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Meeting Reports

Highlights of 2019 Protein Engineering Summit (PEGS) in Boston, USA: advancing antibody-based cancer therapies to the clinic

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Abstract

The 15th Annual Protein Engineering Summit (PEGS) organized by Cambridge Healthtech Institute was held in Boston, USA, from 8 to 12 April 2019. This report highlights the presentations in the Oncology Stream of this meeting with a focus on bispecific antibodies (BsAbs). A variety of BsAb formats with different target antigens (CD3, CTLA4, PD-1, PD-L1, EGFR, HER2, BCMA, CD19, CD20, CD38, CD123, TGFβ, PSMA, etc.) have been discussed, in which the T-cell engaging (anti-CD3) BsAb is the most studied construct to exhibit promising immunotherapeutic activities. The BsAb formats include IgG-like structures or antibody fragments composed of antigen-binding sites only. Preclinical and clinical data from different BsAbs demonstrated the potential therapeutic applications in various solid tumors and hematological malignancies. The ongoing development of BsAb formats will help overcome current clinical issues, such as tumor selectivity and antigen coverage. This report also covers several presentations about emerging targets (e.g. mesothelin, CD47) and new technologies in the field of antibody engineering and therapeutics.

Statement of Significance: The 15th Annual Protein Engineering Summit (PEGS) was held in Boston, USA, from 18 to 12 April 2019. This meeting report highlights about 20 presentations with a focus on bispecific antibodies (BsAbs) for treating solid tumors. Among BsAbs, T-cell redirection is the most common design.

KEYWORDS: antibody platform; bispecific antibodies; bispecific T-cell engager; bivalent; cancer immunotherapy; cytotoxicity; T-cell engager; tumor-associated antigen

ANTIBODIES FOR CANCER THERAPY

8 April 2019: Day 1—opening keynote session

Soldano Ferrone (Harvard University School of Medicine) chaired the opening session. Dr F. Stephen Hodi (Dana-Farber Cancer Institute) presented their recent research work and provided insights into the mechanisms of anti-CTLA-4 and anti-PD-1 blockade resistance in metastatic melanoma. Combination of CTLA-4 and PD-1 blockades improves outcomes in advanced melanoma patients as compared with either monotherapy. However, the mechanism of biologic effects of inhibiting two checkpoints is still not fully understood. By analysis of the biopsy samples from patients enrolled in two independent Phase II clinical trials (CheckMate 064 and CheckMate 069, respectively), Dr Hodi's group found that patients with lower MHC I expression (<30%) were more likely to be resistant to anti-CTLA-4, but not to anti-PD-1 therapy. The response to anti-PD-1 therapy was predicted by MHC II expression (>1%) on melanoma cells. In addition, Dr Hodi showed that IFNγ gene signature, innate immunity signaling and IL-15 were associated with response to

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anti-PD-1 blockade, but not to anti-CTLA-4 blockade. These findings indicate that MHC I expression level may predict the response to anti-CTLA-4 blockade for cancer immunotherapy, while MHC II expression level may serve as a biomarker for anti-PD-1 blockade therapy [1]. These findings may also explain why combination of anti-CTLA-4 and anti-PD-1 is more effective in certain cancers.

Dr Daniel Chen from IGM Biosciences discussed the biological problems in cancer immunotherapy and the engineering solution to induce broader anti-tumor activity. He introduced three immune phenotypes: (1) immune excluded, CD8+ T cells absent from tumor and its periphery, (2) immune excluded, CD8+ T cells present but not effectively infiltrated and inflamed and (3) CD8+ T cells infiltrated, but inhibited. He also gave several examples of potential solutions to these problems [2]. The bifunctional anti-PD-L1/TGFβ Trap fusion protein (M7824) [3] is used to target the cancer microenvironment to increase T cell filtration. The anti-FAP-4-1BBL fusion protein could potentially stimulate T cells only in tumors [4]. Finally, he presented the preclinical development of IgM antibody platform for cancer treatment. The IgM-based anti-CD20-CD3 BsAb has higher affinity to CD20-expressing cells compared to IgG based anti-CD20-CD3 BsAb through avidity and shows higher potency in CD20-expressing cell killing assays.

8 April 2019: Day 1—targeting B7/H3: comparison of different approaches

Dr Daniel A. Vallera (University of Minnesota Masonic Cancer Center) introduced their trispecific NK cell engager (TriKE) platform which is genetically modified to add hIL-15 to the bispecific NK cell engager (BiKE) molecule. Due to the bioactivity of IL-15, TriKE dramatically augments NK cell expansion, survival and cytotoxicity *in vitro*. In addition, they replaced the anti-CD16 scFv engager portion with humanized camelid VHH which improved the affinity, yield and NK cell expansion while avoiding intramolecular disulfides. By using their TriKE platform, they developed several tumor-targeting products, including CD16 × IL15 × CD33, CD16 × IL15 × B7H3 and CD16 × IL15 × EpCAM. All these molecules are currently in the discovery and preclinical stages. CD16 × IL15 × CD33 TriKE has shown enhanced *in vivo* protection against myeloid malignancies in mouse models [5]. CD16 × IL15 × B7H3 TriKE shows impressive anti-tumor activity across a wide range of carcinomas and demonstrates efficacy against ovarian cancer. Dr Vallera's group plans to move CD16 × IL15 × B7H3 TriKE forward to IND stage for the Phase I trial.

Dr Deryk Loo (Macrogenics) presented their preclinical development of MGC018, an ADC comprised of the cleavable linker-duocarmycin payload, valine-citrulline-seco-DUocarmycin hydroxyBenzamide Azaindole (DUBA), conjugated to a humanized anti-B7-H3 (also called CD276) antibody through interchain disulfides. The DUBA linker-payload technology and conjugation were provided by Synthon Biopharmaceuticals. By screening more than 1400 tumor samples, they found that majority of B7-H3-positive tumors express high levels of B7-H3. MGC018 showed potent tumor killing activity and bystander effect *in vitro*. Single doses or repeat doses of MGC018 have efficacy both in tumor models derived from cell lines, e.g. MDA-MB-468 (breast cancer), A375.2 (melanoma), Calu-6 (lung cancer) and PA-1 (ovarian cancer), and in patient-derived xenograft models (prostate cancer and triple-negative breast cancer). Although MGC018 has fast PK in rodents due to the rodent-specific carboxylesterase, CES1c, which degrades DUBA, it is highly stable in cynomolgus monkeys. A GLP toxicology study shows that the highest tolerated dose is 10 mg/kg. Based on those preclinical studies, a Phase I clinical study of MGC018 (NCT03729596) has been initiated.

April 9: Day 2—mesothelin-targeted therapies in solid tumors

Dr Mitchell Ho (National Cancer Institute, NIH) chaired the session on mesothelin-targeted therapies in solid tumors and emerging targets. Mesothelin is a tumor differentiation antigen with limited expression on normal mesothelial cells, but is highly expressed in various cancers [6,7].

Dr Prasad Adusumilli (Memorial Sloan-Kettering Cancer Center) presented his work on the development of anti-mesothelin chimeric antigen receptor (CAR) T cells to treat malignant pleural mesothelioma [8]. They designed several different CAR constructs with a fully human anti-mesothelin antibody and picked the most promising candidate. His group has found that intrapleural administration of CAR T cells has better efficacy compared to intravenous administration. Phase I clinical trials showed that mesothelin-targeted CAR T cell therapy is well tolerated, and the results were presented at the 2019 AACR annual meeting [9].

Dr Raffit Hassan (National Cancer Institute) presented his clinical work on the recombinant immunotoxins targeting mesothelin [10,11]. Dr Mark O'Hara (University of Pennsylvania) presented his clinical research on the pancreatic cancer therapy with mesothelin-redirected CAR T cells [12].

April 9: Day 2—emerging targets

Dr Ira Pastan (National Cancer Institute) presented their work on the new anti-BCMA recombinant immunotoxins in the morning session [13]. In the afternoon session, Dr David D. Roberts (National Cancer Institute) gave a comprehensive review of CD47 target for cancer therapy: "Strategies and challenges for targeting CD47 to enhance antitumor immunity". His presentation covered (1) the anti-tumor mechanism of CD47 blockades, (2) anti-tumor activities of CD47 antibody and related ongoing therapeutics in clinical trials, (3) challenges of anti-CD47 blockade as the anti-cancer therapeutics and (4) strategies to enhance the anti-tumor effect of targeting CD47. As of April 2019, at least five humanized CD47 antibodies (Hu5F9-G4A, CC90002, IBI188, SRF231, AO-176) and two signal regulatory protein α (SIRPα) fusion proteins (TTI-621, TTI-622, ALX148) are at the stages of clinical trials. Dozens of CD47-related blockades and bispecific antibodies are under preclinical

development. The basis of CD47 blockade inhibiting tumor growth is the "don't eat me" SIRPα mechanism on macrophages. However, accumulating evidence shows that CD47 is also a T cell and NK cell immune checkpoint by thrombospondin-1 (TSP-1, another CD47 ligand) signaling. The efficacy of CD47 blockade in several types of tumor models requires T cells. Therefore, not only innate immune but also adaptive immune participates in the anti-tumor effect of CD47 blockade. In addition of the immune modulation effect, CD47 antibodies directly alter signaling in cancer cells. CD47 is expressed on normal cells, in particular on erythrocytes; TSP-1, a ligand of CD47, can bind to CD47. Thus, not surprisingly, CD47 blockade with Hu5F9-G4 from a Phase 1 clinical trial results (NCT02216409) showed side effects (57% anemia and 17% thrombocytopenia) [14]. To enhance the efficacy and avoid adverse side effects, ligand-specific CD47-blocking antibodies were developed. CC2C6 only binds to the pyro-Glu isoform of CD47 which is the SIRPα-binding site and does not interact with TSP-1 [15]. Targeting this specific posttranslational modification of CD47 may provide an approach to avoid side effects induced by TSP-1 binding.

ADVANCING BISPECIFIC ANTIBODIES AND COMBINATION THERAPY TO THE CLINIC

April 10: Day 3—what is working in the clinic: new innovations in bispecific antibodies

The morning session on 10 April 2019 entitled "Advancing Bispecific Antibodies and Combination Therapy to the Clinic" was chaired by Dr Rakesh Dixit (AstraZeneca) (Table 1). Dr Dixit gave an update of bispecific antibodies (BsAbs) and antibody-drug conjugates (ADCs). He emphasized the advantage of oncology immune-mediated therapy of cancer (IMTC) and presented that anti-tumor activities can be promoted by targeting critical phases in cycles: (1) antigen presentation + innate immunity, (2) T cell activation and (3) tumor microenvironment. In addition, he compared the BsAb versus combination of biologicals. In the end, he indicated that the future direction for BsAb research will be on right target, right format/design and right translational strategy[16].

Dr Charlotte Russell (Alligator Bioscience AB) introduced ATOR-1015, the tumor-directed CTLA-4 × OX40 BsAb. He presented preclinical data about ATOR-1015, including the design, rational, binding to both targets simultaneously, T cell activity induction and its superior effect compared to monoclonal antibodies. He demonstrated that the dual targeting directs the effect to the tumor area, allowing ATOR-1015 to induce enhanced anti-tumor effects with expected lower systemic toxicity compared to CTLA-4 monotherapy. The mode of action (MOA) is a combination of effector T cell activation and regulatory T cell depletion [17]. He also showed the preclinical safety study results. The Phase I clinical study was already started, and the first patient was dosed on 9 March 2019 (NCT03782467).

Dr Yariv Mazor (AstraZeneca) introduced MEDI5752, a monovalent bispecific IgG1 antibody (DuetMab) which targets both PD-1 and CTLA-4. He showed that MEDI5752 preferentially targets and saturates CTLA-4 on dual-positive cells, suggesting selective targeting of tumor-infiltrating lymphocytes (TILs) over peripheral T cells. MEDI5752 induces significant downregulation and degradation of the PD-1 receptor upon coupling to recycling CTLA-4. He also presented that MEDI5752 leads to the internalization and subsequent degradation of PD-1 by tethering CTLA-4 to PD-1 [18]. MEDI5752 induces antigen-specific T cell-dependent tumor killing in mouse model. MEDI5752 is currently in the Phase I trial in selected patients with advanced solid tumors (NCT03530397).

Dr Eugene Zhukovsky (Biomunex Pharmaceuticals) introduced a versatile modular bispecific antibody platform (BiXAb), for the development of innovative therapeutics. The BiXAb platform has a tetra-Fab IgG1 antibody structure and enables plug-and-play BsAb formatting from any pair of monospecific mAbs. The platform involves minimal antibody engineering and eliminates the need for screening. It can be tetravalent or bivalent with or without Fc. He illustrated the properties of this BsAb platform by presenting two case studies, BMX-002 (anti-EGFR and HER2) and BMX-101 (anti-CD38 and PD-L1). The indications for these two BsAbs are solid tumors (head and neck, gastric, colon cancers) and hematological malignancies, respectively. The design rational for BMX-002 is to block all signaling pathways of the ErbB family to overcome resistance. He indicated that this BsAb can be expressed and purified with high purity. For BMX-101, Dr Zhukovsky showed that this BsAb can be expressed in CHO cell at high levels without aggregation. BsAb also possesses excellent thermal stability (T_{onset} = 63.3°C) and binds to the cell and soluble cognate antigens comparable to parental monoclonal antibodies (mAbs). Biomunex Pharmaceuticals has signed a licensing agreement with Sanofi in January 2019, and Sanofi will use their platform to develop bi-specific and multi-specific antibody therapeutics.

Dr Ronald Herbst (AstraZeneca) gave a keynote speech "The Need for More Effective Combination Therapies". He remarked that combination approaches are the key to improving clinical response. From preclinical immune-oncology (IO) mouse models to patients enrolled in clinical trials, novel high-throughput technologies enable researchers to understand the mechanisms underlying the complex interactions between the immune system and cancer, identify predictive biomarkers for the patients who will most likely benefit from current immunotherapies, avoid immune-related adverse events and guide the future combination cancer immunotherapy. He listed the checkpoint combinations with other checkpoints (PD-1/PD-L1 + CTLA4), TNF family agonists (CD40, CD137, OX40, CD27), targeted therapies, vaccines, adoptive cellular therapy, etc. Dr Herbst summarized the considerations for IO combinations: safety, dose and schedule, differences between mouse and human immunology, target patient population and co-expression of targets and tumor mutation burden (TMB).

Prof. Wijnand Helfrich (University Medical Center Groningen) introduced a tetravalent BsAb PD-L1

Table 1. Summary of the presentations about bispecific antibodies in 2019 PEGS

Speakers	Companies/ institutions	Drug names	Targets	Formats	Indications	Status
Rakesh Dixit	AstraZeneca	**Chairperson's Opening Remarks (4/10/2019)**				
Charlotte Russell	Alligator Bioscience AB	ATOR-1015	CTLA-4 × OX40	bsAb	Solid tumor neoplasms	Phase I NCT03782467
Yariv Mazor	AstraZeneca	MEDI5752	PD-1 × CTLA-4	DuetMab	Advanced solid tumors	Phase I NCT03530397
Eugene Zhukovsky	Biomunex	BMX-002	EGFR × HER2	BiXAb	H&N, gastric, colon cancers	Preclinical
		BMX-101	CD38 × PD-L1	BiXAb	Hematological malignancies	Preclinical
Ronald Herbst	AstraZeneca	**KEYNOTE PRESENTATION**				
Wijnand Helfrich	Univ Medical Center Groningen	NA	PD-L1 × EGFR	Tetravalent	Tumor	Discovery
Eric Smith	Regeneron	REGN1979	CD20 × CD3	NA	CD20+ B-cell malignancies	Phase II NCT03888105
		REGN5458	BCMA × CD3	NA	MM	Phase I/II NCT03761108
		REGN4018	Muc16 × CD3	NA	Ovarian cancer	Phase I NCT03564340
Jing Li	WuXi Biologics	NA	CD3 × CD19	WuXiBody	Tumor	Preclinical
Timothy Xia	GenScript	NA	PD-1 × CTLA-4	SMAB	Tumor	Discovery
Tony Polverino	Zymeworks	ZW25	HER2 ECD4/ECD2	Azymetric	Gastroesophageal adenocarcinoma	Phase II NCT03929666
		ZW49	ZW25 ADC	Azymetric ZymeLink	HER2-expressing cancers	Phase I NCT03821233
Sebastian Grimm	Molecular Partners	MP0250	VEGF × HGF	DARPin	MM in Relapse	Phase 2 NCT03136653
		MP0274	Her2 2 epitopes	DARPin	Her2+ cancer	Phase I NCT03084926
Nathan D. Trinklein	TeneoBio	Many	TAA × CD3	biTE	Cancers	Discovery, preclinical, IND
Roland Kontermann	University of Stuttgart	**KEYNOTE PRESENTATION (4/11/2019)**				
James L. Gulley	NCI, NIH	M7824	TGFβ × PD-L1	bsAb	Advanced solid tumors	Phase I/II NCT03493945
Brian McGuinness	Crescendo Biologics	CB307	PSMA × CD137 × HSA	Humabodies	PSMA-positive tumor	Preclinical
Thomas T Poulsen	Symphogen A/S	Sym015	Nonoverlapping MET epitopes	Antibody mixture	Oncology, NSCLC	Phase I/II NCT02648724
Rajkumar Ganesan	Janssen	JNJ-63709178	CD123 × CD3	Genmab's Duobody	AML	Phase I NCT02715011
		JNJ-64007957	BCMA × CD3	Genmab's Duobody	Hematological malignancies	Phase I NCT03145181
		JNJ-64407564	GPRC5D × CD3	Genmab's Duobody	Hematological malignancies	Phase I NCT03399799
Christine E. Engeland	NCT diseases, Germany	NA	CD20 × CD3	MV-BiTE	Solid tumor	Preclinical
		Antibodies for Cancer Therapy (April 8)				
Daniel A. Vallera	Univ. of Minnesota	NA	CD16 × IL15 × CD33 CD16 × IL15 × B7H3 CD16 × IL15 × EpCAM	TriKEs	cancers	Discovery and preclinical
		Emerging antibodies (April 11)				
Christoph Spiess	Genentech	BTRC4017A	Her2 × CD3	TDB	Solid tumors	Phase I

× EGFR, which simultaneously binds to PD-L1 and EGFR resulting in enhanced avidity towards PD-L1+/EGFR+ cancer cells. He demonstrated that PD-L1 × EGFR blocks PD-1/PD-L1 interaction in an EGFR-directed manner, blocks oncogenic EGFR-signaling and promotes antibody-dependent cellular cytotoxicity (ADCC) of EGFR+ tumor cells. He suggested that this BsAb may be useful to enhance selectivity, efficacy and safety of PD-1/PD-L1 checkpoint inhibition.

Dr Eric Smith (Regeneron) described Regeneron's bispecific platform and preclinical data on several new bispecifics being developed for solid and liquid tumor indications. He presented the status of Regeneron's multiple clinical stage BsAbs: REGN1979, REGN5458 and REGN4018.

Regeneron's bispecific platform combines a single "common" light chain and one heavy chain (HC*) with two mutations in CH3 (H435R, Y436F, corresponding to IgG3 amino acid) to introduce asymmetric protein A binding. The modified IgG1 backbone allows selective elution of heterodimers (HC × HC*) from Protein A at mild pH >4 (HC* × HC* does not bind to Protein A). Common light chain ensures correct light chain pairing, and the Fc region can be modified to reduce effector function. He displayed the construct of REGN1979, a fully human CD20 × CD3 BsAb. REGN1979 was designed to eliminate CD20+ B cell lymphomas by engaging T cells to directly kill the CD20 expressing B cell [19]. This drug is under Phase II clinical trial now (NCT03888105). The second BsAb Dr Smith explained is REGN5458, a BCMA × CD3 BsAb for the potential treatment of multiple myeloma (MM). BCMA × CD3 BsAb shows potent *in vitro* and *in vivo* activity against MM cell lines and primary cells, and it is well-tolerated and depleted in BCMA+ plasma cells in cynomolgus monkeys. Both REGN5458 and anti-BCMA CAR T cells show similar anti-tumor activities *in vitro* and *in vivo*. A Phase I/II trial has been initiated for REGN5458 in MM (NCT03761108). The third one is REGN4018, a Muc16 × CD3 BsAb, which showed potent *in vitro* activity against ovarian cell lines. REGN4018 was generally well tolerated in GLP toxicology studies and demonstrated efficacy in multiple *in vivo* ovarian tumor models. A Phase I trial was initiated for REGN4018 in ovarian cancer in 2018 and dose escalation is ongoing (NCT03564340).

Dr Jing Li (WuXi Biologics) introduced the WuXiBody platform which is an innovative and versatile BsAb format by utilizing the constant region of human TCR α and β to facilitate correct VH and VL pairing. At the beginning, Dr Li talked about the limitations of many new BsAb platforms, such as yield, purity, stability, solubility, half-life and immunogenicity. Aiming to solve these issues, WuXi Biologics has generated WuXiBody™, a flexible, proprietary BsAb format that can reduce the development time by 6–18 months and decrease cost of goods by 90%. Through the case study of BsAb (CD3 × CD19), Dr Li demonstrated the developability, binding to target cells, T cell directed killing of tumor cells, *in vivo* efficacy and PK in cynomolgus monkey. In the end, Dr Li summarized that WuXiBody™ is a universal bispecific platform with good CMC feasibility and valency flexibility, and anti-CD3 × CD19 WuXiBody™ is a potent and safe BsAb with good developability and PK/PD profile.

Luncheon Presentation I. Dr Mark Paris (Mitra Biotech) introduced "CANscript™, a Phenotypic-based, Tumor Modeling Platform for Drug Discovery and Development". CANscript™ is an *ex vivo* platform technology using human patient materials (tumor tissues, autologous ligands and immune cells) to predict treatment efficacy of drugs across several classes. It is a unique platform that delivers powerful insight into mechanism of action, drug response and resistance. It can rule out ineffective treatments or treatment combinations early to avoid losing time and money and elucidate possible toxicity associated with failed courses of treatment. This test will add substantial guidance and valuable efficiency to the drug research and development process.

Luncheon Presentation II. Dr Timothy Xia (GenScript) presented GenScript's bispecific platform SMAB (Single-domain antibody fused to Monoclonal AntiBody). The key advantages of this platform are being "natural", symmetric design, stability and solubility, high affinity (pM range), superior ability to bind "hidden" epitope (GPCR, ion channel), high expression and flexible bi- or multi-specific designs. Dr Xia also introduced the Phage Display service for single domain antibody discovery and their own BsAb (PD-1 x CTLA-4). Since the mAb is the "keystone" to building SMAB, he further introduced GenScript's strong mAb R&D capability using a case study of Claudin 18.2, which is a difficult target with high homology to Claudin 18.1. He showed the strong binding affinity of humanized anti-Claudin 18.2 mAb relative to the benchmark-IMAB362 and superior binding specificity against Claudin 18.2 versus 18.1 by FACS.

Dr Tony Polverino (Zymeworks) gave a talk about the Azymetric™ platform, which consists of a suite of proprietary, transferable, amino acid changes that can be introduced to generate bispecific IgG-like antibodies binding two antigens. The core technology consists of amino acid changes in the monoclonal antibody CH3 domain to facilitate interaction of two distinct heavy chains. He showed that the proprietary amino acid changes are also introduced at the heavy-light-chain interfaces to facilitate the correct pairing of the heavy chains with their respective cognate light chains. The different chains can be co-transfected into mammalian cells, and the final BsAbs are correctly assembled, secreted and purified in high purity. Azymetric™ antibodies are compatible with glyco-engineering and other Fc modifications (e.g. the EFECT™ Platform) to enhance therapeutic activity. In addition, Dr Polverino showed two BsAb products from their pipeline. The first one is ZW25, a BsAb directed against two distinct epitopes (biparatopic, ECD4 and ECD2) on HER2. ZW25 has been successfully engineered using the Azymetric™ IgG1 antibody scaffold. In Phase I clinical studies (NCT02892123), ZW25 is well tolerated and has demonstrated promising single-agent anti-tumor activity in heavily pretreated HER2-expressing breast, gastric and other cancers. The second one is ZW49, a biparatopic ADC based on the unique design of ZW25 and armed with their proprietary ZymeLink™ cytotoxic payload. A Phase 1 study of ZW49 in patients with locally advanced (unresectable) or metastatic HER2-expressing cancers is ongoing (NCT03821233).

Dr Sebastian Grimm (Molecular Partners) introduced a novel T cell engager platform based on DARPin® (Designed Ankyrin Repeat Proteins) molecules. DARPin proteins are ideal modules for multi-specific binding due to the small size (15 kD), simple repetitive architecture (one polypeptide with one domain) and target binding via rigid surface structure. The format is flexible for multi-specific, half-life-extended constructs, such as CD3+TAA1+TAA2+HSA. High homogeneity and

stability are the key properties of CD3 DARPin® platform. Dr Grimm presented several products from their pipeline. MP0250 is a bispecific VEGF/HGF antagonist blocking cancer escape pathways. It is currently evaluated in a Phase 2 study in combination with bortezomib and dexamethasone in patients with relapsed refractory multiple myeloma (NCT03136653). MP0274 (Her2) binds simultaneously to two different epitopes on HER2, thereby inducing cancer cell suicide, a novel mode of action (MoA) compared to other available drugs. MP0274 is currently under a Phase I clinical trial (NCT03084926) for HER2-positive cancer patients. Finally, Dr Grimm concluded that the ongoing format development will address current limitations in the clinic, such as tumor selectivity or antigen coverage.

Dr Nathan D. Trinklein (TeneoBio) first gave a company overview of TeneoBio, which covered the transgenic rat platform (UinRat and OmniFlic), high-throughput sequence-based discovery engine and multi-specific therapeutic antibody products. He then focused on the T cell engaging BsAb platform. There are several bispecific T cell engager products in their pipeline, including BCMA × CD3, CD19 × CD3, PSMA × CD3, CD79b × CD3. All the BsAbs are in discovery and preclinical stages, and some of them are at IND stages. Dr Trinklein showed that around 75% of BsAbs in development use an anti-CD3 derived from SP34, OKT3 or UCH1 [20]. Teneobio's goal is to discover new anti-CD3 antibodies that in bispecific format are well tolerated and efficacious, which means efficient tumor cell lysis, low toxicity and avoid T-cell exhaustion and activation-induced cell death (AICD). Using their own proprietary NGS-based discovery approach, bioinformatic and high-throughput screening, they have identified a diverse set of leads targeting tumor antigens, which helps them to develop next-generation T cell engaging anticancer therapeutics [21].

April 11: Day 4—bispecific antibodies off the beaten path: fusions, non-antibody scaffolds, etc.

Prof. Roland Kontermann (University of Stuttgart) gave the second keynote sspeech "Bispecific Antibodies: Overview of formats and therapeutics applications". First, he listed what we have achieved on antibody studies: among approximately 80 approved antibodies, 29 human, 36 humanized, 30 chimeric and 5 mouse/rat. Only three BsAbs have been approved (one withdrawn) till now. Second, he talked about why we need BsAb. BsAb formats normally have extending activities, means doing better (1+1>2) or doing different (1+1=3) than combining activity (1+1=2 or 0). Third, Dr Kontermann summarized the major methods for making BsAb: (1) hybrid–hybridoma (somatic hybridization), (2) chemical cross-linking and (3) genetic engineering. Fourth, he overviewed the formats and clinical development of BsAb: about 30 different formats utilized in around 80 different BsAbs; around 70 BsAbs are cancer-related, whereas 15 BsAbs are non-cancer-related. Among the cancer-related BsAbs are about two-thirds in hematological malignancies (e.g. targeting BCMA, CD19, CD20, CD33, CD38, CD123) and one-third in solid tumors (e.g. targeting CEA, EpCAM, HER2, PSMA) [22]. Finally, Dr Kontermann introduced BsAb engineering at University of Stuttgart, including a tetravalent BsAb targeting EGFR and HER3 and a bivalent BsAb targeting HER2 and HER3.

Dr James L. Gulley (NCI, NIH) is an internationally recognized expert in cancer immunotherapy. He has authored numerous papers and has conducted a variety of clinical trials at the NCI, including the Phase I/II study of M7824 (NCT03493945). At this meeting, he presented the clinical progress of bifunctional TGFβ trap/PD-L1 (M7824) in solid tumors. M7824 is a first-in-class bifunctional fusion protein composed of the extracellular domain of two TGF-β receptor 2 molecules that serve as a TGF-β sequestering or trap molecule fused to a fully humanized monoclonal antibody against PD-L1. Preclinical data demonstrated that M7824 enables immune-cell infiltration and can overcome resistance seen with other PD-L1 antibodies through phenotypic modifications. Dr Gulley discussed the dose escalation study, which demonstrated safety, saturation of peripheral PD-L1 and sequestration of all released plasma TGFβ1, -β2 and -β3 throughout the dosing period at doses >1 mg/kg. M7824 1200 mg IV has been tested in multiple cohorts including in HPV-associated cancers (ORR 35%) and non-small cell lung cancer (NSCLC) (ORR 28% with ORR 41% in patients with ≥1% of tumor cells PDL1+). M7824 is now in more than 15 clinical studies (https://clinicaltrials.gov/) for multiple difficult-to-treat cancers. Among these studies are a Phase II trial designed to compare M7824 with Keytruda (pembrolizumab) as a first-line treatment in patients with PD-L1 expressing advanced NSCLC, and several Phase I studies assessing M7824 in solid tumors [23,24]. A recently announced deal to co-develop M7824 between EMD Serono and GSK was valued at up to $4.2B. In addition to being used as a single agent, M7824 is also being considered to be combined with other pipeline candidates from both companies.

Dr Brian McGuinness (Crescendo Biologics) presented CB307, a novel T cell co-stimulatory Humabody therapeutic for PSMA-positive tumors. In the beginning of his talk, Dr McGuinness introduced the company's background, platform (Humabody®), and pipeline. He then compared the conventional antibody and Humabody. Humabodies are fully human, single VH domain building blocks generated using Crescendo's proprietary transgenic mouse. *In vivo* maturation in the absence of light chains optimizes Humabody® potency and develops superior biophysical properties. Small size (13 kDa) and high stability permit Humabody® assembly into an almost limitless array of multifunctional formats optimally configured for therapeutic efficacy. This fully modular plug and play approach coupled with Humabodies'® superior biodistribution and absence of Fc-receptor-driven toxicity offers many advantages over traditional mAbs for targeted payload delivery. CB307 (PSMA × CD137 × HSA) is a tri-specific Humabody®, targeting prostate-specific membrane antigen (PSMA), the potent co-stimulatory molecule CD137 (4-1BB) and human serum albumin (HSA). Simultaneous binding to PSMA is required for the activation of CD137. The molecular weight of CB307 is less than 50 kDa,

and it does not contain an Fc domain, thereby avoiding interaction with Fc receptors. Half-life extension is achieved through the inclusion of a VH domain with specificity for HSA. Co-incubation of primary human T cells from healthy individuals or cancer patients together with PSMA-positive tumor cells and CD3 stimulation induces T cell activation and cytokine release. In an *in vivo* model using NSG mice engrafted with human PBMCs, the growth of PSMA-positive DU145 prostate tumor cells is inhibited by a surrogate BsAb. Taken together, these data support the further preclinical development of CB307 [25,26].

Dr Thomas Tuxen Poulsen (Symphogen A/S) reported on a highly efficacious antibody mixture against MET-dependent tumors. He introduced that increased receptor tyrosine kinase MET activity is linked with poor prognosis in several human cancers currently lacking targeted therapies. In his talk, he showed the characterization of Sym015, an antibody mixture composed of two humanized IgG1 antibodies against nonoverlapping epitopes of MET. Sym015 was selected through high-throughput screening of antibody mixtures with superior growth-inhibitory activity against MET-dependent cell lines [27]. Sym015 is well tolerated and strongly inhibits tumor growth *in vivo*; it also induces high ADCC and CDC activities and is superior to a clinical stage single MET antibody. An ongoing clinical trial (NCT02648724) demonstrates that Sym015 is safe and well tolerated, showing promising signals of clinical activity in a subset of MET-dependent patients.

Dr Rajkumar Ganesan (Janssen Biotherapeutics) gave a talk about engineering BsAbs for specific targeting of tumor cells. Janssen's BsAb portfolio includes Zymeworks' technology and Genmab's Duobody platform, both of which were in-licensed to Janssen through license agreements in 2017 and 2012, respectively. Dr Ganesan presented three Duobodies: JNJ-63709178 (CD123 × CD3), JNJ-64007957 (BCMA × CD3) and JNJ-64407564 (GPRC5D ×CD3). All three products are in clinical Phase I studies with indications of acute myeloma leukemia (AML) and MM (NCT02715011, NCT03145181, NCT03399799). In one slide, Dr Ganesan listed a diversified T cell engager (TCE) BsAbs which are under clinical development, such as Fc-scaffold BsAbs (CLEC12A × CD3, CD20 × CD3, CD123 × CD3, BCMA × CD3, Glypican 3 × CD3, CEA × CD3) and non-Fc scaffold BsAbs (targeting DLL3, PSMA, CD33, EpCAM, etc). Dr Ganesan concluded that the major challenge for T cell engager approach is to find "clean" tumor-restricted antigens. He summarized that intuitive screening and engineering methodologies are the keys to overcoming the challenges of specific tumor targeting.

Dr Christine E. Engeland (National Center for Tumor Diseases, Heidelberg, Germany) introduced "Oncolytic vaccines to augment BiTE efficacy against solid tumors". First, she gave an introduction about oncolytic viruses and principles of virotherapy and indicated that viruses can infect both cancer and normal cells, but only replicate and lyse cancer cells. She showed the vector design for oncolytic measles viruses (MV) encoding bispecific T cell engagers MV-BiTE (CD3 × CD20). Then, she presented the therapeutic efficacy of MV-BiTE in immunocompetent mouse models and patient-derived NGS mouse xenograft models. The data showed that the MV-BiTE treatment was effective in two distinct models of solid tumors without showing signs of toxicity [28]. Furthermore, she showed the MV-checkpoint inhibitors vector design and the synergistic potential of combination therapeutics (pembrolizumab and MV) for *in situ* tumor vaccination. She concluded that this study represents proof of concept for an effective strategy to treat solid tumors with MV-BiTEs, and vector-mediated oncolysis serves as an *in situ* tumor vaccine, inducing synergistic anti-tumor immune responses. This talk highlights the versatility of the MV vector system and avenues for clinical translation.

ENGINEERING ANTIBODIES

April 11: Day 4—emerging technologies in antibody engineering

Dr Christoph Spiess (Genentech) presented their work entitled as "Engineering a T Cell Dependent Bispecific (TDB) Antibody to Broaden the Therapeutic Index for Solid Tumor". They engineered their anti-HER2/CD3 TDB to achieve tumor specificity. HER2 expression level is higher in HER2+ breast cancer while sparing cells in normal tissues express lower amounts of HER2. Thus, there is a treatment window for targeting HER2. Drug dosage must balance the therapeutic effect and toxic effect. They tried to lower the affinity of anti-HER2/CD3 to HER2 to avoid the side effect. By engineering the anti-HER2/CD3 TDB, they selected low-affinity anti-HER2 and designed a bivalent anti-HER2 arm. This novel bivalent, low-affinity anti-HER2 increases selectivity to HER2 tumor cells, but not HER2-amplified normal cells. Their 4D5-H91A 1Fab-IgG TDB improves the selectivity for HER2-overexpressing cells more than 1000-fold and show 300-fold potency than 4D5 WT IgG form HER2 TDB [29].

ENGINEERING BsAbs

April 11: Day 4—bispecifics for CNS and compartmental delivery

Dr Mark Dennis (Denali Therapeutics) introduced their brain-blood barrier (BBB) Transport Vehicle (TV) platform which enables the delivery of large molecule therapeutics such as antibodies and enzymes across BBB into the brain. Denali collaborated with F-Star and utilized its Fc engineering technology to develop an engineered Fc region that binds the transferrin receptor (TfR) which renders IgG to cross the BBB by receptor-mediated transcytosis. Along with two Fab arms specific for a therapeutic target, they developed antibody-based ATV:BACE1 and delivered it efficiently across the BBB to reduce Abeta in non-human primate brains. Also, they developed enzyme-based ETV:IDS for Hunter Syndrome therapy. This TfR-binding engineered Fc format of transport vehicle provides a novel brain delivery platform.

Dr Karin Smith (Alligator Bioscience AB) presented ATOR-1017, a 4-1BB (CD137) agonistic antibody for immunotherapy of cancer [30]. ATOR-1017 was designed

to overcome limitations observed in other 4-1BB antibodies to have better efficacy and safety. ATOR-1017 was shown to bind to a unique epitope of 4-1BB receptor, which is different from BMS's urelumab and Pfizer's utomilumab. ATOR-1017 binds to 4-1BB with high affinity and activates cytotoxic CD8 T cells and NK cells. Although ATOR-1017 and urelumab are both IgG4 subsets, they differ in functional activity. Unlike urelumab, ATOR-1017 is dependent on FcγR-mediated cross-linking. Thus, co-expression of 4-1BB and FcγRs is a potential biomarker for ATOR-1017 patient selection. ATOR-1017 was also demonstrated to induce a potent and dose dependent anti-tumor efficacy in an MC38 mouse model. The first-in-human Phase I clinical trial for ATOR-1017 is expected to be initiated in 2019.

DISCUSSION AND PERSPECTIVE

This report covers about 20 presentations and highlights the development of over 20 BsAbs with a variety of formats (Table 1). More than half of the presented BsAbs are being developed for treating solid tumors. About 30 and 40% of these BsAbs are in preclinical and Phase I clinical trials, respectively. T-cell redirection is the most common design with nearly half of the reported BsAbs contain CD3 targeting. REGN1979 (CD3 × CD20) has been advanced in Phase II clinical study. From the perspective of cancer targets, Her2 is the most popular tumor target; in particular, ZW25 (bind to two Her2 epitopes ECD4/ECD2) is in Phase II, and ZW49 (ZW25+ADC) is in Phase I clinical study. This meeting report represents only a small part of the entire field of bispecific immunotherapy. As of March 2019 [31], there are two BsAbs on the market, with more than 85 BsAbs in clinical trials with 86% targeting cancer and T cell redirection being the most common denominator.

Bispecific immunotherapy has the potential to improve clinical efficacy and safety, but the development of BsAb is more challenging than the development of monospecific antibodies in many aspects including target paring, format selection, affinity, valency, epitope specificity, developability, manufacturing, Fc-mediated effector functions, *in vivo* half-life, efficacy and safety, etc. Development of a successful BsAb relies on multiple factors, including its targets in cancer and immune cells, engineered formats for optimal antibody pairs and biological functions based on synergistic and/or maximum anti-tumor activities. Nevertheless, ongoing preclinical and clinical studies will help define the utility of BsAbs as a major group of cancer therapeutics, in particular for treating solid tumors.

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