BEYOND SMALL MOLECULES: DEVELOPING BIOLOGIC THERAPIES

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Introduction
The goal of this session is to learn about Biologics therapeutics, with a focus on monoclonal antibodies. We will be learning what a biologic is, and then focus on some of the key features of the discovery and early development of monoclonal antibodies. Monoclonal antibodies have exquisite specificity for their targets and have a long half-life in the body resulting in less frequent dosing than a typical small molecule.

Defining Biologics
Biologics are defined by their method of production. A biologic is produced as the product of a cell. These cells can be bacteria, yeast or mammalian cells including Chinese Hamster Ovary cells, the platform of choice for producing monoclonal antibodies. Biologics are usually based on a naturally occurring molecule in one form or another. These include enzymes, growth factors, cytokines, and antibodies. Biologics are also referred to as 'large molecules'. A typical small molecule drug may be around 0.5 1-.0 kDa in size; a cytokine or growth factor may be 15-30 times as big, and a monoclonal antibody drug is usually around 150 kDa in size. The size of monoclonal antibodies impacts their elimination from the body – it is a factor for a much longer half-life than a typical small molecule drug as they do not get filtered out of the blood in the kidney glomeruli.

Features of Monoclonal Antibodies
Monoclonal antibodies are produced from the non-covalent binding association in a symmetrical manner of two heavy chains (approximately 50 kDa each) and two light chains (approximately 25 kDa each). The intact antibody structure has two main regions, the fragment antigen binding (Fab) and the fragment crystallizable (Fc). The exquisite sensitivity of monoclonal antibodies comes from the Fab regions via amino acid sequences called 'Complementarity Determining Regions' (CDRs). The CDRs are unique to each monoclonal antibody, being created by recombination of various gene segments in the Immunoglobulin genes. Each antibody chain (heavy and light) has three CDRs, and contacts the antigen with contributions from each CDR leading to a three dimensional region known as the 'paratope'. The region on the antigen that the monoclonal antibody binds to is called the 'epitope'. The Fc region of an antibody is important for binding a class of receptors on immune cells called Fc receptors. These receptors often signal to the immune cell to respond to the antibody bound cell, often with the induction of 'antibody dependent cellular cytotoxicity' (ADCC), resulting in the death of the cell expressing the target antigen. Fc receptors may also mediate phagocytosis (e.g. of bacteria) and release of chemokines and cytokines that may regulate immune function. The Fc portion of antibodies also binds complement, which is also involved in immune function (e.g. complement dependent cytotoxicity CDC). Another type of Fc receptor, FcRn, is involved in recycling of antibodies within the body, resulting in prolonged survival of the antibody. FcRn binding is another mechanism whereby antibodies show long half-lives within the body. Fc portions of antibodies are classified by their 'isotype'. For human therapeutic antibodies, most are of the IgG1 or IgG4 isotype, with the IgG2 isotype also used.

Why are monoclonal antibodies good therapeutics?
Monoclonal antibodies are good therapeutics due to their specificity and their ability to inhibit many types of cellular function, especially those where the function is mediated by the interaction of two proteins binding each other (protein-protein interaction). They are of limited utility in targets expressed in side cells. They also have limited penetration of the blood brain barrier, so novel strategies are being devised for brain expressed targets to chaperone monoclonal antibodies across the BBB. Monoclonal antibodies can act as therapeutics in many ways – as antagonists (e.g. blocking a ligand-receptor interaction), as agonists (e.g. mimicking a natural ligand), to target cells for death by the immune system (via ADCC or CDC), or by carrying a toxic payload into the cell (antibody-drug conjugates).

Discovering Monoclonal Antibodies
There are many ways to discover a new monoclonal antibody. A typical approach is the 'Hybridoma' approach, where mice or other species are immunized with the antigen. B cells (from spleen, lymph node or peripheral

blood) are fused with a malignant plasma cell (a myeloma) to form a hybrid cell (hybridoma) which has the antibody-producing characteristics of the B cell and the ability to continuously grow in culture from the myeloma cell. Antibodies produced in non-human species have more likelihood of being recognized as foreign when administered to human subjects. A strong anti-drug antibody response arises as a consequence of being recognized as foreign, which can lead to many adverse events and/or neutralization of drug activity. To alleviate this, the process of 'humanization' was developed to make the therapeutic candidates as human-like as possible. Humanization involves the grafting of the CDR regions of antibodies into a human framework. As these have less rodent-derived sequences, they have less chance of being seen as foreign by the human immune system. Other technologies are also used to make antibodies that look more 'human' than traditional rodent-derived antibodies. Transgenic rodents expressing human immunoglobulin genes have been generated which produce fully human antibodies. These can be excellent starting points for therapeutic antibody generation. Other technologies using human antibody libraries displayed in bacteriophage (phage display), yeast, or mammalian cells have also been employed successfully as a starting point to obtain fully human antibodies. A second consequence of making antibodies in rodents via immunization is that is difficult to obtain antibodies which recognize the rodent orthologs of the target due to the immunized rodents being tolerant of epitopes of the antigens that are shared with immunized rodent. As most disease models are rodent-based, this can mean that target validation can require other reagents to provide proof of concept in the animal model. 'Surrogate' antibodies, which have the same mechanism of action, often need to be generated. Ideally, surrogate antibodies should recognize an equivalent region on the rodent ortholog as the potential human antibody does on the human target. Matching should also be performed for the isotype of the rodent antibody. This is to ensure that the surrogate has similar interactions with Fc receptors in the rodent as would be expected for the potential human therapeutic. Human and rodent isotypes have different characteristics to human in terms of binding to Fc receptors and complement, so matching isotypes is of great importance. Use of display technologies can often provide cross-reactive antibodies as they have not been subject to tolerance.

Enhancing Monoclonal Antibody Activity

Antibody activity can be manipulated by engineering of the structure of molecule to enhance the functional activity of the molecule. Examples of functional manipulation include modifying Fc binding Fc receptors or complement to change the ability of the Fc to interact with the immune system. This can enhance ADCC or CDC to obtain a much more potent antibody. In situations where interactions with the immune system may be not desirable, the isotype of the antibody may be engineered as an IgG4 or an IgG1 with mutations to block binding to Fc receptors. Antibodies can also be grown in cells that change the glycan make-up of the antibody, again with the ability to enhance or diminish the interactions with Fc receptors. To improve the half-life of antibodies, mutations can be made in the Fc to enhance binding to the FcRn. Antibodies can be linked to various drugs to produce what is known as 'Antibody drug conjugates' (ADC). ADCs have been employed extensively in a cancer setting, where potent cytotoxics with a low therapeutic index can be targeted to cancer cells using the exquisite sensitivity of the antibody to the target. Keys for a success in a traditional oncology ADC program are 1) a potent antibody against a target that is selective for cancer cells over normal tissue, 2) internalization of the target upon antibody binding and 3) a potent drug stably linked to the antibody (using a synthetic linker) which will reach the appropriate part of the cancer cell and kill the cell. Antibodies can also be linked to radio-isotopes to target cancer tissue and kill antigen-positive cells.

Bispecific Antibodies

A recent advance in antibody technology is to add the specificity of one antibody to a second antibody to create a 'bispecific' antibody, which has the ability to simultaneously bind two antigens. There are many ways to engineer bispecific antibodies. Some of these formats include Fc regions, whereas other, smaller formats use modular Fab-like domains lacking Fc, with theoretical advantages to these formats having better tissue penetration due to their smaller size. There is a great deal of effort in the antibody discovery community to constructing bispecifics that have on specificity to penetrate the BBB and the other specificity against the CNS-expressed disease target.

Developing a monoclonal antibody

Once a candidate mAb has been discovered that has the specificity for disease target required with no off-target binding, mechanistic activity in a disease relevant assay and appropriate reactivity against a relevant toxicology species, the antibody can be considered for entering development. There a number of steps to ready an antibody for development. The biophysical properties of the antibody must be carefully analyzed in both normal and stressed conditions. Certain amino acid sequences in the protein structure may be problematic in the development and scale up of an antibody. Stress testing of the antibody using exaggerated physical and

chemical insult can de-risk the potential sequence liabilities of antibodies. Sequence modifications can be made if the sequence liability may reduce antibody quality in the long term. To understand the properties of antibodies once they have been used in an in vivo setting, a series of bioanalytical assays are developed. These assays focus on measuring drug levels in samples from the in vivo setting as well as measurement of the levels of anti-drug antibodies (ADA). As described previously, ADA can severely impact the activity of the antibodies as well as increase the potential for adverse events. Measurement of target engagement with antibodies with peripherally expressed targets is fairly straightforward, especially for targets that are expressed on immune cells. Here, simple flow cytometry assays can be used to assess target binding on these cells. Safety pharmacology assays are also important to assess the potential risks of these antibodies in activation of the immune system either mechanistically via Fab binding to the antigen or via the Fc portion of the antibody. These assays examine the potential of the antibody to elicit ADCC and CDC, as well as to mediate cytokine release.

Chinese Hamster Ovary (CHO) cells are the typical host for manufacturing large quantities of antibodies. Cells are grown in large volumes for antibody scale-up under Good Manufacturing Practice (GMP) conditions. If antibody batches are of appropriate quality, they are released for further studies, including toxicology (often performed in non-human primates). Antibodies are formulated to allow either infusion for intravenous administration or in a concentrated form for subcutaneous delivery. Antibodies are not typically able to be delivered via the oral route.