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Review

The safety of therapeutic monoclonal antibodies: Implications for cardiovascular disease and targeting the PCSK9 pathway☆

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ABSTRACT

Monoclonal antibodies (mAbs) are established therapies for many conditions, including cancers, autoimmune conditions and infectious diseases. mAbs can offer benefits over conventional pharmacotherapy in terms of potency, dosing frequency and specificity for their target antigen. Mouse-derived antibodies were initially used in humans; however, patients often developed human anti-mouse antibodies, resulting in rapid antibody clearance (and a resulting loss of efficacy) and hypersensitivity reactions. Chimeric, humanized, and fully human antibodies were thus developed, with increasing amounts of human sequence, to reduce immunogenicity. Although generally well tolerated, mAbs may be associated with adverse events (AEs). Many AEs are target-related, and will be specific to the antibody target and the therapeutic area of use. However, off-target AEs, such as hypersensitivity reactions, are observed with many antibodies.

Within the realm of cardiovascular medicine, new antibody-based therapies are under investigation to reduce low-density lipoprotein cholesterol (LDL-C) levels. Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates plasma LDL-C levels by increasing degradation of the LDL receptor (LDLR). Therefore, inhibition of the interaction between PCSK9 and the LDLR with mAbs targeting PCSK9 has great potential for patients with hypercholesterolaemia. Early clinical phase studies suggest these mAbs are effective and well tolerated; however, further studies are required to assess their long-term safety.

Contents

☆ Purpose of the paper: To provide an overview of the literature on the safety of monoclonal antibody therapy and to address cardiovascular physicians' lack of familiarity with monoclonal antibodies as a potential therapeutic option.

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1. Introduction

Cardiovascular diseases (CVDs) are a leading cause of morbidity and mortality worldwide, accounting for approximately 30% of all deaths in 2008 [1]. A correlation between the level of low-density lipoprotein cholesterol (LDL-C) and the rate of cardiovascular (CV) events has been demonstrated in several studies, and lowering LDL-C levels dramatically reduces the incidence of CV events [2–4]. Consequently, international treatment guidelines recommend lowering LDL-C to <2.5 mmol/L (<97 mg/dL) in most patients with established CVDs and to <1.8 mmol/L (<70 mg/dL) in those at very high risk for CV events [5–7]. Recent studies also suggest that significant additional reductions in CV risk can be obtained by reducing LDL-C levels beyond those currently recommended [8–11].

Despite the widespread availability of effective lipid-lowering agents such as statins, 16–53% of patients worldwide fail to achieve their lipid targets [12–14], with even higher rates (79%) in patients with familial forms of hypercholesterolaemia [15]. Approximately 20 million patients in America take statins [16] and, approximately 10–20% of patients are unable to tolerate statins, particularly at the higher doses required to achieve LDL-C goals [17]. New treatments that aggressively reduce lipid levels in patients with severe hypercholesterolaemia, or those unable to reach their lipid targets, are therefore required. Some newer therapies in development for hypercholesterolaemia are utilizing monoclonal antibodies (mAbs), rather than small molecule inhibitors, to address novel targets.

Since their initial introduction into clinical practice in 1986, mAbs have become established therapies for a wide range of conditions, including cancers, transplant rejection, autoimmune conditions and infectious diseases [18–26]. Advances in molecular biology techniques and mammalian cell-line expression systems during the 1980s and 1990s led to the production of better tolerated, more target-specific mAbs with reduced immunogenicity and longer half-lives [19,27]. This class of biotherapeutic drugs has obvious advantages over conventional small-molecule pharmacotherapy in terms of potency, specificity and dosing frequency. Due to their large size, mAbs are not expected to inhibit cardiac potassium ion/human ether-à-go-go related gene (hERG) channels, thus may not lead to QT interval changes [28]. Furthermore, mAbs do not traditionally interact with the cytochrome P450 isoenzyme or other transport proteins in the body, resulting in a greatly reduced potential for drug–drug interactions with other agents. Generally mAbs are well tolerated [29,30]; however, as with all agents, mAbs may be associated with adverse events (AEs) as a result of enhancing/inhibiting the activity of the target molecule on the target tissue, or due to interactions of the mAb with target molecules on tissues other than the intended ones [18,29,31–33]. It is therefore important to understand the mechanism of action by which each mAb elicits its therapeutic effect, to reduce its potential immunogenicity through genetic engineering [18,30,34,35] and to thoroughly assess the efficacy and safety of each mAb in well-defined pre-clinical studies before embarking on clinical trials [35,36].

So far, more than 30 mAbs have been approved for clinical use by European and United States regulatory agencies, and more than 200 are currently undergoing pre-clinical or clinical investigations [19,26]. To date, the only mAb licensed for the treatment of patients with CV-related disorders is abciximab, which targets the glycoprotein IIb/III receptor and is used after percutaneous coronary intervention and for unstable angina [21]. Small-molecule therapies remain the most frequently used drug type in the CV therapy area; however, a new class of mAb—the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor—is being developed for the reduction of LDL-C in subjects with hypercholesterolaemia [37,38]. If successful, PCSK9 mAbs have the potential to dramatically improve the management of CV risk in patients who fail to achieve their therapeutic targets using existing lipid-lowering strategies.

This review examines the long-term safety evidence for mAbs and discusses the implications for PCSK9 mAbs in the treatment of hypercholesterolaemia.

2. The evolution of mAbs

mAbs rely on one of three functionalities to achieve their clinical efficacy: (i) target-specific binding by the Fab domain (antigen-binding site) to enhance or suppress an important biological effect, (ii) interaction of the Fc domain (constant domain) with cell-surface receptors leading to immune-mediated effector functions, including antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, or antibody-dependent phagocytosis [18] and (iii) deposition of complement on multimeric immune complexes between the mAb and the target and subsequent activation of complement-dependent cytotoxicity. The Fc determines the functional characteristics of the antibody, including effector function activity. In situations where a mAb effector function is not wanted, mAbs can be engineered in such a way that the Fc region is modified or isotypes can be selected that are relatively poor at inducing effector functions [18]. However, in general, soluble monomeric targets are not subject to effector functions because there is no multimerization of the antibody.

The production of specific antibodies from hybridoma cell lines was first described by Köhler and Milstein in 1975 [39]. The first mAb to be approved for use in humans was muromonab, a mouse anti-CD3 immunoglobulin G2a antibody for the prophylaxis or treatment of allograft rejection [40]. However, differences between the human and mouse immune systems meant that patients treated with full mouse sequence mAbs often developed human anti-mouse antibodies (HAMAs), resulting in rapid mAb clearance, hypersensitivity, poor target-site penetration and reduced efficacy via stimulation of cytotoxicity [24,40].

To reduce the risk of these events, chimeric and humanized mAbs were produced by genetic engineering [41]. Chimeric mAbs, such as abciximab [21], infliximab (an anti-TNF used in rheumatoid arthritis) [42] and rituximab (an anti-CD 20 used in rheumatoid arthritis, non-Hodgkin's lymphoma and chronic lymphocytic leukaemia) [43] contain the antigen-binding variable domain (Fab) from the species used for immunization (typically mouse), fused onto the human constant domains (Fig. 1). Humanized mAbs, such as daclizumab (an anti-CD 25 in development for multiple sclerosis) [44] and omalizumab (anti-IgE used in asthma) [22], contain Fab sequences with smaller amounts of mouse sequence fused on to human constant domains (Fig. 1). Thus, compared with mouse and chimeric mAbs, humanized molecules contain a greater proportion of human sequence, which reduces the likelihood of HAMA

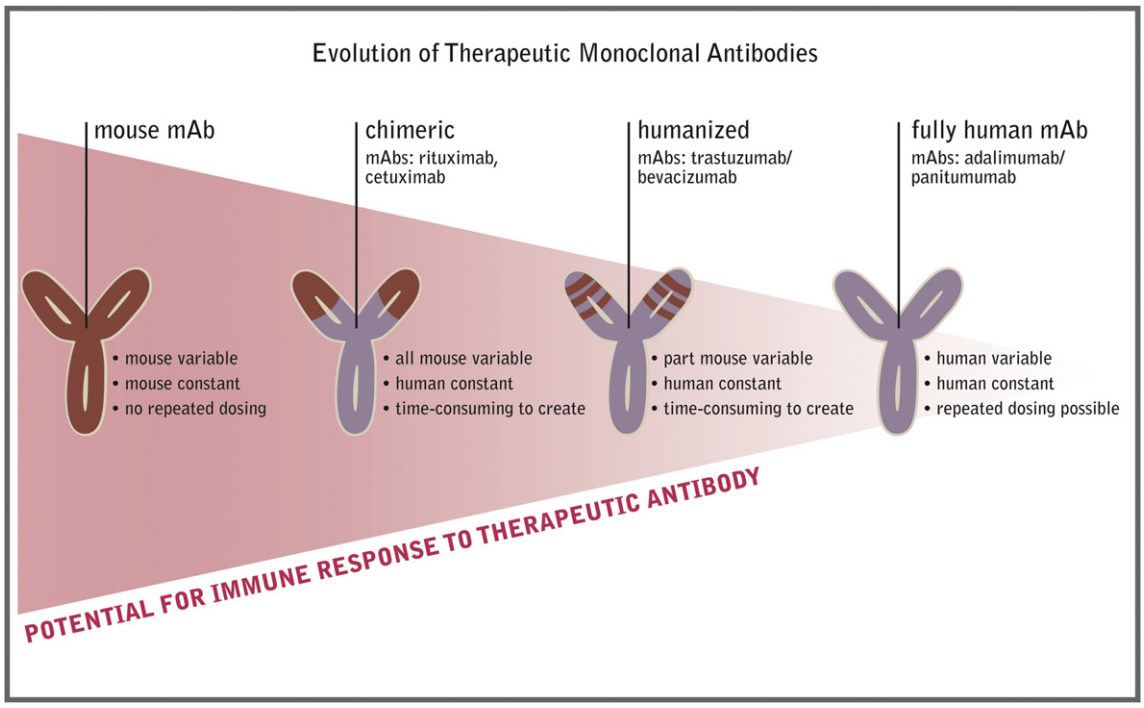

Fig. 1. The evolution of therapeutic mAbs.

development [24,45]. However, in many cases, humanization of mouse mAbs can result in reduced binding affinity for the therapeutic target. This necessitates the need for additional engineering to increase affinity through mutation of amino acids constituting the complementarity-determining regions that bind the target [46].

More recently, fully human mAbs have been produced which, compared with chimeric and humanized mAbs, generally have reduced immunogenicity (Fig. 1) [19]. Fully human mAbs can be produced from *in vitro* systems such as phage display libraries [47,48] or from *in vivo* platforms, such as transgenic mice [49–51]. The first of these techniques involves the recombinant expression of human Fabs in a bacteriophage and the subsequent selection of mAbs based on the required antigen-binding properties. The second technique uses transgenic mice and involves the introduction of human immunoglobulin genes into the murine genome. The transgenic mouse is then immunized against a specific antigen, which stimulates the production of human mAbs that can subsequently be isolated and cloned from the mouse B cells. Selection of antibodies *in vivo* has an inherent advantage over *in vitro* selection by phage display, because the antibody must fold and express efficiently, and have reasonable pharmacokinetic properties in order to compete effectively with other antibodies for binding to antigen.

Overall, nine fully human mAbs are currently approved for use in Europe and the United States [19,26] (Table 1).

Another, raxibacumab (an antibody against *Bacillus anthracis* protective antigen for the treatment of anthrax), has been filed for approval and numerous other fully human mAbs are being developed.

Additionally, the delivery method of antibodies has evolved. Previously, most mAbs were injected intravenously as this results in 100% bioavailability, rapid delivery to the systemic circulation, and high final plasma concentrations. However, intravenous administration has limitations, such as the need for regular clinic visits, which greatly increase the cost of therapy. Also, the rapid nature of intravenous mAb delivery can cause more serious hypersensitivity reactions in some patients. Many antibodies can now be delivered subcutaneously. Furthermore, pre-filled syringes in auto-injector devices can allow self-administration.

Table 1
Fully human monoclonal antibodies approved for use in Europe and the United States.

International non-proprietary name	Brand name	Company	Indication
Adalimumab [52]	Humira	Abbott	Rheumatoid arthritis
Panitumumab [53]	Vectibix	Amgen	Colourectal cancer
Golimumab [54]	Simponi	Centocor	Rheumatoid and psoriatic arthritis, ankylosing spondylitis
Canakinumab [55]	Ilaris	Novartis	Muckle–Wells syndrome
Ustekinumab [56]	Stelara	Johnson & Johnson	Psoriasis
Ofatumumab [57]	Arzerra	Genmab	Chronic lymphocytic leukaemia
Denosumab [58]	Prolia	Amgen	Bone loss
Belimumab [59]	Benlysta	Human Genome Sciences	Systemic lupus erythematosus
Ipilimumab [60]	Yervoy	Bristol-Myers Squibb	Metastatic melanoma

3. Primary safety concerns with mAbs

In general, mAbs are well tolerated and, as noted earlier, have some advantages over small molecules; they are more specific for their target and do not interact with cytochrome P450 or other transport proteins in the body, resulting in reduced potential for drug–drug interactions.

Toxicity associated with mAbs can be related to the pharmacological activity of the mAb, due to a direct consequence of engaging the target molecule (protein or receptor) or process within the intended tissue. For example, the platelet aggregation inhibitor abciximab is associated with an increased risk of minor bleeding [25,31]. This AE is driven by the intended pharmacological action of

abciximab (inhibiting platelet aggregation via blocking glycoprotein IIb/IIIa). Alternatively, mAbs may cause toxicity by interacting with the target antigen on tissues other than the intended tissue. For example, skin toxicity associated with cetuximab—a mAb that inhibits epidermal growth factor receptor (EGFR) and is approved for colorectal and head and neck cancer—is thought to be related to expression of the target antigen, EGFR, in human keratinocytes [30]. Many of the safety profiles of mAbs are antigen/target-related and will be specific to their target and therapeutic area of use.

Off-target, non-specific toxicity can also be observed with mAbs; for example, hypersensitivity reactions are commonly observed and are thought to be related to the immunogenicity of mAbs. It is important to note that the main factor affecting mAb immunogenicity is the proportion of human versus non-human sequence [30,35]. Those mAbs with a high proportion of non-human sequence are likely to be recognized as 'foreign' and therefore induce a host immune response. This can result in reduced efficacy of the mAb, due to increased clearance, and AEs, such as infusion or injection-site reactions. Production of mAbs from human germline sequences and the development of fully human mAbs can reduce the risk of immunogenicity but may not completely eliminate it. This is because factors other than the primary sequence, such as mAb formulation, mAb aggregation induced upon storage, protein conformation, glycosylation, impurities arising from the production method, the container system and storage conditions all contribute to immunogenic potential [35]. The cell line used to manufacture the antibody can also make a difference; for example, cetuximab produced in NS0 cells contains alpha-galactose carbohydrate that can cause hypersensitivity reactions in some allergic individuals, while antibodies manufactured in the widely-used Chinese Hamster Ovary (CHO) cells do not contain this allergenic carbohydrate epitope [61].

Overall, the risk of serious adverse drug reactions (ADRs) with mAbs is generally low [29,62–64]. A meta-analysis of studies assessed the rates of adverse effects of nine biologics, alone or as add-on therapy, in any indication (except HIV/AIDs) compared with any other therapy or placebo in 163 randomized controlled trials (RCTs) (n = 50,010) and 46 extension studies (n = 11,954). This analysis suggested that biologics were associated with a significantly higher rate of total ADRs versus other therapy or placebo (odds ratio [OR] 1.19, 95% CI [confidence interval] 1.09–1.30); however, the rates of serious adverse events (SAEs), serious infections, lymphoma and heart failure were not significantly different from what was seen with the control arm (placebo or other therapy) in these studies [29]. It should also be noted that this analysis does not separate on-target and off-target AEs. It is likely that the majority of the ADRs reported in the studies were related to on-target toxicity, which is specific to the mAb used and the therapy area it was used in. Although the studies included in this analysis were of short duration (median 6 months for RCTs and 13 months for open-label extension studies), previous studies suggest that mAb safety profiles remain relatively unchanged during the long term [62–64]. Additionally, there are some real-life data indicating that mAbs for rheumatoid arthritis and Crohn's disease are well tolerated over the long-term [65,66]. Some examples of the better-known mAb safety concerns, both on-target and off-target, are described in Table 2.

4. mAbs for the treatment of CVD

As noted earlier, CVD is one of the major causes of death and disability in Western societies. To date, few mAbs have been developed for the treatment of CVD or its risk factors; however, with several agents in development, this is likely to change in the near future.

4.1. Abciximab

Abciximab, a Fab fragment from a chimeric mAb, was licensed in the United States in 1994 for use in patients undergoing percutaneous coronary intervention (PCI). It inhibits platelet cross-linking and aggregation by targeting glycoprotein IIb/IIIa, thereby reducing blood coagulability [75,76]. Abciximab is an intravenously administered drug that is used only in hospitals by interventional cardiologists. Abciximab is associated with off-target, non-specific AEs, such as the formation of HAMAs leading to hypersensitivity, a likely consequence of not being a fully human mAb. The agent also leads to on-target AEs, such as an increased risk of bleeding and thrombocytopaenia [21,77].

4.2. PCSK9 mAbs

PCSK9 plays an important role in the regulation of plasma levels of atherogenic LDL-C. In healthy humans, approximately 70% of LDL particles are removed from the circulation via hepatic uptake, more than 90% of which is mediated by the transmembrane LDL receptor (LDLR) [78]. Each LDLR binds a single LDL particle and is internalized by endocytosis [78]. A drop in pH causes the LDL to separate from its receptor in the endosomes and the unoccupied receptors are returned to the cell surface for reuse. At the same time, the lipoprotein is degraded and the released cholesterol is stored in the cell and used for a variety of cellular activities. In the hepatocyte, these are primarily the production of bile acids and very-low-density lipoprotein (VLDL). Each cycle takes about 12 min and, since the LDLR has a lifespan of about 30 h, each LDLR may recycle around 150 times [79]. As a result, minor decreases in LDLR activity can result in major increases in plasma LDL-C levels.

The level of hepatic LDLR is controlled at the transcriptional level via sterol regulatory element-binding protein 2 (SREBP-2) and at the post-transcriptional level via PCSK9 (Fig. 2A) [37,38,80–83]. PCSK9 is the ninth member of the subtilisin family of serine proteases and is mainly expressed in the liver and intestine [84]. Following secretion, it binds to LDLRs and stimulates receptor internalization. This process leads to increased LDLR degradation, reduced availability of transmembrane LDLRs, and increased levels of plasma LDL-C [85]. As described below, people with a mutation in PCSK9 that prevents its ability to interact with LDLR (loss of function in PCSK9) have lower LDL-C and, more importantly, a dramatic reduction in CV events. Thus, blocking the interaction between LDLRs and PCSK9 is likely to reduce CV risk in people with hypercholesterolaemia by increasing the availability of cell-surface LDLRs and reducing serum LDL-C (Fig. 2B).

Since the lipid-lowering efficacy of statins is limited, at least in part, by a statin-induced increase in PCSK9 expression [83,84], it is anticipated that inhibiting the PCSK9–LDLR interaction may also increase the lipid-lowering efficacy of statins. The enzymatic activity of secreted PCSK9 is not required for degradation of the LDLR, making the development of small molecules that target this enzyme difficult [86]. Current approaches to inhibiting circulating PCSK9 include mimetic peptides, nucleic acid technologies and mAbs. Of these, nucleic acid technologies and mAbs have entered clinical development.

Among the nucleic acid-based therapies, SPC5001 (Santaris Pharma A/S), a locked nucleic acid-based inhibitor, and BMS-844421 (Bristol-Myers Squibb), an antisense RNA therapy, have terminated clinical trials [87,88]. ALN-PCS02 (Alnylam), an RNA interference inhibitor, is currently in phase 1 clinical trials [89].

To date, clinical trials conducted for mAbs targeting PCSK9 have been quite successful. Phase 2 trial results are available for the mAbs developed by Sanofi/Regeneron (SAR236553/REGN727) [90–92] and Amgen (mAb1/AMG145) [93–96]; the Sanofi/

Table 2
Examples of safety concerns with monoclonal antibodies, across therapy areas.

AE	Description	Frequency and severity	Mechanism
AEs due to inhibition of target antigen			
Infections	Ranging from mild upper respiratory tract infections to serious infections such as tuberculosis and invasive opportunistic infections	Mild infections common with immunosuppressive antibodies	Generally reported for mAbs designed to reduce the activity of T cells and B cells, such as infliximab, adalimumab and etanercept
Drug-induced thrombocytopaenia	Low levels of platelets, often associated with abnormal bleeding [67]	In patients treated with abciximab, profound thrombocytopaenia occurs in ~1% with first infusion and in ~4% after the first exposure [68].	Generally reported for antibodies used in the CV therapy area, such as abciximab
Autoimmune diseases	Includes lupus-like syndromes, vasculitis, nephritis, demyelinating syndromes, thyroid disease and autoimmune colitis [18,69]	Uncommon, and symptoms are often reversed when therapy is stopped [69]	Often reported for mAbs used in chronic inflammatory diseases (due to their interference with the immune system) [32]
Tumour lysis syndrome	Group of metabolic abnormalities, including hyperuricaemia, hyperkalaemia	Rare, but potentially serious	Reported for mAbs used in cancer therapy, such as rituximab. Large number of tumour cells lysed; if these are not eliminated the intracellular content cause metabolic disturbances
Off-target, non-specific AEs			
Infusion reactions/injection-site reactions	Includes mild injection-site skin reactions, pyrexia and influenza-like symptoms [18,33] Acute infusion reactions include anaphylactic and anaphylactoid reactions, and systemic inflammatory response syndrome [70]	Mild reactions common; can be managed by early risk factor recognition, appropriate monitoring and prompt intervention [18,33] Acute events are uncommon and risks can be reduced by slowing the rate of infusion [18,33]	Off-target, non-specific Immunogenicity thought to be less likely to occur for mAbs with more human versus non-human sequence [30] Cell line used to manufacture antibody can also have an effect; for example, cetuximab produced in cell containing alpha-galactose carbohydrate can cause hypersensitivity reactions [61]
AEs due to antigen expression on normal tissue			
Malignancies	Some mAbs (e.g. infliximab, tositumomab and ibritumomab) have off-target tumour-promoting anti-inflammatory effects [20]	Rare	Exact link remains controversial [18,71]
Cardiotoxicity	Cardiac dysfunction, such as an asymptomatic decrease in left ventricular ejection fraction	Heart failure observed in up to 4% of women treated with trastuzumab and 10% of treated patients experience a decrease in cardiac function. [72] Usually reversible and patients respond well to standard medical management [73]	Thought to be due to the role of the antigen HER2 in cardiomyocyte survival [30]
Cytokine release syndrome	Uncontrolled hypercytokinaemia	Can cause influenza-type symptoms, swelling and redness, and can be fatal	Caused by positive feedback loops between cytokines and immune cells resulting in highly elevated levels of proinflammatory cytokines [74] Three mAbs identified that cause cytokine release syndrome in humans: alemtuzumab, muromonab and TGN1412 (withdrawn from development) [18]

mAb, monoclonal antibody; HER2, human epidermal growth factor 2.

Regeneron compound has now entered phase 3 of clinical development. A number of additional PCSK9 mAbs, in earlier clinical development, are currently being investigated for potential use in humans, including 1B20 (Merck & Co.; pre-clinical) [97], PF-04950615/RN-316 (Pfizer; phase 2) and LGT 209 (Novartis; phase 2) [38] and MPSK3169A (Roche-Genentech, Phase 2 [98].

5. Efficacy and safety of PCSK9 mAbs

5.1. Epidemiological studies

The correlation between PCSK9 and LDL-C levels has been associated, in part, with genetic variation in PCSK9 and more than 50 amino acid variants of PCSK9 have been demonstrated to influence plasma cholesterol levels [99]. Gain-of-function (GOF) mutations in the gene encoding PCSK9 have been identified in families with autosomal dominant familial hypercholesterolaemia (FH). These mutations, which increase the affinity of PCSK9 for LDLR, result in high cholesterol levels and a significantly increased incidence of coronary heart disease [100]. For severe mutations, the onset of coronary artery disease may occur 10 years sooner than in heterozygous FH patients with severe LDLR mutations [101]. Furthermore, a polymorphism within the PCSK9 gene that is associated with higher LDL-C levels has also been associated with increased carotid artery intima–media thickness [102].

Conversely, in people with one PCSK9 allele with loss-of-function (LOF) mutations that prevent productive interaction with

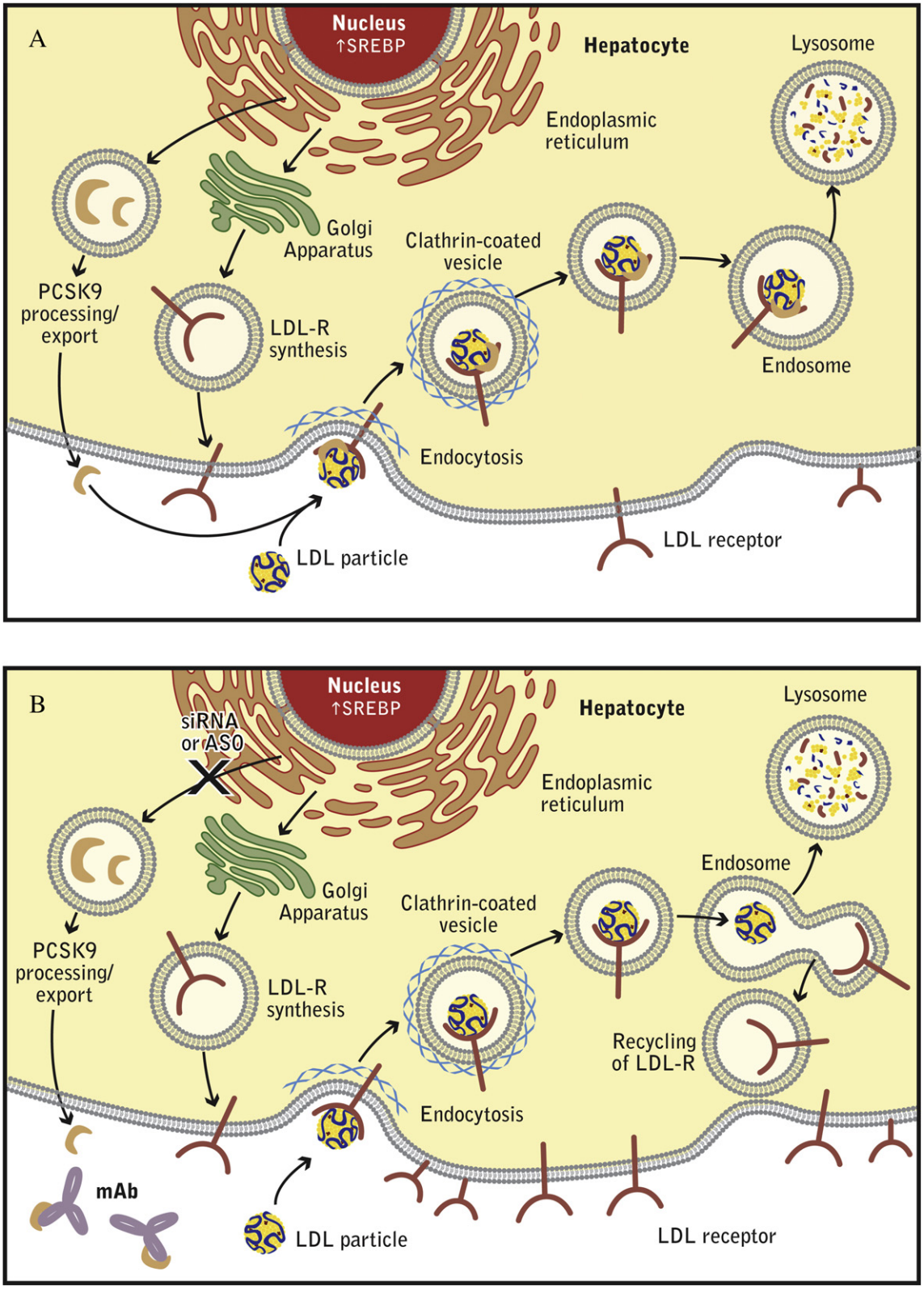

Fig. 2. The role of PCSK9 in the regulation of the LDLR. A. Levels of hepatic LDLRs are controlled by sterol regulatory element-binding protein 2 (at the transcriptional level) and by PCSK9 (at the post-transcriptional level). PCSK9 binds to LDLRs and, upon internalization, directs the receptor to the lysosome for destruction, thus decreasing the level of LDLRs at the cell surface. B. PCSK9 antibody prevents binding of PCSK9 to the LDLR-LDL complex, increasing the availability of cell-surface LDLRs.

LDLR, LDL-C levels are reduced by up to 40% and the risk of coronary heart disease is reduced by up to 88% [103–106]. An analysis of three studies conducted in Copenhagen showed that the PCSK9 R46L allele is associated with lower LDL-C levels than those without this allele. This was observed in all age groups, and the PCSK9 R46L genotype was also associated with a reduced risk of ischaemic heart disease and mortality [107]. The ARIC study also showed that carriers of PCSK9 variants that were associated with lower LDL-C levels had a lower incidence of peripheral arterial disease [108].

Patients with LOF PCSK9 mutations are generally healthy with no other apparent metabolic abnormalities. Three examples of individuals with no detectable circulating PCSK9 have been reported. One subject who had inactivating mutations in PCSK9 in both alleles had an LDL-C of 14 mg/dL, but was otherwise healthy, normotensive and had normal liver and renal function tests [104]. Another individual with a double mutation affecting the same PCSK9 allele, which had dominant negative activity, had an LDL-C of 16 mg/dL; this individual had diabetes, of uncertain aetiology, but had normal hepatic enzyme levels and liver function tests. Additionally, results from this individual showed for the first time that a PCSK9 loss-of-function mutation resulted in increased LDL catabolism in humans [109]. A third subject was homozygous for the C679X mutation of PCSK9 and had an LDL-C of 15 mg/dL [110]. Additionally, no obvious phenotypic abnormalities have been identified in PCSK9 knockout mice [111]. This currently suggests that use of PCSK9 mAbs is unlikely to be associated with on-target AEs [38].

Aside from specific GOF or LOF mutations, overall serum PCSK9 levels in subjects have also been directly correlated with serum concentrations of LDL-C and with total cholesterol [112,113]. A study of patients with FH who have a pathogenic mutation in the LDLR or ApoB gene, and were not receiving cholesterol-lowering treatment, suggested that PCSK9 may contribute to the phenotypic severity of FH. In this study, PCSK9 plasma levels were significantly lower in patients with an LDL-C level below the 75th percentile than in patients with LDL-C above the 90th percentile [114]. PCSK9 levels were also found to be closely associated with LDL-C levels across all groups [114].

5.2. Pre-clinical studies

Pre-clinical studies in wild-type mice show that a single intravenous injection of AMG145 (Amgen's fully human PCSK9 mAb) can increase hepatic LDLR protein levels roughly two-fold and lower serum total cholesterol by up to 36%, an effect not seen in LDLR knockout mice [115]. In cynomolgus monkeys, a single intravenous injection of the same mAb reduced serum LDL-C by up to 80% and maintained a significant decrease for 10 days [115]. Similar results were also obtained in rodents and primates using PCSK9 mAbs from Sanofi/Regeneron (SAR236553/REGN727) [116], Pfizer (J10, J16) [117,118], and Merck & Co (1D05-IgG2 and 1B20) [97,119,120]. Moreover, whereas a twice-daily dose of atorvastatin had little effect on serum LDL-C levels in statin-resistant male Syrian hamsters, a single subcutaneous injection of SAR236553/REGN727 dose-dependently neutralized PCSK9 activity and reduced LDL-C by up to 58%. In this study, SAR236553/REGN727 was well tolerated, with no clinically relevant effects on safety parameters, liver weight, hepatic cholesterol, triglycerides or phospholipid concentrations. This suggests that, in addition to dose-dependently reducing LDL-C by neutralizing PCSK9 activity, SAR236553/REGN727 can overcome the statin resistance observed in hamsters [121].

5.3. Phase 1 studies

Phase 1 studies in humans have shown that PCSK9 mAbs are well tolerated and significantly reduce LDL-C levels in healthy subjects and patients with hypercholesterolaemia [122–125]. Single dose–response studies with Sanofi-Regeneron's fully human mAb SAR236553/REGN727, for example, showed that intravenous or subcutaneous injections dose-dependently reduced mean LDL-C levels by up to 65% in healthy volunteers with LDL-C >2.6 mmol/L (>100 mg/dL), an effect that was maintained for at least 30 days with the higher doses [124]. A further phase 1 multiple-dose study assessed SAR236553/REGN727, administered by subcutaneous injection (50, 100 or 150 mg) in patients with hypercholesterolaemia (FH or non-FH) receiving statins and those with non-FH receiving diet therapy [124]. As an add-on therapy to a statin, SAR236553/REGN727 was associated with reductions from baseline in LDL-C of 41–58% in patients with FH, and reductions of 38–65% in those without FH. In non-FH patients receiving diet therapy only, SAR236553/REGN727 was associated with a 57% reduction from baseline in LDL-C levels [124].

A phase 1a study of Amgen's AMG145 mAb showed that single subcutaneous and intravenous injections dose-dependently reduced LDL-C by up to 64% versus placebo ($P < 0.0001$) in healthy volunteers [125]. In a phase 1b dose-escalation study, patients with hypercholesterolaemia (FH or non-FH) were given repeated doses of AMG145 subcutaneously as add-on therapy to statins (low- to moderate-dose or high-dose) [125]. Over 6–8 weeks, administration of AMG145 resulted in dose-dependent reductions in LDL-C up to 75% versus placebo in patients receiving low- to moderate-dose statins [125]. Similar reductions in LDL-C were observed in patients with heterozygous FH (−65% versus placebo) and those receiving high-dose statins (−63% versus placebo) [125].

In each of the phase 1 studies for SAR236553/REGN727 and AMG145, treatments were generally well tolerated, with no clinically meaningful elevations in liver transaminases or other safety parameters [124,125]. During the multiple-dose study of SAR236553/REGN727 no SAEs were reported. Although two patients in the single-dose SAR236553/REGN727 studies experienced SAEs (abdominal pain with rectal bleeding and a small-bowel obstruction in a patient with prior appendectomy), neither resulted in study discontinuation, nor were they considered related to study treatment [124]. In the SAR236553/REGN727 intravenous study, the incidence of treatment-emergent adverse events (TEAEs) was similar among subjects treated with study drug and those treated with placebo [124]. Compared with placebo, a higher proportion of patients treated with single dose and multiple doses of subcutaneous SAR236553/REGN727 experienced an AE. No specific pattern of AEs was identified, but headache was the most common event. The few reported injection-site reactions were mild in severity and most were transient [124]. For AMG145, no SAEs were reported and the incidence of TEAEs was similar among subjects treated with AMG145 and those treated with placebo [125]. Furthermore, no patients discontinued due to AEs and there did not appear to be any relationship between the incidence of AEs and the dose of AMG145 [125].

5.4. Phase 2 studies

Phase 2 clinical studies evaluating SAR236553/REGN727 have recently completed. The first of these studies compared the effects of five different dosing regimens of SAR236553/REGN727 versus placebo in patients with primary hypercholesterolaemia (LDL-C ≥2.6 mmol/L) who were on stable atorvastatin therapy [90]. This was a double-blind, parallel-group, placebo-controlled trial in which 183 patients were randomized to placebo or one of the SAR236553/REGN727 regimens (50, 100 or 150 mg every 2 weeks, or 200 or 300 mg every 4 weeks) for 12 weeks [90]. After 12 weeks, LDL-C levels decreased significantly from baseline by

40–72% in those receiving SAR236553/REGN727 50 mg, 100 mg and 150 mg every 2 weeks ($P < 0.0001$ for all dose groups versus placebo). In those receiving SAR236553/REGN727 200 mg or 300 mg every 4 weeks, the reductions in LDL-C observed 2 weeks after each dose (67% and 70% reductions from baseline) waned over the following 2 weeks to 39% and 53% reductions from baseline. This suggests that the every-2-weeks regimen may have the maximum effect and be the most favourable regimen, delivered using a single subcutaneous injection [90]. Between 89% and 100% of SAR236553/REGN727 recipients achieved the target LDL-C of <100 mg/dL, compared with 16% of those receiving placebo. Furthermore, LDL-C levels of <70 mg/dL were achieved by between 46 and 100% of SAR236553/REGN727 recipients versus 3% of placebo recipients [90]. Although patients were receiving different atorvastatin doses (10, 20 or 40 mg), LDL-C reductions were similar irrespective of atorvastatin dose [90].

The second of the phase 2 trials compared the LDL-C-lowering efficacy and safety of co-administering SAR236553/REGN727 versus placebo with high-dose atorvastatin (80 mg) in patients with LDL-C ≥2.6 mmol/L (≥100 mg/dL) despite treatment with stable-dose atorvastatin 10 mg for at least 6 weeks prior to randomization [92]. After 8 weeks, patients receiving atorvastatin 80 mg/day plus a single subcutaneous dose of SAR236553/REGN727 administered every 2 weeks achieved a least-squares mean LDL-C reduction of 73%, versus 17% for those on atorvastatin 80 mg plus placebo ($P < 0.001$) [92]. The least-squares mean reduction from baseline in LDL-C after 8 weeks with SAR236553/REGN727 plus atorvastatin 10 mg was 66% [92].

LDL-C reductions were similar irrespective of statin dose. It has been shown that statin treatment results in an increase in PCSK9 levels that limits their lipid-lowering efficacy [126]. As such, co-administration of SAR236553/REGN727 with atorvastatin may benefit patients that fail to achieve their LDL-C target using high-dose statins. Moreover, since the additional reduction in LDL-C with SAR236553/REGN727 appears to be similar irrespective of atorvastatin dose, co-administration of SAR236553/REGN727 with low-dose statins may benefit patients who fail to achieve their LDL-C targets owing to an intolerance of high-dose statins.

A third phase 2 study investigated the addition of various dosing regimens of SAR236553/REGN727 to statins in patients with FH [91]. Patients with FH on stable diet and statin dose, with or without ezetimibe, were randomized to SAR236553/REGN727 150, 200 or 300 mg every 4 weeks, or 150 mg every 2 weeks, or placebo every 2 weeks for a total of 12 weeks [91]. Administration of SAR236553/REGN727 resulted in dose-dependent LS-mean LDL-C reductions from baseline to week 12 of 29–68%, compared with 11% with placebo [91].

In all of the phase 2 studies, SAR236553/REGN727 was generally well tolerated over the treatment period, with no drug-related AEs on liver function tests or other laboratory parameters, and no serious TEAEs related to treatment [90–92]. Injection-site reactions (including erythema, pruritus, swelling, haematoma and rash) were the most common AEs in two of the phase 2 trials and were generally mild in severity [90]. However, in the phase 2 study assessing SAR236553/REGN727 for treatment of FH, one patient, receiving 300 mg every 4 weeks, terminated the study after one dose due to an injection-site reaction and generalized pruritus. No treatment was required for this reaction and the patient did not experience any residual effects [91]. Muscle complaints, including pain and weakness, were infrequently reported in the first phase 2 study, and the incidence was similar across treatment groups. Furthermore, increases in muscle-related enzymes were not observed [90].

There was a single case of cutaneous leukocytoclastic vasculitis (CLV) reported in one patient 9 days after initiation of SAR236553/REGN727 (300 mg). This event was diagnosed by biopsy and was not associated with other organ involvement; the patient responded quickly to steroid therapy initiation and SAR236553/REGN727 discontinuation [90]. Anti-drug antibodies were not found following the event; however, they were present at the 20-week follow-up, although at a titer of 30, the minimum titer detectable by the assay. CLV is a generally benign disease that occurs at the rate of 40–60 cases/million persons/year [127]. It appears to be caused by immune complex deposition in vessel walls, leading to cellular infiltrates, cytokine release and vessel damage with bleeding. Drugs implicated in the development of CLV include small molecules and protein therapy (i.e. mAbs) [128]. In a review of articles published between 1990 and 2008, there were 118 reported cases of CLV in patients receiving mAbs against tumour necrosis factor [129].

AMG145 has been assessed in four phase 2 trials. AMG145 was investigated as monotherapy in 406 patients with hypercholesterolaemia (LDL-C between 2.6 and 4.9 mmol/L) [94]. In this trial, patients received AMG145 70, 105 or 140 mg every 2 weeks or 280, 350, 420 mg every 4 weeks or matching placebo or ezetimibe 10 mg/day. At week 12, the change from baseline in LDL-C ranged from −39% to −51% with AMG145 compared with −3.7% and +4.5% for the two placebo regimens (every 2 weeks and every 4 weeks, respectively) and −15% with ezetimibe ($P < 0.0001$ for all doses versus placebo or ezetimibe) [94].

AMG145 was also assessed in 631 patients with hypercholesterolaemia (LDL-C > 2.2 mmol/L) on a stable dose of statin, with or without ezetimibe [93]. Patients were randomized to AMG145 every 2 weeks (70, 105 or 140 mg) or every 4 weeks (280, 350 or 420 mg) or matching placebo. At week 12, AMG145 was associated with dose-dependent reductions in mean LDL-C versus placebo of 42–66% with the every-2-week regimen and 42–50% with the every-4-week regimen [93].

In an another trial, 160 patients who were statin-intolerant due to muscle-related side effects were randomized to treatment with AMG145 280, 350 or 420 mg alone; AMG145 420 mg plus ezetimibe 10 mg or ezetimibe 10 mg plus placebo [96]. AMG145 or placebo was administered every 4 weeks. After 12 weeks of treatment, least-squares mean percentage changes in LDL-C from baseline ranged from −41 to −51% in the AMG145 alone groups, −63% in the AMG145 420 mg/ezetimibe group and −15% for those receiving placebo/ezetimibe [96].

Finally, AMG145 has also been assessed in patients with heterozygous FH. A total of 167 patients with heterozygous FH with LDL-C ≥2.6 mmol/L despite statin treatment, with or without ezetimibe, received treatment with either AMG145 350 or 420 mg or placebo administered every 4 weeks [95]. At week 12, AMG145 350 and 420 mg were associated with a least-squares mean reduction in LDL-C of 43% and 55%, respectively, compared with a 1% increase with placebo [95].

Overall, AMG145 was generally well tolerated throughout the phase 2 trials, with a similar incidence of treatment-related AEs across treatment groups, and no evidence of a relationship between the incidence of any particular AE and AMG145 dose [93–96]. Injection-site reactions were observed with AMG145 but were generally infrequent and not severe [93–96].

Other phase 2 studies have also completed enrolment, including a multiple-dose study of PF-04950615 [130].

While the development of mAbs targeting PCSK9 for the treatment of hypercholesterolaemia is still in its early stages, current data indicate that a fully human mAb for this target demonstrates clinically meaningful reductions in LDL-C in patients treated with and without statins. Current data also have not revealed potential on-target adverse effects of blocking PCSK9 and appear to avoid the apparent on-target effects of inhibiting HMG CoA reductase with statins: muscle toxicity and liver aminotransferase elevations. The

use of fully human mAbs reduces the level of injection-site reactions and immunosensitivity reactions but some potential for these AEs still exist. However, the current conclusions are based upon phase 1 and 2 trials of limited exposure and from relatively small patient populations. Clinical trials of larger size and longer duration, in more varied patient populations, are ongoing to further assess the efficacy and safety of mAbs targeting PCSK9. For example, the ODYSSEY phase 3 programme will assess SAR236553/REGN727 in more than 22,000 patients [131]. This includes trials assessing the effect of SAR236553/REGN727 on lowering LDL-C levels in varied patient populations, including those at elevated cardiovascular risk [132], those unable to tolerate statins [133], those with FH [134] and a cardiovascular outcomes study [135].

6. Conclusions

mAbs have been used in clinical practice for many years, and are established therapies for cancers, autoimmune conditions and infectious disease. mAbs offer clear benefits over conventional pharmacotherapy in terms of their potential for potency, dosing frequency, and specificity to their target antigen. Initially, mouse antibodies were developed, but many patients treated with these developed HAMAs, resulting in rapid clearance of the mAb, loss of efficacy and hypersensitivity reactions. Chimeric and humanized mAbs, with increasing amounts of human sequence, have thus been developed to reduce the risk of immunogenicity. Most recently, fully human mAbs have been developed; it is anticipated that these may reduce immunogenicity further. Although generally well tolerated, mAbs are associated with AEs. Some of these, such as mild hypersensitivity reactions, are commonly observed with many mAbs, while more serious hypersensitivity reactions occur less frequently. In contrast, other AEs are antigen related and will be specific to the target of the mAb and the therapeutic area of use. Therefore, it will be important to consider the mechanism of action of each mAb and fully assess their efficacy and safety in clinical trials.

mAbs are now being investigated for the reduction of LDL-C in subjects with hypercholesterolaemia by targeting PCSK9. PCSK9 plays a major role in the regulation of LDL-C levels via its interaction with the LDLR. The reduced incidence of CV events in healthy patients with PCSK9 LOF mutations suggests that inhibiting the interaction between PCSK9 and LDLR has great potential for the management of patients with severe and/or refractory hypercholesterolaemia. Initial safety assessments of PCSK9 mAbs showed that these molecules were generally well tolerated. However, further studies are required to assess the long-term safety of this potential new drug class in a wider range of patients. Based on the current findings, PCSK9 mAbs may be of particular use for patients with hypercholesterolaemia who are unable to achieve LDL-C targets or those who are intolerant to currently available treatment options.

Conflict of interest

Professor Catapano has acted as a consultant for Sanofi and Dr Papadopoulos is an employee of Regeneron Pharmaceuticals Inc.

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