MEETING REPORT

Engineering antibodies for targeting

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A two day workshop, entitled "Recombinant Antibody Technologies" took place at Nov. 3./4. 1999 in Heidelberg, Germany. It brought together experts for phage display technologies, antibody engineering and therapeutic antibody applications from academia and industry. Major players in commercial library technology (Morphosys, CAT, Dyax) were represented, as well as companies having successfully brought therapeutic antibodies to the market. A significant part of the workshop was devoted to round table discussions bringing together developers and users of the technology - focussing on what we have learned in the first decade and what we can expect from the next ten years.

The interest in antibody-based therapeutics illustrates a substantial change in the paradigms of pharmaceutical development: by utilising the body's own capabilities as a source for a drug rather than the chemists reagents vessel. However, after more than ten years of phage display technology and the use of human antibody libraries, we still cannot find a single product on the targeting market. Despite the approval of the first therapeutic antibody (Orthoclone) by the FDA in 1985, it took until 1994 to add the next one to the list, and until Nov. 1999, only a total of 9 antibodies were approved by FDA for clinical use. Of these, none is a fusion protein and none was initially made by recombinant technology. All of them are IgGs, derived from mouse hybridomas (2 original mouse IgG, 4 mouse V region / human C region chimeras and 3 humanised mouse monoclonals). This is not surprising, however, due to the typical lag phase of 10 years after proof of principle a pharmacon needs to reach the market, as exemplified for a therapeutic anti-TNF antibody by A. Möller (BASF, Ludwigshafen, Germany). So, the above figures should not discourage us in this respect. In contrast to hybridoma technology, which has clearly reached the endpoint of its technological development, antibody engineering is still developing rapidly, as illustrated by the presentations at this workshop. Some possible key technologies of the next decade, like ribosomal display (presented by A. Plückthun, University of Zürich, Switzerland) or selecting recAbs on the surface of intact cells (e.g. using the ProxiMol strategy of CAT, Cambridge, UK, as presented by S. Lennard), have just started to unfold their potential. But even without these new promising approaches, a plethora of different fusion proteins and targeting concepts employing recombinant antibodies emerged in the first decade and has already reached clinical trials. Currently, more than 80 phase I-III studies are going on, including about 60 on cancer therapy.

The mostly empirical approach in the development of tumor targeting agents that dominated the first decade of antibody technology was mirrored in the excellent presentation of S. Rybak (NCI, Bethesda, USA) who summarised her work on antibody targeted RNases for tumor therapy. Being
small molecules of human origin or at least nonimmunogenic, RNases are different from the bacterial or plant toxins used in immunotoxin approaches so far. S. Rybak consequently pointed out that antibody targeted RNase fusion proteins (ATR’s) should not be considered as immunotoxins - since they are not toxic without a targeting component. Only after transfer into the cytoplasm of a tumor cell do they very efficiently kill this cell by destroying the tRNAs. In the future, further improvement of the therapeutic efficiency of ATRs might be possible if we learn more about the intracellular delivery pathways of the fusion proteins. The use of bispecific antibodies for the targeting of the RNases (e.g. using the binary immunotoxin concept as presented by the author) might allow to further increase the tumor to blood ratio and to reduce unspecific uptake.

A promising new method for radioactive labelling of recombinant antibody fragments was described by A. Plückthun. Using Carbonyl-Tc(I) complexes, Technetium-99m can be conjugated to a His5 tag in a stoichiometrically and topologically defined manner.

D. Moosmayer's (University of Stuttgart, Germany) presentation illustrated the power of recombinant technology to shape an antibody for the needs of therapy. Starting from a mouse hybridoma antibody to tumor stroma protein, he presented a set of derivatives humanised by chain shuffling, which allowed a delicate fine tuning of the specificity. An interesting approach is the combination of this antibody fragment with tissue factor to achieve targeted induction of the coagulation cascade inside of the tumor - proved to work in xenograft mice so far.

Presented on this meeting for the first time (by the author), the successful production of disulphide stabilised bispecific antibodies was described, combining the proven serum stability of dsFvs with the capabilities of diabodies, and further minimizing the fraction of non-human sequences in the fusion protein.

The presentations on the use of antibody fusion proteins for tumor targeting demonstrated that new and promising concepts are still emerging. The same was observed for the area of antibody generation and selection methods. Several strategies were presented to optimise complexity and quality of antibody gene libraries. I. Tomlinson (MRC, Cambridge, UK). D. Neri and A. Plückthun (both University of Zürich, Switzerland) presented concepts of libraries based on one or a few proven framework structures which allow a dramatic increase of the fraction of correctly folded / complete scFv fragments (this fraction was far from optimal in the "first generation" libraries). A. Bradbury (Los Alamos National Laboratory, USA) approached the problem from another side, by presenting an in vivo recombination system for scFv fragments, allowing impressive library sizes up to $10^{11}$. The trends in library technology clearly point in a new direction - after heading for complexity in the first generation libraries, the emphasis in the generation of the recent "second generation" libraries is put into optimisation of folding efficiency (to improve the yield) and improvement of the selection methods to reduce unspecific enrichments. The spearhead of these technologies is the ribosomal display, which for the first time allows us to avoid the bottleneck of E. coli transformation efficiency since the whole process is carried out in vitro. In combination with completely synthetic antibody gene fragments, which were described by B. Krebs (Morphosys, München, Germany), much higher complexities can be achieved than it is possible in E. coli based systems. A second glimpse of what will come in the next decade was the presentation by A. Skerra (Technische Universität München, Germany) of the "Anticalins" which may obviate antibody fragments for targeting in the future. He added four randomised loops to the core structure of lipocalins, to finally obtain a library of incredibly stable small molecules which are far less complex than antibodies. This library can be screened for binders using phage display in a usual manner. Further, it was already possible to
produce bispecific anticalins (duocalins). A potential drawback for the use of anticalins in targeting approaches, however, might be their high immunogenicity.

There was general agreement that while the making of antibodies is getting more easy, we still have to learn a lot more about the biological background of the targeting setups we put these antibodies in. A. Plückthun, in his brisk closing lecture on the future of the technology, pointed out that it is still not clear what exact properties make an antibody a good therapeutic agent - many opinions are currently perpetuated, but real data are few. This was illustrated by two reports (by W. Wels, Chemotherapeutisches Forschungsinstitut, Frankfurt, Germany, and A. Magener, University of Heidelberg, Germany) pointing out that the therapeutic effect was not influenced by the development of antibodies against the injected protein by the patients. Even the influence of affinity (the factor everybody tries to maximise in current screens for therapeutic antibodies) is not evaluated to a point allowing reliable predictions below a certain threshold. Affinity constants up to a few picomolar have been achieved by genetic engineering, but it still remains unclear whether these ultra-high affinities provide any advantage in the final biological setup when compared to the "conventional" nanomolar candidates provided by a native immune system. To solve these problems, two solutions are possible. First, we have to find out much more about the influence of the antibodies' biochemical properties and ultrastructure details on pharmacology and activity in vivo. Second, we may try to develop methods which allow the selection of antibody or peptide libraries much closer to the physiological situation faced by the assigned therapeutic protein. For example, a screening system which directly provides survival of a target cell only if an antibody expressed by this cell binds to the right target (e. g. by blocking a surface receptor which induces apoptosis), might yield antibodies much more capable to fulfil their dedicated task in a "real" physiological environment than those selected from libraries for high affinity only. It might be much faster, too.

After the first decade of antibody engineering and phage library technology, the basic methods to raise nature's treasures have been established, and we were able to make them robust and reliable. In the next decade, getting human antibodies against an antigen of interest will be more and more outsourced to specialised companies or central service groups in academic institutions - probably much faster than observed for the generation of mouse hybridomas since the technological basis is far more complex. Efficient use of phage display and follow-up methods to generate human antibodies requires large libraries, preferentially handled by robotics. This effort, however, is justified by the lesser effort (and shorter time) necessary to obtain a particular antibody once the system is set up. This is the reason why still only a quite restricted number of labs generates and screens "universal" antibody libraries - for the standard lab application, it is just too much work. Consequently, until now, recombinant antibody selection methods have been employed preferentially where a maximum of commercial value can be created downstream - as in case of therapeutics. In the future, however, the availability of universal library selection services will be strongly promoted by the demands of proteomics. The human genome project will supply the information for about 120 000 human genes. Making antibodies for their analysis with conventional methods would require a quarter of a million mice or rabbits - only recombinant antibody library technology allows us to approach this task at reasonable cost.

In conclusion, there is still an urgent need for further development of recombinant antibody technologies. Selection of new antibodies and design of fusion proteins should be done in closer relation to the physiological setup to provide tumor targeting agents which better meet the
requirements of the in vivo situation they finally have to work in.