Antibody Therapeutics:
Challenges, Strategies and Innovations for the Next Generation

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What comprises the bulk of approved and in-development biologics? Antibody-based therapeutics. R&D strategies involving these molecules have led to clinical results and efficacy data that reveal their promise as the basis for effective protein-based drugs. But this common goal cannot be reached without expertise from multiple R&D stages. Protein engineering and development. Formulation and stability. Expression and production, via both traditional and alternative hosts. Analytics and impurities. Process technologies and purification.

Antibody-based therapeutics, a central focus in the protein science space, and the R&D stages mentioned above are in turn the central focus of the pipeline themes of "PepTalk: The Protein Science Week". For the 16th year, 1300 scientists and engineers, biotech and pharma peers, and academics and industry leaders will gather in San Diego, California from January 9-13, 2017 to address protein therapeutics R&D from all directions: up and down process streams, back to recent developments and forward to new technological advances.

As a preview, this white paper provides an in-depth look at antibody therapeutics, as covered at the event. We’ll also reveal the most promising innovations in protein R&D, according to industry experts representing each pipeline topic during the event’s Closing Plenary Panel Discussion.

Engineering Next-Generation Cancer Immunotherapies

Immune checkpoint inhibitors have shown incredible promise with four therapies FDA-approved for use in patients with select types of skin cancer, head and neck cancer, bladder cancer,
non-small cell lung cancer, kidney cancer and lymphoma. [1] [2] Immune checkpoints CTLA4 and PD-1/PDL-1 have attracted much attention industry-wide. However, the issue remains that the majority of patients with solid tumors in most cancer types do not respond to checkpoint inhibitors, hence the need for development of novel immunotherapies.

As a preview to our 7th PepTalk pipeline, “Antibody Therapeutics,” we will explore some of the exciting technologies and advancements that leading organizations are developing. This includes state-of-the-art research into novel applications and techniques for engineering next-gen cancer immunotherapeutics, including strategies for target selection. Also on the agenda are multispecifics, which are designed to target multiple immunosuppressive pathways in the tumor microenvironment for optimized tumor-specific immunity.[3] We’ll discuss bispecifics that fight cancer, engineering and platform innovations, and moving bispecifics into the clinic. We’ll also give a broad overview of antibody-drug conjugates and combination therapies, which are being explored to overcome some of the hurdles faced with single targeted immunotherapies, such as the immunomodulatory effects that they initiate.[4]

**Strategies for Target Identification for Novel Immunotherapies**

State-of-the art personalized medicine approaches to cancer immunotherapy will require novel biomarker development. Currently, the only FDA-approved companion diagnostic for anti-PD-1/PD-L1 agents is PD-L1 immunohistochemistry. “There are numerous PD-L1 IHC antibodies with varying cutoffs and performance characteristics. Tumor mutational burden and RNA transcriptional signatures are likely to enter the clinical arena soon and can help discern responders from non-responders,” states Sandip Patel, Assistant Professor, Cancer Immunotherapy Program, University of California, San Diego who will be speaking about “Emerging Predictive Biomarkers for Cancer Immunotherapy.”

“More sophisticated biomarkers combined with novel therapeutics allow for the development of personalized immunotherapy based on each patient’s unique immunologic deficit. This may require combinations of targeted therapy, cellular therapy, and novel immunologics to maximize anti-tumor responses. The best use of these agents will require novel biomarker methods to interrogate a patient’s tumor-immune microenvironment,” said Patel.

One way to approach target selection is through a computational approach, and in one example of this researchers were able to select candidate targets overexpressed in breast cancer. [5] They computationally identified 50 cell membrane targets for antibody-drug conjugates (ADCs). The ADC for one of these targets is actually in clinical use, and for six other targets, the ADCs are in clinical trials. Beyond the cell membrane targets, the strategy revealed an additional 50 extracellular targets for non-internalizing methodologies as well as alternative strategies. Their methodology involved computational methods to select for targets overexpressed in breast cancer versus normal tissues. They used publically available microarray data from 8000 tissue samples, and classified the breast tumor samples using an iterative ensemble approach which used three feature selection techniques and six classification algorithms. [5]

Another exciting area which is seeing progress with potential new targets for immunotherapy is with tumor necrosis factor superfamily (TNFSF) and their receptors, the TNF receptor superfamily. The TNFSF receptors play a critical role in the anti-tumor immune response. [6] They are expressed
by a variety of immune cells: T cells, dendritic cells and macrophages, in addition to tumor cells. TNFSF-mediated signaling produces a wide range of biological effects, which include proliferation, differentiation and apoptosis, as well as tumor growth. The natural TNFSF ligand structure is trivalent, with three receptor binding sites. In order to successfully transmit signal, there is a clustering of receptors to their respective ligand. Apogenix AG has a proprietary platform with which they construct agonists for treatment of cancer. At PepTalk, Apogenix AG will describe how they’ve engineered a unique single-based fusion protein construct to mimic the 3-D structure of the natural ligands Tumor Necrosis Factor Superfamily (TNFSF) proteins. These are novel hexavalent TNFSF receptor agonists, and have shown promise in preclinical studies in stimulating the immune system.

Another outstanding opportunity lies in the expanding array of evidence for using tumor neoantigens as targets for immunotherapy.[7] Recent clinical data has confirmed that antigenic peptides, or epitopes, processed and expressed on tumor cells, are recognized by tumor-specific T cells and that efforts to enhance this immune response has the potential to prevent tumor regression in advanced stage patients. Mutated cancer proteins, processed as peptides, display themselves on the surface of tumor cells, and these are recognized as “non-self” or foreign by the immune system. [8]

The complexity involved in using these neoantigens for cancer vaccines/immunotherapy lies in the fact that there is a wide range of mutational frequency among patients and across tumor classes. The majority of mutations, in fact, are specific to each patient’s tumor, which leads to the need for more precision-medicine based approaches. Whole exome sequencing and epitope prediction programs are key to capitalizing on this potential ‘goldmine’ of cancer neoantigen targets. [3, 7]

**Novel Strategies for Bi- and Multispecifics**

Bispecific antibodies are uniquely engineered to overcome some of the deficiencies of monoclonal antibodies. These constructs are designed with dual specificities and high bivalent affinity for two antigens. Some are designed to redirect T cells to kill tumor cells, while others function to bind two different disease mediators. More than 30 bispecific antibodies are in clinical trials for a wide variety of cancer types and diseases. [3]

There are significant advantages in using next generation biotherapeutics such as bi- and multispecifics and antibody-drug conjugates over the traditional monoclonal antibody therapeutics, such as overcoming the multiple and/or redundant pathways, including cross-talk between pathways, which result in a tumor’s resistance to monoclonal immunotherapies. However, along with the benefits, there are significant challenges in design, cloning, expression, purification, and analytics. At PepTalk, we will examine these challenges across the R&D spectrum. Some high-throughput technologies, such as Genedata’s platform, automate the engineering, production, and testing of large panels of candidate therapeutic molecules, with built-in tools for developability and manufacturability assessments. We’ll hear from Christopher Smith, Senior Scientific Consultant, Biologics at Genedata on this topic at PepTalk. In another talk, by Syd Johnson, VP Antibody Engineering, MacroGenics, we’ll hear about strategies involving avidity, affinity, and valency of the binding domains, and how they can influence and help optimize bi- and tri-specific antibodies.

Researchers are also designing computational tools for developing the next generation of anti-cancer bispecifics. With the development of a mathematical model that incorporates key
features of bispecific antibodies and their targets, researchers can optimize the affinity of
bispecific arms for maximum potency in binding. These features include the binding kinetics and
the spatial distribution of the binding receptors. [9]

Combination Immunotherapies

Another promising avenue, addressed with a block of talks at PepTalk, is to use combination
therapies, involving bispecific antibodies or chimeric antigen receptor (CAR) T cells with
checkpoint inhibitors. This approach is designed to overcome the general problem of extremely
heterogeneous tumors that have been found in preclinical and clinical studies to develop
resistance to targeted monotherapies. Combination therapies have the potential to be more
effective as preclinical studies have shown the benefit in hitting different targets concurrently. [3]

Combination strategies can tackle tumors using a variety of mechanisms. These include
induction of immunogenic cancer-cell death; removal of immune suppression; enhancement of
antigen presentation or adjuvanticity; activation of memory T-cells or induction of macrophage
effectors.[3] The goal of all of these strategies is to boost T-cell response to antigens expressed
by cancer cells or, alternatively, to engage macrophages and myeloid components in the
destruction of tumor stroma.

One area where combination therapies have seen progress is with CAR T cell therapy. In in
vivo studies, upon repeated antigen encounter, CAR T cells are inhibited by their cytolytic and
cytokine secretion functions. PD-1 and PD-L1 have been shown to play a role in this repeated
antigen stimulation–induced exhaustion. However, researchers have shown that PD-1 antibody
checkpoint blockade can reverse this PD-1–mediated CAR T cell exhaustion. Furthermore,
researchers have been successful in the cotransduction of a CAR with a PD-1 dominant negative
receptor, which results in enhanced functional persistence of T cells in addition to reduced
tumor-mediated T cell inhibition. Simultaneous costimulation and checkpoint blockade prevents
tumor-mediated T cell inhibition in mesothelin -expressing solid tumors. [10]

Engineering Antibody-Drug Conjugates for Clinical Success

One of the areas gaining considerable momentum is with antibody-drug conjugates (ADCs),
which are being evaluated in over 100 open clinical trials for cancer patients. [11] We will explore
the latest research around design and development of ADCs and some strategies for moving
them into the clinic.

With a variety of options for cytotoxic payloads being investigated, the top two are tubulin-
targeting anti-mitotic agents and DNA-damaging drugs. Auristatins and maytansinoids, both
tubulin-targeting anti-mitotic agents, comprise the majority of payloads currently in clinical
development. These payloads cause G2/M mitotic arrest by inhibiting microtubule and spindle
dynamics during interphase.[12, 13]

There is a heated search to find the next generation of ADCs as alternatives to the tubulin
targeting anti-mitotic agents. One strategy is in the development of DNA-damaging agents such
as pyrrolobenzodiazepine (PBD), which functions by binding as a dimer to the minor groove of
DNA. PBD has had promising pre-clinical results. [11]
Leading organizations are benefiting from the numerous advancements in protein engineering that allow them to manipulate the number and position of payloads bound to the antibody with site-specific conjugation. A variety of techniques are used to affect the pharmacokinetic characteristics of the antibody construct, antigen binding, ADC aggregation and safety profile.[11]

Site-specific ADC conjugation techniques include cysteine engineering (THIOMAB), amber suppression (UAA incorporation), and chemoenzymatic systems. Companies are tackling instability and heterogeneity issues through cysteine and other constructs. Thiologics is using cysteine-bridging dibromomaleimides and polytherics, bis-sulfone reagents. With these unique constructs, they effectively increase stability and reduce the maximum number of cytotoxic drugs required for attachment. [14]

Ulf Grawunder, CEO and Founder of NBE-Therapeutics will present methods for developing next-gen ADCs with ultra-potent toxins. Their strategies involve looking at some critical factors such as ADC homogeneity, stability, binding specificity and incorporating immune-mediated cell death.

**Expert Strategies across Seven R & D Stages**

To capture a broad perspective on all seven R&D stages of protein therapeutics - which are covered in depth with talks across seven PepTalk pipelines - we have invited experts that represent these stages to discuss some key big picture topics. In our “It’s a Wrap PepTalk 2017 Closing Plenary Panel,” we’ll hear from these experts, who will share presentation highlights, key findings, and future trends.

We’ve asked our Plenary Panel experts the same question: “What do you see as the next, most promising innovation in your field?”

We spoke with Dr. Tilman Schlothauer, Principal Scientist, Biochemical and Analytical Research, Large Molecule Research, Roche Pharma Research and Early Development, Roche Innovation Center Penzberg. He represents the Protein Engineering & Development pipeline at PepTalk, and discussed the need for incorporating technologies that facilitate “really high throughput, getting access to a lot of different binders, studying the epitope characterization on these and really getting direct information of the cellular event.” Dr. Schlothauer also emphasized “the need for scientific crosstalk, like being at conferences such as PepTalk, getting in touch with other people, sharing their concepts and ideas. Really this is the challenge of our time.”

We also spoke with Dr. Thomas Laue, Professor of Biochemistry and Molecular Biology and Director, Biomolecular Interaction Technologies Center, University of New Hampshire representing our Formulation & Stability pipeline. “I believe that the most important thing is to come up with a better predictive means of determining the solubility and viscosity of protein solution at high concentrations,” says Laue. In particular, we need better data on viscosity of protein solutions as it relates to other physical properties, such as the charge on the protein or on the dipole moment of the protein. Incorporating instruments for making these measurements would move the candidate molecules through the pipeline faster, and lower the costs, says Laue.

Our next speaker, Bjørn Voldborg, Director, CHO Cell Line Development at The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark represents our Biotherapeutic Expression & Production pipeline. Voldborg spoke of the continuous development
of the gene-editing CRISPR-based tools having the greatest innovation possibilities, by being able to manipulate the cells’ behavior in a very refined way, through knocking out genes and by targeting transcription factors and silencers to specific areas of the genomes. Voldberg spoke of the CRISPR-based tools in the future making it possible to potentially induce “different responses during the production of the proteins, so during the different phases we can actually turn on or turn off genes that might or might not be beneficial for the production.”

Elaborating on these same tools, we also heard from our plenary panelist, Dr. Andrew Fosberry, Senior Scientific Investigator in Protein and Cellular Sciences at GlaxoSmithKline, representing Process Technologies & Purification. “Where I think things are going for the future that will enhance discovery in my field is the area of what I call designer microbes,” made possible with molecular biology techniques such as Gibson Assembly and CRISPR/Cas9, said Fosberry.

“We can knock out pathways. We can knock in pathways using CRISPR/Cas9. We can move pathways from one organism or microbe to another, which may be beneficial.” We can also use the new evolution technologies that are out there with higher screening capabilities and explore the whole diversity of our evolution libraries to zero in on the mutants or the enzymes that we really want, said Fosberry.

We heard from another expert, Diane Paskiet, Senior Director, Global Scientific Affairs at West Pharmaceutical Services, representing the Analytics & Impurities topic. “In the field of drug delivery and containment, it’s becoming increasingly important to be able to incorporate more of the patient experience into the drug development process. Protecting biologics and effectively delivering them are two key areas for advancement,” said Paskiet. “Verification of important factors such as adherence, the delivery of the biologic, and patient response to dosing are all possibilities to … providing optimal healthcare and evidence of patient outcomes,” said Paskiet. Technologies such as wearable patch injectors for self-injection in the home setting can be considered as part of the biologic design process, said Paskiet.

Representing our Alternative Expression and Production Pipeline, T.K.S. Kumar, Professor, Chemistry & Biochemistry, University of Arkansas, said “The real challenge is the development of new expression hosts and vectors for the overexpression of hydrophobic peptides and membrane proteins. The non-availability of expression hosts for the large scale production of membrane proteins/peptides has severely impaired structural biology studies and the design of effective drugs against a multitude of membrane proteins involved in a wide range of pathogenesis,” said Kumar.

Join us at PepTalk 2017

To explore many of these ideas further and to hear expert discussions that cross all R & D phases in the protein space, visit us at PepTalk 2017. You’ll have the opportunity to explore a diverse array of strategies to support your own efforts in R&D, along with access to exceptional networking opportunities, short courses, training seminars, and our expansive exhibit hall. To hear more from our plenary speakers, see our Podcast here.
References