue to be translated into clinical programs and immunotherapies in the future.

AFTERNOON PANEL DISCUSSION: THE TRENDS, CHALLENGES AND OPPORTUNITIES OF THERAPEUTIC ANTIBODY CMC

Although many therapeutic antibodies developed in China are moving to the clinical stage, CMC becomes more important and interests China's biopharmaceutical professionals. A topic focused on therapeutic antibody CMC was discussed. The panel moderator Dr. Steve Lee, the CEO of BioGENEXUS LLC, first introduced CMC and the current trends and challenges of CMC. CMC is an FDA term used globally to describe the data for the manufacture and testing of a medicinal product. It is an integral part of entire life cycle management of a medicinal product including drug development, regulatory submission and marketing. As drug development moves from concept to commercialization, the breadth and depth of CMC documentation required in submissions increases in parallel. All regulatory submissions, including IND, BLA, CTA, MAA, NDA and annual reports, require CMC data.

The critical CMC elements of regulatory submission includes characterization of the drug substance (DS) and drug product (DP), raw materials used to manufacture the DS and DP, description of process development and manufacturing processes, release and stability testing data for both DS and DP, analytical methods and specifications used for testing and release, in-process controls and container and closure systems. Because of the complexity of therapeutic antibody, CMC is essential for antibody drug development from cell line development, cell culture process development to purification and formulation.

With fast development of CMC in the past 10 years in the USA and China, current trends of CMC are single-use technology (SUT), continuous manufacturing, automation or artificial intelligence for quality and operations and process analytical technology systems. Four panelists—Dr. Gang Chen, VP from Regeneron, Dr. Christopher Hwang, CTO from Transcenta, Dr. John (Jack) Prior, Head of Manufacturing Science from Sanofi and Dr. Chun Zhang, senior VP from Evelo Bioscience—shared their experiences and insights in CMC, especially cell line development, SUT and continuous manufacturing.

Chinese hamster ovary cell is the major cell line used for the production of biological drugs. It is always a challenge to transition drug candidates to the clinic while assuring clonality and stability of cell lines with robust therapeutic protein production. Master cell banks may be a strategy to minimize the risk. In addition, a significant barrier to access such biologics as antibody drugs is the expensive price of biologics for the countries in Asia and Africa. SUT in CMC is a trend to adapt the fast development of therapeutic antibody. Comparing with the traditional stainless-steel tank, it has many advantages, such as disposable facility without cleaning requirement, fast batch turn-around, low capital investment and improved process flexibility. Moreover, SUT may be more appropriate for production of drugs treating small patient population with rare diseases. Continuous manufacturing in CMC was also discussed. Automation in biomanufacturing process could significantly reduce time and cost although integration of manufacturing sub-processes still remains a challenge.

ACKNOWLEDGMENTS

Dr. Mitchell Ho's work is supported by the Intramural Research Program of the National Institutes of Health, NCI and the Center for Cancer Research. The authors thank the conference speakers for reviewing the manuscript and Jessica Hong (NCI) for editorial assistance. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products or organizations imply endorsement by the US Government.

ABBREVIATIONS:

ADC	antibody–drug conjugate;
AEP	Antibody Engineering Program;
ALL	acute lymphoblastic leukemia;
ATF	alternating tangential flow;
BCR	B-cell receptor;
BCMA	B-cell maturation antigen;
BiTE	bispecific T-cell engagers;
BLA	biologics license applications;
BsAb	bispecific antibody;
CAR-T	chimeric antigen receptor T-cells;
CAS	Chinese Antibody Society;
CPB	checkpoint blockade;
CGRP	calcitonin gene-related peptide;
CMC	chemistry, manufacturing and controls;
CTA	clinical trial application;
CTLA-4	cytotoxic T lymphocyte-associated protein 4;
DCs	dendritic cells;
FDA	US Food and Drug Administration;
GPCs	cell-surface glypicans;
HCAb	heavy-chain-only antibody;
HCL	hairy cell leukemia;
IO	immuno-oncology;
IND	investigational new drug;
MAA	marketing authorization application;
MOA	mechanism of action;
MSLN	mesothelin;
NDA	new drug application;
NSCLC	non–small-cell lung cancer;
PD-1	programmed cell death protein 1;
PD-L1	programmed death-ligand 1;
PE	Pseudomonas exotoxin A;
PK	pharmacokinetics;
RIT	recombinant immunotoxin;
SUT	single-use technology;
TCR	T-cell receptor;
TSLP	thymus stromal cell-derived lympopoirtin;

REFERENCES

1. Ho, M. Advances in liver cancer antibody therapies: a focus on glypican-3 and mesothelin. *BioDrugs* 2011; **25**: 275–84.
2. Gao, W, Ho, M. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. *Cancer Rep* 2011; **1**: 14–9.
3. Li, N, Gao, W, Zhang, YF *et al.* Glypicans as cancer therapeutic targets. *Trends Cancer* 2018; **4**: 741–54.

4. Li, N, Wei, L, Liu, X *et al.* A frizzled-like cysteine-rich domain in Glypican-3 mediates Wnt binding and regulates hepatocellular carcinoma tumor growth in mice. *Hepatology* 2019.
5. Feng, M, Gao, W, Wang, R *et al.* Therapeutically targeting glypican-3 via a conformation-specific single-domain antibody in hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2013; **110**: E1083–91.
6. Gao, W, Tang, Z, Zhang, YF *et al.* Immunotoxin targeting glypican-3 regresses liver cancer via dual inhibition of Wnt signalling and protein synthesis. *Nat Commun* 2015; **6**: 6536.
7. Li, N, Fu, H, Hewitt, SM *et al.* Therapeutically targeting glypican-2 via single-domain antibody-based chimeric antigen receptors and immunotoxins in neuroblastoma. *Proc Natl Acad Sci U S A* 2017; **114**: E6623–31.
8. Feng, M, Bian, H, Wu, X *et al.* Construction and next-generation sequencing analysis of a large phage-displayed VNAR single-domain antibody library from six naive nurse sharks. *Antibody Therap* 2019; **2**: 1–11.
9. Brinkmann, U, Kontermann, RE. The making of bispecific antibodies. *MAbs* 2017; **9**: 182–212.
10. Labrijn, AF, Janmaat, ML, Reichert, JM *et al.* Bispecific antibodies: a mechanistic review of the pipeline. *Nat Rev Drug Discov* 2019; **18**: 585–608.
11. Bargou, R, Leo, E, Zugmaier, G *et al.* Tumor regression in cancer patients by very low doses of a T cell-engaging antibody. *Science* 2008; **321**: 974–7.
12. Muller, MR, Saunders, K, Grace, C *et al.* Improving the pharmacokinetic properties of biologics by fusion to an anti-HSA shark VNAR domain. *MAbs* 2012; **4**: 673–85.
13. Shancer, Z, Liu, XF, Nagata, S *et al.* Anti-BCMA immunotoxins produce durable complete remissions in two mouse myeloma models. *Proc Natl Acad Sci U S A* 2019.
14. Leshem, Y, O'Brien, J, Liu, X *et al.* Combining local Immunotoxins targeting Mesothelin with CTLA-4 blockade synergistically eradicates murine cancer by promoting anticancer immunity. *Cancer Immunol Res* 2017; **5**: 685–94.
15. Zydney, AL. Continuous downstream processing for high value biological products: a review. *Biotechnol Bioeng* 2016; **113**: 465–75.
16. Chen, WC, Murawsky, CM. Strategies for generating diverse antibody repertoires using transgenic animals expressing human antibodies. *Front Immunol* 2018; **9**: 460.
17. Berek, C, Berger, A, Apel, M. Maturation of the immune response in germinal centers. *Cell* 1991; **67**: 1121–9.
18. Jacob, J, Kelsoe, G, Rajewsky, K *et al.* Intraclonal generation of antibody mutants in germinal centres. *Nature* 1991; **354**: 389–92.
19. Liu, YJ, Joshua, DE, Williams, GT *et al.* Mechanism of antigen-driven selection in germinal centres. *Nature* 1989; **342**: 929–31.
20. Grouard, G, Durand, I, Filgueira, L *et al.* Dendritic cells capable of stimulating T cells in germinal centres. *Nature* 1996; **384**: 364–7.
21. Rissoan, MC, Soumelis, V, Kadowaki, N *et al.* Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 1999; **283**: 1183–6.
22. Gilliet, M, Cao, W, Liu, YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. *Nat Rev Immunol* 2008; **8**: 594–606.
23. Cao, W, Rosen, DB, Ito, T *et al.* Plasmacytoid dendritic cell-specific receptor ILT7-fc epsilonRI gamma inhibits toll-like receptor-induced interferon production. *J Exp Med* 2006; **203**: 1399–405.
24. Soumelis, V, Reche, PA, Kanzler, H *et al.* Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol* 2002; **3**: 673–80.
25. Leonard, WJ. TSLP: finally in the limelight. *Nat Immunol* 2002; **3**: 605–7.
26. Corren, J, Parnes, JR, Wang, L *et al.* Tezepelumab in adults with uncontrolled asthma. *N Engl J Med* 2017; **377**: 936–46.
27. Ito, T, Wang, YH, Duramad, O *et al.* OX40 ligand shuts down IL-10-producing regulatory T cells. *Proc Natl Acad Sci U S A* 2006; **103**: 13138–43.
28. Voo, KS, Foglietta, M, Percivalle, E *et al.* Selective targeting of toll-like receptors and OX40 inhibit regulatory T-cell function in follicular lymphoma. *Int J Cancer* 2014; **135**: 2834–46.