Panel 1: Identifying Antibody Targets

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Panel moderator, Susan Lacy (Abbott) set the stage by noting that the panel discussion would focus on the process of antibody target evaluation and that the term ‘target’ needs to be defined. In some cases a target is defined as a whole protein, and so includes the complete sequence from N-terminus to C-terminus. However, she pointed out that a more practical definition of a target is important to consider, e.g., a specific epitope within a whole protein such as the active site of an enzyme. In cases where unique target domains have been elucidated via functional screening methods, the target may be defined by a unique series of epitopes formed only by the co-expression of two or more interacting proteins. In addition, any given target theoretically exists in a “natural” environment in vivo, and failure to faithfully replicate this natural environment in vitro may alter the target to such an extent that screening could generate disparate or meaningless data. Thus, the term ‘target’ is rather general and may more appropriately be used to discuss target classes rather than the actual region(s) of a protein that may need to be engaged by an antibody to generate a pharmacodynamic (PD) effect.

Panelist Christopher Bond (Oncomed) noted that targets can also be defined in terms of biological pathways and combinatorial screening efforts against these pathways can entail shotgun approaches where multiple elements for each pathway are made and screened in parallel. If the goal is antagonizing a pathway where much of the initial signaling is mediated by receptor-ligand interactions, then the target is ultimately defined as a protein-protein interface. However, atomic level structural information for many receptor-ligand complexes is not always available. In these cases we are forced to define targets based on functional domain boundaries when that information is available. In many cases even this level of information is incomplete and so the target definition becomes one of practicality, e.g., what extracellular portions of the specific gene product, complete domains, or stretches of individual domains can be made. Panelist Phil Hammond (Theraclone Sciences), who mentioned that he has a chemical genomics background, commented on the utility of doing functional screening first and then going back to find the target, which is an approach that has not classically been done with antibodies.

Panelist Partha Chowdhury (MedImmune) discussed the characteristics of molecules that make them attractive targets. He first mentioned the limitation that the target needs to be
assessable outside the cell and noted that three important factors should be considered: whether there is a clear mechanism of action, whether the target has homology to any other human proteins and whether there is redundancy in the biologic pathway, i.e., another protein can take over if the targeted molecule is neutralized.

With respect to the numerous classes of well-explored (e.g., soluble, transmembrane) and emerging (e.g., G-protein coupled receptors, ion channels, enzymes) target proteins that may be considered for therapeutic antibody intervention, the panel agreed that targets must be physically accessible to an antibody in vivo. Even if only a portion of a given target class physically resides outside a cell membrane (e.g., GPCR and other membrane-spanning molecules), the industry is moving toward prosecution of these types of targets with therapeutic antibodies. However, once committed to a new target type, resourcing investments must be made to ensure a screening platform is robust and reliable, and, in some cases, this investment in time and money may make the prosecution of new target classes less attractive than other familiar antibody target classes (e.g., soluble proteins). Importantly, antibody platforms to novel target classes may allow antibodies to enter clinical studies in disease states underserved by current antibody therapies.

In addition to the issue of accessibility, the panel felt that a thorough understanding of the target biology, expression pattern, regulation and the potential for therapeutic-associated toxicity were important considerations during target selection. In general, the decision to commit resources to prosecuting a given target depended on the therapeutic application (e.g., oncology or infectious disease), the challenges associated with the expression of the target, the ability to set up reliable screening assays, and even issues such as the target-associated intellectual property landscape.

In some cases a particular target class may be more or less attractive to industry given the successes or failures of antibody therapies to the various target types. Regardless of whether the clinical outcome was success or failure, clinical data can be tremendously informative to discovery scientists who desire to generate second-generation antibodies having a “best-in-class” clinical opportunity. In fact, the lack of efficacy demonstrated by small molecule therapies to certain target classes could inadvertently set the stage for a follow-on antibody approach that may have not been considered in the absence of clinical data. For example, adverse events associated with pan matrix-metalloproteinase (MMP) inhibitors in clinical studies lead discovery scientists to propose MMPs as targets for antibody therapy.

The panel also discussed what considerations drive the choice between using an antibody or an antibody fragment for selected targets. Three principal considerations were mentioned: target accessibility, the need for immune-effector functions and differences in PK/PD. Target accessibility can be taken to mean the distribution of the target within the organism, tissue or tumor, and the cellular location of the target, or the physical location of the epitope. The use of full length antibodies for intracellular targets is impractical due to the difficulty of getting large molecules past the cell membrane, and the reducing environment within the cell. However, one can take advantage of single chain variable fragments or domain antibodies for intracellular targets and imaging by utilizing either gene delivery approaches or active transport mechanisms to get the antibody fragments into cells. In these approaches antibody fragments have an advantage as they are more likely to remain intact in the somewhat reducing environment of the cell. Antibody fragments may also allow access to partially buried epitopes that are inaccessible to a full length antibody.

If the goal is to recruit immune effector function, either ADCC or CDC, the most common approach is to use full length IgG1 antibody. Optimization of these immune effector functions can then be tailored by modulating the nature of the carbohydrate attached to the antibody. There are also efforts to couple immunomodulating domains directly to antibodies or antibody fragments. Antibodies, as compared to antibody fragments, can also have dramatically different serum half-lives. Both because of size and FcRn binding, full length antibodies have half-lives on the order of weeks. Typically these long half-lives are seen as an advantage. However there are settings, e.g., in acute conditions or with highly toxic compounds, where short half lives may be advantageous.

An audience member asked why so few antibodies have been raised against infectious disease targets. The panel agreed that in general antibodies have not proven to be potent against many infectious agents. These organisms have evolved to avoid detection by the human immune system, and commonly have redundant or compensatory biological pathways. The panel noted that the biological mechanisms and enzymology of infectious agents are very different from those in humans and targets associated with organisms have been amenable to small molecule therapeutics development. In contrast, antibody therapeutics have proven useful in cases where subtle differences in targets are important, e.g., discriminating neo-epitopes from normal ones.
Panel 2: Strategies to Assess Immunogenicity

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The topics for discussion, strategies to minimize immunogenicity, the impact of manufacturing process (e.g., variation in purity or glycosylation) on immunogenicity, the effect of immunogenicity on PK and PD, and strategies for predicting immunogenicity were introduced by panel moderator, Patrick Lui (Genentech). The panel discussed the fact that industry is primarily developing either humanized or human antibodies as a consequence of issues with the immunogenicity of murine antibodies. There was some debate surrounding the levels of immunogenicity of chimeric molecules compared to humanized or human antibodies. An audience member recommended a review article by Hwang and Foote (Methods 2005; 36:3–10) on the topic.

The question of the frequency and timing of immunogenicity measurements was raised by the audience. Measurements done once per quarter may give different results for an annual rate than measurements done once per month due to fluctuating background levels. More frequent measurements may be more variable and so small variations in annual rates may be meaningless. Especially in cases of chronic disease where a patient might be checked once per month for years, the presence of even low levels of false positives could yield questionable immunogenicity rates.

Panelist Albert Torri (Regeneron) then noted that transient antibody response is a related issue. He emphasized that the US Food and Drug Administration and the European Medicines Agency are taking a risk-based approach to immunogenicity. Decisions concerning how to measure immunogenicity and how to set up assays are based on the assessment of risk. Some molecules are extremely low risk, e.g., antibody antagonists, and low response to such an antibody should be inconsequential, considering the fact that the antibody is a non-endogenous protein. He commented that development programs have to be designed based on an assessment of the types of adverse events that might be generated and how dangerous these might be to a patient.

The panel discussed the fact that there is no reliable way to predict immunogenicity in humans. An audience member shared the experience of Abgenix in using the Xenomouse model, which has endogenous human immunoglobulin components, to predict immunogenicity. They used the mouse model to test antibodies with known immunogenicity in humans, but the model was not consistently predictive of the human experience. They thought that this was due to issues with the major histocompatibility complex (MHC), i.e., even though the antibody repertoire was part of the immune response, the MHC presentation to T cells is also a critical part and that was not human in the mouse model. The panel also concluded that data from monkeys was not a good predictor of immunogenicity either, but that companies are more careful when starting human studies if monkeys show an immunogenic response.

Dr. Torri then discussed the problem of aggregates, which are known to increase the risk of immunogenicity. He noted that the manufacturing process needs to be carefully monitored, and should be modified if aggregation issues are identified. He further pointed out that the route of administration can affect immunogenicity also. In his experience, subcutaneous (SC) dosing is more likely to cause immunogenicity compared to intravenous dosing. However, the route of administration may be dictated by the marketing potential of the SC dosing. This depends on factors such as the current standard of care in the indication studied. Also, if the immunogenicity associated with SC dosing is low-level and transient, then it may not be relevant.

Dr. Lui asked the panel to discuss deimmunization, which focuses on T cell epitope removal. The technology was developed by Biovation (now part of Merck KGaA). Panelist Geoffrey Davis (Angelica Therapeutics) commented that the approach does seem to work, although there are a limited number of examples of such molecules entering clinical study. To date, approximately 600 people have been exposed to the deimmunized antibodies with little immunogenic response. The technology seems on par with humanization. He also noted that Antitope utilizes a composite antibody approach to reduce immunogenicity. In this case the antibody comprises fragments of human antibodies that resemble the mouse version. The company monitors the potential for generating T cell epitopes at the overlaps.

The panel also discussed technology used to assess immunogenic response, including BIACore, electrochemiluminescence and ELISA. The selection on which to use will depend on what fits with in-house capacity. Dr. Torri noted he prefers the MSD platform, but that BIACore is good for characterization of antibody response. However, an audience member questioned how closely one would want to examine this, since an immune response would interfere with development of the product. It does not seem beneficial to examine the minute details and look for small differences. Dr. Torri agreed, but noted that immune complexes are important to look for, and that the BIACore platform was useful for this purpose.
Panel 3: Antibody Drug Conjugates

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Panel moderator Dennis Benjamin (Seattle Genetics) introduced the discussion topics, which included critical features of successful chemo-types, screening for suitable antibodies and successes in drug linker development. The recent dramatic clinical successes of SGN-35 (brentuximab vedotin) and trastuzumab-DM1 were noted, and the panelists focused on what technical advances would be required to bring about additional successes. Panelists Robert Lutz (Immunogen) and Hans-Peter Gerber (Wyeth) agreed on the current scarcity of drugs that can be successfully conjugated to antibodies and related their experience in searching for novel compounds. In general, highly potent small molecules are required because antibody-mediated delivery of drug is an inefficient process, and the drug must be able to exert cytotoxic effects at relatively low intracellular concentrations. That being said, extreme potency has also resulted in antibody-drug conjugates (ADCs) that demonstrated increased toxicity; the difficulty in finding the right balance between these contrasting requirements was identified as a major obstacle to finding new chemo types.

The panel noted that increasing evidence suggests that minor changes in the linker chemistry can drastically alter the pharmacological properties of an ADC. As an example, Dr. Lutz pointed to recently described maytansine derivatives that include charged groups in the released drug. They discovered that these molecules circumvent the multidrug-resistance (MDR)-mediated efflux of drug observed with other maytansines. Dr. Gerber emphasized that drug linker development in the past was mostly based on rational drug design, whereas success was rather empirical. Molecular biology- and cell biology-based screening have made contributions to a better understanding of signal transduction pathways or intracellular trafficking, and these approaches may be instrumental for the identification of novel payloads and linkers for the next generation of ADCs.

A related topic was the relationship between the drug component of an ADC and the target indication. Tubulin binding agents such as maytansine in trastuzumab-DM1 or MMAE in SGN-35 have a selective mechanism of action that requires active cell division, while DNA binding agents such as calicheamicin in gemtuzumab ozogamicin (Mylotarg) can exert a cytotoxic effect on resting cells as well. Dr. Gerber pointed to the potential use of ADCs in anti-angiogenic therapy as an example where the selectivity of the tubulin binding agents may be advantageous, as cells in normal vasculature are not rapidly dividing. In contrast, in certain solid tumor indications where tumor cells proliferate at lower rates, or when targeting tumor stem cells that display generally lower proliferation rates, ADC compounds based on DNA-binding agents may be most effective. Most importantly, tubulin inhibitor-based ADCs induce cell death in neoplastic cells representing various hematopoietic malignancies and endothelial cells are of hematopoietic origin as well. Therefore, endothelial cells may represent the Achilles’ heel of solid tumors that may be exploited by ADC compounds targeting tumor vasculature.

A number of questions related to identifying suitable antibodies during early stages of development were raised, and there was general agreement of a lack of surrogate measurements that strongly correlated with the ultimate activity of a given ADC in the clinic. The panel recommended that antibody affinities, internalization rates, antigen copy numbers and other parameters of the unmodified mAbs alone have failed to predict potent activity as an ADC. However, improvements in culture conditions of human tumor cells, including three-dimensional matrixes, may improve the tissue architecture and morphology, and the predictive value of in vitro experiments. Dr. Benjamin indicated that direct screening of primary ADCs was the most effective way to identify promising antibodies for ADC therapy. Ultimately, the best data for lead selection may be derived from experiments in animals, combining safety and efficacy endpoints to determine the therapeutic index. A related topic was the interdependence of pharmacokinetics and linker stability. The panel agreed that slow clearance of an ADC was desirable, and that linker strategies need to take advantage of the long serum half lives. Previous linkers with half lives of 1–2 days were seen to be inadequate and the current linkers with half lives of 1–2 weeks provided superior therapeutic indices. The panelists agreed that further improvements in linker stability yielded diminishing returns and the focus for future work on linker technology would be on aspects other than stability.
Panel moderator Laurent Audoly (Merck) noted that the objective of the panel was to elicit the current thinking on topics related to novel antibody or antibody-like molecules because the field is evolving rapidly and descriptions in the literature may not reflect the present state of the industry. The topics for discussion included properties of new scaffolds, screening and selection techniques, engineering drug like properties and the immunogenicity of scaffolds.

Dr. Audoly noted that full size monoclonal antibodies certainly have advantages and more than 20 are approved in the US and other countries (Table 1). The molecules were originally designed by nature, which has had some 65 million years to optimize the mammalian Ig repertoire, and although immunogenicity is always a concern, much progress has been made to ameliorate the problem. Compared to murine molecules, human and humanized versions are much improved in their adverse event profiles.

However, the large size and complexity of antibodies are disadvantages because these characteristics raise potential barriers to manufacturing and maintenance of process consistency. The potential immunogenicity of even the human versions is still a concern. The question to be addressed is whether next generation molecules can be an improvement over full-size IgG. Development of next generation molecules is also attractive because manufacturing and cost of goods of full-size molecules are potential barriers to companies that wish to enter the antibody therapeutics space. Intellectual property also may keep some companies from penetrating the biotechnology space because they have not leveraged the appropriate licenses.

The first topics of discussion were the opportunity to access novel biology through novel scaffolds, targeting new epitopes and epitopes that had previously been inaccessible otherwise, engagement of multiple targets with a single agent and enhancement of tissue distribution or penetration. Kendall Mohler (Trubion) discussed Trubion’s small modular immunopharmaceuticals (SMIPs) and bispecific molecule technologies. SMIPs include a single-chain variable fragment (scFv), hinge and CH2 and 3 domains. The small size of the molecules may enhance tissue penetration. Trubion has learned that the molecules have different properties compared to full size antibodies. For example, the same binding domain in SMIP format will elicit different signaling response in cells and this can be controlled by how the scFv is attached to the Fc portion.

The bispecific technology is built on the SMIP platform; the SMIPs have a scFv, hinge, Fc domain, linker and another scFv. To reveal the full potency of the bispecific technology, attention must be paid to how the scFv is attached. For Trubion, the issue is not protein engineering, but the determination of which two molecules to target with the scFv, and identification of the most efficient path to the best combinations. In theory, the bispecs can be used to target two cell surface molecules, a cell-surface and a soluble target, or two soluble targets. Of these combinations, the easiest to model is the two soluble target combination, for which Trubion uses a centered search strategy. One end is kept constant, while the other is varied. In this way new molecules can be generated, then studied in vivo models to determine if they are improvements of the parent molecule.

On the topic of using next generation molecules to target new epitopes or antigens, Dimiter Dimitrov (National Institutes of Health) suggested that fragments should be well suited to target sterically restricted epitopes that cannot be accessed by full-size antibodies. He noted that good examples of these are epitopes on the HIV gp120 induced by its interaction with the receptor CD4, and that viruses might have evolved protected epitopes because full-size antibodies are part of the human defense system. The concept may also apply to cancer-related proteins, which might also have size-restricted epitopes accessible only by antibody fragments.

Industry-based panelists pointed out that these new targets, which are being characterized mostly by academic researchers, might have limited commercial appeal because of relatively small-size markets (e.g., anti-virus therapies) or limited knowledge of biology (e.g., cryptic cancer epitopes). With new scaffolds, companies might decide to focus on validated targets to mitigate the risk of introducing new scaffolds into an unclear biological pathway. Whether fragments will match the probabilities of success for full-size antibodies is an open question.

The question of differentiating molecules in the crowded anti-TNF space, where full-size IgG, receptor fusion proteins and pegylated Fab products are already approved, was raised. Steffen Nock (Arana) noted that it might be possible to differentiate based on response in subsets of patients that do not respond, or lost response, to other therapies. There may be other commercial advantages such as higher production yields, lower immunogenicity, better tissue penetration or novel intellectual property. The latter might provide needed freedom to operate, e.g., regarding Genentech’s Cabilly patent. However, Dr. Mohler noted that producing just another anti-TNF agent does not make sense for a company such as Trubion because of the difference in their company’s technology and objectives. However, utilizing a new mode of action might be attractive. In this case, the risk is associated more with the biology and less with the structure of the molecule. In addition, combinations of monotherapies have lead to increases in serious adverse events without concomitant increases in potency. The challenge in the preclinical area is to understand which combination to explore and what will provide therapeutic benefit relative to safety issues.

For the second topic, selection and screening, Dr. Audoly then asked panelists to comment on their ability to do better than established methods such as phage display or classic hybridoma technology in the time from lead discovery to the start of GLP safety studies. Dr. Mohler commented that this was difficult to assess because Trubion uses a variety of domain types. For
and it is almost exponentially more complicated on the biology side. The most difficult case is with two cell surface receptors as targets; they have tried setting up screening matrices that are 2 by 2, i.e., two antibodies targeting two cell surface receptors are cross-linked, and then the combination is evaluated for some biological response such as cell death in a tumor cell line. This

Table 1. Therapeutic monoclonal antibodies in FDA review or approved

| Generic name         | Trade name | Target and type | Indication under consideration or first approved | FDA approval year |
|----------------------|------------|-----------------|-------------------------------------------------|------------------|
| Raxibacumab          | ABThrux    | Anti-B. anthrasis PA; Human IgG1 | Anthrax infection | Pending          |
| Tocilizumab          | Actemra    | Anti-IL6R; Humanized IgG1 | Rheumatoid arthritis | Pending          |
| Motavizumab          | Numax      | Anti-RSV; Humanized IgG1 | Prevention of respiratory syncytial virus infection | Pending          |
| Denosumab            | Prolia     | Anti-RANK-L; Human IgG2 | Bone loss | Pending          |
| Ofatumumab           | Arzerra    | Anti-CD20; Human IgG1 | Chronic lymphocytic leukemia | Pending          |
| Ustekinumab          | Stelara    | Anti-IL12/23; Human IgG1 | Psoriasis | 2009             |
| Canakinumab          | Ilaris     | Anti-IL15; Human IgG1 | Muckle-Wells syndrome | 2009             |
| Golimumab            | Simponi    | Anti-TNFα; Human IgG1 | Rheumatoid and psoriatic arthritis, ankylosing spondylitis | 2009             |
| Certolizumab pegol   | Cimzia     | Anti-TNFα; Humanized Fab, pegylated | Crohn disease | 2008             |
| Eculizumab           | Soliris    | Anti-CS5; Humanized IgG2/4 | Paroxysmal nocturnal hemoglobinuria | 2007             |
| Panitumumab          | Vectibix   | Anti-EGFR; Human IgG2 | Colorectal cancer | 2006             |
| Ranibizumab          | Lucentis   | Anti-VEGF; Humanized IgG1 Fab | Macular degeneration | 2006             |
| Natalizumab          | Tysabri    | Anti-α4 integrin; Humanized IgG4 | Multiple sclerosis | 2004             |
| Bevacizumab          | Avastin    | Anti-VEGF; Humanized IgG1 | Colorectal cancer | 2004             |
| Cetuximab            | Erbitux    | Anti-EGFR; Chimeric IgG1 | Colorectal cancer | 2004             |
| Efalizumab           | Raptiva    | Anti-CD11a; Humanized IgG1 | Psoriasis | 2003*            |
| Tositumomab-1131     | Bexxar     | Anti-CD20; Murine IgG2a | Non-Hodgkin lymphoma | 2003             |
| Omalizumab           | Xolair     | Anti-IgE; Humanized IgG1 | Asthma | 2003             |
| Adalimumab           | Humira     | Anti-TNFα; Human IgG1 | Rheumatoid arthritis | 2002             |
| Ibritumomab tiuxetan | Zevalin    | Anti-CD20; Murine IgG1 | Non-Hodgkin lymphoma | 2002             |
| Alemtuzumab          | Campath-1H | Anti-CD52; Humanized IgG1 | Chronic myeloid leukemia | 2001             |
| Gemtuzumab ozogamicin| Mylotarg   | Anti-CD33; Humanized IgG4 | Acute myeloid leukemia | 2000             |
| Trastuzumab          | Herceptin  | Anti-HER2; Humanized IgG1 | Breast cancer | 1998             |
| Infliximab           | Remicade   | Anti-TNFα; Chimeric IgG1 | Crohn disease | 1998             |
| Palivizumab          | Synagis    | Anti-RSV; Humanized IgG1 | Prevention of respiratory syncytial virus infection | 1998             |
| Basiliximab          | Simulect   | Anti-IL2R; Chimeric IgG1 | Prevention of kidney transplant rejection | 1998             |
| Daclizumab           | Zenapax    | Anti-IL2R; Humanized IgG1 | Prevention of kidney transplant rejection | 1997             |
| Rituximab            | Ritetax    | Anti-CD20; Chimeric IgG1 | Non-Hodgkin’s lymphoma | 1997             |
| Abciximab            | Reopro     | Anti-GPlIb/IIa; Chimeric IgG1 Fab | Prevention of blood clots in angioplasty | 1994             |
| Muromonab-CD3        | Orthoclone Okt3 | Anti-CD3; Murine IgG2a | Reversal of kidney transplant rejection | 1986             |

Note: Information current as of October 1, 2009; *Proposed trade name; †Voluntarily withdrawn from US market in April 2009; C5, complement 5; CD, cluster of differentiation; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; GP, glycoprotein; IL, interleukin; PA, protective antigen; RANK-L, receptor activator of NFκb ligand; RSV, respiratory syncytial virus; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor; Source: Tufts Center for the Study of Drug Development.
helps to determine whether targeting those specific antigens in a bispecific format makes sense. The approach has had limited utility, but has provided some guidance.

Ray Camphausen (Adnexus) noted that Adnexus uses an in vitro selection technology that, as compared to phage display, affords larger libraries, e.g., on the order of trillions. This provides the potential to hit unique epitopes within a particular target. As the method does not require passage in cells, Adnexus can determine in two weeks whether a target is amenable to the technology or not. This helps with triage of targets that might be of interest. With the wealth of targets now available, it is an advantage to be able to quickly select targets that might be most accessible to a given technology.

Dr. Nock noted that Arana mitigates risk by having a toolbox of different technologies available. They can do humanization, ribosome display, and also phage display to select for fully human antibodies. He noted that the decision on what technology to apply really depends on what molecule you are targeting and what your starting point is; making the right decision allows you to come to the best lead in the shortest time. Dr. Dimitrov agreed and commented that for the same target he might use various libraries, but also various formats like scFvs, Fabs and single chain Fabs, and various displays as phage, yeast and ribosome display. He typically starts with Fab phage libraries and if no useful binders are found, he continues with scFv yeast display and others. He recommended using all available libraries, formats and displays available if initial selection was not successful or improvement is needed.

Dr. Audoly’s next point for the panel to consider focused on the fact that key components of immunoglobulins (e.g., Fc portion) have been removed from the next generation molecules, and so they no longer have some drug-like properties of full-size antibodies. He asked about what modifications might be made to achieve target product profiles, e.g., half life extension. Dr. Nock noted that Arana’s anti-TNF single domain, which is 12 kDa, has been given a long half life in humans through fusion with Fc. The pharmacokinetics (PK) of the fusion molecule are almost exactly the same as those for a full-size antibody. He suggested that pegylation would also be an option; however, this approach is more complicated due to the need for chemical conjugation. An audience member asked about the consequences of increasing the size of the molecule, i.e., making fusion proteins with relatively large molecules including Fc increases the size of the antibody fragments, and may decrease penetration into tissue or accessibility of sterically restricted epitopes. In response, Dr. Dimitrov discussed his experience with modifications using small albumin binding peptides that do not seem to affect neutralizing activity, but increase half life. He noted also that in some cases a short half life might be desirable, e.g., for radiolabeled, drug-conjugated or diagnostic molecules.

Dr. Camphausen noted that one thing that makes scaffolds attractive is the flexibility with regard to PK. He agreed that, with some of the more exotic biologic targets, long half life may not be desired. The options mentioned (e.g., albumin-binding peptides, pegylation) allow PK properties to be fine-tuned. Dr. Dimitrov suggested that optimal PK and optimal half-life should be the goal, and the need for a long half-life should not be assumed.

Dr. Audoly then asked about steps that have been taken to ensure that organizations can develop a reliable process that maximizes the probability of success with respect to stability and formulation, or that might reduce the cost of goods. Dr. Camphausen commented that the attractiveness of alternative scaffolds, especially as afforded by some of the display technologies, is that many leads are provided for a particular program, which means more choices that might fit selection criteria, e.g., stability, are available. The display technology of Adnexus provides hundreds, if not thousands, of leads, which provides them with the latitude to select not only for potency, but also affinity, monomericity and other criteria. These criteria can be applied very early on in discovery, which facilitates downstream processes.

Dr. Audoly inquired as to whether deliberate steps had been taken to remove methionine or asparagine, or to improve biophysical stability so that the resulting leads will have a better chance of becoming drug candidates. Dr. Camphausen noted that the Adnexus scaffold does not have any endogenous cysteines or natural methionine. However, methionines are still kept in the libraries for binders, so they occasionally appear in the loops. Once in preclinical models or in incubation in serum, some cleavages may be observed, but these are readily corrected through engineering. Dr. Mohler commented that Trubion looked for manufacturing issues early on and this allowed them to narrow down to the parts of the scaffold that they consider most robust. They have been able to make formulations that are stable for up to one year.

An audience member observed that ‘function first’ screening is desirable for drug development. The earliest screening of antibodies from hybridomas were function first, but scaffolds require modification, e.g., Fc fusion, before function can be assessed. He then asked whether there was a way to do function first screening with any of the scaffold technologies being discussed.

Dr. Dimitrov noted that the lack of function first screening is an acknowledged problem in panning of libraries for selection of highest activity agents, and that years ago he tried to develop functional panning for selection of potent neutralizers from phage display antibody libraries, but without success. He also wanted to know if anyone knows about successful functional panning. Dr. Camphausen noted that Adnexus takes the basic approach of selecting binders first, then they focus on developing high throughput assessments of the many binders coming out of the process for the properties of interest, e.g., antagonist activity. He commented also that the advantages of automation allow one to circumvent the need for development of a highly functionalized primary assay, as opposed to relegating it to a secondary or tertiary assay. An audience member wondered about the utility of mathematical modeling in the process, but Dr. Dimitrov emphasized that fine-tuning and optimization are empirical processes that, at the present level of our knowledge, cannot be satisfactorily modeled using mathematics.

Dr. Audoly raised the question of the potential immunogenicity of the scaffolds, since they are unnatural molecules, and asked about the steps being taken to de-risk the scaffold candidates. Dr. Camphausen discussed the fact that the Adnexus molecules comprise domains from human fibronectin and are synthetic loops from libraries based on potentially unnatural
amino acid sequences. The company is indeed concerned about immunogenicity and they have several approaches to address the problem. One approach is to take leads and run them through algorisms that predict T cell epitopes and discard those that do not have good scores. He suggested that it might also be worthwhile to invest resources into having molecules assessed using T cell proliferation assays. Dr. Mohler noted that Trubion’s candidates are not natural, but they try to decrease immunogenicity by utilizing natural structures such as human protein components to the greatest extent possible to lower the overall risk of immunogenicity.

To conclude, Dr. Audoly summarized the key points made: validated targets might be safest, but novel targets can be pursued if the available tools are leveraged appropriately; screening approaches, steps and perhaps time are similar to other platforms, but screening is a numbers game; PK is tunable, but it is important to know when ‘good’ is good enough; immunogenicity is a concern, but there is little clinical experience with the scaffolds and it is too early to tell if the preclinical models will be predictive of human experience.