Current and emerging trends in immunogenicity research

In November 2011 Drug Discovery World hosted a collegial style roundtable discussion on the present and emerging developments in this fundamental area of drug discovery and development. The participants are drawn from the biopharmaceutical, biotechnology and vendor community. Although somewhat sketchy in places this is a transcript of what was discussed in what was a lively and interesting debate.

PARTICIPANTS

Robert Jordan – Publisher & Editor in Chief, *Drug Discovery World*
Dr Young Wang – Clinical Laboratory Manager, Macrogenics, Inc
Hua Li – Associate Director of Research, Macrogenics, Inc
Dr Shannon Marshall – Director of Research, Amplimmune, Inc
Krystyna Hohenauer – Global Product Manager for Life Sciences and Technology, PerkinElmer, Inc
Matthew Reuter – Account Manager for Life Sciences and Technology, Perkin Elmer, Inc

Robert Jordan (Chair): Immunogenicity continues to be a major concern for the biologics industry in terms of its impact on safety and efficacy. Immunogenicity assessment of a biopharmaceutical drug's immunogenic potential is a challenging task as it involves the development of a long and labour-intensive range of assays specific for that biologic. Tests that must be designed for transfer to CROs and regulatory bodies and companion diagnostics later in the process. With that in mind, I am pleased to be joined by a panel of experts in this field who, I hope, will share their experiences and discuss present and future developments in this fundamental area.

Please introduce yourselves and tell how your work relates to immunogenicity research and how that work links into other departments within your companies?

Shannon Marshall: Amplimmune, Inc currently has its first programme in Phase 1 clinical trials. It's an FC fusion protein, and my role in immunogenicity for that project was to develop the immunogenicity assays that went along with our toxicology studies for assessing immune response in non-human primates. We then developed screening confirmatory and titer neutralisation and transferred those assays and built some new assays to a

CRO who is doing the immunogenicity testing for clinical samples. I have been involved in managing the relationship with that CRO and co-ordinating some of the work they are doing and we have been partnered with that programme with GSK and have been linked to the immunogenicity team at GSK in touch with the CRO who is running the testing so everyone understands what the assays are, how are they validated, how are they developed and how are they performing.

Young Wang: I am Clinical Lab Manager at Macrogenics, Inc. We do antibody drug manufacturing specialising in autoimmune disease, infectious disease and oncology. My role is the outsourcing of immunogenicity testing and biomarkers to the CRO central laboratories for all these studies. My role is to audit performance of the lab, review the validation report and sample analysis on this type of work. On a day-to-day basis on the oncology studies we try to set up the immunogenicity and PK at our own facility. My teammate Hua Li oversees the transfer to the CROs.

Hua Li: We have multiple areas in the company's programmes – autoimmune, oncology and infectious diseases, so for many of those assays basically I'm in charge of the GLP lab operation in the company and the development of those assays in supporting those studies and also perform the immunogenicity assays as well as neutralising assays in the lab.

Krystyna Hohenauer: At PerkinElmer we don't do research ourselves, what we look to do is enable researchers by developing instruments and technologies that will help you in your research. I have a couple of roles within PerkinElmer. The primary role is global product manager for the Alpha technology this is the bead-based, or ELISA alternative technology. The other role is developing our strategy for the biotherapeutics market, what I mean by that is understanding the biotherapeutics market, what researchers are doing for the market, what your needs are and what we can do to help you with those needs.

Robert Jordan: In your opinion, from an enabling technology standpoint, what is the current state of immunogenicity research for biotherapeutics and is there any technology that you would dream of having that would enable you to improve your research process as a whole?

Hua Li: This is a very interesting and evolving field – at the beginning there were not very many regulations although currently we have more and more publications/white papers in the area. Also, the understanding of the immunology allows people to understand the cause of ADA, all in all a very exciting area, all the immunogenicity studies are growing with the industry and at the beginning people may not have heard of the risk-based approach, but now it seems like everyone is using this design. It is work in progress, but as you can sense it is a very complex area not a cookie cutter that you already have the solutions – we are all learning along the way.

Shannon Marshall: On the technology side there are two things – one, is that I think screening assays basically work but the one limitation on them is that they are not truly quantitative because you have a convolution of antibody concentration and antibody affinity and ideally it would be nice to know both of those things and not just to have titer for present or absent as the thing that you know. Neutralisation is another area where there is a lot of room for improvement and a lot more variability in what people do. There aren't as many clear area white papers and guidance and one of the challenges that I have seen is that, ideally, people want to be using cell-based activity assays for neutralisation because they are more biologically relevant. But a lot of the time those assays are not very sensitive to small amounts of anti-drug antibody so you can move to something where it is a binding assay and do neutralisation in a binding format and then you have the sensitivity you want, but maybe not the physiological relevance. It would be nice to be able to have cell-based assays that are truly a functional assay and not just a binding assay that are sensitive for neutralisation.

Matthew Reuter: Would you say the main technology right now is traditional calorimetric ELISA for neutralisation and immunogenicity assays that are being used?

Shannon Marshall: A lot of people are running MSD for immunogenicity. For neutralisation people do many different things.

Hua Li: We have Phadia.

Shannon Marshall: It really depends how it is that you can measure your drugs activity.

Shannon Marshall: Another thing that would be nice is better positive controls for all of the assays.

There's always an argument right now as to whether your positive control is actually representative and you can get different performance characteristics in your assay depending on whether you pick a low affinity antibody, high affinity antibody, monoclonal/polyclonal...

Young Wang: The positive control is another issue. For all the immunogenicity, positive control is about what type of controls you are using, is it spiking or the use of the real patients samples, how do we do that?

Shannon Marshall: Especially early on before you have patient samples is the real challenge, because the type of antibodies you get when you immunise an animal or what you can buy as a commercial reagent are not necessarily going to be similar to what you get from your patients. If you immunise a rabbit you are putting in lots of adjuvents and you are trying really hard to get a strong immune response and that's not necessarily going to look the same in the rabbits.

Matthew Reuter: Do you feel it is difficult to detect for ADA assays, ADAs that are also in conflict with free drugs and setting up positive controls based on that as well, such as a cross-linked ADA antibody plus your drug as a positive control to start?

Shannon Marshall: That is another one of the things that depends a lot on what your controls are, if you are going in with a low affinity antibody as your control which is more difficult to pick up in screening assays then drug tolerance isn't as much of a problem because it dissociates readily and you compete it. If you take a high affinity antibody as your positive control then you have no problem adding great sensitivity to your screening but then drug tolerance can be an issue. We've managed to get a reasonable free drug tolerance, but it is also going to depend on what is the expected free drug concentration in your patient samples at the times you are sampling for immunogenicity, especially if you have a drug that has a long half life and you are administering at a reasonably high level then it becomes more of a problem. Again, you don't know if your positive controls are reflective, is the dissociation rate of the antibodies in your patients – are they similar to your positive controls or not?

Hua Li: It's a very complex area, obviously there are two meanings for the positive control. One, you use to control the quality of your assay to see if you can repeat consistently over time and the other one you want to mimic as much to the real ADA in the patient. However, the nature of the ADA is very complex from individual to individual and there are differences even with same individual as the immunology changes. So you are going to have a different repertoire along the way so how can you find a positive control to mimic such a great variety in a patient population? I'm working in assay development so my main focus is to move more towards assay consistency and obviously we are going to use those to provide some of the key parameters of the assay like sensitivity, and the sensitivity is relevant to the positive control and how that relates to the ADA in the patient we don't know. And also most likely the positive control we use is IgG or purified IgG or purified monoclonal antibody. However, the true ADA in the patient could be a combination of all sorts of things which can really be a challenge. Practically, we mention rabbit or some people use goat but how about non-human primates? That's the area to think of twice to see what we can figure out and I do notice some big pharma are using non-human primates and that's closer to humans, but if that's really relevant I don't know.

Robert Jordan: The majority of biotherapeutics developed to date have been antibodies and there seems to be growing area of research in the development of peptide biotherapeutics. Do you think this is going to continue to increase and do you have any experience in the development of this kind of therapeutic?

Shannon Marshall: I haven't done too much work with peptides, I worked with antibodies and recombitant proteins that aren't antibodies, but they are all bigger than peptides.

Matthew Reuter: What would be your thoughts on fusion proteins and that rivalling monoclonal antibodies or biospecific antibodies?

Shannon Marshall: They are all a little different, there have always been a niche for fusion proteins and there are some individual targets that are better reached by a fusion protein than by an antibody so you get different specificity and different function in some cases. There are certainly examples on the market of both antibodies and fusion proteins that are very successful drugs. And I think that will continue to be the case in new drugs.

Krystyna Hohenauer: Is there a specific decision process that is taken as to whether you are going to develop a fusion protein or a protein or an antibody as the therapeutic to the target?

Shannon Marshall: Yes, it has been a conscious decision on projects I have worked on. In some cases it has to do with what is the specificity that you can reach so with a lot of molecules you have multiple receptors and multiple ligands interacting with each other and if you have an antibody for example, antibodies tend to be more specific in their binding patterns. So if you have a family of four receptors the antibody will in many cases bind to one of them, where you may have a fusion protein that may bind to three of the receptors and that gives you a different biological effect. The structures are a little bit different, the geometries that you get are different, so in some cases if you make it one way you will have an agonist, if you make it another way you get antagonist and they all have different consequences. But it's very dependent on the individual target, there are plenty of targets you can hit with an antibody that there isn't really a relevant fusion protein that you can make also.

Hua Li: I think in that sense, if we start with a monoclonal antibody and then as you mentioned that is one of the extreme the peptide, I have no idea. But I can also draw your attention as Shannon mentioned – it's science driven, we really need to listen to what the patient needs. In this case, it is the biospecificity for the fusion proteins or therapeutics that draw a lot of attention recently. At Macrogenics we have a technology called DART platform. It's a dual affinity retarget platform so a small fusion protein, let's say it is a biological, has two specificity which could act on two different targets and in that sense we have the platform used in different areas, for instance, like oncology. We can use one arm to engage the effective cells and the other arm to engage the target cells so we can engage the T-cells to kill the tumour targets and we can also use that for autoimmune disease because we can target two cytokines or you can target two targets on the cell surface in a cyst or trans behaviour. I think that's a growing area and there are a number of molecules already in the trial. Indeed, molecules other than monoclonal antibody are emerging and this is one of the examples.

Krystyna Hohenauer: In those terms, you would say around monoclonal antibody if you develop that, that's brutally targeting a receptor that's involved, whereas if you take the approach of fusion proteins you are actually utilising the patient's immune system to have a greater effect – is that the thinking behind it?

Shannon Marshall: Well it really depends on the individual target, antibodies can certainly engage the immune system as well via the FC portion, and there are many examples where that's the case. The FAB region of the antibody usually has one binding partner you can make like specific antibodies where the two arms are binding to two different things. With a fusion protein, the thing you are fusing to is an endogenous protein in the body so you are taking advantage of whatever it is that the protein has evolved to do. It's functional in the body normally and in some cases the fusion protein will recapitulate the function of the endogenous protein and in other cases it will inhibit it. But it's very much on a product by product basis what the differences are, I think it's been best fleshed out with the TNF inhibitors where you have Enbrel as a fusion protein and multiple anti TNF antibodies and they function differently from each other they have different binding modes, their efficacy and different disease indications are different and toxicity profiles aren't the same but it will be idiosyncratic for each of the individual targets.

Young Wang: From a business perspective the antibody drug it's pretty mature, there are multiple successful stories of products on the market and it's still growing. You have a good assay to go with the other antibody drug product and the companion diagnostic kit. I see that fusion protein is a good area but my personal opinion is that I still like the antibody drug that's the humanised chimeric antibodies.

Robert Jordan: Looking at biosimilars and biobetters, a few of these are being developed to reduce the rate of immunogenicity and prove or improve drug efficacy. Would you like to comment on the impact the development of these drug types will have on the market in the future.

Shannon Marshall: I think there is a long way to go before they are really a big threat. From the point of view of immunogenicity it will take a while to prove that a new biobetter truly has less immunogenicity and that that gives you a patient benefit and its going to take a lot of money to prove that the first time round and a lot of time. And until it's truly proven clinically, I hear a lot of skepticism

from people about are you really going to make it a lot better that it's going to make a difference?

Matthew Reuter: Are they describing being better by being less immunogenic or more effective?

Shannon Marshall: I think more effective, if you have something where the efficacy was the same and all it was less immunogenic – I think it's an open question how much of a market advantage it would be. And how much less immunogenic would it really be? Would doctors switch patients on to this less immunogenic version and change their prescribing choices enough to really create a new market. And there is always a big risk because you are trying to make something less immunogenic and maintain the safety profile otherwise but there are no guarantees. It's still going to be a new drug so there is going to be a high bar on those molecules for not showing toxicity in any other way.

Krystyna Hohenauer: Reading some of the reports, outside of Europe and North America there are a huge number of companies set up and their main focus seems to be biosimilars and reporting that their focus is as a biosimilar company. I'm intrigued to see how they do in the market place – if their main strategic focus is just to develop a biosimilar.

Shannon Marshall: It's also what markets they are going after, there are a lot of world markets where a lot of US and European companies are probably not focusing their efforts as much but not sure if they are trying to break into the US market but if they see really good results then I imagine that they might. Certainly there are existing drugs, less of the antibodies but some other protein therapeutics where immunogenicity is very high and I certainly imagine that there is room for improvement. It remains to be shown clinically in people that you can predictively lower immunogenicity and get a significant patient benefit as a result.

Krystyna Hohenauer: The other thing I am see occurring more in the future, which has been mentioned a few times, is companion diagnostics. We are seeing that with some of the newer biotherapeutics they have a companion diagnostics test to go along with them to see whether the genetic markers in a patient are going to show if a drug will actually be more effective, I know for some drugs in some of the cancer treatments, there actual effectiveness was only 30-50% but with the companion diagnostics that rate has gone up in some cases to 85%. So do you have any thoughts on companion diagnostics in the research field?

Shannon Marshall: For patient selection and patient monitoring it's a big issue. With immunogenicity specifically I'm not as sure. An open research question is for a lot of drugs, what does it really mean for a patient to have an ADA response? There are definitely some drugs where you just dose through it, it gets better and the patients respond again. There are other drugs where you have really serious side-effects and need to stop immediately, there's others where you lose efficacy and you would want to switch to a different therapeutic so that patients are being treated effectively. Especially, as assays get more and more sensitive you find more and more stuff – so the question is how to interpret that and how do you use that information to guide clinical decision making?

Krystyna Hohenauer: Every company is gathering a body of research that is specific to a population – do you think there will ever be a time where there will be more sharing of information so that if, for example, as you are developing new drugs you know in a specific population that you might have this type of immuno response, do you think this will happen in the future – really opening up and sharing of information?

Shannon Marshall: It's always going to be limited – there are some big picture things, such as in general people report lower rates of immunogenicity with oncology patients. But it's always hard to tell with a lot of the comparison studies because not only are the patient populations different – you also have differences in assay methodology, differences in the drug dose levels and frequency so it's hard to make some of those comparisons.

Hua Li: Again, it is a complex issue, with the knowledge we gain from immunogenicity studies – especially in the basic immunology studies then people start to get sophisticated immunology knowledge to apply to the immunogenicity area. For instance, in the HRA related to the immunogenicity whether that could come along with pre screening. But again, it's a complicated situation because it's a question of whether the patient has lost the right to be treated or not. Another thing I sense is in the area for inducing intolerance so maybe those patients are prone to ADA so maybe the intolerance induction would be an approach and then that knowledge can be applied to this area.

Young Wang: I have been thinking about the companion diagnostics. This is useful for immunogenicity and its useful information to guide a physician on a drug. If we are doing clinical trials we do the antibody then the clinical trial, I think it's a good idea to do the mutual diagnostic kit for the trial parallel. This is for the drug antibody and it's for a companion diagnostic then it turns into two sets of data to submit to the FDA and then you can approve the drug and that would be on the market so it would be a win-win.

Shannon Marshall: Back to your question about people from industry talking more, the white papers that have been put out have been very successful in guiding what people do in immunogenicity and getting a lot more uniformity among the way different companies are testing. If a similar thing is done in the future especially if there is a specific topic of interest to those in the field. There is some history of people from different companies productively working together on this topic.

Krystyna Hohenauer: If there was another white paper to be produced is there a specific topic you would like it to be based on?

Shannon Marshall: Neutralisation hasn't been well covered, for example.

Krystyna Hohenauer: Neutralisation assays – are those more problematic?

Shannon Marshall: There's more variability and with the other assays it's pretty straightforward to see if this meets the standards of the industry. Is this an appropriate assay that is performing to the level that it should be and with NAB assays there is more ambiguity about what its performance is suppose to look like and what the format of the assay is suppose to be.

Krystyna Hohenauer: For the neutralising assay is there one format that you use at the moment or is it dependent on the project or therapeutic being studied?

Shannon Marshall: I think it's based on the individual therapeutic and what assays are available to measure its activity.

Robert Jordan: I'm seeing an increase in animal imaging, how much of an interest is that to you?

Shannon Marshall: We are definitely interested in imaging developments. The main limitation for us in animal imaging is that it's very expensive for the instrumentation. For biodistribution it's interesting to us as well as in terms of running animal models of efficacy. For example, if you are running tumour models or metastasis models where you don't have a palpable tumour and if you want to measure disease severity as a function of time – you can do it with an imaging modality otherwise you can't measure in the live animal. Some of these are more physiologically relevant models than running a subcutaneous tumour model and that's very useful. Also with some of the autoimmune models you can do measurements for paw thickness for CIA, you can measure some of the abnormalities in EAE? and it also gets around some of the bias from the individual person who is measuring especially for things where it's a subjective disease spore if you can get it to be an objective disease spore where you are measuring something that is a subjective if you can get it to be an objective. It creates a more solid data set so it is interesting to us for those reasons.

Krystyna Hohenauer: Also looking at a mouse that has a tumour, then looking at imaging it, then treating it without having to sacrifice the animal, then you are going to get truer information about that tumour and instead of sacrificing at different points to get the data.

Shannon Marshall: You can get a lot more power out of your study. Otherwise if you are sacrificing them at different points then measuring it's going to be a really large study but if you can get all the data points from one mouse because you don't have to kill them to get data that's much better.

Hui Li: It's test results in real time, even in human, these animal studies are needed the commonalities in human study before Phase I to get all the equivalents. Label the isotope or antibody and you know if it goes to the liver or kidney, you know the distributions. The FDA would like it.

Krystyna Hohenauer: That's where we see the future is in those techniques of imaging animals. Untimely also having more sophisticated labels or enabling customers so that when you are going into patients if you want to remove a tumour in the surgery, having it labelled so that maybe it is luminescent, when the surgeon is going in it ensures that they get the full tumour, the problem with surgery is in the follow up then treatment because you may have removed the full tumour but have gotten all of the surrounding tumour cells.

Matthew Reuter: Working with biotechs with all of our technologies we see a need and importance to partner closely with the CROs as well. A case in point being Amplimune and Macrogenics for any assays that are transferred out – does the CRO have the relevant technology that you are currently using or can they offer you something better?

Shannon Marshall: We have switched, for example we did all of our preclinical on DELFIA because we have DELFIA. The CRO did their's on an MSD because that's their standard platform, so its inefficient because we double developed those assays but at the same time the plate reader that does DELFIA is a work horse for us because it runs lots and lots of assays. So from a small starting company it was much more cost-effective for us to do it.

Robert Jordan: What advice you would give industry vendors?

Shannon Marshall: In terms of technology, I would like to be able to go from mouse to rat to non-human primate to human but there are very few companies that let me do that. **DDW**