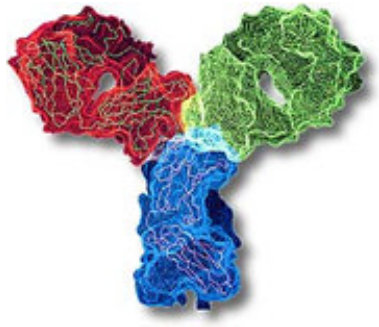
Therapeutic and Diagnostic Antibody Sector: Current Status and Future Directions

March 2006



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Why is ITI Life Sciences Interested in Monoclonal Antibodies?

ITI's mission is to be a catalyst for growth and sustainability of the Life Science Industry in Scotland.

ITI Life Science conducts foresighting to identify commercially attractive areas that can be exploited through technical innovation and the generation of protectable intellectual assets.

ITI's previously released environmental scan on the monoclonal antibody sector identified next-generation technologies as a key area for innovation with significant market opportunities.

This report is one of a series of foresighting documents (stem cells, biomarkers, cell-based assays, nanotechnology, liquid biofuels and molecular imaging) that help ITI define and prioritise strategic funding in order to best meet the ITI Mission.

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Key Findings

A multi-faceted protein, the antibody has been through a number of incarnations and is likely to undergo further engineering to address current challenges and to broaden its utility.

Mabs have made a significant impact on the management of several diseases and their success in terms of sales revenues has led many pharma companies to either collaborate or acquire their way into the mab space, witness the recent acquisition of mab company Abgenix by Amgen.

ITI believes the next chapter in the mab story will be about addressing some of the drawbacks of mabs including the need for parenteral administration and the significant costs associated with manufacturing these protein therapeutics. The latter is critical as many countries try to contain their drug spend by introducing measures such as health economics to look at cost as well as clinical effectiveness. As a result health services and insurers may refuse to reimburse certain expensive antibodies based on a cost-benefit analysis.

Key Findings

In addition to improving current or developing alternative manufacturing processes there are various technologies being developed that could result in lower cost mab therapeutics. Moreover, novel technologies offer the tantalising promise of amongst other things oral administration and use of mabs against previously intractable targets.

One of the most exciting trends within the mab sector is the refinement of existing, and development of new, mab fragment technologies. A spin out of this work has been the emergence of molecular scaffolds that are not immunoglobulins but like immunoglobulins are able to bind with high affinity and specificity to targets. ITI believes these scaffolds present a significant opportunity to create attractive therapeutics retaining the key attributes of mabs but also incorporating other attractive characteristics. The main obstacle is appraising the various scaffolds in development particularly as many have yet to demonstrate proof-of-concept.

ITI is keen to explore the potential of mab fragments and scaffolds in applications such as molecular imaging (as potential tracers) and protein arrays (diagnostics and research tools).

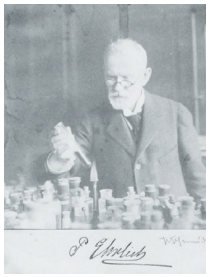
Key Findings

Ultimately it is likely that those developing therapeutics or diagnostics will be able to adopt a mix and match approach depending on the application, choosing first the antibody format required (whole IgG or one of the myriad of fragments and scaffolds) and then if required the molecule to which it is conjugated (cytotoxic, radionuclide, peptide, fluorescent probe…).

ITI believes Scotland is well placed to participate in the development and commercialisation of the next wave of mab technologies. Scotland has the expertise and skills required to develop new mab technologies and therapeutics indeed companies such as Haptogen and Viragen have demonstrated that Scottish companies can commercialise their endeavours in this area. Scotland also has world renowned biologists and this expertise will be crucial if we are to identify novel targets for new antibody-based therapeutics.

Introduction

- At the end of the 19th century, immunologist, Paul Ehrlich proposed using antibodies as ‘magic bullets’ to target tumour cells and pathogens



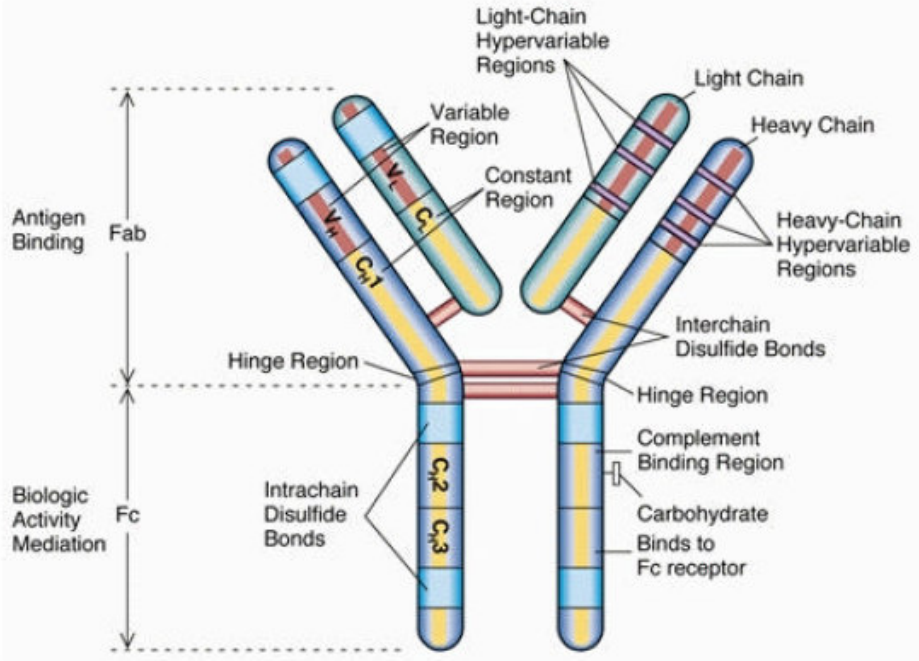
- However, it was not until the introduction of the hybridoma technique by Kohler and Milstein in 1975 that monoclonal antibody (mab) production was possible

- Despite having the technology available to produce antibodies, it took a further decade before Johnson & Johnson’s OrthoClone was launched

- Mabs now represent one of the most exciting and valuable tools for both the diagnosis and treatment of disease

Defining Antibodies

- Antibodies (IgG, IgM, IgA, IgE) are highly specific targeting agents
- They are the body's key defence against pathogenic organisms and toxins
- IgG, the main serum antibody and the format almost exclusively used in therapeutic antibodies, is a Y-shaped multidomain protein with antigen-binding sites located on the two Fab tips and recruitment of effector functions mediated by the Fc domain



- IgG antibodies are bivalent and the ability to bind to two antigens greatly increases their avidity (i.e. total affinity when multivalent antigens and antibodies) to many cell-surface receptors and polyvalent antigens
- The Fc domain recruits cytotoxic effector function through complement and/or through interactions with Fc receptors and can provide long serum half-lives

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Status of Antibody Sector

Products Speak for Themselves

- 24 antibody products have been approved in the US and Europe, 19 therapeutic and 5 diagnostic
- Amongst these are four blockbusters (annual sales over $1 billion): Rituxan, Remicade, Herceptin and Humira
- Several other mabs are expected to gain blockbuster status in the near future
- There are approximately 200 mab-based medicines in clinical trials

SIMULECT®
(basiliximab)

Mylotarg®

ORTHOCLONE
OKT®3
(muromonab-CD3)

Panorex® (17-1A monoclonal antibody)

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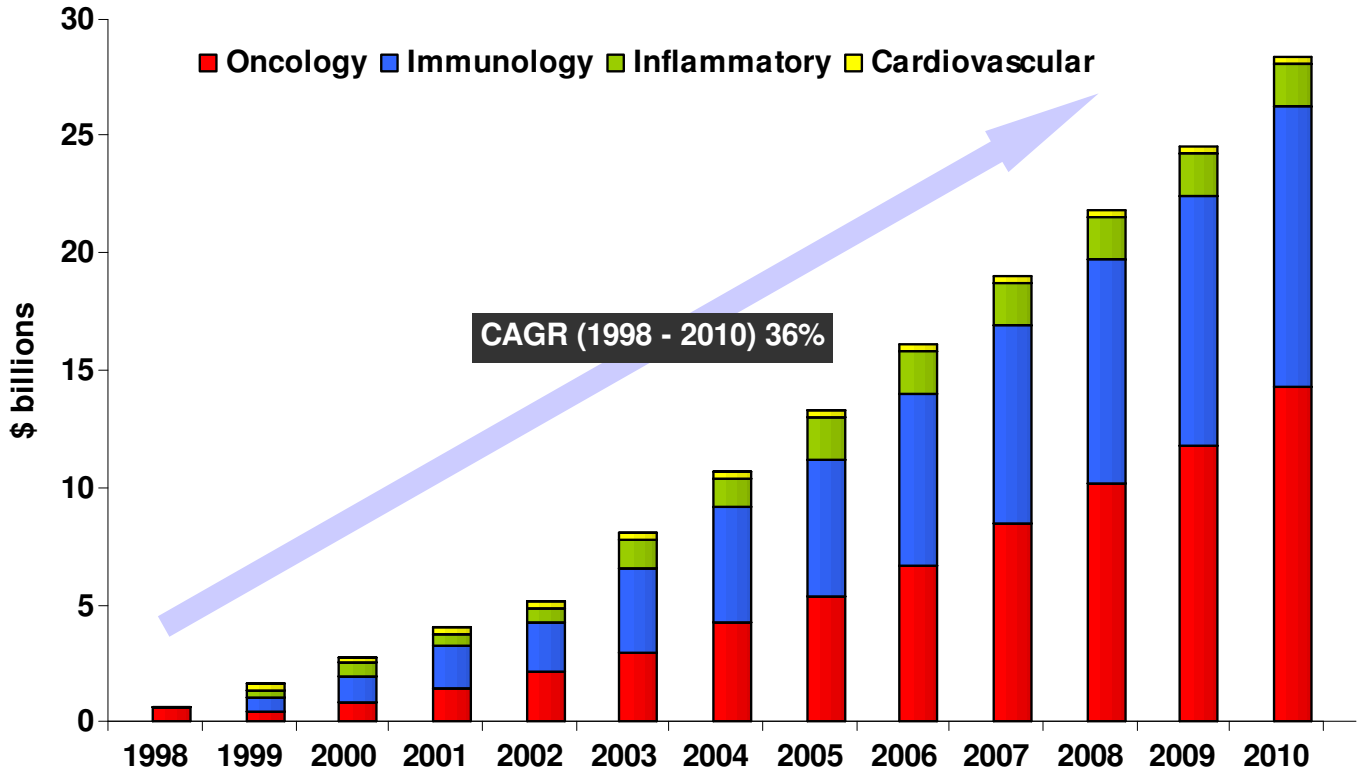
Products Speak for Themselves

Product	Target	Product Type	Generic	Product category	Originator / Partner	Launch
OrthoClone OKT3	CD3	Murine IgG2a	muromonab - CD3	transplant rejection	Johnson & Johnson	1986
Panorex	17-1A	Murine	edrecolomab	anti-cancer	Centocor (J&J) / GSK	1995
ReoPro	GpIIb/gpIIIa	Chimeric Fab	abciximab	gpIIb/IIIa antagonist	Centocor (J&J) / Eli Lilly	1995
Zenapax	CD25	Humanized IgG1	daclizumab	transplant rejection	PDL / Roche	1997
MabThera / Rituxan	CD20	Chimeric	rituximab	anti-cancer	Biogen IDEC / Genentech / Roche	1997
Herceptin	Her-2	Humanized IgG1	trastuzumab	anti-cancer	Genentech / Roche	1998
Synagis	RSV	Humanized IgG1	palivizumab	RSV	MedImmune / Abbott	1998
Simulect	CD25	Chimeric IgG1	basiliximab	transplant rejection	Novartis	1998
Remicade	TNF-α	Chimeric IgG1	infliximab	Crohn's & RA	Centocor / Schering-Plough	1998
Mylotarg	CD33	Humanized IgG4	gemtuzumab	acute myeloid leukaemia (AML)	Celltech Group / AHP	2000
Campath	CD52	Humanized IgG1	alemtuzumab	chronic lymphocytic leukaemia (CLL)	Millennium / ILEX / Schering AG	2001
Zevalin	CD20	Murine	ibritumomab tiuxetan	non-Hodgkin's lymphoma (NHL)	Biogen IDEC / Schering AG	2002
Humira	TNF-α	Human IgG1	adalimumab	Rheumatoid arthritis	Abott / CAT	2002
Bexxar	CD20	Murine	^{131}I-tositumomab	NHL	GlaxoSmithKline	2003
Xolair	IgE	Humanized IgG1	omalizumab	anti-allergy conditions	Genentech / Novartis / Tanox	2003
Raptiva	CD11a	Humanized IgG1	efalizumab	anti-psoriasis	Genentech / Xoma	2003
Erbitux	EGFR	Chimeric IgG1	cetuximab	anti-cancer	Imclone / Merck KGaA	2004
Avastatin	VEGF	Humanized IgG1	bevacizumab	anti-cancer	Genentech/Roche	2004
Tysabri	CD40	Humanized IgG4	natalizumab	Multiple sclerosis	Elan / Biogen Idec	2005

Growth Sector –Therapeutic Mab Market Will Reach ~ $30 billion by 2010



Source – Boehringer Ingelheim and ITI Life Sciences

Mabs are expected to account for a third of all revenues in the therapeutic biotech market by the end of the decade.

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Transforming Disease Management

- Rituximab, the first mab approved for the treatment of cancer, has demonstrated consistent efficacy, revolutionising the treatment of Non-Hodgkin's Lymphoma (NHL):
  - **First-line** treatment with chemotherapies in both aggressive and indolent NHL
  - **Maintenance** treatment in NHL
  - Single-agent to treat relapsed or refractory low-grade or follicular NHL

- Other cancer mabs such as Herceptin and Avastin have been approved as **first line** treatments in combination with chemotherapy for HER2 over expressing metastatic breast cancer and metastatic carcinoma of the colon or rectum, respectively

- The anti-TNF mabs, Humira and Remicade have proved highly effective not only in improving the signs and symptoms of RA, but also attenuating the progression of joint damage, improving quality of life and preserving functional status

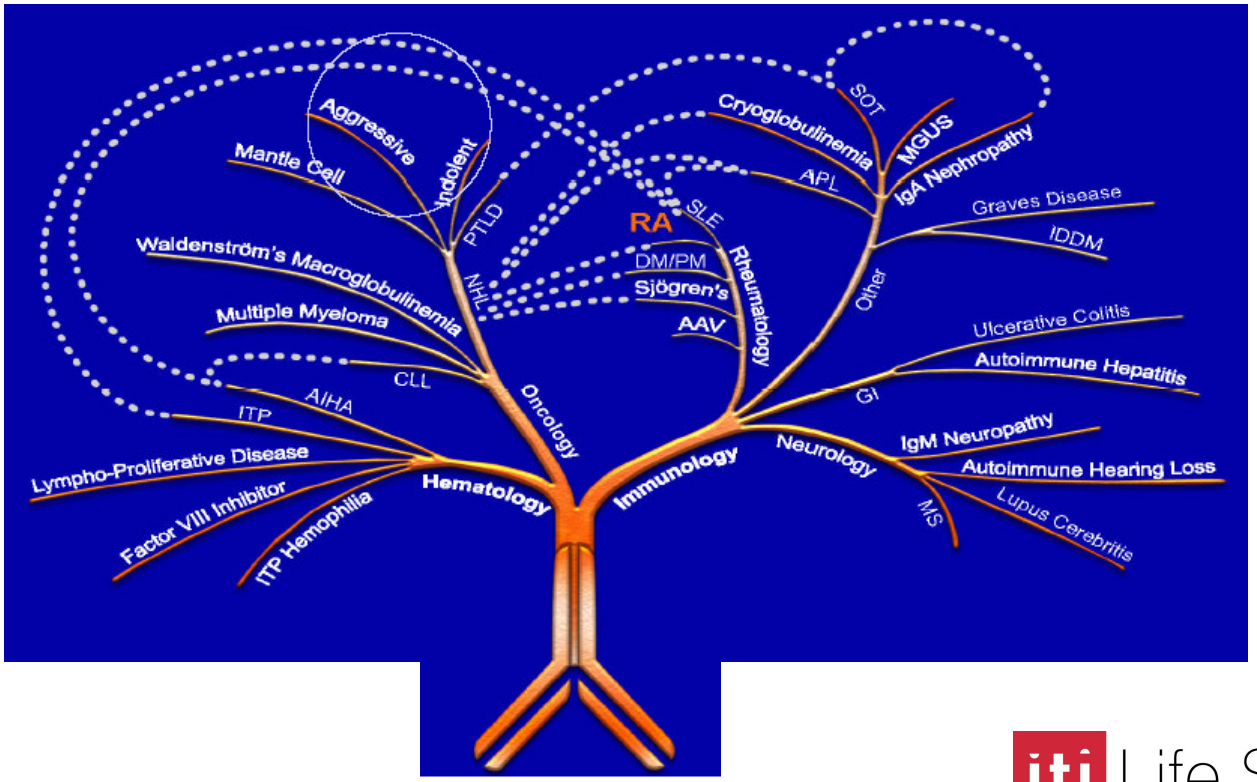
- The anti-respiratory syncytial virus (anti-RSV) antibody, Synagis is the leading agent indicated for the prevention of serious lower respiratory tract disease caused by RSV in pediatric patients at high risk of RSV disease

Multi-indication Therapeutics (MIT)

- Mab therapeutics often secure multiple labels, i.e. they are approved for more than one indication
- Abbott has on several occasions referred to its mab Humira (originally approved for RA) as a “pipeline in a drug”
- Humira is not alone. The other approved anti-TNF antibody Remicade is approved for ulcerative colitis, Crohn’s disease, ankylosing spondylitis and psoriatic arthritis in addition to its approval for RA
- Finally, products such as Avastin and Erbitux are being investigated in many other cancers in addition to the ones they were first licensed to treat

MIT: B-Cell Disease Tree Points to Further Labels for Rituximab

- In addition to the various NHL labels secured, Rituximab has recently been approved (in combination with methotrexate) for the treatment of moderate to severe RA in patients who have had an inadequate response to anti-TNF therapies
- It also appears to be effective in chronic lymphocytic leukaemia (CLL) and other non-malignant B-cell diseases such as idiopathic thrombocytopenic purpura (ITP)



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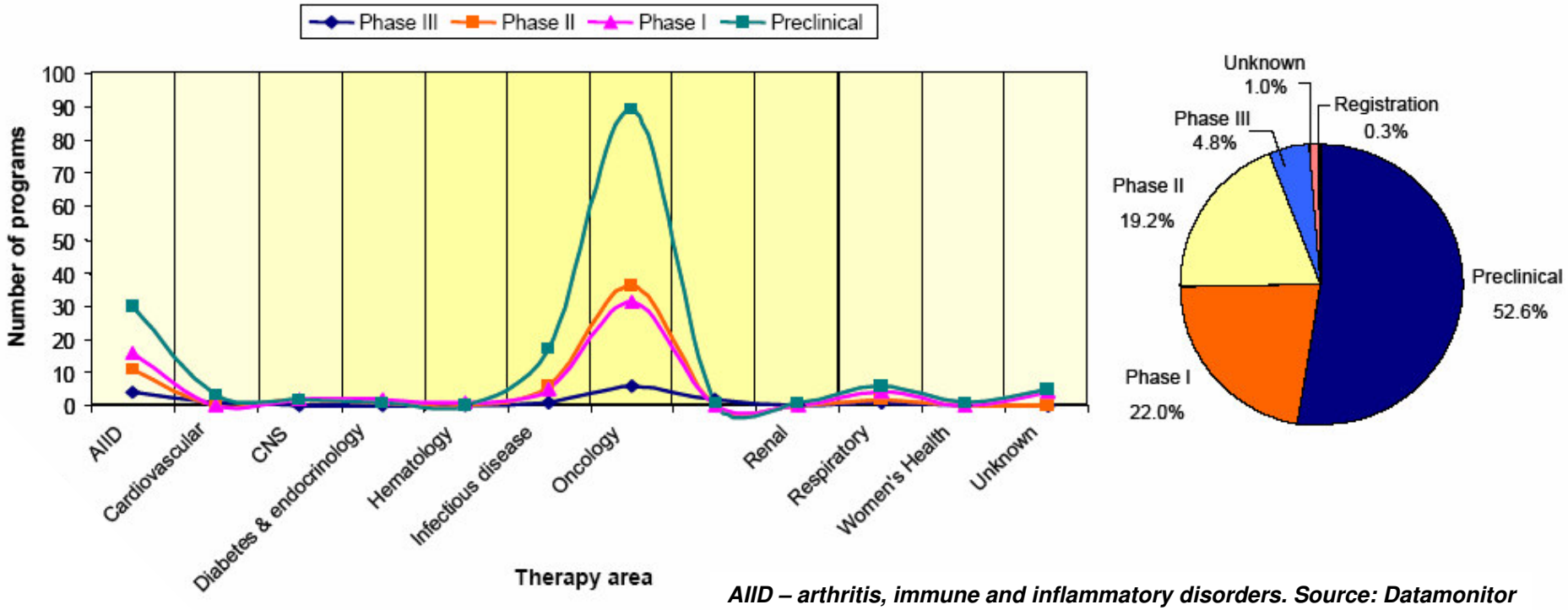
Target Therapeutic Areas



AIID – arthritis, immune and inflammatory disorders. Source: Datamonitor

- Mabs under development have a wider therapeutic focus than those currently on the market – pipeline products span 11 therapy areas, compared to just six therapy areas for the marketed mabs in 2004
- Oncology continues to dominate; however, other areas such as CNS are starting to emerge
- To date, antibody use has been limited to life-threatening diseases because companies must charge a premium to recoup manufacturing and development costs. As the manufacturing costs come down it is likely that antibodies will be used for less life-threatening conditions

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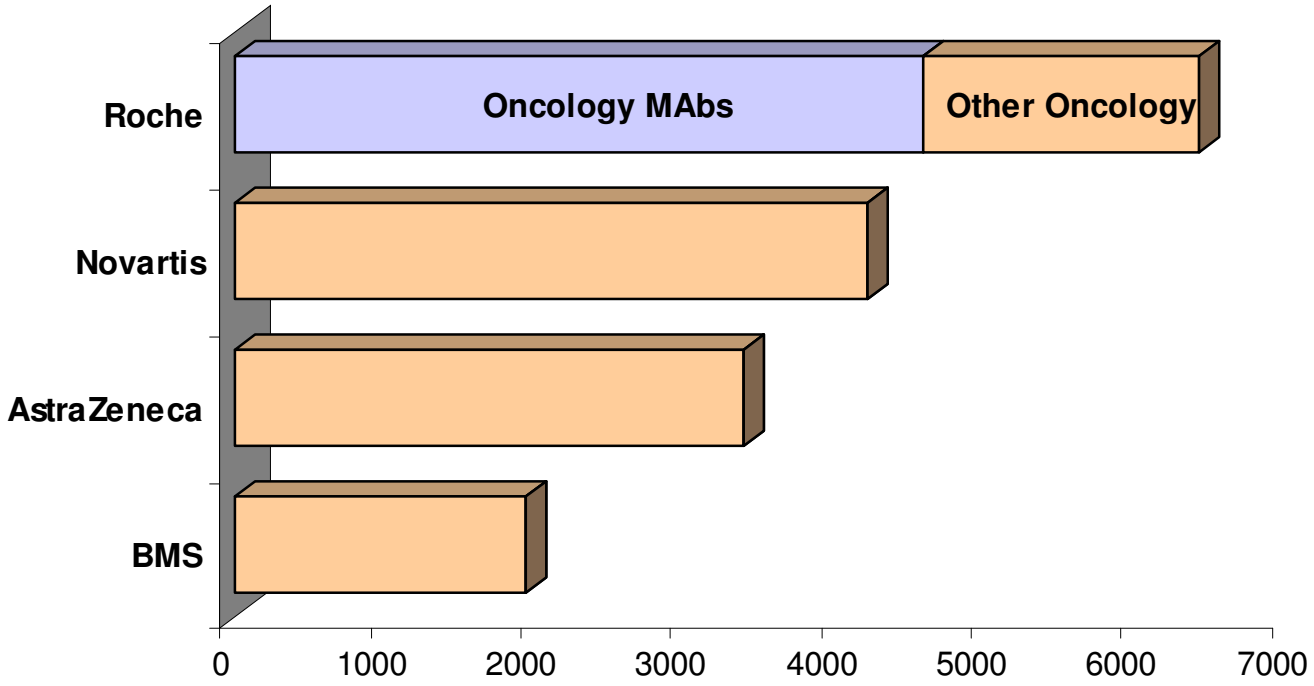
Mabs have Grabbed Pharma's Attention



Oncology Franchise Sales 2004 ($m)
(Source: Company SEC Filings/Annual Reports)

- "We acknowledge that small molecules can't do it all," Jan Lundberg, Head of Discovery, AstraZeneca
- Increasing emphasis among Big Pharma on specialist, niche products
  - costs and risks associated with primary care drugs increasing

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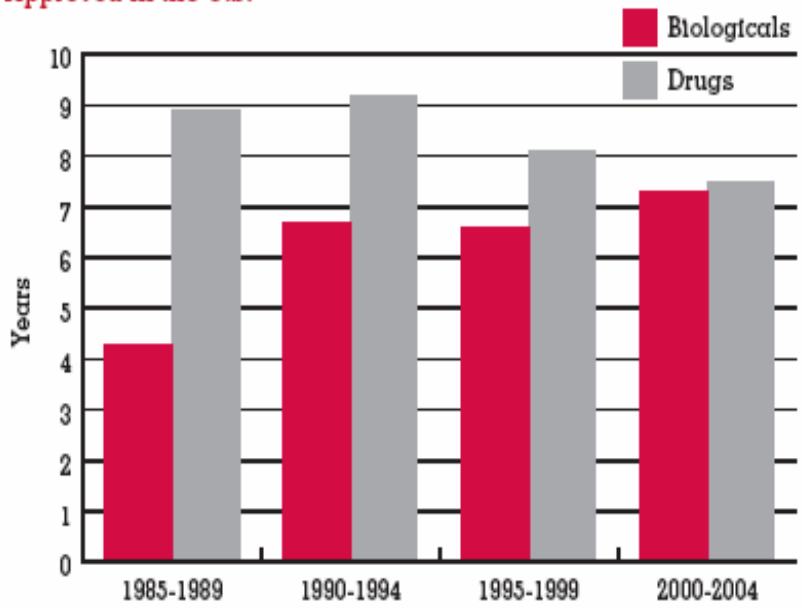
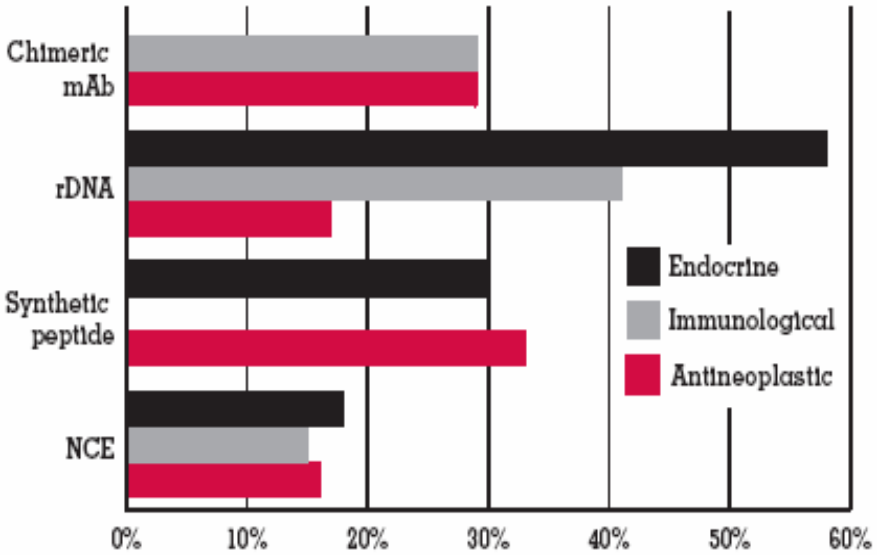
Mabs have Grabbed Pharma's Attention: Development Times Similar but Chances of Approval Better

Total Development Times for Drug and Biological Products Approved in the U.S.



Source: *Tufts Center for the Study of Drug Development*

Comparative Approval Success Rates of U.S. Biopharmaceutical Products and Drugs



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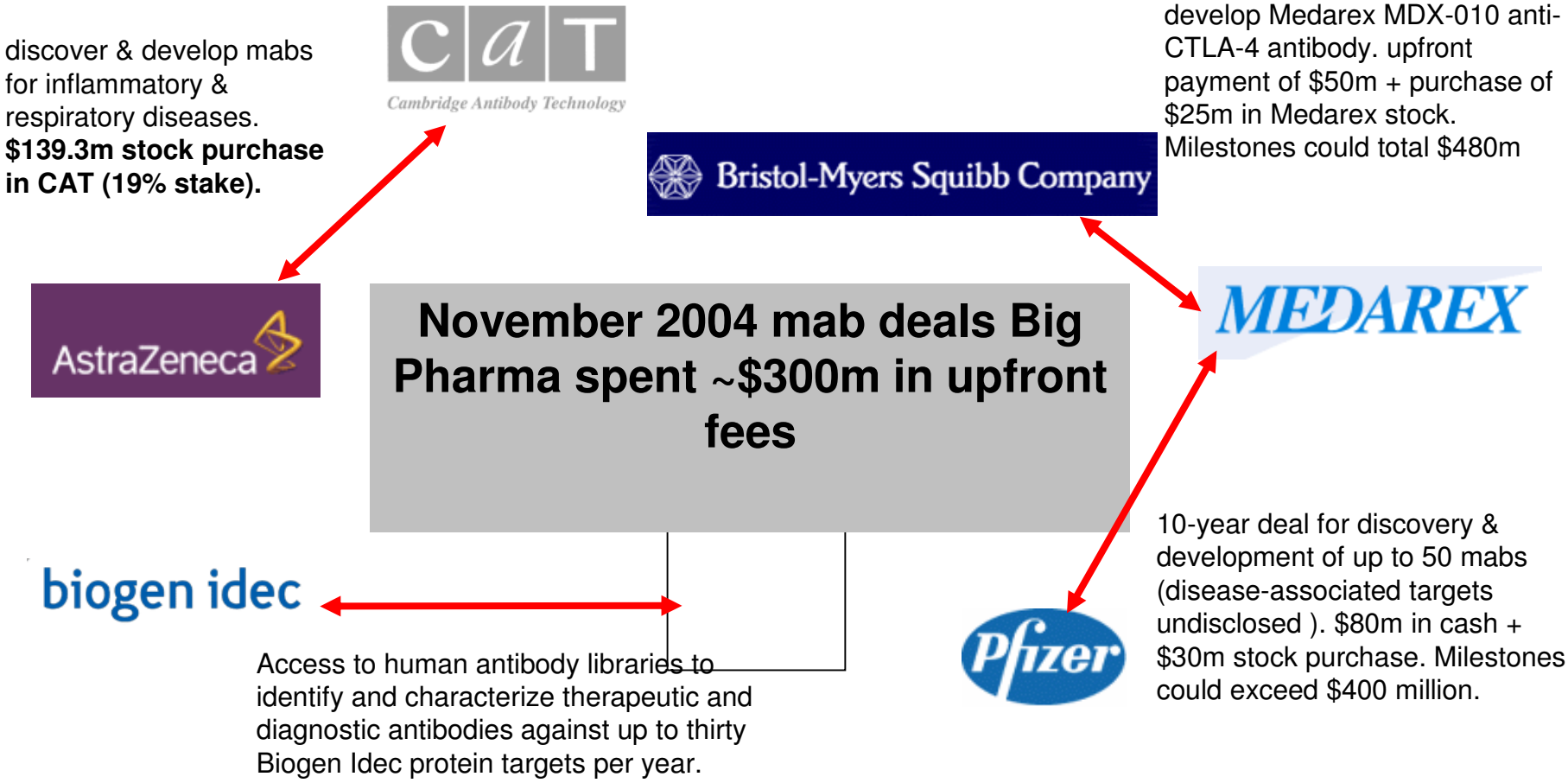
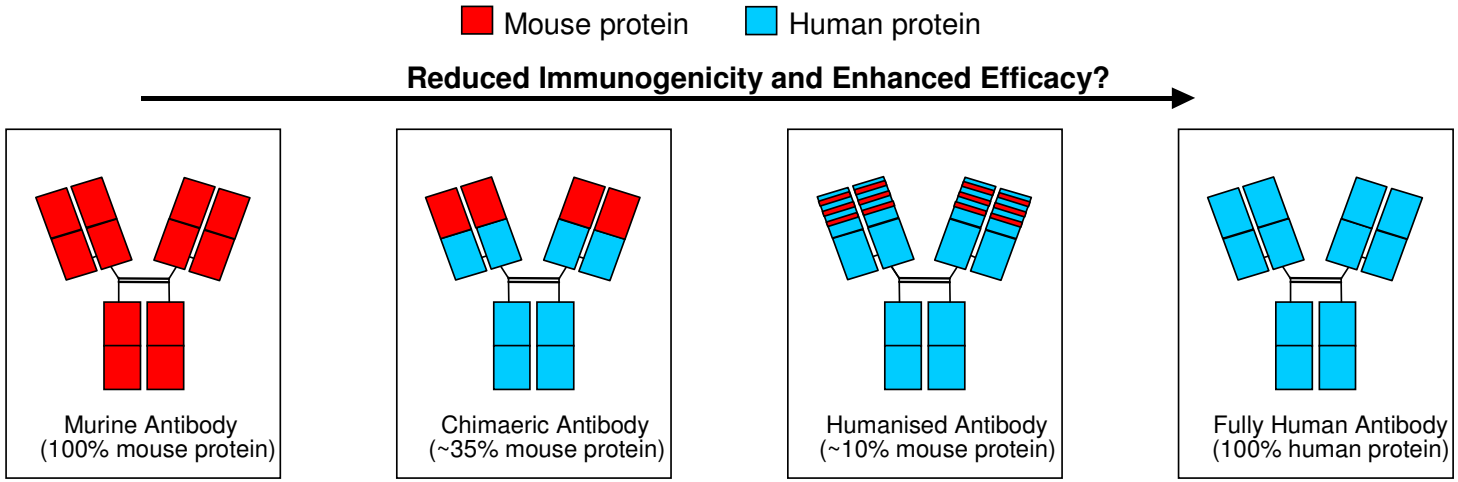
Mabs Grabbed Pharma's Attention: One Month in 2004



Current Technologies

Key Driver over last 30 Years has been to make Therapeutic Mabs more Human



- Immunogenicity has proved to be the most significant barrier to the use of mabs as therapeutics. Consequently, over the last 30 years, a great deal of time and money has been devoted to developing ways to overcome this immunogenicity.
- Murine mabs, when administered more than once, elicit an immunogenic reaction called the human anti-mouse antibody (HAMA) response.
    - This response clears the murine antibodies from the serum, preventing them from reaching their target and producing their therapeutic effect
    - During the clearing process, a set of events is set in motion causing patients to experience flu-like symptoms and allergic reactions which can in severe cases cause death
    - The extent to which immunogenicity presents a problem depends on the therapeutic situation. Where the mab is given only once or administered to immunocompromised patients, the risk of an immune response causing adverse effects and reduced efficacy is less

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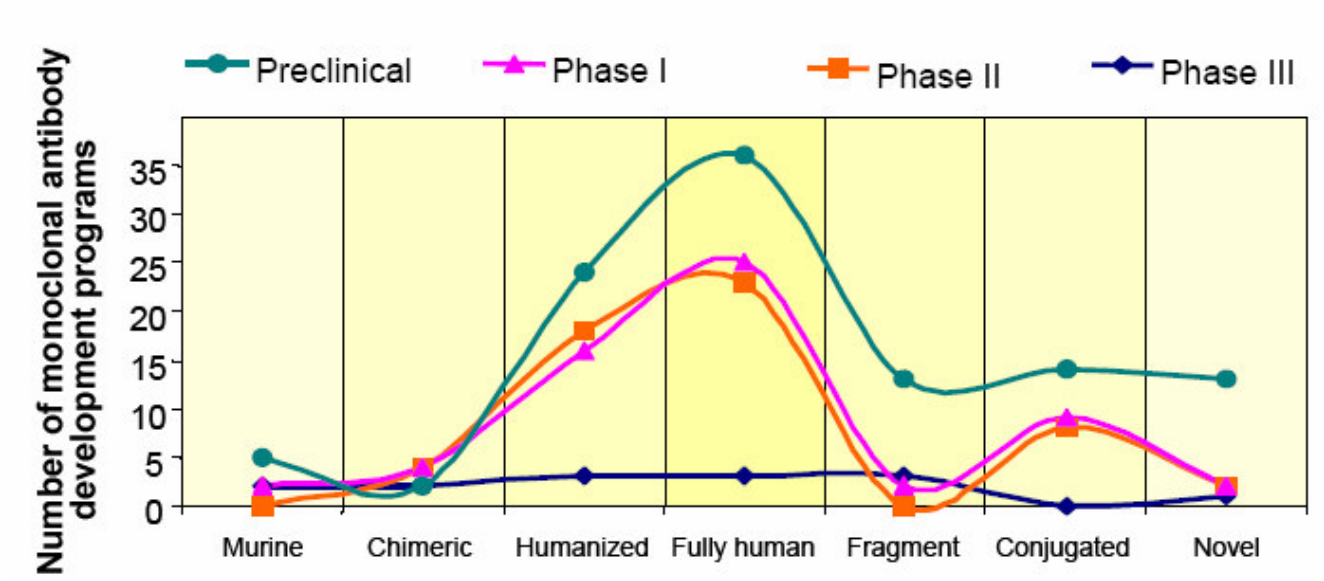
Evolution Translated into Clinic



Source - Datamonitor

- The majority of mabs in development are humanised or fully-human mabs
- This evolution towards more human antibodies and away from murine antibodies appears to have helped reduce immunogenicity, thereby improving the safety and efficacy profiles of mab products.

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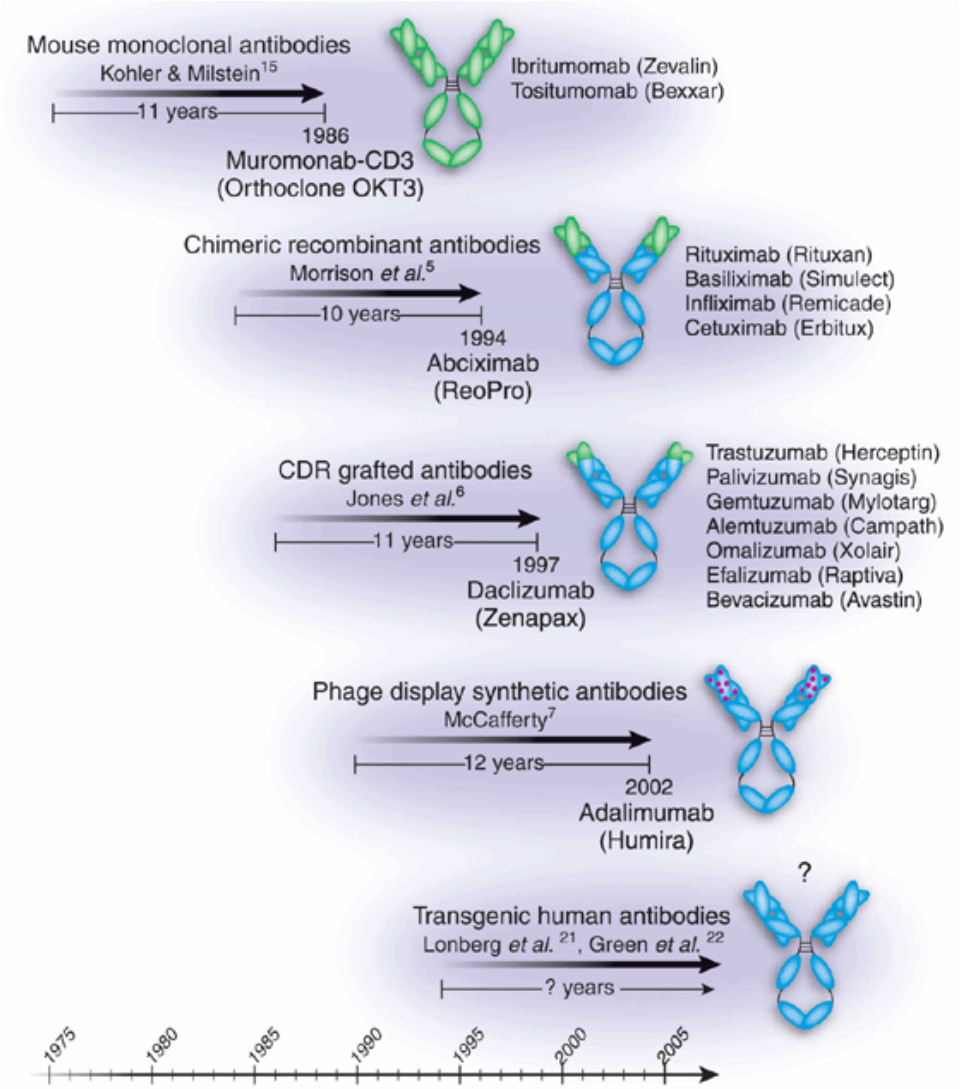
Evolution of Therapeutic Antibody Technology and Progress to the Clinic



Source: Nature Biotechnology, September 2005

- The emergence and refinement of mabs as therapies depends on the development of new technologies
- On average FDA-approved mabs have emerged 10 to 12 years after the date that the new technologies on which they were based were reported in the scientific literature.
- Many of these technologies continue to be used to create new mab therapeutics.
- However, the evolution from murine to fully human mab represents just one technological strategy and we are now seeing new technologies emerging that will address challenges and broaden the application of mabs.

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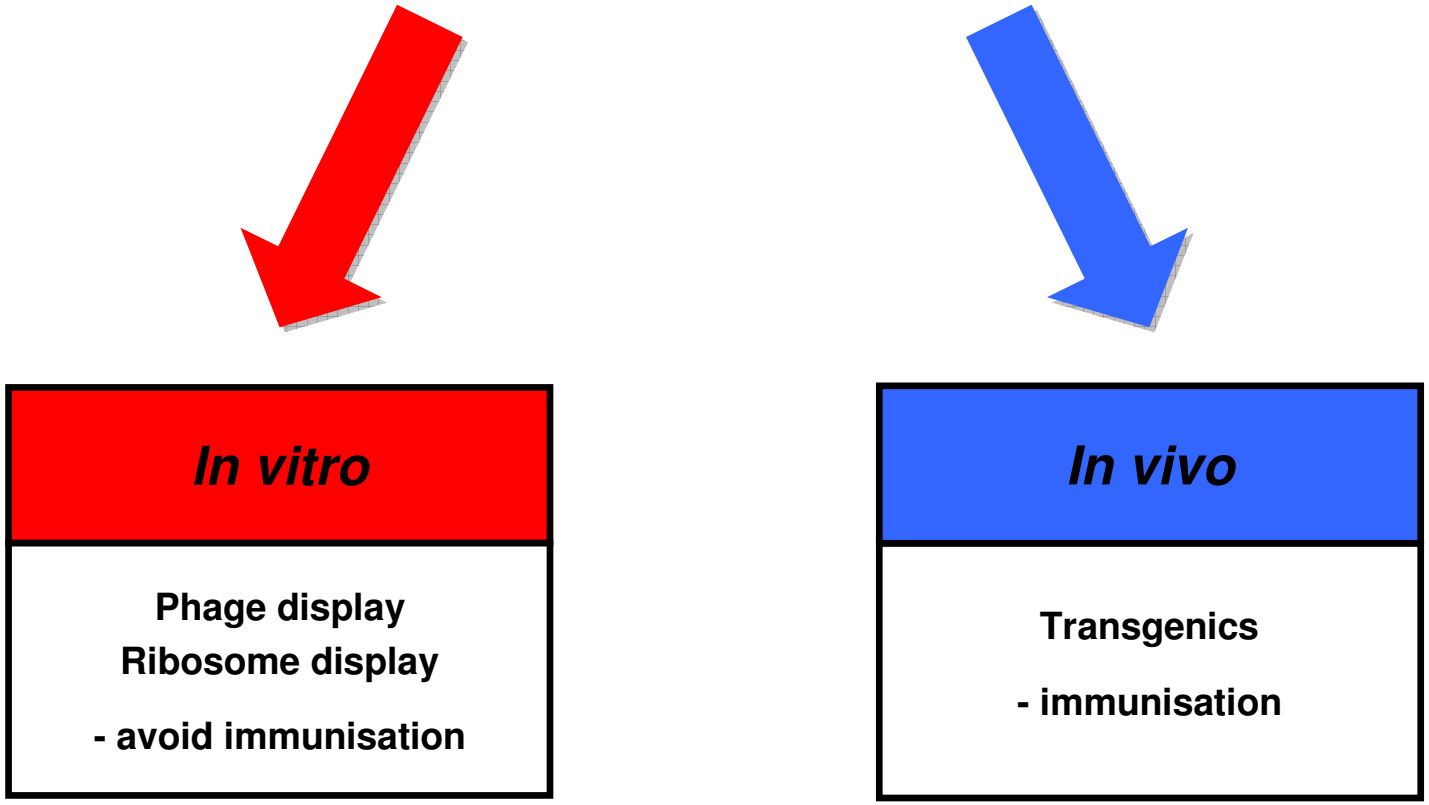
Two Principal Approaches are Widely Used to Generate Fully Human Mabs



In terms of *in vitro* screening systems that link phenotype to genotype there is also yeast display and *in vitro* compartmentalisation

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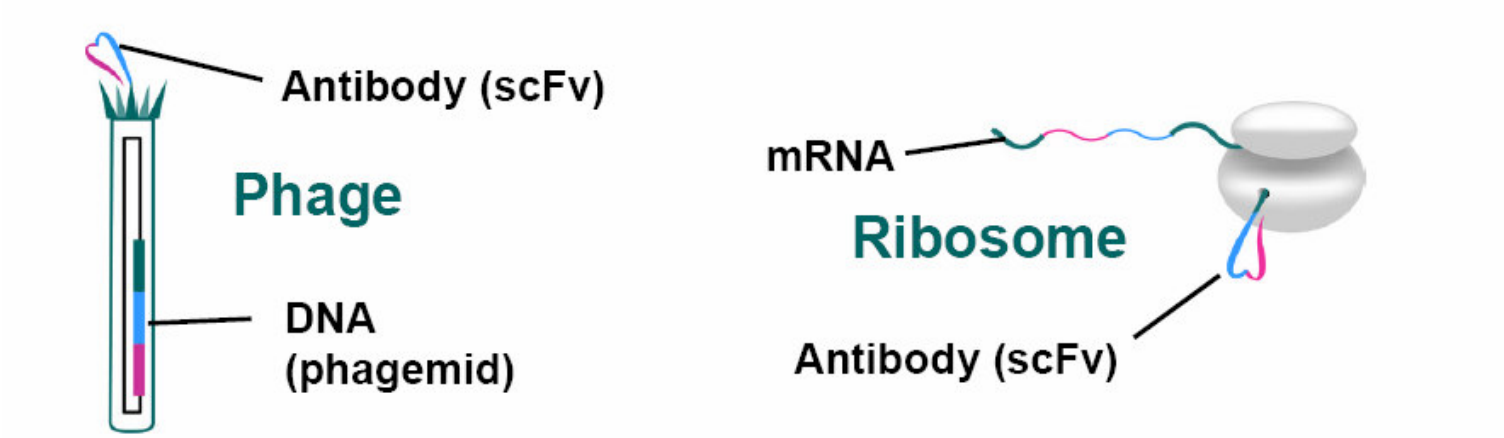
Phage and Ribosomal Display – Coupling Genotype and Phenotype



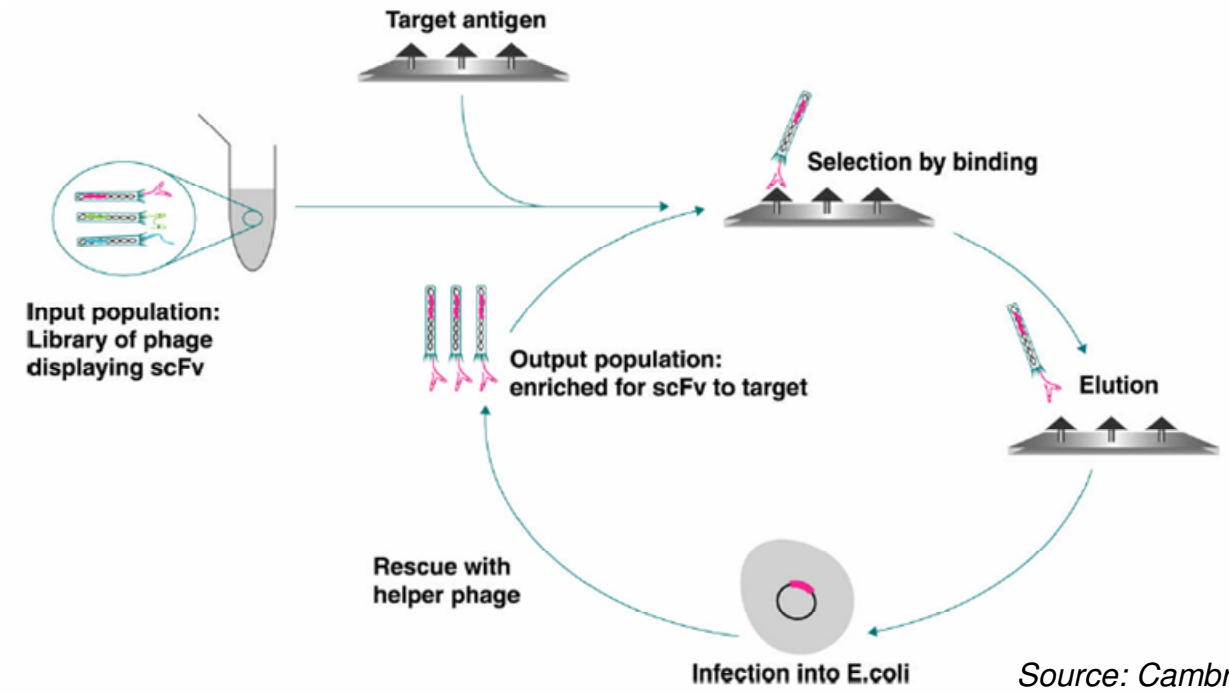
- *In vivo* and *in vitro*
- Library size 10^9 -10^{10}
- Proven track record
- Robust
- Cell-surface selections

- *In vitro* only
- Library size >10^{12}
- Rapid library construction
- Mutagenesis during selections
- Tailored folding conditions

Source: Cambridge Antibody Technology

In its simplest terms phage display involves combining human sequences in thousands upon thousands of variations each of which is inserted into a phage gene. The phage then display antibody-binding regions on their surface, and the entire library can be rapidly screened (biopanning) against particular antigens

The Phage Display Process



Source: Cambridge Antibody Technology

Haptogen
Small is Beautiful™

Within Scotland Haptogen has been leveraging its own phage display technology called **DBDx™** to generate novel mabs that recognise small bioactive compounds (haptens) or the molecular signature (haptenic structure) of larger biological molecules. Together these include: drugs, cell to cell signalling molecules, sugars (glycoproteins), peptides, modification state proteins, polymers and biological toxins.

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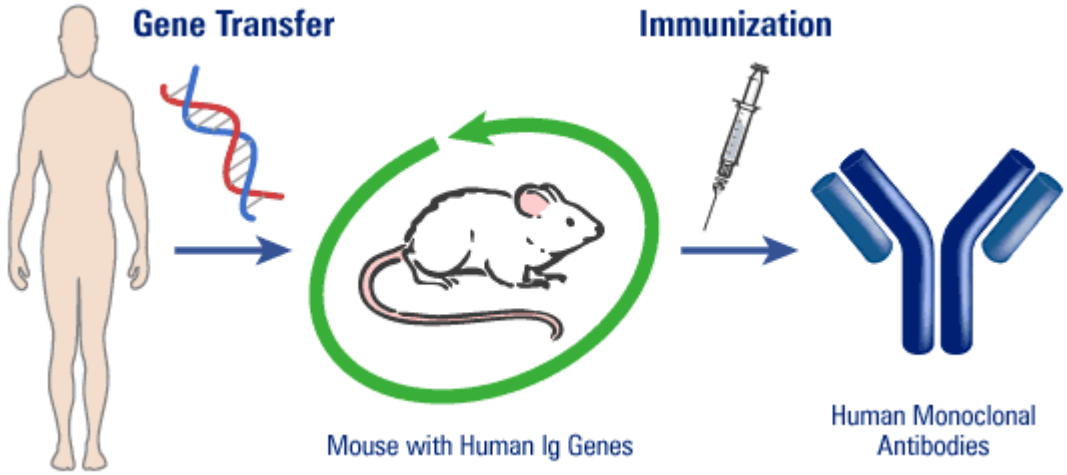
Transgenic Mouse

- Genetically engineered animals such as mice have an immune system in which the animal antibody genes are inactivated and functionally replaced with human antibody genes, while leaving intact the other components of the animal immune system

- These transgenic mice are capable of generating human antibodies to human antigens because the only human products expressed in the mice are the antibodies themselves. By introducing human antibody genes into the mouse genome, it is not necessary to humanize each individual antibody that the mouse generates



Investment By Big Pharma in Human Antibody Technologies

	Abgenix	Medarex	Genmab	CAT	Dyax	Morphosys
Pfizer	✓	✓		✓		✓
GSK						
Sanofi-Aventis						
Merck				✓		✓
AstraZeneca	✓			✓		
Roche/Chugai	✓		✓	✓		
Novartis		✓				✓
BMS						✓
Wyeth				✓		
Abbott	✓	✓		✓		

Transgenic Mouse Phage Display

Pharma and big biotech now generally prefer to enter into broad collaborations or acquisitions to gain access to these technologies

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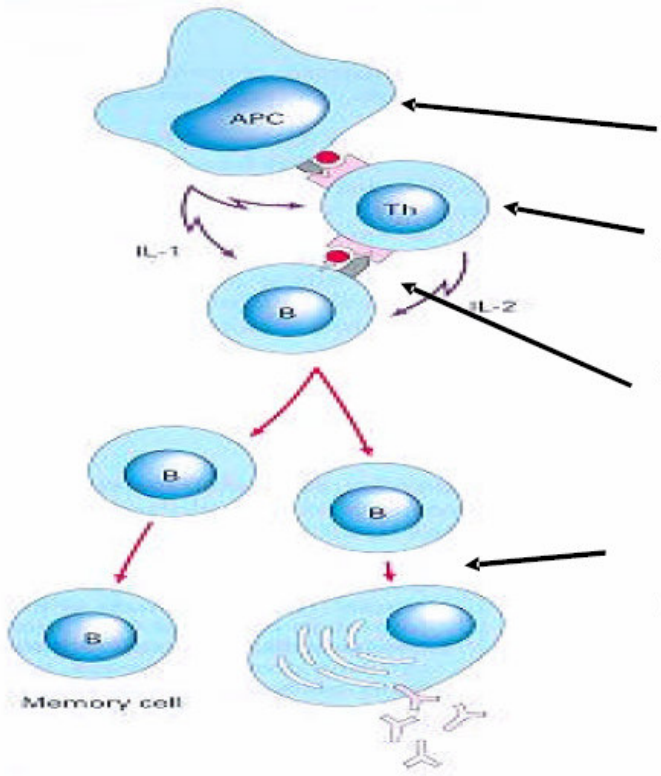
Development of Fully-human Mabs is Not the End of the Road

- In theory we should be tolerant to human antibodies
- However, for several reasons this is not the case:
  - Incomplete tolerance to rare proteins
  - Allelotype differences
  - CDR regions of antibodies
  - Different glycosylation pattern (depending on production method)
- It has been reported that the percentage of patients exhibiting an immunogenic reaction may be higher with the fully-human mab Humira than for several of the humanised mabs (e.g. Synagis and Herceptin)
- Therefore several technologies have been developed to address this issue including for example de-immunisation, gene shuffling and PEGylation
- Intrinsic cause of immunogenicity are CD4+ T cell epitopes located within the mab

Identification of CD4+ T Epitopes

To prevent the CD4+ T helper response (illustrated below) kicking in it is necessary to identify and modify the T cell epitopes



- Antigen presentation by dendritic cells
- CD4+ T cells respond to peptide/MHC
- B cells with the same peptide/MHC are activated by CD4+ T cells
- B cells upregulate Ab, class switch, become plasma cells

A number of companies including the former Merck KGaA unit ***Biovation*** and ***Genencor*** have developed technologies to identify and remove T cell epitopes from proteins including antibodies

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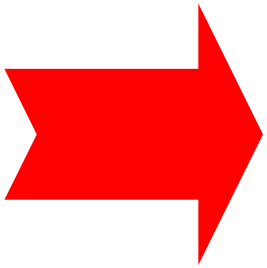
Optimisation of Mabs

After generating any mab further optimisation (through screening and engineering) can lead to more therapeutically valuable mab products

Attributes of Protein that can be optimised

- potency
- affinity
- k_{on}, k_{off}
- thermodynamic stability
- solubility
- serum half-life
- expression
- folding kinetics
- binding specificity
- protease susceptibility
- effector functions
- drug recycling



Benefits

- greater efficacy
- improved pharmacokinetic profile
- enhanced patient convenience
- decreased cost-of-goods
- improved safety
- reduced immunogenicity
- longer shelf-life

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Key Lesson From the Past 30 years

- The development of mabs has not been without its problems
  - In February 2005, Biogen Idec and Elan suspended marketing and clinical trials of Tysabri (natalizumab, a humanized monoclonal antibody against integrin alpha4) after two cases of the often fatal progressive multifocal leukoencephalopathy (PML) were seen in a multiple sclerosis (MS) trial. The FDA has recently allowed Biogen Idec and Elan to reintroduce Tysabri to the market as a first-line therapy for relapsing MS in conjunction with a strict risk management plan.

- One of the key lessons learnt by the sector is that **biology matters**, and some of the initial failures could be due to poor choices of targets. Many of the platform companies have now absorbed this lesson.

- However, rather than building large biology groups and funding costly and time-consuming target discovery many have tended to focus on targets that have been validated by marketed products

- An understanding of disease biology is also key to choosing the appropriate antibody technology for generating the best antibody therapeutic

Intellectual Property

Key Intellectual Property Issues

- A number of parties have patents relating to the generation of antibody products. This has resulted in numerous cross-licensing arrangements among companies seeking to develop antibody-based products:
  - Amgen – Genentech (manufacture and use of antibodies and related technology e.g. under the Cabilly patent family)
  - Abgenix-Medarex (transgenic mouse technology including that covered by the Lonberg patents)
  - CAT – MorphoSys (phage display technology)
  - CAT had also entered into cross-licensing deals with Dyax, Xoma and several other companies

- Royalty Stacking - in many cases, companies will have to license at least two or three patents in order to make a single antibody product adding considerable cost to the process
  - This can become very complex and can create tension between partners as witnessed by the recent dispute between CAT and Abbott over royalties on the anti-TNF mab Humira

IP - Claims Scope

- **Comparison of USPTO and EPO approaches to antibody claims. Broad claim scope for antibodies to novel antigens but claim scope becoming more limited for antibodies to known antigens**
- **The following illustrates what claims are possible in the US and EU for antibodies against novel and known antigens**

PATENT OFFICE	ANTIGEN NOVELTY: Novel Ag	ANTIGEN NOVELTY: Known Ag
	Novel Ag; USPTO An Ab that specifically binds Ag X	**Known Ag; USPTO** An Ab that binds Ag X comprising CDR sequences S
	Novel Ag; EPO An Ab that specifically binds Ag X	**Known Ag; EPO** An Ab that binds Ag X and has property Y

Source Cambridge Antibody Technology

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Highly cited patent families

Patent No. *	Organization	Ist Inventor	Technology
US6075181	Abgenix	Kucherlapai	Human antibodies derived from immunized xenomice
US4816567	Celltech	Boss	Methods of making recombinant antibodies and fragments – vectors and host cells
US5859205	Celltech	Adair	Humanized antibodies
US6808901	Celltech	Neuberger	Production of chimeric antibodies
US4671958	Cytogen	Rodwell	Antibody conjugates for the delivery of compounds to target sites
US5223409	Dyax	Ladner	Phage display
US4946778	Enzon	Ladner	Single chain antibodies
US4816567	Genentech	Cabilly	Recombinant antibodies made in cell culture including genetically altered and constant-variable region chimeras
US5877397	GenPharm	Lonberg	Transgenic non-human animals capable of producing heterologous antibodies
US6132970	Maxygen	Stemmer	Methods of shuffling polynucleotides
US5225539	MRC	Winter	Humanized antibodies in which all or part of the CDRs, are replaced
US6248516	MRC (exc. lic. to CAT)	Winter	Antibody variable domain expression libraries carrying a diversity of CDR sequences
US5885793	MRC/CAT	Griffiths	Method for isolating human antibodies to human proteins by phage display
US5969108	MRC/CAT	McCafferty	Antibody (including scFv) phage display
US6291158	Scripps/Stratagene/ MRC (exc. lic. CAT)	Winter	A method for isolating from the immunological gene repertoire a gene coding for a receptor having the ability to bind a preselected ligand.
US5585089	Protein Design Labs	Queen	Humanized immunoglobulins

* Patent citation analysis helps identify the key patents in a specific area of technology based on the number of forward citations. The above table shows only one member of each cited patent family. The citation count used is the cumulative total of US, European and PCT citations for each patent family.

Thememap of Monoclonal Antibody Technologies

Keywords within an identified set of patents and patent applications in the field of mab technologies are grouped into topics by the 'Themescape' software to produce a 'map'. Collections of documents which share common elements are geographically close together whilst collections with less similarity are further away. The patent landscape is therefore displayed as a series of technology 'mountain tops' and 'valleys' with the higher 'mountains' representing the larger patent collections.

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Current Challenges

Key Challenges

- Mab therapeutics are very expensive and therefore cost containment and reduction will be a major driver in this sector:
  - Their molecular complexity means they are comparatively expensive to manufacture
  - The plasma concentration of mabs required for therapy ranges from 1ug/ml up to >100ug/ml for Rituxan. Therefore the mass of protein to be manufactured is much higher than for other biologics
  - Due to the high development and manufacturing costs involved, most companies adopt a premium pricing strategy for mab products
  - A year's treatment with the oncology mab, Avastin, costs $50,000. The rheumatoid arthritis (RA) mab, Humira, costs $16,000 per year whereas the drug it often replaces, methotrexate, costs $500 for a year's treatment
  - Moreover, many mab drugs need to be used in combination with existing regimens such as chemotherapy (oncology setting), increasing the overall treatment cost.
  - In Europe, although mabs may possess clear advantages over conventional drugs, lower priced small molecule drugs remain the first line treatment choice in many instances. The case for expensive mabs is particularly weak where the condition is perhaps not immediately life-threatening
  - Pharmacoeconomic studies suggest that various mab therapies such as those prescribed for RA are cost effective. Such evidence is critical to secure reimbursement

Key Challenges Cont'd

- Lack of novel, therapeutically relevant antibody targets
  - "*When it comes to antibody targets, the low hanging fruit is gone*" Dr Jim Cornett, Medarex
  - Companies are having to be more innovative in identifying novel targets for new antibody-based therapeutics.
  - Technologies that can open up new classes of targets will be valuable
- Despite possessing desirable properties such as high specificity and relatively low toxicity, mabs have innate limitations:
  - bulky molecules - they can't enter cells, penetrate deep into tissue or reach targets buried in other structures
  - they can't be administered orally
  - their binding to targets is reversible, so that they have to be given in concentrations on the order of 5,000-10,000 times the concentration of their targets to be effective.
  - Their molecular complexity means they are comparatively expensive to manufacture
- Success has created an IP minefield

Strategies for Addressing Challenges

Strategies for Addressing Challenges

- Within the context of this foresighting it is impossible to look at every mab technology

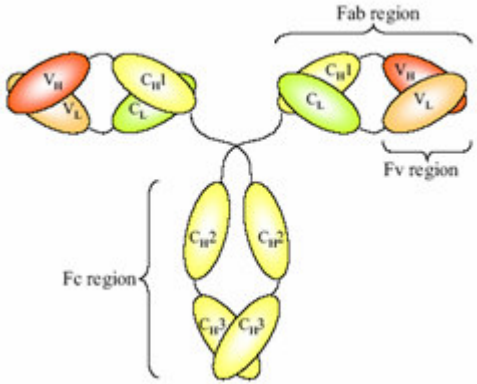
- Instead ITI has focused on technologies that may address the key challenges currently constraining the antibody sector

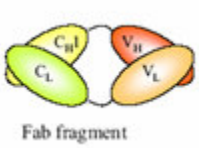
Antibody Fragments

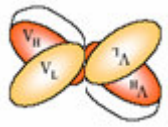
- After a decade of intensive engineering followed by preclinical and finally clinical testing, antibody fragments seem set to join mabs as powerful therapeutic and diagnostic agents

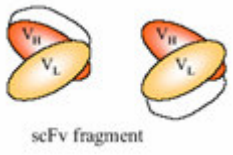
- The creation of large natural and synthetic *in vitro* repertoires of antibody fragments now means that there is a rapid process for generation of specific, high-affinity mab fragments against virtually any target

Immunoglobulin	Antibody Fragment	Bivalent Fragments (diabodies, Minibodies)	Single Chain Antibody	Domain Antibody
IgG (160KDa)	Fab (50KDa)	(50KDa)	scFv (30KDa)	dAb (13KDa)









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Advantages & Disadvantages of Antibody Fragments

Advantages

- High solubility and stability
- Amenable to pulmonary and oral administration
- Reduced costs of goods through manufacture in yeast and bacteria
- Small size leads to better tissue penetration and allows access to cavities on receptor targets
- Freedom to operate

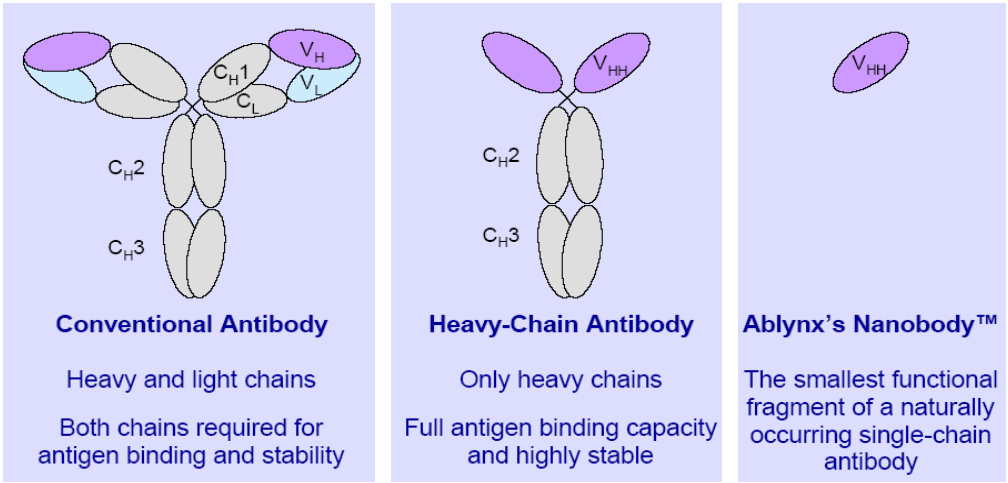
Disadvantages

- Reduced affinity compared to IgGs (poor target retention time)
- Plasma half lives of <1hr (2-3 weeks for an IgG) as a result of size reduction (< 70kD glomerular filtration cutoff) and removal of Fc region (mediates recycling through FcRn receptor)
- No effector function (complement dependent cytotoxicity (CDC) or antibody cellular cytotoxicity (ADCC))
- Potential immunogenicity

Fragments can bind to Epitopes not Recognised by Conventional Mabs

- Many pathogenic viruses have evolved narrow cavities in their surface antigens that bind their target receptor but are poorly accessible to intact antibodies and are therefore largely immunosilent.
- This blind-spot exists because few antibodies have sufficiently long antigen-binding moieties (so-called complementary determining region) to penetrate the target
- Other targets inaccessible to conventional antibodies include G protein–coupled receptors and enzyme active sites
- Single variable domains offer a potential strategy for cryptic epitopes. Unfortunately, such fragments normally don't retain the affinity of the parent antibody and suffer from poor solubility and a tendency to aggregate, although these issues are starting to be addressed
- Two organisms, the camelids (camels, llamas) and cartilaginous fish, are known to have evolved high-affinity single variable domains mounted on constant domain frameworks as a crucial component of their immune system.
    - The variable domains of these molecules are able to penetrate cavities in target antigens, such as enzyme active sites. Moreover the molecules are in general soluble and stable. The following slides detail the development of camelid domains, better known as nanobodies

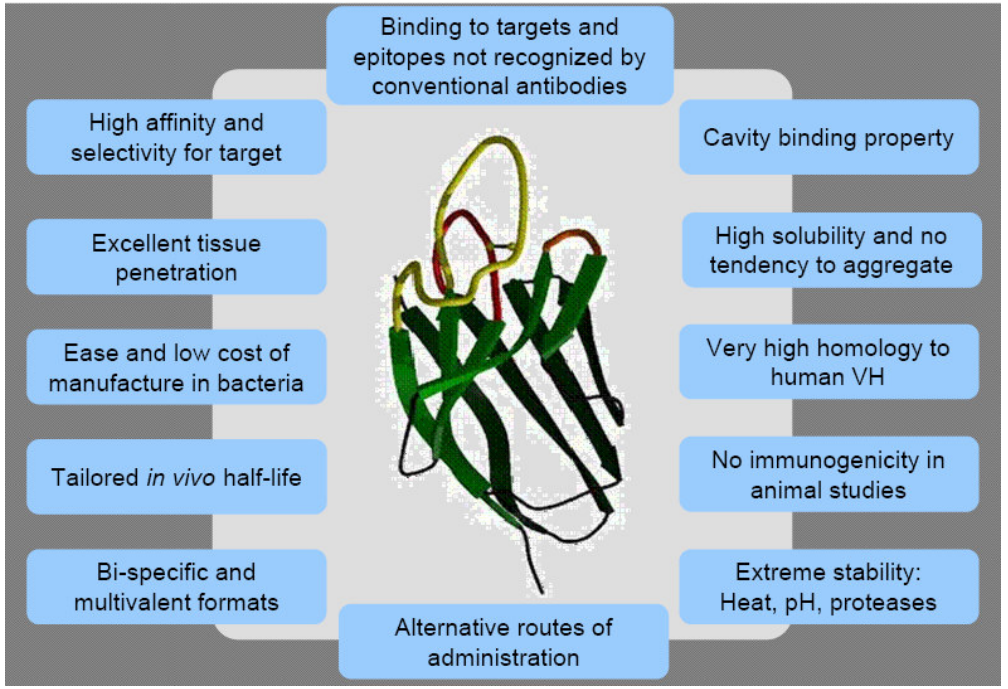
Nanobodies – Potential for Oral Administration



- Nanobodies are single-domain antibody fragments derived from camelid antibodies that lack a light chain, a property that confers potentially significant advantages over both conventional antibodies and their fragments:
    - These include high stability under conditions of high heat, high pH and the presence of enzymes; the potential for oral administration; high solubility; and affinity for antigens.
- An oral anti-TNF alpha nanobody to treat inflammatory bowel disease is expected to enter the clinic by late 2007.
    - Questions remain such as the likely dose required; however, if Ablynx could demonstrate proof of concept then this would be a major milestone for the mab sector and one that could drastically expand the market opportunity
- Another company working on domain antibodies, Domantis, is also investigating oral and pulmonary delivery for topical treatment of GI and respiratory disorders

Nanobodies Cont'd

- Recent deals by Ablynx illustrate the ongoing rush by pharma companies to ensure they are players in the next-generation antibody space
- Ablynx has signed deals with Novartis and J&J subsidiary, Centocor. Other fragment specialists such as Domantis have also signed lucrative deals (e.g. Dec. 2005, multi-target discovery collaboration with BMS)
- Ablynx has generated Nanobodies against more than 20 disease targets and has five therapeutic programs, of which three are in formal preclinical development



- The lead candidate is an injectable Nanobody that selectively prevents thrombus formation in high shear blood vessels by inhibiting the ability of von Willebrand Factor to anchor platelets to collagen. It is slated to enter the clinic by early 2007.
- Ablynx also has research programs involving a topical Nanobody for psoriasis, and injectable Nanobodies targeting solid tumours and Alzheimer's disease (AD).

Market Outlook for Fragments

- Whilst there are a number of therapeutic fragments on the market, including ReoPro (GPIIb/IIIa), DigiFab, Digibind and CroFab, it has been some time since the launch of a new therapeutic fragment
- Moreover, the revenue generated by the launched fragments has been modest
- However, this is likely to change with the approval (expected June 2006) of Genentech and Novartis' Lucentis (ranibizumab) for the treatment of wet age-related macular degeneration (AMD)
- During 2006, approval is also expected for UCB's PEGylated humanized antibody fragment against TNF alpha, Cimzia (certolizumab) to treat Crohn's disease
- If both products are successfully approved then the antibody fragment market will grow significantly
- Library selection strategies for generating high-affinity and high-specificity scFv and Fab fragments have most recently been applied to the selection of diabodies and single-domain antibodies, thus spawning many new biotechnology companies including Ablynx, Domantis and EvoGenix to name just a few

Alternative Scaffolds

- Ideal next-generation technologies will combine advantages of mabs and small molecules
- Molecular recognition scaffolds: a conserved framework region and a highly variable antigen-binding site
- Generally based on naturally occurring protein families with high sequence diversity. Many proteins, other than the immunoglobulins, mediate high-affinity interactions including:
    - proteins from non-immunoglobulin adaptive immune systems and proteins not involved in immunity (see table below)
- Experience gained from working with antibody libraries has enabled researchers to exploit these alternative scaffolds
- Challenge is determining what is possible using these scaffolds and for which applications they may prove to be superior to antibodies

Immunoglobulin-like (hypervariable loops on top of a rigid scaffold):

- lipocalin (Pieris)
- fibronectin (Compound Therapeutics)
- T-cell receptor (Avidex)

Other frameworks:

- crystallin, ubiquitin (Scil)
- ankyrin (Molecular Partners)
- protein A (Affibody)
- tetranectin (Borean)
- avimer sub-domains (Avidia)
- transferrin (Biorexis)
- aptamers (Archemix)

Alternative Scaffolds Cont'd

- Mabs mediate their therapeutic effect either by blocking a target or by exerting effector functions residing in the Fc region to activate complement or cytotoxic cells. Novel scaffolds do not have effector functions and therefore much of the focus has been on target neutralisation
    - However, others have addressed the effector function issue by fusing scaffolds to the Fc region of antibodies or (to avoid re-incorporating the aforementioned antibody problems) they have fused the scaffolds to toxins, cytokines or radiolabels
- Novel scaffolds may also lack the affinity and half-life of the IgG format.
    - However, a number of strategies can be employed to address affinity including oligomerisation and approaches such as PEGylation can extend the scaffolds serum half-life.
- Several scaffolds are now in development including
    - affibodies (derived from an immunoglobulin binding domain of *Staph. Aureus* protein A)
    - Dyax has human lipoprotein-associated coagulation domain 1 (LACI-D1, DX-88), a selective and high affinity inhibitor of human plasma kallikrein in clinical trials
- Since the scaffolds will be non-human or at least an engineered human scaffold then there is the potential for immunogenicity and this will have to be closely monitored in the clinic.
    - Strategies such as PEGylation and T-cell epitope engineering may help combat immunogenicity if this is seen in clinical trials.
- Many drug targets are located in the cytoplasm of the cell and proteins able to bind these would be useful research tools and potential therapies. Unlike antibodies, scaffolds that lack disulphide bonds would remain functional under reducing intracellular conditions (absence of disulfide bonds also facilitates bacterial expression - avoids need for refolding or eukaryotic expression) and may offer a potential intracellular targeting strategy

Alternative Scaffolds – Further Applications

- For novel binding proteins to be used in diagnostic applications they will have to demonstrate similar specificity and sensitivity to existing monoclonal antibody reagents
- Different scaffolds have been successfully tested in experimental diagnostic assays but further work is required
- The simplicity of the scaffolds in comparison to mabs offers several advantages including reduced cost and the ability to introduce in a controlled manner sites for derivitisation or in the case of protein chips for site-specific immobilisation
- Novel scaffolds could provide a lower cost alternative to monoclonal antibodies for affinity chromatography
- There are a group of scaffolds (Src homology domains 2 and 3 and PDZ domains) that offer one of the few peptide-binding alternatives to antibodies. These may represent interesting therapeutic agents
- It has been suggested that scaffolds could be designed that incorporated both binding and intrinsic detection (such as GFP) moieties whereby the signal changed as a function of binding. Therefore unlike classical fusions of the same moieties separation would not be required for detection.

Mab Conjugates

- Since virtually all anticancer agents are associated with dose-limiting toxicities, significant interest has surrounded the possibility of improving drug/radiation efficacy while minimizing systemic toxicity through mab-based targeting strategies
- Antibodies have been conjugated to a variety of molecules including
  - **cytotoxic drugs** (e.g. doxorubicin, paclitaxel, mitomycin C, camptothecin)
  - **toxins** [e.g. calicheamicin, ricin-A chain, diphtheria toxin, maytansine derivatives and auristatins]
  - Others – **cytokines/chemokines, peptides, proteins, enzymes (e.g. ribonuclease), liposomes and viruses**
  - **radionuclides**

  - Immunoconjugates

  - Radioimmunoconjugates
- However, it has only been in the past few years that the critical parameters for optimization have been identified and have begun to be addressed. These include:
  - physiological barriers to mab extravasation and intratumoural penetration,
  - mab immunogenicity,
  - normal tissue expression of the targeted antigen and specificity of the mab for tumour-associated antigens,
  - low cytotoxic potency,
  - linker stability
  - inefficient drug release from the mab and difficulties in releasing drugs in their active states
- Several promising new agents comprising potent anticancer drugs attached to mabs through optimized linker technologies are showing unprecedented activities in preclinical models, and many of these agents have entered into clinical trials
- One of the remaining challenges facing this field surrounds the metabolic fate of circulating conjugate that does not localize within tumour masses

Immunoconjugates

- Early antibody conjugates proved to be inefficient in the clinic, largely as a result of not getting sufficient cytotoxic drug into cells.
  - The cytotoxic potency of immunoconjugates has been augmented by either increasing the number of molecules that can be delivered or by taking advantage of highly cytotoxic compounds such as calicheamicin and maytansine derivatives
- Considerable attention has also been directed toward the development of linkers that are relatively stable at neutral pH but undergo hydrolysis under the mildly acidic (approximately pH 5) conditions within the cell.
- The use of peptides as stable linkers for drugs to mabs has gained a great deal of interest because enzymatic hydrolysis allows greater systemic stability than linkers that are chemically cleaved by water or low molecular weight reducing agents.
  - The peptides are designed for high serum stability and rapid enzymatic hydrolysis, once the mab-drug conjugate is internalized into lysosomes of target cells. Proteases that lead to drug release are mainly intracellular and are not nearly as active outside the cells because of their pH optima and inhibition by serum protease inhibitors

Radioimmunoconjugates

- Clinical evaluation of a spectrum of radioimmunoconjugates continues in a variety of diseases and clinical settings. In haematologic malignancies, the approval of Biogen-Idec's Zevalin and Corixa's Bexxar led many to conclude that these agents would have broad applicability. However, this is unlikely to hold true for a number of reasons:

  - The central issue is balancing the dose delivered to tumour against exposure of normal organs and tissues to radiation. Toxicity remains a key challenge
  - Shipment, handling, administration and disposal of a radioactive biological agent introduce complexities beyond the use of conventional drugs
  - The use of these agents also requires the co-operation of a multidisciplinary team (oncologists and nuclear medicine)
  - The challenges are even greater when treating solid tumours not only because of delivery issues (i.e. achieving sufficient uptake within the target tissue), but also because of the apparent radioresistance of solid tumours relative to lymphomas and leukaemias

- Strategies have been directed toward enhancing efficacy, including stem cell support or combination regimens (e.g. with chemotherapy)

- Use of the first generation of radioimmunoconjugates appears to be most useful in settings of minimal residual disease or of micrometastases, or in an adjuvant setting, and as one component in multimodality treatment of cancer.

Mab-Peptide or -Protein fusions

- Amgen is developing so-called peptibodies - peptides fused to the Fc fragment of an antibody. These fusion molecules can be produced in *E.coli*
- A similar technology is being developed by CoVx, except it uses a whole mab rather that a fragment and the peptide is fused to the mab's binding pocket.
- Companies such as EMD Lexigen (part of Merck KGaA) have also developed mab-protein fusions. This technology called Fc-X once again involves a fusion between the constant region of the mab and the protein
- These approaches are all designed to increase the half life of the protein or peptide thereby allowing for less frequent dosing
- This technology may be particularly useful for developing peptide therapeutics as they undergo rapid clearance from the body, with half-lifes often measurable in minutes
    - peptides are interesting as their interaction with protein targets is highly specific, more so than with small molecules and they may be a useful strategy in those situations where it is difficult to create small molecules against specific targets
- Syntonix also has a drug-Fc fusion technology. In addition to optimising pharmacokinetic and pharmacodynamic properties the company believes that through binding to the neonatal Fc receptor (FcRn) the Fc fusion will be transported across epithelial cell barriers such as those present in the lungs and intestines. Syntonix is therefore developing an interferon-beta therapy for multiple sclerosis (MS) that can be administered by inhalation

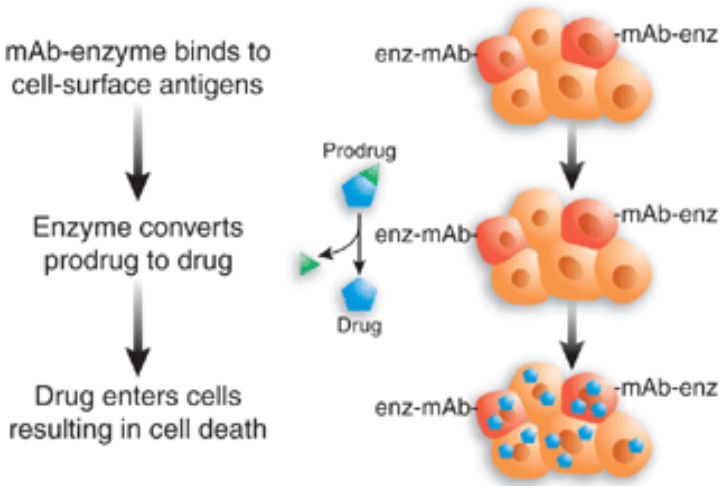
Pre-targeting Strategies

- The aim of cancer treatment is to achieve high tumour-to-nontumour ratios, since non-targeted conjugates can lead to dose-limiting toxicities. However, uptake of macromolecular drugs into solid tumours is a slow process requiring sustained levels in the blood to overcome the barriers to extravasation and penetration
- In some applications such as therapy with unlabeled mabs and stable mab-drug conjugates, non-tumour-associated mabs and mab-drug conjugates may not bring about marked toxicity, providing that there is minimal cross-reactivity and metabolism within normal tissues
- This is not the case with mab-radionuclide conjugates and certain other conjugates as their cytotoxicity is independent of intratumoural uptake and antigen binding
- Several approaches have been tested to provide high tumour-to-nontumour ratios including so-called pretargeting. For example rather than direct radiolabeling of mabs to provide targeted delivery to tumours, mab-directed localization and radionuclide delivery are separated physically and temporally:
    - a mab conjugate is administered that binds to tumour-associated antigens. If necessary, a clearing agent is applied that removes unbound mab conjugate from the blood
    - Once the tumour-to-nontumour conjugate ratio is very high, a low molecular weight radioactive ligand that binds to the localized mab conjugate is administered
    - The approach effectively separates the slow distribution of the mab moiety from rapid binding and elimination of the radioisotope-tagged ligand. This should result in very high ratios of tumour uptake compared with normal tissue activity

Pre-targeting Strategies Cont'd

- However significant challenges have yet to be addressed including the immunogenicity of the components, preparation of two or three injectables, optimization of the dose and timing of each step, length of procedure and administration of large amounts of radiolabeled ligand as the final step

- An alternative is to use bispecific mabs (see bispecific slides), with one arm specific for the tumour and the other arm recognizing the radiolabeled ligand



Source: Nature Biotechnology

- Another strategy (pictured above) is referred to as antibody-directed enzyme prodrug therapy (ADEPT). A mab-enzyme conjugate is administered that localizes within the tumour mass and clears from the systemic circulation over time. Once the tumour-to-nontumour ratio is sufficiently high an anticancer prodrug is given, and this is converted to the active drug by the targeted enzyme

- Finally an alternative to pre-targeting to circumvent poor tumour penetration is to target the endothelium of tumour blood vessels. The vasculature is more accessible, relatively stable and targeting it rather than the tumour directly means that the antibody is likely to be active on more than one tumour

Mab Fragment Conjugates

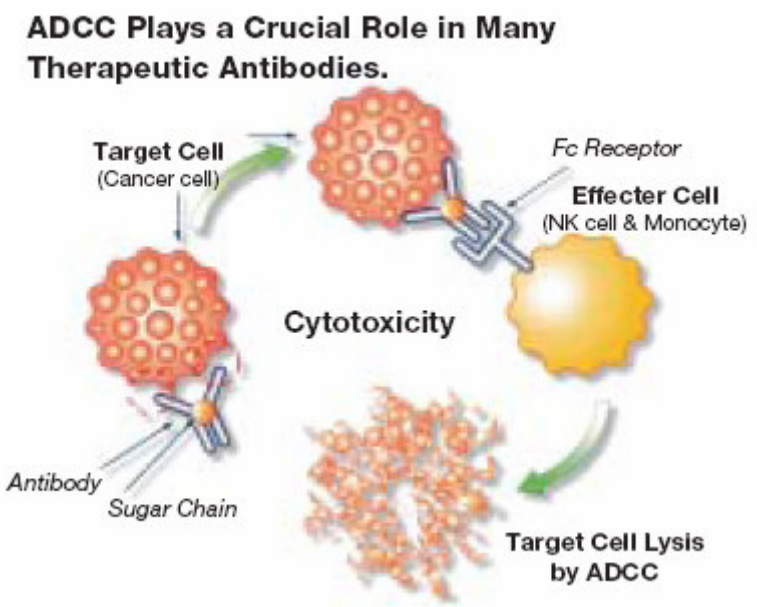
- Intact mabs, the current 'gold standard,' are capable of delivering cytotoxics to tumours, but as described:
    - because of their size (150 kDa) they have been found to penetrate solid tumours only slowly, are non-uniform in their final distribution and have high serum levels and
    - the activity that can be administered is limited by their prolonged circulation time, which results in dose-limiting toxicity
- Conversely, small scFv fragments (30 kDa) are cleared extremely rapidly reducing normal tissue exposure but also reducing tumour accretion, a classic trade-off
- Furthermore, rapid clearance and retention in liver and kidney must also be considered in the overall toxicity profile
- The ideal tumour-targeting reagents may be intermediate-sized multivalent molecules (for example, bivalent diabodies, 55 kDa), which provide rapid tissue penetration, high target retention and rapid blood clearance
- The pharmacokinetics of mab fragments can also be modified using other strategies, such as linkage to polyethylene glycol (PEG) or noncovalent interaction with long-lived serum proteins, such as albumin or serum immunoglobulin. If desired, systemic clearance can be accelerated by mannose glycosylation a result of manufacture in yeast
- The further engineering and evaluation of mab fragments should reveal the optimal fragment for targeted delivery of cytotoxic agents whether they be toxins, drugs or radionuclides

Imaging Using Antibody Fragments

- For *in vivo* imaging, contrast (signal-to-noise ratio) is key. A long serum half-life results in poor contrast in imaging applications
- Smaller fragments, such as diabodies and minibodies, reach their maximum tumour uptakes within 1–6 h of administration. Because of rapid blood clearance, tumour-to-blood ratios increase steadily over time and reach high values (>20:1) by 24 h, making these fragments promising candidates for imaging
- Clinical imaging studies have been conducted using tumour-targeting scFv dimers (diabodies) and minibodies. A ^{123}I-labeled anti-CEA minibody has been used to visualise colorectal tumours including lesions that were not visible by computed tomography
- What remains to be seen is whether antibody technologies can be exploited to take full advantage of next-generation imaging technologies such as positron-labeling chemistries and quantum-dot conjugations to open new opportunities for medical imaging
- The cost of using labelled mabs as tracers may be an issue when compared with metabolic tracers such as FDG or labelled small molecules

Increasing ADCC

- Antibody-dependent-cell-mediated-cytotoxicity (ADCC) is an immune response in which antibodies coat target cells making them vulnerable to attack by immune cells such as NK cells and monocytes
- ADCC activity is one of the major anticancer mechanisms of launched therapeutic mabs such as Herceptin and Rituxan.
- It has long been known that specific mutations in either the polypeptide sequence of the Fc region (part of mab that interacts with immune cells causing ADCC) or the sugars that decorate it can alter the Fc's ability to recruit NK cells
- ***BioWa*** (wholly-owned subsidiary of Kyowa Hakko Kogyo) and ***GlycArt*** (part of Roche) have achieved dramatically increased ADCC activity by using engineered CHO cells to lower the content of fucose in an antibody's carbohydrate chains



- Mabs with this technology applied exhibit more than 100x greater anticancer efficacy than a conventional mab in animal studies.
- This approach is also expected to lower costs and reduce side effects since such enhanced efficacy enables low-dosage treatment.
- Other companies such as ***GlycoFi*** and ***Xencor*** are also involved in Fc region optimisation to modify the immune response. The latter used protein-engineering to create a library of different Fcs with enhanced tumour killing power whilst the former has used engineered yeast strains to produce antibodies with optimised sugar chains

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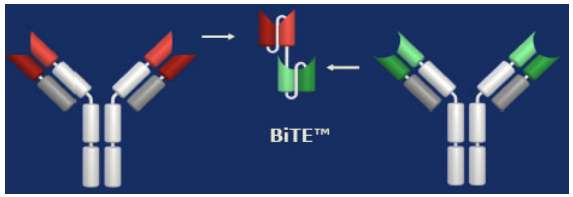
Intracellular Antibodies - Intrabodies

- Functional expression of intracellular mab fragments (termed intrabodies), which can be used to ablate or modify crucial transcriptional and translational regulators and controls.
- Potentially provide a powerful way to ablate viral-encoded genes and products or influence cellular function in malignancies, for example by inhibiting the transcription of oncogenes, inducing apoptosis
- Significant progress has recently been made both in framework designs that achieve functional folding of the mab scaffolds in the reducing intracellular environment and also in the isolation of functional intrabodies directly from repertoires. Together, these new molecular designs and selection technologies should facilitate and accelerate generation of potent intrabodies
- Like their extracellular counterparts, intrabodies may be engineered into multivalent and multispecific forms to enhance their efficacy. For example, a bispecific intrabody (intradiabody) has recently been described that allows the simultaneous knockdown of two cell-surface receptors.
- However, as with gene therapy strategies the main obstacle is likely to be delivery of the expression vector
- An alternative to gene delivery could involve targeting intracellular antigens directly by fusion of the intrabody to a membrane translocator sequence, a naturally internalizing mab or by direct selection for internalization.
    - PoC has been achieved with a membrane translocating peptide conjugated to an anti-caspase-3 mab. mab inhibited *in vitro* apoptosis-related events, such as caspase-3 activity, DNA fragmentation and spectrin cleavage.

Bispecific Antibodies

- Bispecific antibodies (bisAbs) comprise two different binding specificities fused into a single molecule
  - they can be designed to bind either two adjacent epitopes on a single antigen, thereby increasing both avidity and specificity, or
  - to bind two different antigens for numerous applications, but particularly for recruitment of cytotoxic T- and natural killer (NK) cells or retargeting of toxins, radionuclides or cytotoxics
- BisAbs can be based on whole mabs or fragments such as diabodies and scFv
- The ability to design multivalent specificity into mab fragments is also enabling the re-emergence of pretargeting (previously discussed) for cancer therapy
  - A mab fragment bispecific for a tumour marker and a hapten is first infused to specifically localize the bisAb fragment at the tumour site; subsequently, a drug-hapten conjugate or radiolabeled hapten is introduced, which then binds to the bisAb.
- One company specialising in bispecific antibodies is Micromet.
  - Generates bi-specific antibody derivates, called *BiTE* molecules, composed of two scFv antibody fragments fused together.
  - Micromet is focusing on recombinant bi-specific antibodies where one fragment targets a cell surface antigen whilst the other engages a receptor such as CD3 on the surface of killer T cells eliciting a T-cell response
  - *BiTE* molecules appear to be extremely potent at sub-ng/ml levels, which could translate into patient doses of just a few micrograms (much less than for conventional mabs)



Non-therapeutic Applications: Antibody Microarrays

- To obtain an integrated view of disease mechanisms and cellular processes at the protein level, proteomic methods are required
- The prize is replacing 2D gels (time, expense and reproducibility issues) with antibody arrays that can analyze the expression of one or more proteins in a mixture such as serum without a prior separation stage
- Although technology is at an early stage of development, several applications in areas such as autoantibody profiling, cancer research or signal pathway characterisation highlight their potential
- Antibody arrays hold great promise for accelerating development of both diagnostic biomarkers and therapeutic interventions. They are well suited for parallel screening of potentially clinically useful biomarkers in human blood serum, particularly since in many cases one or a few markers are sufficient for disease diagnosis.
- Several companies are working to bring antibody chips to market
    - **Zyomyx** is using photolithography to etch wells on the surface of silicone chips so as to maintain mab hydration and avoid denaturation. Zyomyx offers a human cytokine biochip to provide sensitive protein profiling of 30 cytokines and proteins known to play a key role in inflammatory diseases.
    - **Ciphergen Biosystems** - ProteinChipR arrays in combination with SELDI (surface-enhanced laser desorption/ionization) has been used for differential profiling and protein marker discovery.

Non-therapeutic Applications: Antibody Microarrays - Challenges

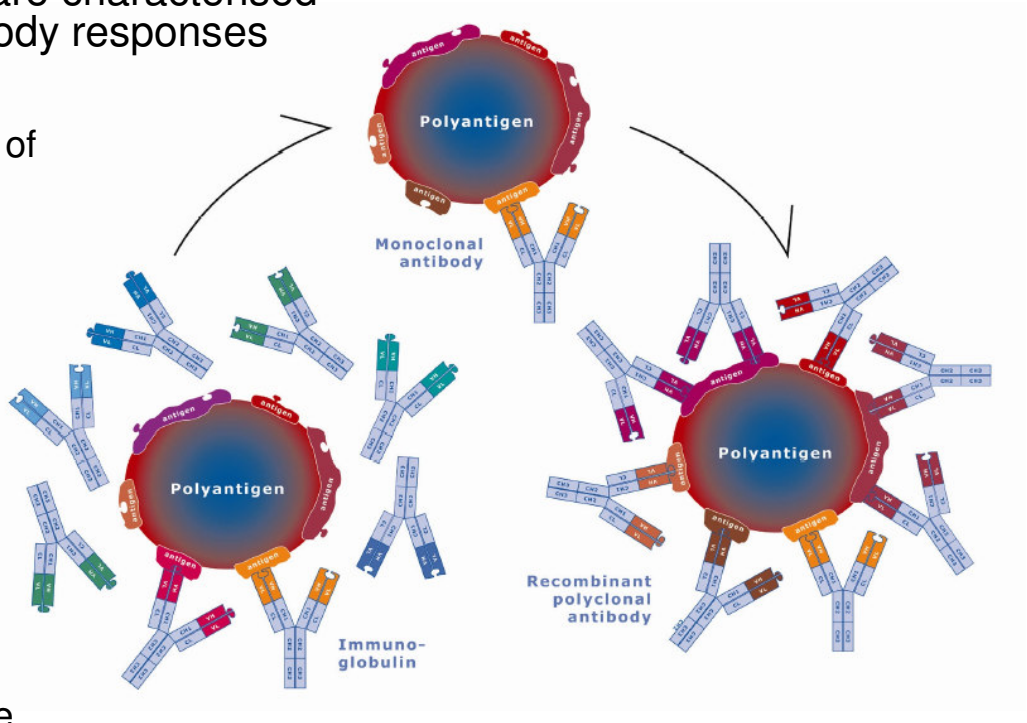
- **Large linear range required** (contrast with DNA microarrays) - detection of low and high abundance proteins in samples such as human serum on one antibody chip is not an easy task
- Antibodies **cannot be synthesised** on the surface of the chips, as is done for DNA arrays; the probes have to be spotted onto the chip surface in an array format
- Although several tens of thousands of monoclonal antibodies are commercially available, **hundreds of thousands are required** for large-scale protein profiling. The introduction of so-called display technologies are helping to address this requirement.
- More work must be done to address the challenge of **fixing** (print) the antibody probe onto the chip surface in a biologically active form.
- Must also protect antibody binders against **denaturation**, which is often caused by evaporation during spotting and subsequent incubation with a target
- The development of sufficiently **sensitive detection methods** applicable to protein microarrays with large numbers of different antibody specificities is a challenge in itself. Particularly in relation to clinical samples the technology must be able to cope with body fluid samples, tissue cell aspirates or biopsies that are usually available in very low amounts
- It is unclear how long it will be before antibody arrays transition from proteomic research tool to diagnostic

Non-therapeutic Applications: Antibody Fragment Microarrays

- Formats for antibody microarrays are rapidly diversifying from immobilization of intact mabs onto glass-surface microarrays to other new, more protein-friendly surfaces
- Several such platforms have recently come on the market including:
  - Biosite's rapid enzyme immunoassay (Triage) system; Zyomyx's Protein Profiling Biochip for human and murine cytokines; PerkinElmer's Hydrogel microarray substrate for optimizing protein activity and accessibility; and most recently, Pointilliste's Molecular Canvas arrays, which comprise high-density spots of full-length mabs for use in capturing small molecules, peptides or proteins.
- Arguably, antibody fragments are ideally suited for incorporation into biosensing devices such as microarrays since they provide small, stable, highly specific reagents against the target antigen
- Moreover, we may start to see alternative scaffolds (described earlier) being incorporated into microarrays
- We anticipate that new platforms will become increasingly available over the next few years, driven by the demand for new reagents to diagnose the vast array of biomarkers stemming from proteomics discovery programs.

Polyclonal Antibodies

- Antibodies derived from human blood are characterised by the natural diversity of human antibody responses
  - ***Ig from human donors***
    - Used to boost the immune system of immunocompromised patients
    - Leading players include CSL, Mitsubishi Pharma, Bayer and Baxter
  - ***Hyperimmune Ig***, collected from individuals with a high titre of antibodies against a particular disease-associated antigen
    - Often used as prophylaxis or treatment for the same disease (most commonly anti-Rhesus D, transplant rejection and viral infections)
    - Leading players include Nabi, Cangene, Protherics and Genzyme



- Recombinant mabs demonstrate high specificity toward a single, often well-characterised antigen
- Recently interest is growing in developing human recombinant antigen-specific polyclonal antibodies

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Polyclonal Antibodies Cont'd

Polyclonal antibodies offer:

- Polyclonals like the natural immune system consist of antibodies with different specificities. This multivalency of binding means that polyclonals may be more effective than mabs at eliminating targets as they can work through a combination of actions (neutralisation, improved phagocytosis or ADCC and increased complement activation)
- Also unlike mabs polyclonals should not compete for binding to the same epitope
- Infectious agents and cancer cells are less likely to escape immune recognition by polyclonals through mutation.
- The ability to combat infectious diseases caused by diverse strains of pathogens or that require neutralisation of multiple epitopes for successful treatment. Also potential for neutralising snake and insect venoms that comprise multiple toxic components
- Also reduced sensitivity to natural target variation
    - Strain or allelic variants
    - Conformational variation

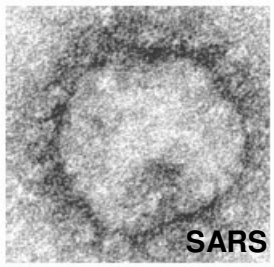
Polyclonal Antibodies Cont'd

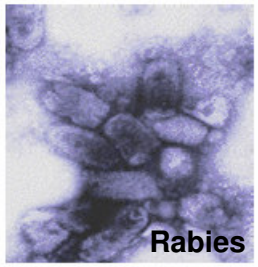
- Widespread use of polyclonals from blood has been limited due to constrained supply, problems with batch-to-batch variations as well as safety issues.
    - Supply of human-derived Ig is restricted to diseases where sufficient numbers of donors can be identified carrying a particular immune response

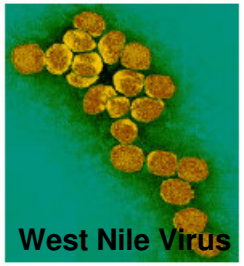
- For other antigens, some companies have immunised animals to create Ig products
    - E.g. Genzyme's Thymoglobulin – hyperimmune Ig purified from the blood or rabbits immunised with human T-lymphocytes. Used to induce T-cell immunosuppression in the treatment of prevention of kidney transplant rejection. However, there is a risk of immune response to this animal Ig
    - Other companies such as Hematech (part of Kirin Brewery) are developing technologies to produce human-like polyclonal antibodies in transgenic cows
    - These transgenic animal technologies have limitations, such as the need for purification (to remove unwanted animal proteins) and the fact that the transgenic hyperimmune Igs will contain a significant fraction of irrelevant, normal non-specific Ig

Recombinant Polyclonal Antibodies

- Recombinant polyclonal antibodies offer an alternative to animal of human derived antibodies
  - Companies such as Symphogen and Merus have developed technologies for identifying and reproducibly manufacturing recombinant polyclonals.
  - These polyclonals should be active against complex antigens and retain activity in the event of antigen mutation. They will also have a better safety profile than plasma-derived products
  - The major focus is on creating therapeutics for infectious disease, transplant rejection and cancer. They may also be a useful biodefence agent.
  - Regulatory authorities are struggling with multi-component therapies and are likely to adopt a rather conservative view
  - Merus's technology platform permits the production of mixtures of 3-5 mabs by a single, clonal cell line, potentially allowing for a simplified regulatory view
  - Other companies such as Crucell, are working on cocktails of mabs; once again the focus is infectious diseases such as rabies (cocktails ensure strain coverage)







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Manufacturing

Manufacturing Introduction

- The development of very efficient expression systems is essential to the full exploitation of the antibody technologies
- The expression of functional, correctly folded antibodies or antibody fragments and its scale-up to commercial level is a major goal in therapeutic antibody development
- The choice of the most suitable expression system is function of the antibody format (whole IgG, monovalent or bivalent fragment) and the application (therapeutic, diagnostic, experimental tools),
- but factors such as scale-up, total annual production, post translational modification and regulatory issues comes also into play.
- The production of recombinant proteins involves three main steps that contribute about equally to the cost of the final product:
  - **protein production (that is, fermentation);**
  - **protein purification;**
  - **'fill and finish' - the filling and packaging of a formulated and stabilized product.**
- As the second and third steps are essentially fixed expenses that are required irrespective of the production process, most efforts to reduce costs have focused on improving the fermentation process itself or by considering alternative production systems. This is being driven by the huge demand for large amounts of mabs and the need for reducing COGs (cost of goods) to increase uptake of mab therapeutics
- However, significant effort is being devoted to downstream process development and particularly purification. For example can purification using affinity chromatography with staphylococcal protein A be improved upon or alternatives developed

Manufacturing Issues

- Rapid progress in manufacturing technologies:
  - **Increasing yields** through molecular biology techniques. Some companies are starting to push the 5 gram/litre limit and that's not considered the ceiling level
  - **Disposable** plastic bioreactors may eliminate much of the expense and time consumed with cleaning and revalidating steel reactors between batches
  - **Transgenics** (see later) – cost, glycosylation patterns, immunogenicity, downstream processing, scaleability and containment remain the main issues.
    - Rapid progress in traditional cell-line production means there is little immediate pressure for companies to switch to alternative platforms that are as yet commercially unproven?
- Some of the technologies discussed for increasing half-life are also likely to reduce the volumes of product that must be manufactured
- A commercial manufacturing facility carries a price tag of $300m to $500m and takes 4-5 years to build, validate and license. It is therefore vital for CMOs and biotech/pharma companies to make accurate predictions about future demand
  - Rapid advancements in manufacturing technologies make these predictions difficult, making mab manufacturing a risky endeavour. Manufacturing ultimately affects the drug price and the manufacturers' ability to supply the market

Impact of Innovation on Cost of Manufacturing

Innovation is driven by desire to reduce COGs for what are high-dose, long-term and therefore high-price therapies. The following table illustrates the reduction in COGs possible with improvements to manufacturing technologies

Production Indices	No Innovation	With Innovation
Titre	100 mg/L	1000 mg/L
Yield	40%	70%
Capacity required	6.25 million L/year	0.36 million L/year
Number of bioreactors	31	2
Capital	$1550 million	$100 million
CoGs per g	$1500	$260
CoGs per year	$375 million	$65 million

Assumptions: 10,000 L scale, 250kg/year, $50million investment per reactor. Process improvements (increase in yield through improved expression systems and greater yields from better downstream processing) can make a tremendous contribution to lowering the cost of goods.

Alternative Mab Expression Platforms

- A variety of expression systems have or are being developed for the production of therapeutic mabs, including **bacterial**, **yeast (fungal system)**, **algae, insect cells infected with baculovirus**, and **transgenic animals** and **plants** amongst others
  - The use of mammalian cell culture has emerged as the dominant platform for the production of recombinant human glycoproteins for therapeutic purposes
- However, much effort is being focused on protein expression platforms that are able to reduce costs and offer greater control over post-translational processing
  - The latter has become increasingly important as the extent and type of *N*-glycosylation have been found to have a marked impact on the therapeutic properties of therapeutic proteins
- Although yeast have proven to be robust protein-expression platforms for many industrial enzymes and some non-glycosylated therapeutic proteins, the inability to perform human-like *N*-glycosylation reactions has precluded yeast from being used to produce most recombinant human glycoproteins intended for therapeutic use
  - To overcome this shortcoming, several labs have been investigating genetically engineering human *N*-glycosylation pathways into yeast and other fungi and recently GlycoFi reported that human antibodies with specific human N-glycan structures could be produced in glycoengineered lines of the yeast *Pichia pastoris*
  - Moreover, certain glycoforms of Rituxan produced using the engineered yeast had significantly increased antibody dependent cell cytotoxicity (ADCC) compared with Rituxan derived from mammalian cell culture (which is composed of multiple glycoforms)
  - However transfer of sialic acid may prove more challenging as a source of endogenous sialic acid is unknown in yeasts. The importance of terminal sialic acid is highlighted by the often rapid clearance of incompletely sialylated recombinant proteins by the liver

Transgenics Plants Example

- **Biolex** is developing a plant-based expression technology based on Duckweed (*Lemna*). This system has a number of attractive features:

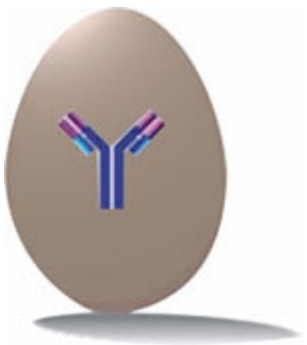
- Rapid proliferation – Lemna doubles its biomass in 36 hours
- Proliferates vegatatively (clonal replication) therefore no seeds or pollen generated
- Unlike microbial fermentation plants are capable of post-translational modification
- Plants are devoid of human infective viruses or prions
- Containment – transgenic Lemna grown in aseptically sealed vessels
- Lemna can secrete the target protein directly into inorganic media, simplifying purification
- Plants grow in synthetic media consisting of water and inorganic nutrients; again no danger of introducing pathogens
- Cost advantages result from simple media, inexpensive facilities and simplified down-stream processing
- High expression: consistently able to produce batches of at least 40g of mab in one processing suite every two to four weeks.
- Can be used to produce proteins that are difficult to manufacture in mammalian cells (e.g. cytokines)
- Lemna produced interferon alpha2b (Locteron) entered the clinic towards the end of 2005
- Success will depend on safety of the plant manufactured product (no more antigenic than proteins from other expression systems)
- Detailed cost analysis comparing this strategy with mammalian and bacterial production is required
- Biolex has a strong IP position which was further strengthened by the company's acquisition of Epicyte Pharmaceuticals (Biolex now has control over plant production of mabs)

Transgenics Animals

- For over a decade, production of proteins in the milk of transgenic animals has offered the promise of significantly reducing the cost of manufacturing biopharmaceuticals. However, these technologies have not yet been widely adopted in industry due to several limiting factors, including:
    - The time required to develop and generate production herds of dairy animals is too long to fit with increasingly rapid biopharmaceutical product development schedules
    - The investment required for line development is high and must be made early in clinical development at a time when product risk is still high
    - Containment of dairy animals for production is not easy.
- The most advanced transgenically produced therapeutic protein is GTC's ATryn®, a recombinant form of human antithrombin (a plasma protein with anticoagulant and anti-inflammatory properties). GTC has produced and purified the protein from the milk of transgenic goats (typical yields are ~1-4 g/L)
- Earlier this year the EMEA rejected GTC's application for marketing authorisation of ATryn® (first attempt by any company to seek approval of a recombinant therapeutic protein produced using transgenic technology)
- Whilst the EMEA was quick to deny that the refusal to approve was linked to the production process it is clear that the bar has been set very high for the regulatory approval of transgenically produced pharmaceuticals.
- Companies investing in transgenic production include Pharming, Hematech, AviGenics, TranXenogen and Viragen

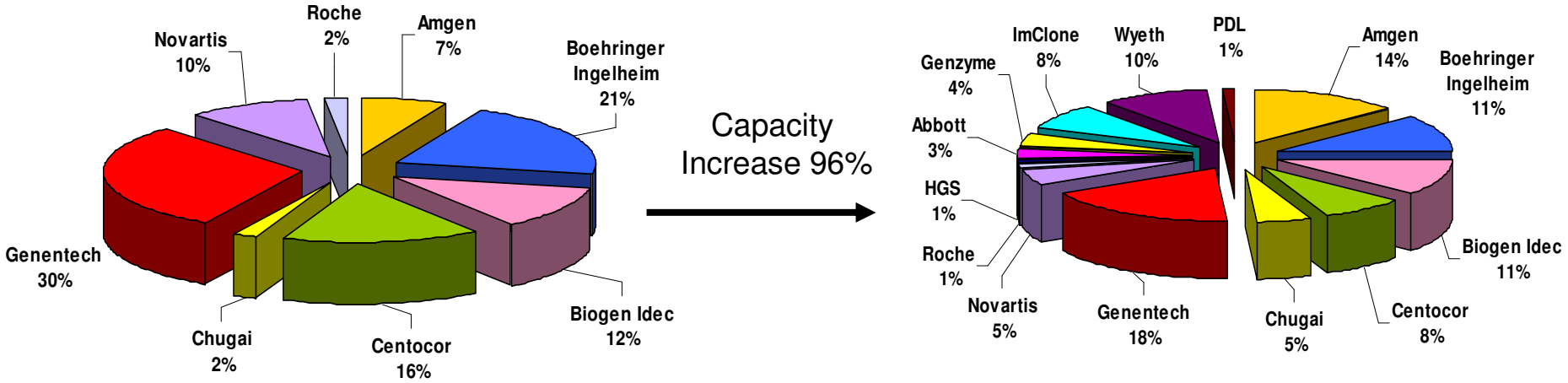
Transgenics Animals Example

- One of the leading transgenic companies, Viragen, is based in Scotland where the company continues to develop a technology licensed from the Roslin Institute called Avian Transgenic Technology
- The technology relies on creating transgenic chickens able to lay eggs expressing therapeutic proteins including monoclonal antibodies
- Viragen and competing companies such as AviGenics believe that producing proteins in chicken eggs has a number of advantages:
    - Proteins can be manufactured faster, cheaper and in large quantities (result of relatively short gestation period and rapid breeding)
    - Chicken eggs have very similar glycosylation patterns to human proteins
    - Chicken eggs have been used in the manufacture of vaccines for more than 30 years – specific pathogen free (SPF) flocks of chickens have been developed and regulatory experience with egg derived biopharmaceuticals established
    - Contained breeding facility



Manufacturing Capacity

- By 2005 demand will exceed manufacturing capacity by a factor of four
  - *The State of Biologics Manufacturing, JP Morgan March 2001*
- Enbrel was seen as the tip of the iceberg. Actually it was a relatively isolated case
- Now appears to be some excess manufacturing capacity. This turnaround can in large part be attributed to a building boom which is set to continue:



2004, Total Volume: 861,000 L

2007, Total Volume: 1,686,000 L

Ramp up in pharma/biotech cell culture capacity. Source: BioProcess Technology, JP Morgan and Boehringer Ingelheim

- Performance of CMOs testament to this:
  - *Diosynth had a difficult year with sales down 24%, reflecting the overall situation in the contract manufacturing industry*. Akzo Nobel 2004 Annual Report
  - *Custom manufacturing activities continued to face a challenging business environment. Sales were down 19.0% YoY.* Lonza 2004 Annual Report

Generic mabs: Biosimilar/Biogeneric question

- Majority of leading mabs have patent protection to 2013. However, Synagis patent expiry is imminent.
- Techniques to demonstrate comparability of monoclonal antibodies (arguably the most complex biological therapeutics) some way off?
- BioPartners have now submitted two MAAs to the EMEA for biosimilar products (Valtropin – rhGH and rINFalpha for HCV).
- Sandoz has been blocked in the EU and the US in its efforts to obtain approval of Omnitrop, another biosimilar hGH. Both EMEA and FDA have still to resolve the legal and regulatory issues.
- So many issues (in addition to regulatory ones):
    - Defence strategies (new indications and formulations, lobbying, litigation, delivery systems…)
    - Comparability
    - Sales, marketing & distribution
    - Price discount/war
    - Emerging markets
    - Pharmacy substitution

Key Sector: ITI Keen to Initiate Dialogue with Interested Parties

- Summary:
  - **Hugely important enabling technology**
  - **Creating large and fast-growing markets**
  - **Competitive but still room to play, particularly in relation to next-generation technologies**

- Scotland has a strong track-record in this field – Biovation, Haptogen, Alba Bioscience, Serologicals, Aquatic Diagnostics, Viragen and Axis-Shield are notable examples of companies commercialising antibodies
- Within this space we believe there is an opportunity to create new technology or build upon existing technology and to examine ways of commercially exploiting such technology and know-how within Scotland
- ITI Life Sciences would very much welcome dialogue with those keen to discuss any aspect of this report or their own interest in the antibody field. We would particularly welcome enquiries from those keen to discuss exciting projects that are aligned with the findings of this report.

To arrange a discussion, please contact ITI Life Sciences at:

email@itilifesciences.com
tel. +44 1382 568060

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Appendix - Patented “next generation” mab technologies

* Patent No.	Organization	Technology
Improved Fc region for increased effector function		
WO06019447	Xencor	Optimized Fc variants and protein engineering methods for their generation
WO0231140	BioWa	Cells producing antibody compositions with a high antibody-dependent cellular cytotoxic activity
WO06014679 WO06014683 WO06014685 WO06014725	GlycoFi	Various antibodies with enriched N-glycan structures that confer a specific effector function
WO04065540	GlycArt	Use of fusion constructs to make antibodies with increased Fc receptor binding affinity and effector function
WO04074455	Applied Molecular Evolution	Novel Fc region variants
WO04035752	Protein Design Labs	Alteration of FcRn binding affinities or serum half lives by mutagenesis

Appendix - Patented "next generation" mab technologies

* Patent No.	Organization	Technology
Small molecule antibody fragments		
WO04003019 WO03002609	Domantis	The use of small stable antibody fragments (Domain Antibodies) to create dual targeting antibodies that have extended half life by binding serum albumin.
WO04041865	Ablynx NV	Stabilised single domain antibody fragments (nanobodies)
WO05037989	Trubion	Small single chain molecules with full target binding activity and effector function expressed at high levels.
WO03097697	Esbatech	Single chain human antibody fragments with enhanced stability
WO05105844	Micromet	Methods of making antibody variable regions comprised in antibody fragments.
WO05003170	Celltech	Modified antibody fragments.
WO05063816	Genentech	Monovalent antibody fragments
Protein scaffolds that mimic antibody function		
WO0232925	Phylos	Protein Scaffolds as antibody mimics
WO06009888	Avidia	Alternative Scaffold (avimer) technology
WO03093321	Affibody	Fusion polypeptide with a non-antibody target recognition moiety.

Appendix - Patented “next generation” mab technologies

* Patent No.	Organization	Technology
Alternative expression technologies		
WO04050847	GTC Biotherapeutics	Modified antibodies stably produced in the milk of transgenic animals
WO03014344	Viragen	Expression of antibodies or humanized functional fragments in avian cells
WO05040395	Alder Biopharm	Yeast expression system using a haploid mating strategy
WO04072266	KaloBios	Method for engineering of high-affinity human antibodies using epitope guided replacement of variable regions using competitive cell-based methods
WO05035768	Biolex.	Optimized antibodies and Fab fragments expressed in duckweed
WO05070962	Novozymes	Methods and constructs for making mabs in a fungal host cell.
WO03081993	Origen Therapeutics	Transgenic aves producing human polyclonal antibodies

Appendix - Patented "next generation" mab technologies

* Patent No.	Organization	Technology
Other		
WO03095491	Affitech	The use of antibody expression libraries derived from immunochallanged individuals to screen and isolate recombinant human antibodies using a filter based assay system.
WO04020404	Biorexis	Modified fusion proteins of transferrin and Fab with increased serum half-life or stability.
WO04047742	Archemix	Aptamer therapeutics having improved pharmacokinetic and pharmacodymanic properties
WO05077090	Seattle Genetics	Improved method of making antibody conjugates
WO05113022	MRC	Superantibodies linked to a contrast agent for MRI
WO04006955	Evogenix	Method of producing "Superhumanized" antibodies with high affinity and low immunogenicity
WO04039995	Evogenix	Mutagenesis system for selection of optimized antibodies by ribosome display
WO0240997	Genencor	Method of neutralizing or reducing the ability of T- cells to recognize epitopes and thus prevent sensitization of an individual to the protein
WO9749800	Columbia University	Engineered antibody having a conformation suitable for degrading cocaine.