# Anti-infective immunotherapy for treating hospital-acquired infections

The frequency of nosocomial (acquired in healthcare facility) pneumonia has experienced a steady increase in recent years, and treatment of these infections has become more challenging and expensive due to the emergence of multi-drug resistant bacterial strains. Fuelling this problem is the declining effectiveness of existing antibiotics and the steady decrease in new antibiotics. The critical need for new anti-infectives can be addressed by exploiting and engineering various components of the immune response such as monoclonal antibodies (mAbs), as these were originally developed by mother nature to combat infections. Anti-infective immunotherapy is an attractive approach to address this global public health problem.

**By Dr Vu Truong, Dr Nadine Weich, Dr Andreas Loos and Dr Eric Patzer**

A good case study which underscores the critical need for new anti-infectives and the potential impact of efficacious anti-infective immunotherapy is in Healthcare-Associated Infections (HAIs), which are among the most frequent medical complications related to current hospital care. These infections not only lead to significant morbidity and mortality, increased hospital stay and long-term disability for individual patients, but also contribute to increased prevalence of antimicrobial resistance and financial burdens for health systems worldwide. Recent figures from the World Health Organization (WHO) revealed that for every 100 hospitalised patients, seven patients in developed and 10 patients in developing countries will acquire at least one HAI. A 2011 survey by the United States Centers for Disease Control and Prevention (CDC) determined that on any given day, about one in 25 hospital patients has an HAI. In aggregate, there were approximately 648,000 patients with 722,000 HAIs in US acute care hospitals in 2011 and an estimated 75,000 HAI-associated deaths during these patient hospitalisations[1]. The most common HAIs are blood-

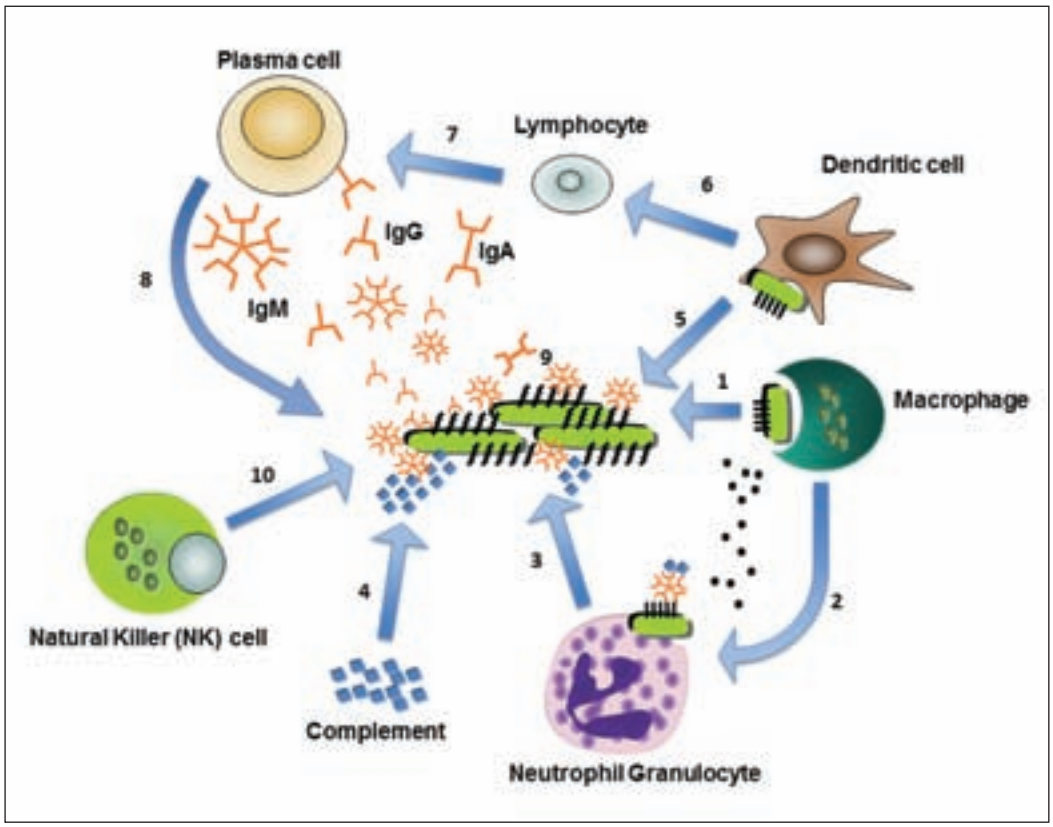


**Figure 1**
When a bacterial pathogen penetrates the outer physical barriers of the body it encounters various cells and soluble factors of the immune system. Immune cells that are already present in the affected tissue such as tissue macrophages (1) recognise the pathogen by virtue of its pathogen-associated molecular patterns (PAMPs). They phagocytose the pathogen and secrete immune mediators that induce a local inflammation. Blood flow and permeability of the blood vessels is increased and leukocytes such as monocytes and neutrophils (2) are attracted by the chemotactic gradient of the mediators and migrate from the blood stream into the tissue. Already present and newly arrived cells co-operate to phagocytose and kill the pathogen (3). Proteins of the complement system opsonise the pathogen and kill it directly (4) or enhance phagocytosis (1,3). Dendritic cells, a special type of phagocytic cell, connect the innate with the adaptive immune response. They phagocytose the pathogen (5) and then present degraded parts to B- and T-lymphocytes (6). Upon this activation, the B- and T-lymphocytes specific for one antigen of the pathogen start to multiply and differentiate. This is the start of the adaptive immune response. B-lymphocytes differentiate to plasma cells (7) that secrete large amounts of antibodies, first mainly IgM antibodies with a later shift to IgG antibodies (8). These antibodies can neutralise pathogens or their toxins (9), activate complement (4), enhance phagocytosis through opsonisation (1,3), induce cell-mediated cytotoxicity mainly by natural killer (NK) cells (10), or agglutinate the pathogen. Over the course of the adaptive immune response, the specificity of cells and antibodies for the antigen increases as the immune system learns to fight the pathogen more effectively. *Components are not drawn to scale

stream infections, pneumonia, urinary tract infections and surgical site infections. Together these account for approximately 80% of all HAIs. Pneumonia, the second most common HAI, accounts for approximately one-quarter of all infections in intensive care units and is foremost in terms of morbidity, mortality and cost[2].

According to the American Thoracic Society guidelines, hospital-acquired pneumonia (HAP) is defined as a lung infection in non-intubated patients acquired 48 hours or more after hospital admission. Pneumonia developing more than 48 hours after intubation is referred to as ventilator-associated pneumonia (VAP). Notably, the risk of developing pneumonia increases six to 20-fold in ventilated patients and is associated with a 46% mortality. HAP and VAP (nosocomial pneumonia) together increase time spent in the hospital by an average of 7-9 days per patient and recent studies have determined the costs attributable to VAP to be $40,000 per case[3].

## Therapeutic strategies for Multi-Drug Resistant (MDR) bacterial infections

The major cause of HAP and VAP are microbial infections caused by a variety of different pathogens with *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Klebsiella ssp* and *Haemophilus influenza* being the most common[1]. Standard of care for these infections consists of appropriate, broad-spectrum antibiotics prescribed at adequate doses. However, multi-drug resistant (MDR) infections are more problematic and result in a significant decrease in successful treatment of HAP and VAP when treated with antibiotics alone. Two of the most common bacteria responsible for multi-drug resistant HAP and VAP infections, *Pseudomonas aeruginosa (P. aeruginosa)* and *Staphylococcus aureus (S. aureus)* are also intrinsically more susceptible to developing antibiotic resistance. *P. aeruginosa*, in particular, has the capability of developing resistance to multiple antimicrobial agents making the treatment of pneumonia caused by this pathogen more difficult and more expensive[4]. Of increasing concern is the rising rate of MDR bacterial pneumonia, which is outpacing the rate of development of new anti-infectives, elevating the need for novel innovative therapies. Furthermore, the 2013 US Center for Diseases Control (CDC) data suggested that the development of antibiotic resistance to newly introduced antibiotics is occurring at a shorter time frame (2-4 years) than at any time since antibiotics have been introduced.

Currently, antibacterial treatments rely almost exclusively on antibiotics, with the exact course of treatment normally dependent on the risk assessment of the patient and the pathogen. *P. aeruginosa* infections in low-risk patients (no MDR risk, early onset of treatment) typically receive aminopenicillins in combination with β-lacatmase inhibitors, cephalosporin or carbepenems. For high-risk *P. aeruginosa* patients, cephalosoporins of the latest generation monobactams, carbapenems, fluoroquinolones or colistin are recommended. In *S. aureus* infections, methicillin-resistant strains (MRSA) pose the biggest challenge. In such cases, the use of either vancomycin or linezolid is recommended. Combination therapy with different classes of antibiotics can be used for very high risk patients (eg ICU patients with a high risk of MDR infection). Furthermore, to attain high drug levels in the lung, inhalation of antibiotic aerosols have been an effective method, particularly for cystic fibrosis patients[5,6].

With the slowing development of new antibiotics, in combination with the continuing emergence of bacterial resistance to both existing and new antibiotics, more attention has been given to other methods of antibacterial treatments. One attractive approach is to exploit the power of the immune system. This is our natural defence against infections and passive transfer of human and animal immune serum was one of the first effective rational therapies for pneumonia, which is still used on a small scale. With modern molecular biology, it is now possible to produce many of these active, curative agents of the immune system *in vitro* and then develop them for use in patients. For this reason companies and research institutions look to the normal immune system to learn which immune molecules are most suitable for therapeutic purposes. In **Figure 1**, a schematic overview of the immune response to a bacterial infection is shown.

## Antibodies as anti-infectives

Theoretically, most of the components of the immune system can be used for therapeutic purposes[7,8]. Strategies that are actively pursued, and many of which are still in their infancy, include the

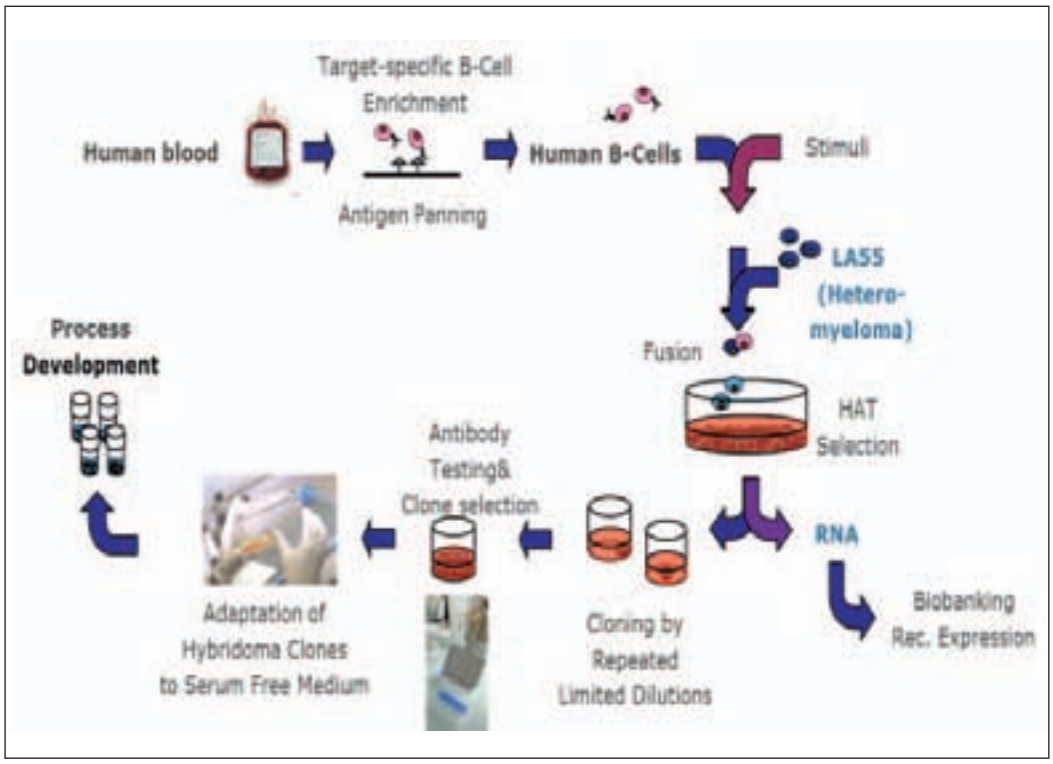


**Figure 2**
Aridis proprietary MAbIgX™ technology enables the culture of human, antibody-producing cells and thereby harnesses the potential of the human immune system to generate highly-effective, well-tolerated antibodies against any given antigen. After target-specific enrichment of B-cells they are fused to specially treated mouse myeloma cells. The resulting stable hybridoma cell lines are cultured, the produced antibodies are tested and optimal clones are selected. Before going to process development, the clones can be adapted to serum free media

isolation and expansion of antigen-reactive T-cells, the administration of recombinantly produced cytokines to influence the immune response, the *ex vivo* targeting of T-cells to the antigen by transfecting antigen-receptors or vaccination with 'antigen-charged' dendritic cells to jump-start the cellular immune response. Immunotherapy with antibodies has taken the leading role in this development aided by the virtually unlimited supply of purified human mAbs, which are among the immune system's most effective weapon against bacterial pathogens. They can bind and neutralise bacterial toxins and virulence factors, opsonise pathogens and at the same time improve recognition and binding by phagocytic cells thereby increasing the phagocytic (killing) efficiency. Antibodies can also activate complement which in turn lyse micro-organisms directly or increase phagocytic activity. In addition, bacteria can be agglutinated by antibodies, which then increases their clearance (see **Figure 1**). In contrast to antibiotics, antibodies are generally well tolerated by the patient. With proper selection of the binding epitope, such that the antibody targets are highly conserved or are required for pathogenesis, bacteria are unlikely to quickly develop resistance since downregulation of these epitopes will result in less virulence. A common strategy used in conventional antibiotic drug development is to target critical pathways involved in bacterial cell replication such as those involving nucleic acids, protein, cell wall synthesis, which are targets that may result in a greater selective pressure for mutations and drug resistance development, as compared to extracellular cell associated structural components. Antibodies can exert their effect immediately after administration and have a long serum half-life, such that durability of action is significantly longer than conventional small molecule antibiotics (serum half-life of days to several weeks versus hours for antibiotics). An added benefit is that passive immunotherapy similar to vaccination generally has minor effects on the specific composition of the normal human flora during treatment, and there is no evidence that either treatment selects for antimicrobial resistance.

For therapeutic purposes, antibodies of the immunoglobulin G (IgG) class are most frequently used. Their advantages include relatively easy production, purification and storage, while at the same time they exhibit high affinities and are well tolerated. However, depending on the indication, other classes of immunoglobulins might be used preferentially, such as IgA antibodies, which are the most effective in a mucosal surface environment. Alternatively, for complement mediated bacterial killing, which is a major mechanism of bacterial killing by the immune system, IgM is typically more effective than other

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antibody subtypes. IgM antibodies can be especially useful in gram-negative bacterial infections since it is the major naturally occurring antibody idiotype directed against bacterial lipopolysaccharides (LPS), which is the most common component found on bacterial cell walls[9]. These bacterial infections are fought by the immune system mainly via complement-mediated killing and complement-mediated phagocytosis. Moreover, IgMs have 10 antigen-binding sites (in contrast to two in IgGs) which allow the antibodies to bind with high avidity to antigens.

Due to a different mode of action as compared to antibiotics, antibodies are effective against antibiotic resistant pathogens such that adjunctive usage of antibodies with antibiotics can be an effective approach to managing difficult to treat MDR infections, including those associated with HAIs. Therefore, they are currently among the leading candidates to be the new 'magic bullet' in this area. Other anti-bacterial treatment options such as nanoparticles, gallium, bacteriophages, anti-sense products, antimicrobial peptides, fusion proteins, etc are also being explored[10].

## Production of anti-infective mAbs

The first monoclonal antibodies developed for human therapy were mouse antibodies. Although very similar to human antibodies, the genetic differences were large enough to evoke an immune response in the patient, leading to a fast removal of the drug and significant inflammation. To overcome this problem, 'chimeric' and later 'humanised' antibodies were generated by grafting the antigen binding 'variable' domains or the complementarity determining regions (CDRs) of the mouse antibody on to human antibody constant and framework regions respectively. Whereas mouse-derived, chimeric or humanised mAbs were commonly used only a few years ago, many companies currently prefer to develop mainly fully human mAbs. Several techniques are available to generate fully human mAbs, such as the use of recombinant mice that are engineered to express only human antibodies. These antibodies contain human constant domains and human framework regions, and although CDRs containing the epitope binding region are defined by the mouse immune system, these antibodies are considered to be human. Another approach is the phage display technology, where variable regions of human heavy and light chains are cloned, randomly paired, expressed and screened for the 'best' binding antibody. However, these methods rely either on the mouse immune system to generate the antigen binding site or *de novo* pair heavy and light chain of the antibody. A new approach to developing fully human antibodies is to employ the naturally occurring, antibody-producing human B-cells as the manufacturing platform. The difficulty in selecting and culturing human antibody-producing B-cells has been stabilisation of the recovered B-cell in *ex vivo* tissue culture conditions. MAbIgX™-technology (Aridis Pharmaceuticals) has overcome this hurdle through cellular immortalisation and allows the culture of highly productive human B-cells (see **Figure 2**). The immortalisation of human antibody producing cells takes advantage of the full therapeutic potential of the human antibody repertoire and enables the production of fully human antibodies. A hallmark of the MAbIgX™-technology is the fusion of antigen-induced plasma cells with a specific heteromyeloma cell line, LA55, resulting in the establishment of stable cell lines capable of expressing fully human antibodies of all isotypes. Thus, this technology allows the isolation and selection of an optimal antibody isotype for a given indication. Due to the high affinity and selectivity of the naturally-generated human mAbs, lower dosages are likely to be effective. Being fully human, these MabIgX antibodies exhibit exceptionally low immunogenicity and have a high potential for long term administration.

## Antibodies in clinical development for hospital acquired infections

Antibodies have great potential to address the shortcomings of current antibiotic therapy. Several antibodies currently in development show great promise in combating the rapidly growing rise in antibiotic resistant pneumonia and other infections (see **Table 1**). There are three complement activating therapeutic antibodies directed against MDR *P. aeruginosa* in clinical development for pneumonia. Aerucin™ is a human IgG1 mAb specific for the surface polysaccharide, alginate, of *P. aeruginosa*. In preclinical studies, it was shown to promote phagocytic killing of both mucoid and non-mucoid strains as well as protect against both types of strains in a mouse model of acute pneumonia[11]. A Phase I study to evaluate safety and pharmacokinetics of Aerucin has recently been initiated in healthy volunteers. Panobacumab ('AR-101') is a monoclonal IgM antibody that directed against *P. aeruginosa* serotype O11 that was generated from lymphocytes isolated from a volunteer immunised with the *P. aeruginosa* serotype O11 O-polysaccharaide-toxin[12]. O11 is one of the most common serotypes among multi-drug resistant *P. aeruginosa* clinical isolates. AR-101 was shown to provide full

**Table 1:** Anti-infective MAbs in development for Healthcare-Associated Infections*

| COMPANY | PRODUCT | INDICATION | PHASE | CLINICAL DEVELOPMENT STATUS |
|---|---|---|---|---|
| BMS | Aurexis (Tefibazumab) Humanised IgG | *Staphyloccus aureus* bacteremia Cystic Fibrosis | Phase II | Completed |
| Aridis Pharmaceuticals | Panobacumab Human IgM | *Pseudomonas aeruginosa* pneumonia | Phase II | Completed |
| Medimmune/Astra Zeneca | MEDI4893 Human IgG | *Staphylococcus aureus* pneumonia | Phase II | Recruiting |
| Aridis Pharmaceuticals | AR-301 Human IgG | *Staphylococcus aureus* pneumonia | Phase I/II | Recruiting |
| Aridis Pharmaceuticals | Aerucin Human IgG | *Pseudomonas aeruginosa* pneumonia | Phase I | Ongoing |
| Medimmune/Astra Zeneca | MEDI3902 Human IgG | *Pseudomonas aeruginosa* pneumonia | Phase I | Recruiting |
| XBiotech | 514G3 | *Staphylococcus aureus* bacteremia | Phase I/II | Open, not recruiting |
| Arsanis | ASN-100 Human IgG | *Staphylococcus aureus* pneumonia | Preclinical | NA |
| Aridis Pharmaceuticals | AR-401 Human IgG | *Acinetobacter baumannii* infections | Preclinical | NA |
| Achaogen | MAb program | *Pseudomonas aeruginosa* infections | Discovery/ preclinical | NA |
| Arsanis | MAb program | *Klebsiella pneumoniae* *Streptococcus pneumoniae* | Discovery/ preclinical | NA |

** This table does not provide an exhaustive list of all antibodies in preclinical development for pneumonia*

protection against lethal challenges with O11 strains of *P. aeruginosa* in preclinical murine lung infection and sepsis models, and to be safe in healthy human volunteers[13]. In a Phase IIa trial with 18 patients with nosocomial pneumonia, resolution of pneumonia occurred at nine days following treatment, with AR-101 compared to 15.3 days in the standard-of-care patients[14]. Medimmune (a member of the Astra Zeneca Group) is currently entering a Phase I study with MEDI3902, a human monoclonal biphasic antibody specific for the Type three secretion system (T3SS) PcrV protein and the Psl exopolysaccharide of *P. aeruginosa*. MEDI3902 has been shown to mediate protection in multiple animal models[15]. A Phase I study to assess the safety and pharmacokinetics of MEDI3902 is currently recruiting. Previously, another anti-PcrV humanised mAb, developed by Kalobios Inc, completed Phase IIa trials in cystic fibrosis patients which failed to meet its primary endpoint[19].

Methicillin-resistant *S. aureus* (MRSA) lung infections are also a current focus of therapeutic antibody development. Three companies are separately developing human mAbs against *S. aureus* alpha-toxin (see **Table 1**). This toxin is an

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extracellular, conserved virulence factor that is secreted by almost all *S. aureus* strains. AR-301 was assessed in a murine lung infection model and was found to protect against disease when administered prior to the onset of *S. aureus* infection and against lethal pneumonia when administered therapeutically at various time points after the infection[16]. A Phase I/IIa clinical study with AR-301 to assess safety, tolerability, pharmacokinetics and therapeutic efficacy in patients with severe pneumonia caused by *S. aureus* is currently ongoing. MEDI4893, being developed by Medimmune, is a human mAb that is also directed against the *S. aureus* alpha-toxin[17]. It was found to be safe and well tolerated in a Phase I study with healthy human volunteers, and has just entered a Phase II safety and efficacy study in non-symptomatic *S. aureus* colonised patients. A humanised mAb, Aurexis, that binds to the surface-expressed adhesion protein clumping factor A is being developed by Bristol-Myers Squibb as adjunctive therapy for *S. aureus* infections[18]. A Phase II safety and pharmacokinetics study in cystic fibrosis patients who have *S. aureus* in their lungs was just completed.

## Conclusion

Resistance to new antibiotics is emerging at an ever-increasing pace, and at the same time the rate of introducing new antibiotics has slowed substantially. Multi-drug resistant bacterial strains cause multiple problems for public health, and especially for those patients already hospitalised. The urgent need for new therapeutic options has increased the interest in antibody-based immunotherapy for bacterial pneumonia. Several antibodies addressing this need are currently in clinical studies, some with preliminary signs of efficacy. Among the advantages of adjunct antibody therapy that is combined with standard-of-care antibiotic therapy are the excellent safety profiles, no side-effects, long serum half-life, little impact on normal human flora, a new mode of action and a low risk of emergence of resistance. These therapeutic advantages of mAbs, along with improved production technologies, provide a solid foundation for continued growth of the field of antibody-based anti-infectives. DDW

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