mRNA as novel technology for passive immunotherapy

Thomas Schlake1, Andreas Thess1, Moritz Thran1, Ingo Jordan1

Received: 18 June 2018 / Revised: 13 September 2018 / Accepted: 3 October 2018 / Published online: 17 October 2018
© The Author(s) 2018

Abstract
While active immunization elicits a lasting immune response by the body, passive immunotherapy transiently equips the body with exogenously generated immunological effectors in the form of either target-specific antibodies or lymphocytes functionalized with target-specific receptors. In either case, administration or expression of recombinant proteins plays a fundamental role. mRNA prepared by in vitro transcription (IVT) is increasingly appreciated as a drug substance for delivery of recombinant proteins. With its biological role as transient carrier of genetic information translated into protein in the cytoplasm, therapeutic application of mRNA combines several advantages. For example, compared to transfected DNA, mRNA harbors inherent safety features. It is not associated with the risk of inducing genomic changes and potential adverse effects are only temporary due to its transient nature. Compared to the administration of recombinant proteins produced in bioreactors, mRNA allows supplying proteins that are difficult to manufacture and offers extended pharmacokinetics for short-lived proteins. Based on great progress in understanding and manipulating mRNA properties, efficacy data in various models have now demonstrated that IVT mRNA constitutes a potent and flexible platform technology. Starting with an introduction into passive immunotherapy, this review summarizes the current status of IVT mRNA technology and its application to such immunological interventions.

Keywords mRNA technology · Antibody · Immune globulin · Adoptive cell transfer · CAR T cell · Passive immunization · mRNA design · Tolerability

Abbreviations
AAV Adeno-associated virus
ADA Anti-drug antibodies
ARCA Anti-reverse cap analog
bi-(scFv)2 Bivalent single chain variable fragment
BiTE Bi-specific T cell engager
CAR Chimeric antigen receptor
CD Cluster of differentiation
CTL Cytotoxic T lymphocyte
DC Dendritic cell
DNA Deoxyribonucleic acid
E. coli Escherichia coli
Fab Fragment antigen binding
Fc Fragment crystallizable
FcRn Neonatal Fc receptor
FPLC Fast protein liquid chromatography
GMP Good manufacturing practice
HIV Human immunodeficiency virus
HPLC High performance liquid chromatography
IFIT Interferon induced protein with tetratricopeptide repeats
IgG Immunoglobulin G
IRES Internal ribosome entry site
IVT In vitro transcription
LNP Lipid nanoparticle
mAb Monoclonal antibody
MHC Major histocompatibility complex
mRNA Messenger ribonucleic acid
NSG NOD (non-obese diabetic) scid gamma
ORF Open reading frame
Poly(A) Polyadenylic acid
PKR Protein kinase R
RIG-I Retinoic acid inducible gene I
scFv Single chain variable fragment
TAA Tumor-associated antigen
TCR T-cell receptor
TLR Toll-like receptor
UTR Untranslated region
VEGF Vascular endothelial growth factor

© Springer
**Introduction**

Our bodies are continuously exposed to molecules that may indicate disease or parasitic invasion. The immune system is responsible for the detection and clearance of such molecules and for control of the underlying causes. Immediate discrimination between self and foreign upon first exposure is mediated by innate immunity. Signals that induce innate immunity are called pathogen-associated and damage-associated molecular patterns (PAMPs and DAMPs) [1, 2]. The innate immune system is characterized by induction of generic defenses against a broad spectrum of infectious agents and is mainly aimed at clearance of tissue damage at the site of infection and interruption of further pathogen replication. Responses against specific targets following prolonged or repeated exposures are processed by the adaptive immune system [3]. Important effectors of adaptive immunity are highly specialized cells: B cells secrete antibodies against soluble or cell-associated antigens [4]. Cytotoxic CD8$^+$ T cells (CTLs) recognize and kill infected or neoplastic cells [5]. Regulatory CD4$^+$ T cells augment B cell maturation or inhibit auto-reactive immune cells [6]. Dendritic cells (DCs) process and present antigen for the transition from innate to adaptive immunity [7]. Some B and T cells progress towards persisting memory cells that react faster and with greater affinity to the foreign molecules upon re-exposure. Compared to innate immunity, adaptive immunity usually requires weeks (as opposed to minutes) until an effective response against novel targets is achieved. Advantages of the adaptive immune system include the ability to launch specific immune responses also against antigens of endogenous (not only microbial) origin that may be associated with degenerative or neoplastic disease.

Manipulation of the immune system is an important component of many prophylactic and therapeutic applications against infectious, degenerative and neoplastic diseases. The diverse repertoire of methods can be roughly divided into four approaches: active immunization (vaccination) prepares adaptive memory responses, usually prior to first exposure, and constitutes one of the most efficacious and cost-effective medical interventions. Immunization with antigens generally leads to the induction of antibody production, whereas for instance inoculation with attenuated viruses also elicits cytotoxic effector cells [8]. In addition, active immunization can be accomplished by adoptive cell transfer. In a typical setting, dendritic cells are loaded with tumor antigens ex vivo and subsequently infused into patients to induce an adoptive immune response against cancer cells [9]. In contrast, passive immunotherapy circumvents the initial steps required for immune responses to be launched and directs the immune system efficiently to the desired medical targets. For cellular approaches, CTLs are equipped with recombinant receptors ex vivo. These cells are designed to attack neoplastic cells that express the cognate tumor-associated antigen immediately after infusion [5]. Passive immunization by administration of processed antibodies derived from human or animal donors is a well-established emergency procedure for treatment of snake-bite envenomation or post exposure prophylaxis against, for example, rabies [10]. The advantage of passive immunization is that protective antibodies can be provided in a very short time. Application of recombinant antibodies further expands the number of available targets and is increasingly important for augmentation of conventional therapies against cancer [10, 11].

Passive immunotherapies either require or can exploit modern nucleic acid-based methods, among which mRNA is the latest technology. The present review is dedicated to provide an overview on mRNA in passive immunotherapy after introducing the immunological approach as well as mRNA technology as such.

**Passive immunotherapy: general overview**

**Antisera and polyclonal immune globulin preparations**

It is well known that protection raised by most of today’s licensed vaccines is primarily antibody-dependent (Fig. 1a) [8, 12]. Basically, this explains the long and successful history of passive immunotherapy (Fig. 1b). The protective capacity of serum against bacterial toxins was discovered in the early 1890s [13]. The avoidance or control of infection by such passive immunization is based on the transfer of serum and later polyclonal immune globulin (= antibody) preparations from convalescent or vaccinated humans or animals [14, 15]. Prior to the discovery of antibiotics, serum was the only antidote for bacterial diseases [16]. Thus, following successful passive immunization against diphtheria toxin [13], a whole plethora of serum or immune globulin therapies for viral and bacterial diseases as well as to neutralize snake toxins was developed [17]. Clinical benefits of serum and immune globulin therapy were demonstrated for viral diseases such as influenza, measles, and polo and bacterial infections with meningococcus or pneumococcus [18–20].

With the advent of antibiotics, the use of serum or polyclonal immune globulin preparations as antibacterial agent was largely discontinued in late 1940. However, such therapies retained a niche as treatment for venoms, toxins, and...
certain viral infections. In the second half of the twentieth century, polyclonal antibody preparations were developed and in part licensed for the prophylaxis and treatment of hepatitis A and B, cytomegalovirus, varicella-zoster virus, vaccinia virus, rabies, respiratory syncytial virus, West Nile virus, and various hemorrhagic fevers [21]. For instance, passive immunization against the Argentine hemorrhagic fever shows beneficial effects when applied within 1 week after the emergence of symptoms, and post-exposure treatment with human or equine immunoglobulins are recommended for rabies [22–24]. Moreover, botulism is treated with equine antitoxin [25, 26]. Such immune globulin preparations of non-human origin are particularly prone to elicit an immune response that obstructs their therapeutic use or efficacy [27]. Further disadvantages or difficulties associated with the use of serum or polyclonal globulin preparations are the often high content of non-neutralizing antibodies, batch-to-batch variations, and in case of human sources the availability of appropriate immune donors [21, 28].

**Monoclonal antibodies**

In 1975, groundbreaking work described the production of monoclonal antibodies (mAb) by immortalization of B cells [29]. The resulting hybridoma technology was then rapidly exploited for clinical use, for instance, to produce a mAb to CD3 for preventing organ rejection [30]. Recombinant technologies further expanded the available therapies based on mAbs. In vitro antibody selection technologies like phage or ribosome display were developed to enable the generation of highly specific human mAbs out of libraries that may even be naïve for the specific antigens [31–36]. In the early 2000s,
a high throughput technique to amplify and clone antibody genes from single human B cells was described [37, 38].

**Full-size antibodies**

Although recombinant mAb technology is exploiting a large variety of different antibody formats (Fig. 2), the prevalent type is still a full-size antibody of the IgG class. In addition to the variable domains essential for antigen binding, they contain constant domains including the Fc-region. The latter is important for antibody function and can mediate antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-dependent cellular phagocytosis (ADCP) [39]. Furthermore, binding to the neonatal Fc receptor (FcRn) plays a role in controlling the antibody half-life which is 21–28 days for human IgG [40–42]. FcRn rescues bound antibody from degradation by transporting it back to the cell surface where it is released into the extracellular space [43, 44]. Specific mutations in the Fc region that increased the affinity to FcRn have been shown to prolong antibody half-life up to fivefold [45]. In addition to the impact of FcRn binding, half-life...
also benefits from the large size of IgGs. It obstructs antibody clearance by the kidney as well as metabolism by cytochrome P450 [42, 46]. The downside of the large size is the correspondingly low access to and penetration of tissue which can affect therapeutic efficacy [47].

Full-size antibodies are often posttranslationally glycosylated, modulating Fc function. Although aglycosylated IgGs can be produced in bacteria [48], modern production processes usually take advantage of the cellular machinery for advanced posttranslational maturation and secretion of eukaryotic cells [48–51]. The vast majority of approved therapeutic antibodies are currently produced in mammalian cells [52, 53]. Production processes have been optimized especially for the predominant production system, the continuous Chinese hamster ovary (CHO) cell line. CHO cells secrete antibodies with negligible non-human glycoforms [54] and are also amenable to glycoengineering [55]. Differences in glycoforms depend on the production system and affect distribution and stability of the antibody, Fc effector function and immunogenicity in recipients [54].

**Antibody fragments**

Since traditional expression hosts such as *E. coli* do not allow efficient production of full-size antibodies, smaller proteins consisting of fragments derived from the variable domains were developed as promising alternatives. Such single-chain variable fragments (scFv) and various derivatives thereof preserve antigen binding while facilitating manufacturing (Fig. 2b, c) [56]. Another type of antibody fragment is derived from camelids or cartilaginous fish. These animals produce single-domain antibodies devoid of light chains (Fig. 2e) [57, 58]. Since antigens are recognized by a heavy-chain-only V_{H} domain (V_{H}H) in camels [59], the variable V_{H}H fragment can be easily engineered into nanobodies that offer additional advantages such as improved heat and pH stability [60]. Moreover, they can also be assembled into V_{H}H-based neutralizing agents (VNAs) (Fig. 2e) [61]. Various studies demonstrated that multivalent formats were more effective than monovalent single-domain antibodies [62, 63]. Notably, all formats based on antibody fragments can be relatively efficiently produced with less expensive bacterial expression systems, typically employing *E. coli* [64, 65]. The antibody fragments produced in this system are often targeted to the oxidative environment of the periplasm using specific signal peptides to foster disulfide bond formation and proper folding [64, 65]. Moreover, enhanced expression of chaperones and cytoplasmic oxidases has been demonstrated to increase the yield of antibody fragments [48, 66].

Small antibody fragments were also the basis for developing the concept of bispecific antibodies more than 20 years ago. Initially, single chain antibodies with a different binding specificity were fused to the C-terminal ends of heavy chains of IgGs [67]. Generation of first bispecific IgG molecules benefited from the knob-into-hole technology [68]. Today, many different bispecific antibody formats combining two different antigen binding domains in one molecule are available (Fig. 2d) [69–72]. Among them, bispecific diabodies (bi-(scFv)2) and BiTE antibodies are prominent examples [73, 74]. In general, bispecific antibodies can be deployed to target therapeutic substances such as toxins, radionuclides, and drugs as well as effector cells like CTLs to the site of cognate antigen expression [75].

Associated with their small size, many formats using antibody fragments are cleared by renal elimination [76, 77]. Moreover, in the absence of an Fc region, recycling by the FcRn rescue mechanism cannot take place [77]. As a consequence, these formats usually reveal short plasma half-lives [77]. For instance, bi-(scFv)2 antibodies have a serum half-life of less than 2 h which usually requires continuous infusion [78]. In case of the BiTE blinatumomab, the antibody is usually administered daily due to its short half-life [79]. Possible strategies to extend serum half-lives are site-specific PEGylation and fusion to an Fc region [80, 81]. However, the latter approach would negate various advantages of antibody fragments including their better and faster tissue penetration [41, 82]. It has been shown that small single-domain antibodies could even cross the blood–brain barrier [83]. In case of an anti-rabies antibody, this allowed partial rescue of mice challenged with virus injection into the brain in contrast to full-size immunoglobulins [84, 85].

**Clinical status quo**

Today, monoclonal antibodies play an important role in the therapeutic armamentarium. Dozens of antibodies have been licensed to treat cancer, rheumatoid arthritis, multiple sclerosis, psoriasis, allergy, systemic lupus, and other diseases. In addition, mAbs have shown promise in protecting against various microorganisms, viruses, and fungal infections as well as in treating neurodegenerative diseases [86–89]. However, mAbs for infectious disease indications are still scarce among licensed products. Palivizumab for respiratory syncytial virus prophylaxis in high-risk infants was the first antiviral mAb approved by the FDA [90]. Since then, antibodies against anthrax and rabies in India became available. In addition, bezlotoxumab binding to *Clostridium difficile* toxin is used to prevent recurrent bacterial infections [91]. mAbs for treating cancer are still the largest group of licensed products. Here, rituximab, directed against the transmembrane protein CD20 on the surface of B lymphocytes, was the first mAb in clinical use [92]. However, as for many other mAbs in antitumor therapy, high doses are needed to obtain clinical efficacy [93]. A successful example for a bispecific antibody is the first-in-class BiTE against
CD19/CD3, blinatumomab, which is approved for the treatment of acute lymphoblastic leukemia [94–96].

**Cellular immunotherapy**

In addition to the administration of immunoglobulins, passive immunity can also be conferred by transferring functionalized immune cells. Adoptive transfer of CTLs was shown to be a potent therapeutic means to treat both viral infections and cancers [97–99]. To this end, T cells can be equipped with an additional T-cell receptor (TCR) or a chimeric antigen receptor (CAR) [100]. While CARs are limited to the binding of surface antigens, TCRs recognize MHC-presented peptides derived also from intracellular proteins. The first successful clinical trial with an engineered TCR on CTLs demonstrating tumor regression was reported in 2006 [99]. Subsequently, passive immunotherapies with TCR-engineered T cells became an important approach for antitumor treatments [101]. Efficient targeting and killing of cancer cells expressing the respective antigen in patients with various forms of cancer including metastatic melanoma, synovial sarcoma, and colorectal carcinoma have been demonstrated [99, 102–104]. A possible problem specific to the use of TCR-engineered T cells is the presence of an endogenous TCR. Mispairing of endogenous and introduced α- and β-chains may create new specificities with potential reactivity to host molecules [105, 106]. To avoid such mispairing, the use of γ/δ T cells has been suggested, since γ/δ-chains do not pair with α- or β-chains [107–109]. Alternatively, gene editing is now being explored to disable endogenous TCR expression [110, 111].

The concept of CARs was developed in 1989 [112] and then refined using a scFv fragment to obtain antibody-like receptor specificity without the need to transfer multiple genes [113]. First generation CARs consisted of an antigen-specific scFv, fused to a transmembrane and intracellular CD3ζ TCR signaling domain, conferring transient activation and cytotoxicity to T cells [114]. Upon target binding through the scFv domain, the engineered T cell is activated in an MHC-independent manner [115]. Subsequent generations of CARs were improved with respect to cytotoxicity and persistence by including additional co-stimulatory domains such as CD28, OX-40 or 4-1BB [114, 116–118]. T cells engineered with such CARs targeting specific tumor antigens are remarkably successful in treating hematological malignancies like leukemia and lymphoma [119, 120]. For instance, CD19-directed CAR T cells repeatedly revealed complete and durable remissions in patients with B-cell acute lymphoblastic leukemia (B-ALL) [121–123]. In contrast, CAR T cell therapies face various challenges for solid tumors [124].

At present, the most common techniques for generating TCR- or CAR-engineered T cells utilize viral gene transduction with retro- or lentiviral vectors [125]. However, permanent expression of the transgenetic receptor mediated by this efficient technology can be disadvantageous in case of therapy-related severe toxicities due to accidental cross-reactivity [126–128]. On-target/off-tissue and off-target toxicities by engineered T lymphocytes attacking healthy host cells as well as cytokine release syndrome are feared side-effects which were repeatedly reported when virus vector-transduced cells were applied [129–132]. Another concern of using retroviral vectors is the potential to induce insertional mutagenesis and genotoxicity in effector cells [133–136]. Hence, more precise cell manipulations are currently under investigation. Among them, gene editing of primary human T cells was recently demonstrated to be an efficient approach [137].

**DNA-based antibody expression in vivo**

While T cell engineering for adoptive transfer inevitably requires the use of nucleic acids encoding a target-specific receptor, antibody immunotherapy can deploy recombinant proteins. However, as pointed out above, maintaining therapeutically effective levels may require frequent administrations dependent on clearance and indication [138]. Thus, DNA-mediated antibody expression directly in the body may represent an attractive alternative to administration of recombinant proteins (Fig. 1c). Both, plasmids as well as viral vectors have been used for passive immunization. Although efficient in small animal models, strong expression of recombinant genes using unformulated DNA does not scale well to larger animals (including primates) [139]. Consequently, application of recombinant adeno-associated viruses (AAVs) is currently the preferred method for transduction of the antibody gene of interest [140]. Early work reached single digit µg/ml serum titers [141]. Later studies with advanced vector designs reported expression levels in the high µg/ml or even in the single digit mg/ml range [142–144]. Much work has been done in the field of HIV prophylaxis. Here, a single intramuscular injection of recombinant AAV was demonstrated to elicit peak antibody titers above 100 µg/ml in mice [142]. Treated mice revealed substantial anti-HIV antibody levels for more than a year and were protected from HIV-1 challenge.

Although AAV usually maintains an episomal state, this vector still harbors an inherent risk of insertional mutagenesis. In patients with hepatocellular carcinomas integration of AAV2 into known cancer genes was observed [145]. Moreover, AAV-based immunotherapy faces various issues regarding immunogenicity [146–148]. A substantial percentage of the population has already been in contact with the used virus and consequently shows pre-existing immunity limiting the efficacy of treatment [149, 150]. The induction of anti-viral responses during immunotherapy may have
similar consequences if a single virus serotype is used in repeated treatments [148]. Pre-existing or induced immunity could lead to clearance of the viral vector and/or AAV-transduced cells.

Finally, gene delivery by AAV has been reported to induce immune responses against the encoded protein. Even when using an endogenous gene such as erythropoietin (EPO), some macaques receiving EPO-encoding AAV intramuscularly developed severe autoimmunity against the protein [151]. When non-human primates were treated with AAV vectors expressing antibody or antibody-like proteins, titers dropped rapidly in some animals [152]. One reason is the possibility of inducing anti-idiotype antibodies which requires immune suppression to obtain sustained expression [153]. Further studies indicated that primates may be more prone to develop a robust T cell response to the AAV-encoded protein compared to mice [154].

In summary, the risks of insertional mutagenesis and genotoxicity, long-lasting expression without control in case of adverse events, and various potential issues regarding immunogenicity associated with viral vectors that may limit efficacy emphasize the high demand for other vectors for passive immunotherapy than DNA. How mRNA offers a viable option to meet the demands is reviewed in the following sections.

mRNA as novel technology for passive immunotherapy

The cellular machinery uses mRNA as a transient carrier of genetic information for the synthesis of proteins. Based on this fundamental biological concept administering exogenous mRNA represents an alternative to DNA-mediated protein delivery in vitro and in vivo. Using mRNA instead of DNA as therapeutic substance is attractive due to the absent risk of insertional mutagenesis. Moreover, efficient expression is even obtained in non-dividing cells, since mRNA does not require a nuclear phase for activity. Compared to the delivery of proteins and peptides, mRNA may prolong the availability of effector molecules, however, not as much as DNA. In contrast to the latter, mRNA therapy, therefore, has to cope with the short half-life in vivo of exogenously delivered mRNA as for instance indicated by mRNA-mediated VEGF expression in myocardium returning to baseline within 72 h [155]. While this may be a therapeutic disadvantage in various instances, it can be considered advantageous from a safety perspective, particularly in case of adverse events.

mRNA was first employed for the expression of a protein of interest in the early 1970s when RNA preparations were microinjected into Xenopus oocytes and synthesis of the encoded protein was demonstrated [156, 157]. In 1989, the group of Inder Verma presented a reliable method to efficiently introduce RNA into a variety of cells using a cationic lipid [158]. Almost at the same time, mRNA-mediated protein expression in vivo was demonstrated after direct injection into mouse muscle [159]. Much of the early work on a potential therapeutic use of mRNA focused on the development of active vaccination approaches, in part since low amounts of antigen suffice due to the amplifying nature of the immune response. Subcutaneous injection of liposome-encapsulated, antigen-encoding mRNA was the first example of eliciting an antigen-specific CTL response in mice [160]. Gene gun delivery of mRNA into mouse epidermis provided the first evidence of an antigen-specific antibody response [161]. In addition, mRNA turned out to be a potent means to load dendritic cells with antigens to convert them to tailored antigen-presenting cells in vitro and in vivo [162]. Later, a new vaccination protocol was introduced which elicited a complete adaptive immune response consisting of antigen-specific antibodies and T cells with lytic activity without requiring any transfection reagents, special equipment or heterologous boost [163]. From a retrospective view, this event marked the starting point of the commercial development of mRNA vaccines.

Building blocks for therapeutic mRNA

In the minimal structure, mRNA contains a protein-encoding open reading frame (ORF) flanked at the 5′- and 3′-end by two elements essential for the function of eukaryotic mRNA: the “cap”, a 7-methyl-guanosine residue (m7G) bound to the 5′-end of the RNA via a 5′–5′ triphosphate bond, and, with the exception of histone mRNAs, a poly(A) tail at the 3′-end [164–166]. Synthetic mRNA is transcribed in vitro from a plasmid DNA template that contains at least a bacteriophage promoter, the ORF, and a unique restriction site for linearization of the plasmid to ensure defined termination of transcription. Typically, the template also contains a poly(d[A/T]) sequence transcribed into poly(A). Alternatively, the poly(A) tail can be generated by enzymatic in vitro polyadenylation of transcribed RNA. The cap may be incorporated into the transcript during transcription by including an m7GpppG dinucleotide as a structural homolog of the endogenous cap structure in the transcription reaction. Different options for how to derive IVT mRNA are summarized in Fig. 3.

Cap structures

Various elements of an mRNA molecule contribute to the level and duration of expression of the encoded protein. The cap structure is required for efficient translation and stabilizes mRNA towards exonuclease decay [167–169]. Various structures have been repeatedly used for IVT mRNA
(Fig. 4). The basic m7GpppG cap analog is incorporated in both orientations into the RNA by the bacteriophage RNA polymerase [170]. However, reverse incorporation of the cap analog results in mRNA molecules that lack the m7 methylation at the cap and are not recognized by the translational machinery [171]. Substitution of the hydroxyl group in C2′ or C3′ position of the m7G with a methoxy group prevents reverse incorporation of the cap analog by inhibiting elongation at the m7G. The dinucleotide is, therefore, called ‘anti-reverse cap analog’ or ARCA [171, 172]. ARCA-capped mRNA revealed increased as well as prolonged protein expression in cultured cells and enhanced reporter protein expression in mouse dendritic cells up to 20-fold [173, 174]. For further optimization of the cap structure, modifications were introduced within the triphosphate linkage to inhibit decapping. Substitution of a non-bridging oxygen in the β-phosphate moiety of an ARCA by sulfur results in the β-S-ARCA dinucleotide. While β-S-ARCA maintains recognition by the translational machinery, protein expression from mRNA capped with β-S-ARCA was extended in HC11 cells and immature DCs, but not in mature DCs [175, 176].

Fig. 3 Flowchart for the manufacturing of IVT mRNA. a Starting with a DNA template (yellow) harboring all essential mRNA sequences including a poly(A) tail, different processes can be used to generate mRNA with either a cap0 or a cap1 structure (Fig. 4). T7p, T7 RNA polymerase promoter; dTn, poly(dT); T7, T7 RNA polymerase; rNTPs, ribonucleotides; cap0, cap1, cap analogs; VCE, Vaccinia virus capping enzyme; 2′-O-Met, Vaccinia virus methyltransferase. b If a poly(A) tail is not encoded in the DNA template, it can be enzymatically added to the mRNA after IVT and capping by a poly(A) polymerase (PAP).

Sequences

Like the cap structure at the 5′-end, details of the poly(A) tail at the 3′-end influence translation and stability of mRNA [167, 182]. While numerous studies demonstrated a positive effect of a poly(A) tail and some correlation of effectiveness and length of the element, details of observations were remarkably variable. The majority of studies indicate an enhancement of translation when extending the poly(A) length from approximately 60–70 to 100–150 nucleotides or by tail extension using enzymatic polyadenylation [174, 183–185]. However, effects were mostly moderate, but
reached a maximum of a 35-fold increase in one particular setting. In contrast, another study reported an optimum of approximately 60 nucleotides; protein expression declined with further increasing poly(A) length [186]. In addition to poly(A) length, one report suggests a positive role of using a type IIS restriction enzyme for DNA template linearization to obtain a free poly(A) end rather than one extended with unrelated nucleotides [183].

Further elements that can affect mRNA translation and/or half-life are untranslated region (UTR) sequences flanking...
the ORF sequence. Trans-acting regulatory RNA-binding proteins (RBPs) interact with distinct RNA sequence elements, thereby affecting ribosome recruitment and transit [187]. For instance, β globin 5′- and 3′-UTRs, duplicating the β globin 3′-UTR, the 5′-UTR of tobacco etch virus, and a structure of the 5′-UTR of human heat shock protein 70 all enhanced mRNA translation in mammalian cells [158, 183, 188, 189]. According to a very recent survey of a combinatorial UTR library, 5′-UTR sequences appear to be most critical for protein expression [190]. In contrast, 3′-UTRs seem to be the key driver for mRNA half-life as exemplified by the stabilizing effects of the α globin 3′-UTR as well as a duplication of the β globin 3′-UTR [183, 191].

Codon usage of the ORF sequence also affects translation efficacy in many species. Although in humans codon usage bias does not correlate with tRNA levels and gene expression [192, 193], still increased protein expression from mRNA upon codon usage optimization has been reported. For instance, codon usage adaptation of HIV-1 gag improved protein yield from mRNA approximately 1.6-fold in a human T lymphocyte cell line [194]. A more pronounced increase in protein expression as a result of codon optimization was reported upon transfection of mRNA encoding angiotensin-converting enzyme 2 into A549 and HepG2 cells [195]. However, alternatively to a direct effect of codon usage, the enhancement may be an indirect result of the use of modified nucleotides, the content of which was altered by codon optimization. Furthermore, coding sequence engineering exploiting more advanced concepts like codon optimality may prove valuable in designing therapeutic mRNAs of high efficacy. According to recent insights, codon usage may also affect fidelity of translation or the stability of transcripts [196].

In addition, ORF as well as UTR sequences can have an effect on immunostimulation and thus on translational activity. Initially, researchers applied mRNA containing only the four unmodified bases A, U, C, and G [158–160, 197]. In this context, for instance, U-rich sequences, as well as several RNA structural features were described as immunostimulatory due to interactions with various RNA sensors such as Toll-like receptors (TLR), RIG-I, and protein kinase R (PKR) [198–205]. As a consequence, development of mRNA therapies was hampered by the immunogenicity of in vitro transcribed mRNA.

**Immunostimulation**

The consideration of mRNA for therapeutic purposes gained momentum by the finding that incorporation of modified bases into in vitro transcribed mRNA reduced immunostimulation of such preparations. Various modified bases found in natural RNAs suppressed recognition by TLRs in vitro [206]. Among these, particularly pseudouridine increased translation and stability of mRNA [207]. In addition to the effect on TLR binding, replacement of uridine by pseudouridine affected binding to and activation of further sensors such as PKR and 2′-5′-oligoadenylate synthetase, contributing to higher and longer protein expression [208, 209]. However, the effects of mRNA modifications on translation, immunostimulation and resulting protein expression appear to be variable, for instance dependent on the type of target cells. In vitro testing of various modifications and combinations thereof revealed decreased protein yield for any of them in a context dependent manner, while most of them reduced immunostimulation in RAW124.7 macrophages [210].

As found in vitro, pseudouridine modification of mRNAs reduced immunostimulation and increased level and duration of protein expression after intravenous (IV) or intraperitoneal administration of formulated mRNA in mice [188, 207]. In contrast, another study on systemic administration of nanoparticle-complexed mRNA concluded that neither immunostimulation nor protein expression benefited from pseudouridine modification [211]. After intradermal or intramuscular injection of formulated mRNA, only N1-methyl-pseudouridine, but not pseudouridine, substantially enhanced expression [212]. In line, a different study applying lipid nanoparticle (LNP)-formulated mRNA intradermally found that replacement of uridine with N1-methyl-pseudouridine resulted in much increased and longer lasting protein expression [213]. Notably, recent studies revealed that also endogenous eukaryotic mRNA harbors various modified nucleotides [214–217]. However, the total level of modification is rather low which is in strong contrast to the usually 100% replacement of an unmodified nucleotide in IVT mRNA. Moreover, heavy nucleotide modification appears to interfere with the function of translation-enhancing RNA elements such as UTRs and internal ribosomal entry sites (IRESes) [218].

At the time when modified nucleotides were introduced to minimize immunostimulation of IVT mRNA, the importance of stringent purification of such preparations was recognized. Chromatographic purification, particularly HPLC, can separate mRNA according to size, thereby removing smaller or larger by-products such as abortive transcripts, mRNA from traces of non-linearized DNA template or double-stranded RNA (Fig. 5) [219, 220]. Such purification enhanced protein yield, most probably by enriching functional transcripts and depleting contaminants causing detrimental immunostimulation [220, 221]. The latter is corroborated by the finding that stringent purification reduced the beneficial effect of chemical modification or even made it dispensable, particularly in combination with specific sequence-engineering of the mRNA [218, 220]. Importantly, the specifics of mRNA formulation are another layer that can influence activation of the innate immune system.
mRNA as novel technology for passive immunotherapy

by masking mRNA from recognition by sensors, particularly TLRs. For example, immune responses after mRNA administration into the central nervous system were effectively suppressed by the use of a nanomicelle formulation compared to naked mRNA [222].

mRNA expression of therapeutic proteins in vivo

After in vivo administration of mRNA was proven to be feasible, the concept of using mRNA as a basis for therapeutics was pursued almost immediately. The very first report on a therapeutic effect with exogenous mRNA was already published in 1992 and described a temporary reversion of diabetes insipidus in rats by intrahypothalamic injection of vasopressin mRNA [197]. Thereafter, it took almost two decades until further studies started to demonstrate the broad potential of mRNA-based protein therapies. Meanwhile, there is a plethora of publications on a huge variety of indications comprising anemia [188, 218], hemophilia [223, 224], myocardial infarction [155, 225], cancer [226, 227], lung disease such as surfactant B deficiency and asthma [228–230], metabolic disorders [231–235], fibrosis [195], skeletal degeneration [236], tendon impairment [237], and neurological disorders such as sensory nerve dysfunction, Friedreich’s ataxia and Alzheimer’s disease [238–240]. Whereas evidence for the therapeutic potential of mRNA is mostly restricted to mouse models, first data in swine indicate that mRNA-based protein therapies are feasible also in large animals [218, 225]. In view of the various indications, it is hardly surprising that this diversity goes along with different routes of administration and various formulations. Only very few studies looking at local administration used uncomplexed and thus unprotected mRNA [225, 228, 230, 237]. The majority of investigations built on lipid-based formulations with a clear tendency to the application of LNP [223, 224, 231–233, 239]. Most if not all groups purified their IVT mRNA before in vivo administration. While some simply precipitated the mRNA [195, 227, 236], most used commercial purification kits. Only a few researchers applied HPLC purification [188, 218, 232]. With respect to the mRNA, the vast majority of studies used long poly(A) tails of at least 100 nucleotides. Likewise, there is a clear prevalence to chemically modified mRNA, although various examples suggest that this is not mandatory [218, 223, 229, 239]. While most mRNAs harbored 5-methyl-cytosine and/or pseudouridine initially [155, 188, 226], there appears to be a trend towards the use of N1-methyl-pseudouridine at present [224, 225]. Regarding the cap structure, almost all early studies cotranscriptionally generated cap0 mRNAs using ARCA [226, 228, 229]. However, since about 2 years, research groups prefer to apply mRNAs with a cap1 5′-end [223, 227, 231].

mRNA in passive immunotherapy

Passive cellular immunotherapy with mRNA

In vitro characterization

Initial attempts to apply mRNA to passive immunotherapy focused on cellular approaches for various reasons. Adoptive transfer of CTLs equipped with either an additional TCR or a CAR had shown great promise in cancers and viral infections. In contrast to typical scenarios of antibody therapy, receptor expression usually requires much lower protein levels. Furthermore, T cells are loaded with receptor-encoding nucleic acid (DNA or mRNA) ex vivo. Hence, passive cellular immunotherapy does not require sophisticated and highly efficient formulations for in vivo delivery but can build on the armamentarium of cell transfection methods. Previous work on active cellular vaccination with antigen-presenting cells that had been transfected with antigen-encoding mRNA revealed electroporation as easy and efficient means to load cells [241]. Comparison to passive cell pulsing and lipofection demonstrated that electroporation was also most efficient for transfection of T lymphocytes [242]. RNA electroporation had up to 90% efficiency without eliciting any critical toxicity [243]. Onset of transgene expression was very rapid and lasted about 7 days [243]. Receptor transfer into T cells by mRNA electroporation has now been well established for many years [243, 244]. Moreover, GMP-compliant protocols for manufacturing receptor-expressing...
Electroporation of human T lymphocytes with various antigen-specific TCRs redirected them to recognize cancer cells in an MHC-dependent manner in vitro [243]. mRNA-mediated TCR expression conferred in vitro cytotoxicity to T cells for at least 72 h [244]. The lytic efficacy of such cells was comparable to retrovirally transduced lymphocytes [244, 247]. Likewise, transfection of CAR-encoding mRNAs generated cells that were lytically active in vitro. Using an optimized IVT mRNA for a CD19-specific CAR, surface expression and cytotoxic function were detectable for up to 10 days [248]. To avoid as many manipulation steps as possible in generating T cells for adoptive transfer, it was demonstrated that human peripheral blood lymphocytes instead of purified T lymphocytes could be used as well to elicit strong cytotoxicity in vitro upon electroporation of a CAR mRNA [249]. Currently, most CAR approaches deploy αβ T cells. However, γδ T lymphocytes are an attractive target as well due to their antitumor effector function which is not MHC restricted and does not require co-stimulation. Accordingly, mRNA-mediated TCR and CAR expression in such cells was investigated very recently and shown to kill target cells in an antigen-specific manner [246].

To the best of our knowledge, there is so far just one study that started to systematically analyze the role of different mRNA elements for receptor expression in T cells. To this end, the group of Carl June built on previous findings in dendritic cells which revealed the superiority of a duplicated β globin 3′-UTR over a single copy of the same element and of a long (120 nt) over a short (16–51 nt) poly(A) tail [183]. With respect to receptor expression, 150 As enhanced expression compared to 64 As [184]. A tandem repeat of the β globin 3′-UTR had also a beneficial effect, particularly in combination with a long poly(A). In contrast, the VEGF translational enhancer as 5′-UTR element had even detrimental consequences. The authors speculated that this may be due to reduced capping efficacy but did not provide data corroborating their hypothesis. However, they demonstrated the important role of the cap structure. Co-transcriptional cap0 using ARCA and an enzymatically generated cap1 structure were equivalent and outperformed the basic cap analog as well as an enzymatic cap0. Besides expression level, capping also appeared to have an effect on the persistence of expression. Modification of the ORF sequence by removing all internal ORFs had no effect on receptor production.

**In vivo findings**

T lymphocytes transfected with TCR- or CAR-encoding mRNA proved to be functional also in vivo. Robust antitumor effects were observed in various preclinical models [184, 249]. They were mRNA-specific, since mock-transfected T cells had no or very little unspecific impact. Although mRNA-mediated receptor expression is transient, a single injection of CAR T cells against CD19 was sufficient to prolong survival of mice [248]. Using peripheral blood lymphocytes instead of purified T cells for CAR mRNA transfection (see above) also enabled a strong antitumor response in vivo although those cells could not persist long-term in vitro [249]. Very recently it was shown that mRNA cannot only be used to drive receptor expression, but can support the generation of T cells for adoptive immunotherapy. By expressing a chimeric membrane protein against CD3, cells could be efficiently stimulated and expanded in vitro [250]. After transfection with an mRNA for an anti-CD19 BiTE, those cells mediated sustained reduction in tumor burden upon intraperitoneal injection.

Based on encouraging preclinical data, adoptive T cell therapies using mRNA were already subjected to first clinical testing. In a phase 1 trial on solid tumors addressing the safety and feasibility of using such cells, CAR-transfected lymphocytes migrated to tumor sites after IV injection [251]. In addition, the study appeared to provide initial evidence of antitumor activity. Due to the transient nature of mRNA expression, subjects received repeated infusions of T cells. This led to an anaphylactic response in one patient who developed antibodies specific to the scFv domain of the CAR [252]. However, this could be a consequence of the murine origin of this domain. In another trial, mRNA-transfected CAR T cells were injected intratumorally in metastatic breast cancer patients [253]. Treatment was well tolerated and elicited an inflammatory response within tumors.

As discussed above, the use of viral vectors for adoptive T cell therapy has potential safety issues. In various studies, authors ascribed toxicities particularly to the persistence of receptor-expressing T cells. Regarding such concerns, mRNA-mediated TCR or CAR expression offers at least two advantages. First, mRNA does not integrate into a cell’s genome, thus excluding genotoxicity. Second, due to its transient nature, any potential toxicities accompanying treatment are temporary as well [254]. However, the increased level of safety has a substantial drawback. Apparently, clinical efficacy correlates with long-term persistence of receptor-engineered T cells [255, 256]. As a consequence, mRNA-transfected cells are expected to have limited antitumor activity because of rapidly declining receptor expression. Substantial mRNA translation only lasts about 1 day which translates into efficacious receptor levels on the cell surface for several days [183, 184]. Importantly, clinical studies demonstrated that IV infused T lymphocytes reached tumor sites only 2–3 days after administration [257, 258]. As a consequence, intratumoral or intraarterial administration was suggested to counteract the delayed cell arrival. The problem of transience of mRNA is further enhanced by the
well-known ligand-induced receptor internalization. Upon target recognition, TCRs as well as CARs are rapidly internalized, a mechanism which is important for proper signal transduction [259, 260]. This explains why lentiviral vectors generated a more robust treatment effect than mRNA [184]. mRNA transfection can give rise to high receptor expression, equaling lentiviral vectors [261]. However, mRNA was only similarly effective during the first hours after electroporation. Later, contact to target cells strongly down-regulated receptor on the cell surface while lentiviral expression remained constant [261]. In comparison to a single transfer of cells with retroviral CAR expression, an mRNA-encoded receptor required three consecutive lymphocyte infusions to obtain a comparable antitumor effect [262]. These observations and considerations may be put into perspective at least in part by the finding that transferred T cells can become tolerized rather rapidly, thereby losing their ability to function in the tumor microenvironment [263]. Thus, frequent injection of T cells may be desired even for viral vector transduced T cells. Notably, mRNA was also considered to be of use in settings that require long-term expression for therapeutic efficacy and preclude repeated cell administration. The greater safety of mRNA-transfected T cells may make the initial testing of novel antigens and receptors with unknown on-target/off-tissue toxicity less hazardous [248].

**Antibody therapy with mRNA**

Passive immunization with antibodies often requires considerable amounts of polypeptide to obtain therapeutically active concentrations after systemic administration. This poses a substantial challenge to the broad applicability of any nucleic acid-mediated passive immunization strategy. Thus, compared to the very first attempts regarding in vivo protein expression with mRNA in the 1990s, the optimization of IVT mRNA to enhance and extend expression is a prerequisite for many mRNA-based passive immunotherapies. As reviewed above, great progress towards this goal was made during the last almost three decades. Another potential hurdle for passive mRNA immunization is related to delivery. To obtain high levels of in vivo antibody expression, the mRNA should be targeted to as many “producer” cells as possible which in turn should be transfected with high efficiency. To this end, the mRNA which is prone to degradation by RNases should also be protected against these ubiquitous nucleases in an appropriate manner. Moreover, a viable mRNA complexation reagent needs to be well tolerated. Notably, various commercial transfection reagents can be used to formulate mRNA and suffice research purposes. Among them, TransIT has been repeatedly used for in vivo studies [188, 218, 264]. With respect to potential therapeutic applications, the class of lipid nanoparticles (LNPs) became the most widely deployed means of complexation [223, 224, 231–233]. After IV delivery, LNPs mainly route to the liver on the basis of an apolipoprotein E (ApoE-dependent mechanism [265]. However, such nanoparticles were demonstrated to be also applicable to intramuscular and subcutaneous administration [266].

Advances in mRNA and formulation technology led to a couple of recent intriguing studies as to passive immunization with mRNA (Fig. 6). While the group of Drew Weissman described successful passive mRNA immunization for prophylaxis of viral infections [267], Stadler et al. demonstrated the applicability of mRNA-mediated antibody expression for cancer immunotherapy [264]. The feasibility of using mRNA for such indications was confirmed by Thran et al. who applied different mRNA-encoded antibody formats to diverse biological threats, viruses, toxins, and tumors [268]. Finally, Sabnis and colleagues presented first antibody expression data in non-human primates (NHPs) in a publication dealing with the development of novel LNP formulations [269].

![Fig. 6 Schematic illustration of mRNA-mediated passive antibody immunotherapy. For in vivo administration, mRNA is usually formulated in nanoparticles which for instance can be administered by IV injection. For many formulations, liver is the main target organ. Upon uptake of nanoparticles by hepatocytes and release of the mRNA into the cytosol, it is translated into antibodies that are typically secreted into circulation and finally bind their cognate antigens](image-url)
mRNA design and formulation

Fundamental designs of antibody-encoding mRNAs reveal only a few common features but several differences (Table 1). Among the latter, the exclusive use of chemically unmodified nucleotides by Thran et al. as in a previous publication from the same group [218] may be the most prominent one, since it contrasts to all other reports. Other differences are much more diverse among these studies. Hence, they do not provide an unequivocal guidance for future work, but at least commonalities may be taken as a recommendation. Although obviously not mandatory, mRNA with cap1 structure was clearly preferred. In addition, all mRNAs harbored a poly(A) tail. The use of bipartite poly(A) elements by some groups may be owed to the experience that maintenance of long poly(d[A/T]) vector sequences is challenging and strongly dependent on bacterial strains [186].

Beyond these common RNA elements, the publications suggest that mRNA should be subjected to further optimizations to exploit its full potential for antibody expression. However, different strategies appear to be applicable, but little is known about the interchangeability of individual elements. Finally, chromatographic purification of IVT mRNA appears to be generally recommended as well (Table 1). Whether Pardi et al. actually used FPLC as stated throughout their report instead of HPLC applied by other groups is not fully clear, since they referred to earlier publications describing the use of HPLC [220, 270].

Pardi et al. encoded a well-known, broadly neutralizing antibody against HIV-1, VRC01 [267]. To this end, heavy and light chains of the full IgG antibody were represented on separate mRNA molecules. For delivery, heavy and light chain mRNAs were mixed in a molar ratio of 1:1. Likewise, Thran et al. used separate mRNAs to encode heavy and light chain of various full IgG antibodies [268]. Titration of heavy and light chain mRNA found a molar ratio of approximately 1.5:1 to be optimal for co-delivery. Neither report provides a rationale for encoding chains on separate molecules. In principle, a bicistronic construct separating heavy and light chain by an IRES sequence or an mRNA for a polypeptide where a 2A sequence between heavy and light chain would lead to separate antibody chains by ribosome skipping could have been used [271]. For Pardi et al. the observation that modified nucleotides can hamper the function of IRES elements may have affected the selection [218]. Sabnis et al. also worked with a full IgG antibody, directed against influenza A, but did not provide any details on how heavy and light chain were represented [269]. In contrast, Stadler et al. chose BiTE antibodies directed against TCR-associated CD3 and one of three different tumor-associated antigens (TAAs) [264]. They displayed the BiTEs as Fab(scFv)2 or scFv2 molecules but focused on the latter format. Their findings on single-chain antibodies are complemented by Thran et al. whose work covers single domain-derived VNAs in addition to IgG antibodies [268]. For IV administration of antibody-encoding mRNA all but the group of Ugur Sahin used LNP formulations which, however, may differ from each other in composition (Table 1). The latter team exploited TransIT but switched the route of administration which had been intraperitoneal in previous studies [188, 218]. As with LNPs,

Table 1 Overview of the current literature on mRNA-encoded antibodies

| Publication                | Capping (structure) | 5′ UTR                     | 3′ UTR                     | Polyadenylation (structure) | Base modifications | Codon usage            | mRNA purification | Formulation/delivery | Dose (range) | Species                | Maximum titer across experiments (range) |
|----------------------------|---------------------|----------------------------|---------------------------|-----------------------------|-------------------|-----------------------|-------------------|----------------------|--------------|------------------------|-----------------------------------------|
| Pardi et al., 2017 [267]   | Enzymatic (Cap1)    | Tobacco etch virus         | Not disclosed             | Vector-encoded (monopartite; approximately A100) | N1-methyl-pseudouridine | Not disclosed         | FPLC              | LNP/intravenous       | 1–1.4 mg/kg | M. musculus            | 70–200 µg/ml                              |
| Stadler et al., 2017 [264] | Enzymatic (Cap1)    | Tobacco etch virus         | F-I (not yet characterized) | Vector-encoded (bipartite; A30-linker-A70) | N1-methyl-pseudouridine | Sequence provided     | HPLC              | TransIT/intravenous   | Approx. 0.25 mg/kga | M. musculus            | 7 µg/ml                                  |
| Thran et al., 2017 [268]   | Co-transcriptional (Cap0); Enzymatic (Cap1) | Hydroxysteroid (17-β) dehydrogenase 4 | Human albumin             | Enzymatic adenylation (bipartite; A64-linker-PolyA) | Unmodified         | GC enrichment (disclosed in [218]) | HPLC              | LNP/intravenous       | 0.06–2 mg/kg | M. fascicularis         | 20–400 µg/ml                              |
| Sabnis et al., 2018 [269]  | Not disclosed (Cap1) | Unknown origin             | Human alpha globin        | Poly(A) tail (details not disclosed) | N1-methyl-pseudouridine | Not disclosed         | Not disclosed      | LNP/intravenous       | 0.3 mg/kg   | M. fascicularis         | 4 µg/ml                                  |

*aDue to lack of information, the value was calculated here for mice of 20 g in weight*
nanoparticles were shown to mainly target the liver upon IV injection [264].

mRNA-mediated antibody expression

Drew Weissman’s group administered 30 µg of VRC01-encoding mRNA in most in vivo studies [267]. This corresponded to doses between 1 and 1.4 mg/kg due to differences in mouse weight among experiments. Antibody serum titers 24 h after administration, the earliest time of analysis, ranged between approximately 80 and 200 µg/ml in various mouse strains. Obviously, slight differences in dosage, as well as the respective strain contributed to varying peak levels. Notably, increasing the administered mRNA dose in steps of two enhanced serum titers by more than twofold with each step. Moreover, 30 µg of mRNA in LNPs generated higher serum titers than 600 µg of recombinant VRC01 protein. The kinetics of antibody serum titers revealed an accelerated decline after about a week in BALB/c mice. The kinetics in NSG mice appeared to be basically the same, since the level at 1 week after single administration was largely the same as in BALB/c at this time. The observed kinetics may also explain why weekly injections of mRNA-LNPs in NSG mice did not show additive effects on serum titers at the times of analyses. Since measurements were conducted 7 days after each treatment, antibodies from the preceding injection probably dropped to background levels within this 2-week period as observed in BALB/c animals. Such accelerated decline of protein titers after a few days is often indicative of the induction of an anti-drug antibody (ADA) response [139]. The likelihood of such a response may be particularly high for the reported experiments, since the authors expressed a human antibody in mice. The emergence of ADAs cannot be fully ruled out because animals were not analyzed accordingly. However, the apparently similar kinetics in immunocompromised NSG mice which are unable to develop ADAs suggests a different explanation for the pharmacokinetics. Possibly, the mRNA continues to express antibody for a few days which would inevitably lead to a seemingly extended antibody serum half-life during that period. Only after expression ceases, the actual shorter antibody half-life becomes evident. While this could also easily explain the serum profile of repeated treatment of NSG mice, it remains hypothetical due to the lack of respective analyses.

Stadler et al. first characterized their mRNA in vitro demonstrating expression and secretion of functional antibodies [264]. In a PBMC-mediated killing assay, mRNA-derived BiTE antibodies targeted CTLs to tumor cells via binding to CD3 on PBMCs and to the cognate TAA on tumor cells, thereby inducing T cell activation and tumor cell lysis. These antibodies were equally potent as the corresponding recombinant protein. In immunodeficient NSG mice, antibody plasma levels peaked within 6 h, but rapidly declined by more than 50% within the next 18 h. Subsequently, the decrease of BiTE titers became much slower. The authors did not provide an explanation of this striking kinetics. A pharmacokinetic analysis in non-tumor-bearing mice could have elucidated whether the initial kinetics reflects the trapping of antibody in the engrafted tumor until saturation of binding sites. BiTE plasma levels above background for a few days were in accordance with the sustained ex vivo cytotoxicity of plasma from mRNA-treated mice. In contrast to the antibody plasma kinetics, cytotoxicity showed a steady and slow decline during the observation period. 0.05 µg of mRNA were already sufficient to obtain strong plasma activity in the ex vivo killing assay. 5 µg of mRNA (approx. 0.25 mg/kg) were comparable to 4–7 µg of recombinant antibody with respect to peak plasma concentrations that were in the range of 6.5 µg/ml in NSG mice. As opposed to this modified and HPLC-purified mRNA, antibody plasma levels were almost undetectable with mRNA preparations without modification and chromatographic purification. Plasma titers declined much faster for recombinant protein compared to mRNA, thereby demonstrating the substantial impact of mRNA on BiTE pharmacokinetics. Consequently, only mRNA was able to maintain a sustained cytotoxic activity of plasma by weekly administrations.

The various mRNA-encoded antibodies of Thran et al. included VRC01 which had been used in Drew Weissman’s work [267, 268]. However, analyses were limited to in vitro characterization, preventing a direct comparison between studies. As observed for BiTEs, IgG and VNA antibodies produced from mRNA in vitro revealed potencies comparable to that of the respective recombinant proteins. IV administration of 40 µg of unmodified mRNA (approx. 2 mg/kg) gave rise to antibody serum titers between 15 and 400 µg/ml in immunocompetent mice. This contrasts strongly with the finding of Stadler et al. who found unmodified mRNA to be basically inactive. Differences in purification and mRNA design may be responsible for this striking discrepancy. Similar to the Weissman work, Thran and colleagues observed a disproportionate increase of antibody serum titers with elevated mRNA doses. Onset of antibody expression was rather rapid, being already substantial 2 h after injection and reaching peak levels after approximately 4 h. This confirms findings on other mRNA-mediated protein therapies showing that mRNA starts accumulating in hepatocytes within minutes after administration and leads to substantial protein levels within a couple of hours [223, 231]. Serum half-life of IgG antibodies appeared to be in the range of 1 week and thus slightly longer compared to Pardi et al. [267]. As in the latter study, one of two IgGs showed an accelerated decline after about 1 week, however, only in approximately half of the animals. Here, the expedited clearance could be assigned to the development of an ADA response against the
mRNA-encoded antibody. Importantly, this response was antibody-dependent and not intimately linked with the use of mRNA. As expected, VNAs revealed a much shorter serum half-life of about 1–2 days. Compared to published kinetics data on recombinant VNAs, mRNA appeared to contribute to extended antibody availability during the first days after administration as it has been observed for BiTE-expressing mRNA by Ugur Sahin’s group. However, the lack of a head-to-head comparison hampers a detailed analysis.

While previous in vivo studies on mRNA-mediated antibody expression were limited to mice, Sabnis et al. presented expression results in NHPs using a proprietary LNP formulation [269]. A 0.3 mg/kg dose of mRNA gave rise to antibody serum titers of about 4 µg/ml 24 h after IV administration which is at least at the lower end of the range of efficacy observed in mouse studies. However, data on a different protein suggest that efficacy of the formulation may be slightly lower in NHP than in mouse. Whereas a 0.5 mg/kg dose induced protein levels of approximately 7 µg/ml in mice, a 0.2 mg/kg dose generated protein titers between 200 and 800 ng/ml in NHPs.

In vivo efficacy of mRNA-encoded antibodies

All mouse studies on mRNA-encoded antibodies investigated their therapeutic efficacy. Pardi et al. used two different humanized mouse models to demonstrate that mRNA-derived VRC01 protects from HIV-1 challenge [267]. mRNA encoding a reporter protein was utilized as control. mRNA-LNPs were administered 24 h prior to challenge with one of two HIV-1 isolates. In the authors’ first model, a VRC01 mRNA dose of 0.35 mg/kg was ineffective, but 0.7 mg/kg already reduced viral RNA copies in the plasma to undetectable levels as assessed by quantitative real-time polymerase chain reaction (qRT-PCR). The latter dose is well below the 10–20 mg/kg doses that are typically used for prophylactic immunization with recombinant antibody in humanized mice to reach therapeutic concentrations [272, 273]. However, the authors did not titrate the dose of recombinant VRC01 but used a 28 mg/kg dose as control which was sufficient to completely eradicate viral RNA copies in the plasma. mRNA efficacy could be also demonstrated in the second mouse challenge model.

To show in vivo efficacy of mRNA-mediated BiTE expression, Stadler et al. implanted tumor cells subcutaneously in immunodeficient NSG mice [264]. About 1 week before mRNA treatment, human PBMCs were engrafted into these animals. 3 µg of BiTE mRNA (approx. 0.15 mg/kg) given three times with an interval of 1 week could eliminate tumors entirely. In contrast, tumors progressed in control animals that received mRNA encoding a reporter protein. The recombinant BiTE required three injections per week and a total of ten injections of 4–7 µg each to obtain a comparable antitumor effect as with BiTE mRNA. The need for a more frequent administration corroborated the previous finding that mRNA substantially improved antibody plasma half-life.

Due to the diversity of antibodies included in their study, Thran et al. utilized various disease models for demonstrating therapeutic efficacy [268]. In contrast to all other studies, the authors applied mRNA encoding irrelevant antibodies instead of a reporter protein as control. A 40 µg dose (approx. 2 mg/kg) of antibody mRNA could protect mice from challenges with either rabies virus or botulinum toxin. In the intoxication model, mRNA was proven to be equally protective as recombinant antibody. However, mice received approximately 0.1 mg/kg of recombinant VNA compared to approximately 2 mg/kg of mRNA. Based on protein expression levels from mRNA dose titrations, lower doses than 2 mg/kg may still confer full protection but this remains hypothetical, since the authors did not conduct an mRNA dose titration in their challenge model. Notably, mRNA was effective in pre- as well as in post-exposure settings. The latter is important for some indications of passive immunization and confirms the aforementioned rapid onset of antibody expression. The post-exposure scenario for botulinum toxin requires very rapid availability of neutralizing antibodies. Whereas recombinant protein can act immediately after administration, mRNA needs more time to provide the antibody. Hence, it may well be that in such instances higher doses of mRNA than of protein are required, not for obtaining the same peak level but for reaching meaningful titers in a timely manner. In a further model, Thran et al. evaluated their mRNA approach with respect to anti-tumor efficacy. Using a disseminated tumor model for Rituximab, they showed efficient tumor growth control with injections of 50 µg (approx. 2.5 mg/kg) of Rituximab mRNA twice a week. Higher doses (200 µg, approx. 10 mg/kg) of recombinant Rituximab were less potent. This finding is reminiscent of results of Drew Weissman’s group and contrasts those of Ugur Sahin and colleagues who required similar doses of mRNA and recombinant protein (but less frequent dosing with mRNA) to obtain equivalent therapeutic effects [264, 267]. Notably, the difference among studies may be related to the use of IgG antibodies on the one hand and a scFv2 protein on the other hand. Moreover, the irrelevant antibody control used by Thran et al. appeared to have a slight unspecific anti-tumor effect. It may have contributed to the superiority of mRNA compared to recombinant protein regarding dosing. Amongst other explanations, the potential unspecific effect may be due to an mRNA-LNP-independent response to repeated treatment or may be the consequence of a weak and transient cytokine response observed after mRNA-LNP administration. However, the phenomenon was not investigated further.
T tolerability of mRNA-based passive immunization

In line with previous reports, Pardi et al. confirmed the importance of highly purified IVT mRNA. In combination with LNPs, only modification with N1-methyl-pseudouridine plus chromatographic purification was sufficient to avoid cytokine release by innate immune activation [267]. For this analysis, however, the authors deployed an mRNA encoding a different protein than the VRC01 antibody which had been used for in vivo expression and efficacy experiments. Tolerability of mRNA-LNPs was also addressed by repeated mRNA treatments. Translation of VRC01 mRNA was not compromised over time, but the analysis was conducted in immunodeficient NSG mice. To overcome this caveat, the authors complemented their study by repetitive treatment of immune competent BALB/c mice. To this end, they switched to an endogenous protein, since human VRC01 may be recognized as foreign and thus elicit an immune response. Again, mRNA injections did not lose efficacy over time. However, the authors also changed the formulation (TransIT instead of LNP) as well as the route of administration (intraperitoneal instead of IV) compared to the use of VRC01 mRNA. Hence, evidence for immune silence and overall tolerability of VRC01 mRNA in LNPs is just circumstantial yet.

Stadler et al. did not observe any liver toxicity upon treatment with mRNA in TransIT according to liver enzyme analyses [264]. Moreover, BiTE mRNA administration did not elevate murine cytokines such as IFNα and TNFα above background in plasma. Likewise, analysis of systemic human cytokine release from engrafted PBMCs did not show any unspecific T cell activation. As opposed to modified and HPLC-purified mRNA, preparations without nucleotide modification and chromatographic purification induced detectable levels of murine cytokines. Similar to the Weissman group, the authors also assessed the tolerability of formulated mRNA by repeated injections. Administrations did not lose efficacy over time, but as in the corresponding Weissman experiment immunodeficient NSG mice were used.

Using chemically unmodified mRNA formulated in LNPs, Thran et al. did not observe any liver toxicity in histopathological analyses [268]. Only a few animals developed an ADA response which was dependent on encoded antibody and was, thus, no intrinsic consequence of treatment with mRNA-LNP. In addition, treatment appeared to elicit a transient weak cytokine release which, however, neither suppressed antibody expression nor induced adverse effects. Since there are ample differences among studies on mRNA-mediated antibody expression and no detailed analyses of the issue, the role of mRNA, LNP, and/or encoded antibody/protein in cytokine induction remains elusive. An earlier study on erythropoietin showing the absence of any appreciable immunostimulation suggests that the use of chemically unmodified instead of modified mRNA is not the decisive parameter [218].

Conclusions

Quite a few in vivo studies provided compelling evidence for the principle feasibility of mRNA-based immunotherapies. As discussed above, challenges and open questions regarding adoptive T cell transfer are less related to the mRNA and its formulation or transfection but more of fundamental character. In contrast to ex vivo loading of cells, mRNA-mediated antibody expression is strongly affected by body size. Thus, while there are now convincing efficacy data in diverse small rodent models, the translation to larger animals and finally humans has still to be demonstrated. First data suggest that substantial expression can be obtained in small NHPs. However, the utilized LNPs appeared to lose some efficacy when switching from mouse to NHP. Hence, the development of human therapies may perhaps require further advancements of the mRNA technology as well as primate-specific formulations with improved efficacy. In addition, tolerability of formulations has to be analyzed further and in more depth in the future. For instance, repeated dosing of nanoparticles can induce complement activation-related pseudoallergy (CARPA) [274]. However, this can in principle be counteracted by optimization towards better biocompatibility. In case of LNPs, fast degradation was shown to be particularly important [231, 232, 269].

While antibodies for cancer treatment were initially developed for IV administration, there is a trend towards subcutaneous injections today. For instance, Rituximab was initially formulated for IV infusion which is typically administered over a period of 1.5–6 h [275]. This treatment schedule poses a substantial burden to patients as well as the healthcare system. Thus, a formulation which reduces the time and required resources would be advantageous. To meet these goals by subcutaneous administration, the antibody solution was concentrated 12-fold [276, 277]. Since this volume was still too large for subcutaneous injection, Rituximab was co-formulated with human hyaluronidase which limits swelling and associated pain by increasing the dispersion and absorption of co-administered substances [276, 278]. Now, median administration time for Rituximab using the subcutaneous route is 6 min. As a consequence, antibody immunotherapy with mRNA does not only require competitive efficacy and costs but also routes of administration to become a viable alternative to recombinant proteins. Although other routes than IV have been shown to be possible for mRNA, there are still a few open questions to be addressed by future studies.
Where are the advantages of using mRNA for antibody immunotherapies? Compared to DNA it may be primarily the safety aspect. Concerning recombinant proteins various points matter. As reviewed above, mRNA provides benefits as to the pharmacokinetics when short-lived antibodies such as scFv, (bi)-scFv2 or VNA are used. Moreover, solving the challenges of antibody cocktails may be easier using mRNA. Different mRNA sequences are much more similar with respect to their physicochemical characteristics than different proteins are. Hence, producing a cocktail may be less demanding for mRNA compared to protein. However, co-delivery and thus co-expression implicates the risk of antibody chimerism and thus requires specific solutions such as knob-into-hole concepts [68]. Last but not least, while proteins are difficult to deliver directly through the cell membrane [279], mRNA-mediated protein expression makes a large number of potential intracellular targets accessible to antibody immunotherapy. Particularly single-chain and single-domain formats are amenable to functional expression in the cytosol and thus suited as intrabodies, since they are less dependent on disulfide bond formation [280, 281]. The value of targeting intracellular proteins has already been demonstrated by various studies. A bispecific scFv could restore p53 function in mutant p53 colon cancer cells and trapping CCR5 in the ER via an intrabody reduced HIV cell entry [282, 283]. Support for the potential of intrabodies as therapeutics also comes from further work in the field of oncology or neurodegenerative diseases [284–286]. Although it has been recently demonstrated that even a full antibody can be delivered into cells in vivo [287], it has been recognized that fusions of cell-penetrating peptides (CPPs) and macromolecules are often trapped in endosomes instead of being released to the cytoplasm [288]. In contrast, nucleic acids including mRNA can be very efficiently transfected into cells, making them ideal for the delivery of intrabodies. However, while LNPs provide very efficient solutions for systemic delivery to the liver, formulations for routing mRNA to other tissues are scarce today. With the recent steps to solve the challenges of antibody cocktails it is likely that there will be much to follow in the near future.

**Acknowledgements** We thank Mariola Fotin-Mleczek for discussion on the manuscript. We are grateful to Nigel Horscroft and Michael Stolz for critical reading of the review. We also thank Bettina Danker for her graphical illustrations. Finally, we apologize to those authors whose work was not cited owing to space limitations.

**Compliance with ethical standards**

**Conflict of interest** The authors are employees of CureVac AG developing therapeutics based on sequence-engineered mRNA.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

**References**

1. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124(4):783–801. https://doi.org/10.1016/j.cell.2006.02.015
2. Rubartelli A, Lotze MT (2007) Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol 28(10):429–436. https://doi.org/10.1016/j.it.2007.08.004
3. Sanchez-Trincado JL, Gomez-Perosanz M, Reche PA (2017) Fundamentals and methods for T- and B-cell epitope prediction. J Immunol Res 2017:2680160. https://doi.org/10.1155/2017/2680160
4. Kato A, Hulse KE, Tan BK, Schleimer RP (2013) B-lymphocyte lineage cells and the respiratory system. J Allergy Clin Immunol 131(4):933–957. https://doi.org/10.1016/j.jaci.2013.02.023
5. Wang M, Yin B, Wang HY, Wang RF (2014) Current advances in T-cell-based cancer immunotherapy. Immunotherapy 6(12):1265–1278. https://doi.org/10.2217/imt.14.86
6. Sakaguchi S, Miyara M, Costantini CM, Hafler DA (2010) FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 10(7):490–500. https://doi.org/10.1038/nri2785
7. Collin M, Bigley V (2018) Human dendritic cell subsets: an update. Immunology 154(1):3–20. https://doi.org/10.1111/imm.12888
8. Plotkin SA (2010) Correlates of protection induced by vaccination. Clin Vaccine Immunol 17(7):1055–1065. https://doi.org/10.1128/CVI.00131-10
9. Palucka K, Banchereau J (2012) Cancer immunotherapy via dendritic cells. Nat Rev Cancer 12(4):265–277. https://doi.org/10.1038/nrc3258
10. Laustsen AH, Johansen KH, Engmark M, Andersen MR (2017) Recombinant snakebite antivenoms: a cost-competitive solution to a neglected tropical disease? PLoS Negl Trop Dis 11(2):e0005361. https://doi.org/10.1371/journal.pntd.0005361
11. Beck A, Wurch T, Bailly C, Corvaia N (2010) Strategies and challenges for the next generation of therapeutic antibodies. Nat Rev Immunol 10(5):345–352. https://doi.org/10.1038/nri2747
12. Amanna IJ, Slifka MK (2011) Contributions of humoral and cellular immunity to vaccine-induced protection in humans. Virology 411(2):206–215. https://doi.org/10.1016/j.virol.2010.12.016
13. Behring E, Kitasato S (1890) Ueber das Zustandekommen der Diptherie-Immunität und der Tetanus-Immunität bei tieren. Dtsch Med Wochenschr 16:1113–1114
14. Both L, Banyard AC, van Dolleweerd C, Horton DL, Ma JK, Fooks AR (2012) Passive immunity in the prevention of rabies. Lancet Infect Dis 12(5):397–407. https://doi.org/10.1016/S1473-3099(11)70340-1
15. Casadevall A, Dadachova E, Pirofski LA (2004) Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2(9):695–703. https://doi.org/10.1038/nrmicro974
16. Saylor C, Dadachova E, Casadevall A (2009) Monoclonal antibody-based therapies for microbial diseases. Vaccine 27(Suppl 6):G38–G46. https://doi.org/10.1016/j.vaccine.2009.09.105
17. Keller MA, Stiehm ER (2000) Passive immunity in prevention and treatment of infectious diseases. Clin Microbiol Rev 13(4):602–614
mRNA as novel technology for passive immunotherapy

18. Janeway CA (1945) Use of concentrated human serum gammaglobulin in the prevention and attenuation of measles. Bull N Y Acad Med 21(4):202–222

19. Hammon WM, Coriell LL, Wehrle PF, Stokes J Jr (1953) Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. IV. Final report of results based on clinical diagnoses. J Am Med Assoc 151(15):1272–1285

20. Casadevall A, Scharff MD (1994) Serum therapy revisited: animal models of infection and development of passive antibody therapy. Antimicrob Agents Chemother 38(8):1695–1702

21. Marasco WA, Sui J (2007) The growth and potential of human monoclonal antibody therapeutics. Nat Biotechnol 25(12):1421–1434. https://doi.org/10.1038/nbt1363

22. Maiztegui JI, Fernandez NJ, de Damilano AJ (1979) Efficacy of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. Antiviral Res 78(1):132–139. https://doi.org/10.1016/j.antiviral.2007.10.010

23. Enria DA, Briggiler AM, Sanchez Z (2008) Treatment of Argentine hemorrhagic fever and association between treatment and a late neurological syndrome. Lancet 2(8154):1216–1217

24. Steele JH (1988) Rabies in the Americas and remarks on global aspects. Rev Infect Dis 10(Suppl 4):S585–S597

25. American Academy of Pediatrics (1997) Clostridial infections. In: Peter G (ed) Red Book Report of the Committee on Infectious Diseases, 24th edn. American Academy of Pediatrics, Elk Grove Village, pp 174–178

26. Shapiro RL, Hatheway C, Sowerlow DL (1998) Botulism in the United States: a clinical and epidemiologic review. Ann Intern Med 129(3):221–228

27. Wilde H, Thipkong P, Sitprija V, Chaiyabutr N (1996) Heterologous antisera and antivenins are essential biologicals: perspectives on a worldwide crisis. Ann Intern Med 125(3):233–236

28. Goudsmit J, Marissen WE, Weldon WC, Niezgoda M, Hanlon CA, Rice AB, Kruij J, Dietzschold B, Bakker AB, Rupprecht CE (2006) Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. J Infect Dis 193(6):796–801. https://doi.org/10.1086/500470

29. Kohler G, Milstein C (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256(5517):495–497

30. Norman DJ (1988) An overview of the use of the monoclonal antibody OKT3 in renal transplantation. Transpl Proc 20(6):1248–1252

31. Hoogenboom HR (2002) Overview of antibody phage-display technology and its applications. Methods Mol Biol 178:1–37

32. Breitling F, Dubel S, Seehaus T, Klewinghaus I, Little M (1991) A surface expression vector for antibody screening. Gene 104(2):147–153

33. Hoogenboom HR (2005) Selecting and screening recombinant antibody libraries. Nat Biotechnol 23(9):1105–1116. https://doi.org/10.1038/nbt1126

34. Thom G, Groves M (2012) Ribosome display. Methods Mol Biol 901:101–116. https://doi.org/10.1007/978-1-61779-931-0_6

35. ter Meul en J, Bakker AB, van den Brink EN, Weverling GJ, Martina BE, Haagmans BL, Kuiken T, de Kruij J, Preiser W, Spaan W, Gelderblom H, Goudsmit J, Osterhaus AD (2004) Human monoclonal antibody as prophylaxis for SARS coronavirus infection in ferrets. Lancet 363(9427):2139–2141. https://doi.org/10.1016/S0140-6736(04)16506-9

36. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng YR, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan YP, Hickey AC, Dimitrov DS, Broker CC, Rocks B (2011) A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Sci Transl Med 3(105):105ra103. https://doi.org/10.1126/scitranslmed.3002901

37. Wardemann H, Yurasov S, Schaefer A, Young JW, Melfire E, Nussenzweig MC (2003) Predominant autoantibody production by early human B cell precursors. Science 301(5638):1374–1377. https://doi.org/10.1126/science.1086907

38. Tiller T, Melfire E, Yurasov S, Tsuji M, Nussenzweig MC, Wardemann H (2008) Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. J Immunol Methods 329(1–2):112–124. https://doi.org/10.1016/j.jim.2007.09.017

39. Chan AC, Carter PJ (2010) Therapeutic antibodies for autoimmune and inflammation. Nat Rev Immunol 10(5):301–316. https://doi.org/10.1038/nri2761

40. Roopenian DC, Akiles J (2007) FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 7(9):715–725. https://doi.org/10.1038/nri2155

41. Keijer J, Huijema AD, Schellens JH, Beijnen JH (2010) Clinical pharmacokinetics of therapeutic monoclonal antibodies. Clin Pharmacokinet 49(8):493–507. https://doi.org/10.2165/11531280-0000000000-00000

42. Wang W, Wang EQ, Balthasar JP (2008) Monoclonal antibody pharmacokinetics and pharmacodynamics. Clin Pharmacol Ther 84(5):548–558. https://doi.org/10.1038/clpt.2008.170

43. Brambell FW, hemmings WA, Morris IG (1964) A theoretical model of gamma-globulin catabolism. Nature 203:1352–1354

44. Junghans RP (1997) Finally! The Brambell receptor (FcRb). Mediator of transmission of immunity and protection from catabolism for IgG. Immunol Res 16(1):29–57. https://doi.org/10.1007/bf02786322

45. Zalevsky J, Chamberlain AK, Horton MM, karki S, Leung IW, Sproule TJ, Lazar GA, Roopenian DC, Desjarlais JR (2010) Enhanced antibody half-life improves in vivo activity. Nat Biotechnol 28(2):157–159. https://doi.org/10.1038/nbt.1601

46. Mould DR, Green B (2010) Pharmacokinetics and pharmacodynamics of monoclonal antibodies: concepts and lessons for drug development. BioDrugs 24(1):23–39. https://doi.org/10.2165/11530560-0000000000-00000

47. Samaranayake H, Wirth T, Schenken W, Ratz J, Yla-Herttuala S (2009) Challenges in monoclonal antibody-based therapies. Ann Med 41(5):322–331. https://doi.org/10.1080/07853890802698842

48. Breitling F, Dubel S, Seehaus T, Klewinghaus I, Little M (1991) A surface expression vector for antibody screening. Gene 104(2):147–153

49. Bossart KN, Geisbert TW, Feldmann H, Zhu Z, Feldmann F, Geisbert JB, Yan L, Feng YR, Brining D, Scott D, Wang Y, Dimitrov AS, Callison J, Chan YP, Hickey AC, Dimitrov DS, Broker CC, Rocks B (2011) A neutralizing human monoclonal antibody protects african green monkeys from hendra virus challenge. Sci Transl Med 3(105):105ra103. https://doi.org/10.1126/scitranslmed.3002901

50. Wurm FM (2004) Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 22(11):1393–1398. https://doi.org/10.1038/nbt1026

51. Chadd HE, Chomow SM (2001) Therapeutic antibody expression technology. Curr Opin Biotechnol 12(2):188–194

52. Jager V, Bussow K, Wagner A, Weber S, Hutt M, Frenzel A, Schirrmann T (2013) High level transient production of recombinant antibodies and antibody fusion proteins in HEK293 cells. BMC Biotechnol 13:52. https://doi.org/10.1186/1472-6750-13-52

53. Walsh G (2014) Biopharmaceutical benchmarks 2014. Nat Biotechnol 32(10):992–1000. https://doi.org/10.1038/nbt.3040

54. Liu L (2015) Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. J Pharm Sci 104(6):1866–1884. https://doi.org/10.1002/jps.24444
55. von Horsten HH, Ogoreck C, Blanchard V, Demmler C, Giese C, Winkler K, Kaup M, Berger M, Jordan I, Sandig V (2010) Production of non-fucosylated antibodies by co-expression of heterologous GDP-6-deoxy-n-lyxo-4-hexulose reductase. Glycobiology 20(12):1607–1618. https://doi.org/10.1093/glycob/cwq109

56. Holliger P, Hudson PJ (2005) Engineered antibody fragments and the rise of single domains. Nat Biotechnol 23(9):1126–1136. https://doi.org/10.1038/nbt1142

57. Konning D, Zielonka S, Grzeschik J, Empting M, Valldorf B, Hultberg A, Temperton NJ, Rosseels V, Koenders M, Gonzalez-Mukherjee J, Dmitriev I, Debatis M, Tremblay JM, Beamer G, Ibanez LI, De Filette M, Hultberg A, Verrips T, Temperton NJ, Aubrey N, Devaux C, Sizaret PY, Rochat H, Goyffon M, Baltimore RB, Hmila I, Vincke C, Benlasfar Z, Pellis M, Dabiri H, Zahar B (2009) Identification of potent nanobodies to neutralize the most poisonous polypeptide from scorpion venom. Biochem Biophys Acta 1431(1):37–46.

58. Hultberg A, Temperton NJ, Rosseels V, Koenders M, Gonzalez-Pajuelo M, Schepens B, Ibanez LI, Vanlandschoot P, Schillemans J, Saunders M, Weiss RA, Saelens X, Melero JA, Verrits CT, Van Gucht S, de Haard HJ (2011) Llama-derived single domain antibodies to build multivalent, superpotent and broadened neutralizing anti-viral molecules. PLoS One 6(4):e17665. https://doi.org/10.1371/journal.pone.0017665

59. Hamers-Casterman C, Atarhouch T, Muylldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R (1993) Naturally occurring antibodies devoid of light chains. Nature 363(6428):446–448. https://doi.org/10.1038/363446a0

60. van der Linden RH, Frenken LG, de Geus B, Harrms MM, Ruuls RC, Stok W, de Ron L, Wilson S, Davis P, Verrits CT (1999) Comparison of physical chemical properties of Llama VHH antibody fragments and mouse monoclonal antibodies. Biochim Biophys Acta 1431(1):37–46.

61. Mukherjee J, Dmitriev I, Debaets M, Beamer G, Kashensteva EA, Curiel DT, Shoemaker CB (2014) Prolonged prophylactic protection from botulism with a single adenovirus treatment promoting serum expression of a VH-based antigen protein. PLoS One 9(8):e106422. https://doi.org/10.1371/journal.pone.0106422

62. Ibanez LI, De Filette M, Hultberg A, Verrits T, Temperton NJ, Weiss RA, Van de Velde W, Schepens B, Vanlandschoot P, Saelens X (2011) Nanobodies with in vitro neutralizing activity protect mice against H5N1 influenza virus infection. J Infect Dis 203(8):1063–1072. https://doi.org/10.1086/658314

63. Cardoso FM, Ibanez LI, Van den Hoecke S, De Baets S, Smet A, Roose K, Schepens B, Descamps FJ, Fiers W, Muylldermans S, Depicker A, Saelens X (2014) Single-domain antibodies targeting neuraminidase protect against an H5N1 influenza virus challenge. J Virol 88(15):8278–8296. https://doi.org/10.1128/JVI.03178-13

64. Aubrey N, Devaux C, Sizaret PY, Rochat H, Goyffon M, Bilal-Miller P (2003) Design and evaluation of a diabody to improve protection against the most poisonous polypeptide from scorpion venom. Cell Mol Life Sci 60(3):617–628

65. Abderrazek RB, Hmila I, Vincke C, Benlasfar Z, Peliss M, Dubois H, Saerens D, El Ayeb M, Muylldermans S, Bouhaouala-Zahar B (2009) Identification of potent nanobodies to neutralize the most poisonous polypeptide from scorpion venom. Biochem J 424(2):263–272. https://doi.org/10.1042/BJ20090697

66. Gaciarz A, Veijola J, Uchida Y, Saaranen MJ, Wang C, Horkko S, Coloma MJ, Morrison SL (1997) Design and production of novel tetravalent bispecific antibodies. Nat Biotechnol 15(2):159–163. https://doi.org/10.1038/nbt0297-159

67. Ridgway JB, Presta LG, Carter P (1996) ‘Knobs-into-holes’ engineering of antibody CH3 domains for heavy chain heterodimerization. Protein Eng 9(7):617–621

68. Choi BD, Kuan CT, Cai M, Archer GE, Mitchell DA, Gedeon PC, Sanchez-Perez L, Pashan B, Bigner DD, Sampson JH (2013) Systemic administration of a bispecific antibody targeting EGFRvIII successfully treats intracerebral glioma. Proc Natl Acad Sci USA 110(1):270–275. https://doi.org/10.1073/pnas.1219817110

69. Fournier P, Schirrmacher V (2013) Bispecific antibodies and trispecific immunocytokines for targeting the immune system against cancer: preparing for the future. BioDrugs 27(1):35–53. https://doi.org/10.1007/s14220-012-0008-z

70. Zitron IM, Thakur A, Norkina O, Barger GR, Lum LG, Mittal S (2013) Targeting and killing of glioblastoma with activated T cells armed with bispecific antibodies. BMC Cancer 13:83. https://doi.org/10.1186/1471-2407-13-83

71. Kontermann RE (2012) Dual targeting strategies with bispecific antibodies. MAbs 4(2):182–197. https://doi.org/10.4161/mabs.4.2.19000

72. Holliger P, Prospero T, Winter G (1993) “Diabodies”: small bivalent and bispecific antibody fragments. Proc Natl Acad Sci USA 90(14):6444–6448

73. Hayden MS, Linsley PS, Gayle MA, Bajorath J, Brady WA, Norris NA, Fell HP, Ledbetter JA, Gilliland LK (1994) Single-chain mono- and bispecific antibody derivatives with novel biological properties and antitumour activity from a COS cell transient expression system. Ther Immunol 1(1):3–15

74. van Spriel AB, van Oijck HH, van De Winkel JG (2000) Immunotherapeutic perspective for bispecific antibodies. Immunol Today 21(8):391–397

75. Lobe ED, Hansen RJ, Balthasar JP (2004) Antibody pharmacokinetics and pharmacodynamics. J Pharm Sci 93(11):2645–2668. https://doi.org/10.1002/jps.20178

76. Tabrizi MA, Tseng CM, Roskos LK (2006) Elimination mechanisms of therapeutic monoclonal antibodies. Drug Discov Today 11(1–2):81–88. https://doi.org/10.1016/S1359-6446(05)03638-X

77. Garber K (2014) Bispecific antibodies rise again. Nat Rev Drug Discov 13(11):799–801. https://doi.org/10.1038/nrd4478

78. Lee KJ, Chow V, Weissman A, Tulpule S, Aldoss I, Akhtari M (2016) Clinical use of bivalentmab for B-cell acute lymphoblastic leukemia in adults. Ther Clin Risk Manag 12:1301–1310. https://doi.org/10.2147/TCRM.S84261

79. Yang K, Basu A, Wang M, Chintala R, Hsieh MC, Liu S, Hua J, Zhang Z, Zhou J, Li M, Phyu H, Petti G, Mendez M, Janjua H, Peng P, Longley C, Borowski V, Mehlig M, Filipula D (2003) Tailoring structure-function and pharmacokinetic properties of single-chain Fv proteins by site-specific PEGylation. Protein Eng 16(10):761–770

80. Kontermann RE (2009) Strategies to extend plasma half-lives of recombinant antibodies. BioDrugs 23(2):93–109. https://doi.org/10.2165/00060300-20092320-00003

81. Harmsen MM, De Haard HJ (2007) Properties, production, and applications of camelid single-domain antibody fragments. Appl Microbiol Biotechnol 77(1):13–22. https://doi.org/10.1007/s00253-007-1142-2

82. Rutgers KS, Nabuurs RJ, van den Berg SA, Schenck GJ, Rotman M, Verrits CT, van Duinen SG, Maat-Schiena ML, van Buchem MA, de Boer AG, van der Maarel SM (2011) Transmigration of beta amyloid specific heavy chain antibody fragments across the in vitro blood-brain barrier. Neuroscience 190:37–42. https://doi.org/10.1016/j.neuroscience.2011.05.076

83. Tregonny S, Francart A, Lamboral S, Hultberg A, Rommelaere H, Wittelsberger A, Callewaert F, Stohr T, Meerschaert K, Ottevaere I, Stortelers C, Vanlandschoot P, Kalai M, Van Gucht S (2014) Protective effect of different anti-rabies virus VHH constructs against rabies disease in mice. PLoS One 9(10):e109367. https://doi.org/10.1371/journal.pone.0109367

84. Tregonny S, Francart A, Rommelaere H, Stortelers C, Van Gucht S (2016) Post-exposure treatment with anti-rabies VHH and...
vaccine significantly improves protection of mice from lethal rabies infection. PLoS Negl Trop Dis 10(8):e0004902. https://doi.org/10.1371/journal.pntd.0004902

86. Teitelbaum R, Glatman-Freedman A, Chen B, Robbins JB, Unameu E, Casadevall A, Bloom BR (1998) A mAb recognizing a surface antigen of Mycobacterium tuberculosis enhances host survival. Proc Natl Acad Sci USA 95(26):15688–15693.

87. Nosanchuk JD, Steenbergen JN, Shi L, Deepe GS Jr, Casadevall A (2003) Antibodies to a cell surface histone-like protein protect against Histoplasma capsulatum. J Clin Investig 112(8):1164–1175. https://doi.org/10.1172/JCI19361

88. Both L, Banyard AC, van Dolleweerd C, Wright E, Ma JK, Fooks AR (2013) Monoclonal antibodies for prophylactic and therapeutic use against viral infections. Vaccine 31(12):1553–1559. https://doi.org/10.1016/j.vaccine.2013.01.025

89. Huang L, Su X, Federoff HJ (2013) Single-chain fragment variable passive immunotherapies for neurodegenerative diseases. Int J Mol Sci 14(9):19109–19127. https://doi.org/10.3390/ijms140919109

90. Boivin G, Caouette G, Carbonneau J, Ouakki M, De Serres G (2008) Human respiratory syncytial virus and other viral infections in infants receiving palivizumab. J Clin Virol 42(1):52–57. https://doi.org/10.1016/j.jcv.2007.11.012

91. Bellottoxumab (Zinplava) for prevention of recurrent Clostridium difficile infection (2017). JAMA 318 (7):659–660. https://doi.org/10.1001/jama.2017.10092

92. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Riepl RL, Newman RA, Hanna N, Anderson DR (1994) Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. Blood 83(2):435–445.

93. Pescevit MD (2006) Rituximab, an anti-CD20 monoclonal antibody: history and mechanism of action. Am J Transpl 6(5 Pt 1):859–866. https://doi.org/10.1111/j.1600-6143.2006.01288.x

94. Topp MS, Kufer P, Gokbuget N, Goebeler M, Klinger M, Neuhaus A (2003) Antibodies to a cell surface histone-like protein protect against metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 21(9):917–924. https://doi.org/10.1200/JCO.2010.32.2537

95. Wu J, Fu J, Zhang M, Liu D (2015) Blinatumomab: a bispecific T cell engager (BiTE) antibody against CD19/CD3 for refractory minimal residual disease in chemotherapy-refractory minimal residual disease in B-lineage acute lymphoblastic leukemia patients results in high response rate and prolonged leukemia-free survival. J Clin Oncol 33(19):2493–2498. https://doi.org/10.1200/JCO.2015.32.7270

96. Wang J, Zhu J, Zhang M, Liu D (2015) Blinatumomab: a bispecific T cell engagement tumor vaccine significantly improves protection of mice from lethal rabies infection. PLoS Negl Trop Dis 10(8):e0004902. https://doi.org/10.1371/journal.pntd.0004902

97. Barrett DM, Grupp SA, June CH (2015) Chimeric antigen receptor- and TCR-modified T cells enter main street and wall street. J Immunol 195(3):755–761. https://doi.org/10.4049/jimmunol.1500751

98. Rosenberg SA, Restifo NP (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348(6230):62–68. https://doi.org/10.1126/science.aaa4967

99. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammla US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA (2009) Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114(3):535–546. https://doi.org/10.1182/blood-2009-03-211714

100. Robbins PF, Morgan RA, Feldman SA, Yang JC, Sherry RM, Dudley ME, Wunderlich JR, Nakhi AV, Helman LJ, Mackall CL, Kammla US, Hughes MS, Restifo NP, Raffeld M, Lee CC, Levy CL, Li YF, El-Gamil M, Schwarz SL, Laurencot C, Rosenberg SA (2011) Tumor regression in patients with metastatic synovial cell sarcoma and melanoma using genetically engineered lymphocytes reactive with NY-ESO-1. J Clin Oncol 29(7):917–924. https://doi.org/10.1200/JCO.2010.32.2537

101. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, Davis JL, Morgan RA, Merino MJ, Sherry RM, Hughes MS, Kammla US, Pan GQ, Lim RM, Wank SA, Restifo NP, Robbins PF, Laurencot CM, Rosenberg SA (2011) T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther 19(3):620–626. https://doi.org/10.1038/mt.2010.272

102. Debets R, Willemsen R, Bolhuis R (2002) Adoptive transfer of T-cell immunity: gene transfer with MHC-restricted receptors. Trends Immunol 23(9):435–436

103. Zhang T, He X, Tsang TC, Harris DT (2004) Transgenic TCR expression: comparison of single chain with full-length receptor constructs for T-cell function. Cancer Gene Ther 11(7):487–496. https://doi.org/10.1038/sj.cgt.7700703

104. Koning F, Maloy WL, Cohen D, Colgan JE (1987) Independent association of T cell receptor beta and gamma chains with CD3 in the same cell. J Exp Med 166(2):595–600

105. Siroti T, Hochstenbach F, Marrusic-Gulesic S, Kruisbeek AM, Brenner M, Germain RN (1988) Surface expression of only gamma delta and/or alpha beta T cell receptor heterodimers by cells with four (alpha, beta, gamma, delta) functional receptor chains. J Exp Med 168(3):1003–1020

106. van der Veken LT, Dodoo E, Zumla A, Maeurer M (2015) T-cell therapy: options for infectious diseases. Clin Infect Dis 61(Suppl 3):S217–S224.

107. Boscia AF, Maramboi T, Brown S, Pinheiro L, Brubaker C, Strobel M, Strickler GD, Storchova Z, Maddon PJ, Plotkin SA, et al. (2015) Janus kinase 3 inhibition of CD4+ and CD8+ T lymphocytes in an antigen-specific model of autoimmunity. J Immunol 194(12):7533–7541. https://doi.org/10.4049/jimmunol.1500751

108. Barrett DM, Grupp SA, June CH (2015) Chimeric antigen receptor- and TCR-modified T cells enter main street and wall street. J Immunol 195(3):755–761. https://doi.org/10.4049/jimmunol.1500751

109. Rosenberg SA, Restifo NP (2015) Adoptive cell transfer as personalized immunotherapy for human cancer. Science 348(6230):62–68. https://doi.org/10.1126/science.aaa4967

110. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, Kammla US, Royal RE, Sherry RM, Wunderlich JR, Lee CC, Restifo NP, Schwarz SL, Cogdill AP, Bishop RJ, Kim H, Brewer CC, Rudy SF, VanWaes C, Davis JL, Mathur A, Ripley RT, Nathan DA, Laurencot CM, Rosenberg SA (2009) Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood 114(3):535–546. https://doi.org/10.1182/blood-2009-03-211714

111. Morris EC, Stauss HJ (2016) Optimizing T-cell receptor gene therapy for hematologic malignancies. Blood 127(26):3305–3311. https://doi.org/10.1182/blood-2015-11-629071

112. Gross G, Waks T, Eshhar Z (1989) Expression of immunoglobulin-T-cell receptor chimeric molecules as functional receptors with antibody-type specificity. Proc Natl Acad Sci USA 86(24):10024–10028

113. Eshhar Z, Bach N, Fitzner-Attas CJ, Gross G, Lustgarten J, Waks T, Schindler DG (1996) The T-body approach: potential...
for cancer immunotherapy. Springer Semin Immunopathol 18(2):199–209.

114. Sadelain M, Brentjens R, Riviere I (2013) The basic principles of chimeric antigen receptor design. Cancer Discov 3(4):388–398. https://doi.org/10.1158/2159-8290.CD-12-0548

115. Eshhar Z, Waks T, Gross G, Schindler DG (1993) Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proc Natl Acad Sci USA 90(2):720–724

116. Curran KJ, Pegram HJ, Brentjens RJ (2012) Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions. J Gene Med 14(6):405–415. https://doi.org/10.1002/jgem.2004

117. Zhao Z, Condomines M, van der Stegen SJ, Perna F, Kloss CC, Gunset G, Plotkin J, Sadelain M (2015) Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell 28(4):415–428. https://doi.org/10.1016/j.ccell.2015.09.004

118. Dai H, Wang Y, Lu X, Han W (2016) Chimeric antigen receptors modified T-cells for cancer therapy. J Natl Cancer Inst 108(7):djv439. https://doi.org/10.1093/jnci/djv439

119. Kalos M, June CH (2013) Adoptive T cell transfer for cancer immunotherapy in the era of synthetic biology. Immunity 39(1):49–60. https://doi.org/10.1016/j.immuni.2013.07.002

120. Restifo NP, Dudley ME, Rosenberg SA (2012) Adoptive immunotherapy for cancer: harnessing the T cell response. Nat Rev Immunol 12(4):269–281. https://doi.org/10.1038/nri3191

121. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin N, Chew A, Gonzalez VE, Zheng Z, Lacey SF, Mahnke YD, Morgan RA, Laurencot C, Rosenberg SA (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119(12):2709–2720. https://doi.org/10.1182/blood-2011-10-384388

122. Turtle CJ, Hanafi LA, Berger C, Gooley TA, Cherian S, Hudecka T, Schlake et al. (2015) Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell 28(4):415–428. https://doi.org/10.1016/j.ccell.2015.09.004

123. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van den Bergh LM, Fijnheer R, Gratama JW, Stoter G, Oosterwijk E (2006) Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retarded against carbonic anhydrase IX: first clinical experience. J Clin Oncol 24(13):e20–e22. https://doi.org/10.1200/JCO.2006.5.9964

124. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van Kriempen B, Groot C, Vulto A, den Bakker M, Oosterwijk E, Debets R, Gratama JW (2013) Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther 21(4):904–912. https://doi.org/10.1038/mt.2013.17

125. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Mari C, Stelet-Cervenka M, Pan GQ, Hughes MS, Sherry RM, Yang JC, Kammula US, Devillier L, Carpenter R, Nathan DA, Morgan RA, Laurencot C, Rosenberg SA (2012) B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. Blood 119(12):2709–2720. https://doi.org/10.1182/blood-2011-10-384388

126. Hartmann J, Schussler-Lenz M, Bondaninza A, Bachholz CJ (2017) Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med 9(9):1183–1197. https://doi.org/10.15252/emmm.20167485

127. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL (2014) Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124(2):188–195. https://doi.org/10.1182/blood-2014-05-552729

128. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL (2014) Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124(2):188–195. https://doi.org/10.1182/blood-2014-05-552729

129. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL (2014) Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124(2):188–195. https://doi.org/10.1182/blood-2014-05-552729

130. Modlich U, Baum C (2009) Preventing and exploiting the oncogenic potential of integrating gene vectors. J Clin Investig 119(4):755–758

131. Hartmann J, Schussler-Lenz M, Bondaninza A, Bachholz CJ (2017) Clinical development of CAR T cells-challenges and opportunities in translating innovative treatment concepts. EMBO Mol Med 9(9):1183–1197. https://doi.org/10.15252/emmm.20167485

132. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA, Mackall CL (2014) Current concepts in the diagnosis and management of cytokine release syndrome. Blood 124(2):188–195. https://doi.org/10.1182/blood-2014-05-552729

133. Modlich U, Baum C (2009) Preventing and exploiting the oncogenic potential of integrating gene vectors. J Clin Investig 119(4):755–758

134. Herder D (2001) Gene therapy. Safer and virus-free? Science 294(5547):1638–1642. https://doi.org/10.1126/science.294.5547.1638

135. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulfraat N, Leboelich P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JL de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabes E, Macintyre E, Sigaux F, Soulager J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M (2003) LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1. Science 302(5644):415–419. https://doi.org/10.1126/science.1088547

136. Nienhuis AW, Dunbar CE, Sorentino BP (2006) Genotoxicity of retroviral vectorization in hematopoietic cells. Mol Ther 13(6):1031–1049. https://doi.org/10.1038/s21503112

137. Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, Haliburton GE, Ye CJ, Bluestone JA, Doudna JA (2015) Pharmacokinetics and immunogenicity of broadly neutralizing HIV monoclonal antibodies in macaques. PLoS One 10(3):e0120451. https://doi.org/10.1371/journal.pone.0120451

138. Schumann K, Lin S, Boyer E, Simeonov DR, Subramaniam M, Gate RE, Haliburton GE, Ye CJ, Bluestone JA, Doudna JA, Marson A (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. Proc Natl Acad Sci USA 112(33):10437–10442. https://doi.org/10.1073/pnas.1512503112

139. Rosenberg Y, Sack M, Montefiori DC, Johnson PR, Clark KR (2015) Pharmacokinetics and immunogenicity of broadly neutralizing HIV monoclonal antibodies in macaques. PLoS One 10(3):e0120451. https://doi.org/10.1371/journal.pone.0120451

140. Schnepp BC, Johnson PR (2015) Vector-mediated antibody gene transfer. J Transl Med 15(1):131. https://doi.org/10.1186/s12967-017-1234-4

141. Simeonov DR, Schnepp BC, Johnson PR (2015) Vector-mediated antibody gene transfer for infectious diseases. Adv Exp Med Biol 848:149–167. https://doi.org/10.1007/978-1-4939-2432-5_8

142. Lewis AD, Chen R, Montefiori DC, Johnson PR, Clark KR (2002) Generation of neutralizing activity against human immunodeficiency virus type 1 in serum by antibody gene transfer. J Virol 76(17):8769–8775
mRNA as novel technology for passive immunotherapy

142. Balazs AB, Chen J, Hong CM, Rao DS, Yang L, Baltimore D (2011) Antibody-based protection against HIV infection by vector-tempered immunophylaxis. Nature 481(7379):81–84. https://doi.org/10.1038/nature10660

143. Balazs AB, Bloom JD, Hong CM, Rao DS, Baltimore D (2013) Broad protection against influenza infection by vectored immunophylaxis in mice. Nat Biotechnol 31(7):647–652. https://doi.org/10.1038/nbt.2618

144. Fang J, Qian JJ, Yi S, Harding TC, Tu GH, VanRoey M, Joos K (2005) Stable antibody expression at therapeutic levels using the 2A peptide. Nat Biotechnol 23(5):584–590. https://doi.org/10.1038/nbt1087

145. Nault JC, Datta S, Imbeaud S, Franconi A, Malten M, Couchy G, Letouze E, Pilati C, Verret B, Blanc JF, Balabaud C, Calderaro J, Laurent A, Letexier M, Bioulac-Sage P, Calvo F, Zucman-Rossi J (2015) Recurrent AAV2-related insertion mutagenesis in human hepatocellular carcinomas. Nat Genet 47(10):1187–1193. https://doi.org/10.1038/ng.3389

146. Baldo A, van den Akker E, Bergmans HE, Lim F, Pauwels K (2013) General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination. Curr Gene Ther 13(6):385–394

147. Fausther-Bovendo H, Kobinger GP (2014) Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what’s important? Hum Vaccines Immunother 10(10):2875–2884. https://doi.org/10.4161/hv.29594

148. Fuchs SP, Desrosiers RC (2016) Promise and problems associated with the use of recombinant AAV for the delivery of anti-HIV antibodies. Mol Ther Methods Clin Dev 3:16068. https://doi.org/10.1038/mtm.2016.68

149. Louis Jeune V, Joergensen JA, Hajjar RJ, Weber T (2013) Pre-existing anti-adeno-associated virus antibodies as a challenge in AAV gene therapy. Hum Gene Ther Methods 24(2):59–67. https://doi.org/10.1089/hgtb.2012.243

150. Suscovich TJ, Alter G (2015) In situ production of therapeutic monoclonal antibodies. Expert Rev Vaccines 14(2):205–219. https://doi.org/10.1586/14760584.2015.1001375

151. Gao G, Lebherz C, Weiner DJ, Grant R, Calcedo R, McCullough B, Bagg A, Zhang Y, Wilson JM (2004) Erythropoetin gene therapy leads to autoimmune anemia in macaques. Blood 103(9):3300–3302. https://doi.org/10.1182/blood-2003-11-3852

152. Johnson PR, Schnepp BC, Zhang J, Connell MJ, Greene SM, Yuste E, Desrosiers RC, Clark KR (2009) Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. Nat Med 15(8):901–906. https://doi.org/10.1038/nm.1967

153. Saunders KO, Wang L, Joyce MG, Yang ZY, Balazs AB, Cheng C, Ko SY, Kong WP, Rudicell RS, Georgiev IS, Duan L, Foulds KE, Donaldson M, Xu L, Schmidt SD, Todd JP, Baltimore D, Roederer M, Haase AT, Kwong PD, Rao SS, Mascola JR, Niedzwiecka A, Mascola R, Darzynkiewicz E, Rhoads RE (2015) Broadly neutralizing human immunodeficiency virus type 1 antibody gene transfer protects nonhuman primates from mucosal simian-human immunodeficiency virus infection. J Virol 89(16):8334–8345. https://doi.org/10.1128/JVI.00908-15

154. Gao G, Wang Q, Calcedo R, Mays L, Bell P, Wang L, Vandenberghe LH, Grant R, Sanmiuel J, Furrh EE, Wilson JM (2009) Adeno-associated virus-mediated gene transfer to non-human primate liver can elicit destructive transgene-specific T cell responses. Hum Gene Ther 20(9):930–942. https://doi.org/10.1089/hum.2009.060

155. Zangi L, Lui KO, von Gise A, Ma Q, Ebina W, Prszasz LM, Spater D, Xu H, Tabebordbar M, Grorbavit R, Sena B, Nahrendorff M, Briscoe DM, Li RA, Wagers AJ, Rossi DI, Pu WT, Chien KR (2013) Modified mRNA directs the fate of heart progenitor cells and induces vascular regeneration after myocardial infarction. Nat Biotechnol 31(10):898–907. https://doi.org/10.1038/nbt.2682

156. Gurdon JB, Lane CD, Woodland HR, Marbaix G (1971) Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. Nature 233(516):177–182

157. Laskey RA, Gurdon JB, Crawford LV (1972) Translation of encephalomyocarditis viral RNA in oocytes of Xenopus laevis. Proc Natl Acad Sci USA 69(12):3665–3669

158. Malone RW, Felgner PL, Verma IM (1989) Cationic liposome-mediated RNA transfection. Proc Natl Acad Sci USA 86(16):6077–6081

159. Wolff JA, Malone RW, Williams P, Chong W, Acscadi G, Jani A, Felgner PL (1990) Direct gene transfer into mouse muscle in vivo. Science 247(4949 Pt 1):1465–1468

160. Martinon F, Krishnan S, Lenzen G, Magne R, Gomard E, Guillet JG, Levy JP, Meulien P (1993) Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur J Immunol 23(7):1719–1722. https://doi.org/10.1002/eji.1830230749

161. Qiu P, Ziegelhoffer P, Sun J, Yang NS (1996) Gene gun delivery of mRNA in situ results in efficient transgene expression and genetic immunization. Gene Ther 3(3):262–268

162. Boczkowski D, Nair SK, Snyder D, Gilboa E (1996) Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J Exp Med 184(2):465–472

163. Hoerl I, Obst R, Ramnensee HG, Jung G (2000) In vivo application of RNA leads to induction of specific cytotoxic T lymphocytes and antibodies. Eur J Immunol 30(1):1–7. https://doi.org/10.1002/1521-4141(200001)30:1%3c1:AID-IMMU1%3e3.0.CO;2-%3c

164. Banerjee AK (1980) 5′-terminal cap structure in eucaryotic messenger ribonucleic acids. Microbiol Rev 44(2):175–205

165. Wickens M (1990) How the messenger got its tail: addition of 5′-terminal cap structure in eucaryotic messenger RNA. Microbiol Rev 44(2):175–205

166. Dominski Z, Marzluff WF (1999) Formation of the 3′ end of histone mRNA. Gene 239(1):1–14

167. Gallie DR (1991) The cap and poly(A) tail function synergistically to regulate mRNA translational efficiency. Genes Dev 5(11):2108–2116

168. Parker R, Song H (2004) The enzymes and control of eukaryotic mRNA turnover. Nat Struct Mol Biol 11(2):121–127. https://doi.org/10.1038/nsmb724

169. Yamashita A, Chang TC, Yamashita Y, Zhu W, Zhong Z, Chen CY, Shyu AB (2005) Concerted action of poly(A) nucleases and decapping enzyme in mammalian mRNA turnover. Nat Struct Mol Biol 12(12):1054–1063. https://doi.org/10.1038/nsmb1016

170. Pasquinelli AE, Dahlberg JE, Lund E (1995) Reverse 5′ caps in RNAs made in vitro by phage RNA polymerases. RNA 1(9):957–967

171. Stepiński J, Wadдел C, Stolar ski R, Darzynkiewicz E, Rhoads RE (2001) Synthesis and properties of mRNAs containing the novel “anti-reverse” cap analogs 7-methyl(3′-O-methyl)GpppG and 7-methyl(3′-deoxy)GpppG. RNA 7(10):1486–1495

172. Jemielity J, Fowler T, Zuberek J, Stepinski J, Lewdorowicz M, Niedzwiecka A, Mascola R, Darzynkiewicz E, Rhoads RE (2003) Novel “anti-reverse” cap analogs with superior translational properties. RNA 9(9):1108–1122

173. Zohra FT, Chowdhury EH, Tada S, Hobarta T, Akaike T (2007) Effective delivery with enhanced translational activity synergistically accelerates mRNA-based transfection. Biochem Phys Res Commun 358(1):373–378. https://doi.org/10.1016/j.bbrc.2007.04.059

174. Mockey M, Goncalves C, Dupuy FP, Lemoine FM, Pichon C, Midoux P (2006) mRNA transfection of dendritic cells: synergistic effect of ARCA mRNA capping with Poly(A) chains in cis
Akira S (2006) Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature 441(7089):101–105. https://doi.org/10.1038/nature04734

203. Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F, Reis e Sousa C (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 314(5801):997–1001. https://doi.org/10.1126/science.1132998

204. Rehwinkel J, Tan CP, Goubau D, Schulz O, Pichlmair A, Bier K, Robb N, Vreede F, Barclay W, Fodor E, Reis e Sousa C (2010) RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell 140(3):397–408. https://doi.org/10.1016/j.cell.2010.01.020

205. Nallagatla SR, Hwang J, Toroney R, Zheng X, Cameron CE, Kariko K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Buckstein M, Ni H, Weissman D (2005) Suppression of PKR by RNAs with short stem-loops. Science 318(5855):1455–1458. https://doi.org/10.1126/science.1147347

206. Kariko K, Buckstein M, Ni H, Weissman D (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity 23(2):165–175. https://doi.org/10.1016/j.immuni.2005.06.008

207. Kariko K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Weissman D (2008) Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 16(11):1833–1840. https://doi.org/10.1038/m.2008.200

208. Anderson BR, Muramatsu H, Nallagatla SR, Bevilacqua PC, Sansing LH, Weissman D, Kariko K (2010) Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. Nucleic Acids Res 38(17):5884–5892. https://doi.org/10.1093/nar/gkq347

209. Anderson BR, Muramatsu H, Jha BK, Silverman RH, Weissman D, Kariko K (2011) Nucleoside modifications in RNA limit activation of 2′-5′-oligoadenylate synthetase and increase resistance to cleavage by RNase L. Nucleic Acids Res 39(21):9329–9338. https://doi.org/10.1093/nar/gkr586

210. Uchida S, Kataoka K, Itaka K (2015) Screening of mRNA chemical modification to maximize protein expression with reduced immunogenicity. Pharmaceutics 7(3):137–151. https://doi.org/10.3390/pharmaceutics7030137

211. Kauffman KJ, Mir FF, Jhunjhunwala S, Kaczmarek JC, Hurtado JE, Yang JH, Webber MJ, Kowalski PS, Heartlein MW, DeRosa F, Anderson DG (2016) Efficacy and immunogenicity of unmodified and pseudouridine-modified mRNA delivered systemically and the evolutionary origin of RNA. Immunity 44(1):101–105. https://doi.org/10.1016/j.immuni.2016.09.006

212. DeRosa F, Guild B, Karve S, Smith L, Love K, Dorkin JR, Kauffman KJ, Zhang J, Yahalom B, Anderson DG, Hearlein MW (2016) Therapeutic efficacy in a hemophilia B model using a bio-synthetic mRNA liver depot system. Gene Ther 23(10):699–707. https://doi.org/10.1038/gt.2016.46

213. Ramaswamy S, Tonnu N, Tachikawa K, Limpfong P, Vega JB, Karmali PP, Chivukula P, Verma IM (2017) Systematic delivery of factor IX messenger RNA for protein replacement therapy. Proc Natl Acad Sci USA 114(10):E1941–E1950. https://doi.org/10.1073/pnas.1619653114

214. Carlsson L, Clarke JC, Yen C, Greigore F, Albery T, Billger M, Eganell A-C, Gan L-M, Jennbacken K, Johansson E, Linhardt G, Martinsson S, Sadiq MW, Witman N, Wang Q-D, Chen C-H, Wang Y-L, Lin S, Ticho B, HSieh P, Chien KR, Fritsch-Danielson R (2018) Biocompatible, purified VEGF-A mRNA improves cardiac function after intracardiac injection 1 week post-myocardial infarction in swine. Mol Ther Methods Clin Dev 5:330–344. https://doi.org/10.1016/j.omtm.2018.04.003

215. Wang Y, Su HH, Yang Y, Hu Y, Zhang L, Blancafort P, Huang L (2013) Systemic delivery of modified mRNA encoding herpes simplex virus 1 thymidine kinase for targeted cancer gene therapy. Mol Ther 21(2):358–367. https://doi.org/10.1038/mt.2012.250

216. Hirschberger K, Jarzabinska A, Kessel E, Kretzschmann V, Aneja MK, Dohmen C, Herrmann-Jansen A, Wagner E, Plank C, Rudolph C (2018) Exploring cytotoxic mRNA as a novel class of anti-cancer biotherapeutics. Mol Ther Methods Clin Dev 8:141–151. https://doi.org/10.1038/mt.2017.12.006

217. Kormann MS, Hasenpusch G, Aneja MK, Nica G, Flemmer AW, Herber-Jonat S, Huppmann M, Mays LE, Illenyi M, Schams A, ... Springer
Griese M, Bettmann I, Handgretinger R, Hartl D, Rosenecker J, Rudolph C (2011) Expression of therapeutic proteins after delivery of chemically modified mRNA in mice. Nat Biotechnol 29(2):154–157. https://doi.org/10.1038/nbt.1733

229. Mahiny AJ, Dewerth A, Mays LE, Alkhale M, Mothes B, Maleksafet E, Lorezt B, Rottenberger J, Brosch DM, Reutschnig P, Surapoloža P, Zeyer F, Schams A, Carevic M, Baktele M, Griese M, Schwab M, Nurnberg B, Beer-Hammer S, Handgretinger R, Hartl D, Lehr CM, Kormann MS (2015) In vivo genome editing using nuclelease-encoding mRNA corrects SP-B deficiency. Nat Biotechnol 33(6):584–586. https://doi.org/10.1038/nbt.3241

230. Mays LE, Ammon-Treiber S, Mothes B, Alkhaled M, Rottenberger J, Muller-Hermelink ES, Grimm M, Mezger M, Beer-Hammer S, von Stebut E, Rieber N, Nurnberg B, Schwab M, Handgretinger R, Idzko M, Hartl D, Kormann MS (2013) Modified Foxp3 mRNA protects against asthma through an IL-10-dependent mechanism. J Clin Investig 123(3):1216–1226. https://doi.org/10.1172/JCI165351

231. An D, Schneller JL, Frassetto A, Li X, Park JS, Theisen M, Hong SJ, Zhou J, Rajendran R, Levy B, Howell R, Besin D, Hasenpusch G (2017) Tendon healing induced by chemically modified mRNAs. Eur Cell Mater 33:294–307. https://doi.org/10.1007/s13238-017-0422-6

232. Birkholz K, Hombach A, Krug C, Reuter S, Kershaw M, Kampgen E, Schuler G, Abken H, Schaft N, Dorrie J, Schaft N (2014) A GMP-compliant protocol to expand and transfen cancer patient T cells with mRNA encoding a tumor-specific chimeric antigen receptor. Cancer Immunol Immunother 63(10):999–1008. https://doi.org/10.1007/s00262-014-1572-5

233. Harrer DC, Simon B, Fujii SI, Shimizu K, Ush U, Schul G, Gerer FK, Hoyer S, Dorrie J, Schaft N (2017) RNA-transfection of gamma/delta T cells with a chimeric antigen receptor or an alpha/beta T-cell receptor: a safer alternative to genetically engineered alpha/beta T cells for the immunotherapy of melanoma. BMC Cancer 17(1):551. https://doi.org/10.1186/s12885-017-3539-3

234. Beatty GL, Haas AR, M准入 MV, Torigian DA, Soulen MC, Plesa G, Arnold A, Zhao Y, Levine BL, Albelda SM, Kalos M, June CH (2014) Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res 2(2):112–120. https://doi.org/10.1158/2326-6066.CIR-13-0170
mRNA as novel technology for passive immunotherapy

252. Maus MV, Haas AR, Beatty GL, Albelda SM, Levine BL, Liu X, Zhao Y, Kalos M, June CH (2013) T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. Cancer Immunol Res 1:26–31

253. Tchou J, Zhao Y, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ, Kulikovskaya I, Brennan AL, Liu X, Lacey SF, Posey AD Jr, Williams AD, So A, Conejo-Garcia JR, Plesa G, Young RM, McGgettigan S, Campbell J, Pierce RH, Matro JM, DeMichele AM, Clark AS, Cooper LJ, Schuchter LM, Vonderheide RH, June CH (2017) Safety and efficacy of intratumoral injections of chimeric antigen receptor (CAR) T cells in metastatic breast cancer. Cancer Immunol Res 5(12):1152–1161. https://doi.org/10.1158/2326-6066.CIR-17-0189

254. Riet T, Holzinger A, Dorrie J, Schaft N, Schulter G, Ahken H (2013) Nonviral RNA transfection to transiently modify T cells with chimeric antigen receptors for adoptive therapy. Methods Mol Biol 969:187–201. https://doi.org/10.1007/978-1-62703-260-5_12

255. June CH (2007) Adoptive T cell therapy for cancer in the clinic. J Clin Investig 117(6):1466–1476. https://doi.org/10.1172/JCI32446

256. Rosenblum SA, Restifo NP, Yang JC, Morgan RA, Dudley ME (2008) Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer 8(4):299–308. https://doi.org/10.1038/nrc2355

257. Meidenbauer N, Marienhagen J, Laumer M, Vogl S, Heymann J, Andreessen R, Mackensen A (2003) Survival and tumor localization of adoptively transferred Melan-A-specific T cells in melanoma patients. J Immunol 170(4):2161–2169

258. Mitchell MS, Darrah D, Yeung D, Halpern S, Wallace A, Volberdt PD, June CH (2006) Interleukin-15 rescues tolerant CD8+ T cells for clinical research. Mol Ther Oncolytics 3:16024. https://doi.org/10.1038/ncomms14630

259. Suzuki Y, Ishihara H (2016) Structure, activity and uptake mechanism of siRNA-lipid nanoparticles with an asymmetric ionic lipid. Int J Pharm 510(1):350–358. https://doi.org/10.1016/j.ijpharm.2016.06.124

260. Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, Bunnett NW, Teague RM, Sather BD, Sacks JA, Huang MZ, Dossett ML, Sorkin A, Von Zastrow M (2002) Signal transduction and endosomes: a legitimate platform for the signaling train. Proc Natl Acad Sci USA 106(42):17615–17622. https://doi.org/10.1073/pnas.1126028106

261. Pardi N, Secreto AJ, Shan X, Debonera F, Glover J, Yi Y, Muramatsu H, Ni H, Mui BL, Tam YK, Shafeen F, Collman RG, Kariko K, Danet-Desnoyers GA, Madden TD, Hope MJ, Weissman D (2017) Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. Nat Commun 8:14630. https://doi.org/10.1038/s41467-017-07678

262. Pardi N, Suytjisme S, Muramatus H, Kariko K, Mui BL, Tam YK, Mohseni, and Jonas T, Senn JJ, Lynn A, Bulychev A, McFadyen I, Chan J, Almarsson O, Stanton MG, Benenato KE (2018) A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Mol Ther. https://doi.org/10.1016/j.mther.2018.03.010

263. Weissman D, Pardi N, Muramatsu H, Kariko K (2013) HPLC purification of in vitro transcribed long RNA. Methods Mol Biol 969:43–54. https://doi.org/10.1007/978-1-62703-260-5_3

264. Chan HY, Sivakamasundari V, Xing X, Krausz P, Yap SP, Ng P, Lim SL, Luktf T (2011) Comparison of IRES and F2A-based locus-specific multicistronic expression in stable mouse lines. PLoS One 6(12):e28885. https://doi.org/10.1371/journal.pone.0028885

265. Klein F, Halper-Stromberg A, Horwitz JA, Gruell H, Scheid JF, Bournaouos S, Mouquet H, Spatz LA, Diskin R, Abadir A, Zang T, Dorner M, Billerbeck E, Labritt RN, Gaebler C, Marcovech P, Incus RB, Eiseichen TR, Bengan PA, Seaman MS, Bjorkman PJ, Ravetch JV, Ploss A, Nussenzweig MC (2012) HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. Nature 492(7427):118–122. https://doi.org/10.1038/nature11604

266. Gauduin MC, Parren PW, Weir R, Barbas CF, Burton DR, Koup RA (1997) Passive immunization with a human monoclonal antibody protects hu-PBL-SCID mice against challenge by primary isolates of HIV-1. Nat Med 3(12):1389–1393

267. Szeben J (2014) Complement activation-related pseudoadalergy: a stress reaction in blood triggered by nanomedicines and biologicals. Mol Immunol 61(2):163–173. https://doi.org/10.1016/j.molimm.2014.06.038

268. Dakhil S, Hermann R, Schreeder MT, Gregory SA, Monte M, Windsor KS, Hurst D, Chai A, Brewster M, Richards P (2014) Phase III safety study of rituximab administered as a 90-minute infusion in patients with previously untreated diffuse large B-cell and follicular lymphoma. Leuk Lymphoma 55(10):2335–2340. https://doi.org/10.3109/10428194.2013.877135

269. Shipilborg O, Jackisch C (2013) Subcutaneous administration of rituximab (MabThera) and trastuzumab (Herceptin) using hyaluronidase. Br J Cancer 109(6):1556–1561. https://doi.org/10.1038/bjc.2013.371

270. Bittner B, Richter WF, Hourcade-Potelleret F, Hertzing F, Schmidt J (2014) Non-clinical pharmacokinetic/pharmacodynamic and early clinical studies supporting development of a novel subcutaneous formulation for the monoclonal antibody rituximab. Drug Res (Stuttg) 64(11):569–575. https://doi.org/10.1055/s-0033-1363993

271. Bookbinder LH, Hofer A, Haller MF, Zepeda ML, Keller GA, Lim JE, Edgington TS, Shepard HM, Patton JS, Frost GI (2006)
A recombinant human enzyme for enhanced interstitial transport of therapeutics. J Control Release 114(2):230–241. https://doi.org/10.1016/j.jconrel.2006.05.027

279. Marschall AL, Zhang C, Frenzel A, Schirrmann T, Hust M, Perez F, Dubel S (2014) Delivery of antibodies to the cytosol: debunking the myths. MAbs 6(4):943–956. https://doi.org/10.4161/mabs.29268

280. Kontermann RE, Muller R (1999) Intracellular and cell surface displayed single-chain diabodies. J Immunol Methods 226(1–2):179–188

281. Schmidt FI, Hanke L, Morin B, Brewer R, Brusic V, Whelan SP, Ploegh HL (2016) Phenotypic lentivirus screens to identify functional single domain antibodies. Nat Microbiol 1(8):16080. https://doi.org/10.1038/nmicrobiol.2016.80

282. Weisbart RH, Wakelin R, Chan G, Miller CW, Koeffler PH (2004) Construction and expression of a bispecific single-chain antibody that penetrates mutant p53 colon cancer cells and binds p53. Int J Oncol 25(4):1113–1118

283. Steinberger P, Andris-Widhopf J, Buhler B, Torbett BE, Barbas CF 3rd (2000) Functional deletion of the CCR5 receptor by intracellular immunization produces cells that are refractory to CCR5-dependent HIV-1 infection and cell fusion. Proc Natl Acad Sci USA 97(2):805–810

284. Amici C, Visintin M, Verachi F, Paolini F, Percario Z, Di Bonito P, Mandarino A, Affabris E, Venuti A, Accardi L (2016) A novel intracellular antibody against the E6 oncoprotein impairs growth of human papillomavirus 16-positive tumor cells in mouse models. Oncotarget 7(13):15539–15553. https://doi.org/10.18632/oncotarget.6925

285. Zhou C, Emaidi S, Sierks MR, Messer A (2004) A human single-chain Fv intrabody blocks aberrant cellular effects of overexpressed alpha-synuclein. Mol Ther 10(6):1023–1031. https://doi.org/10.1016/j.ymthe.2004.08.019

286. Cardinale A, Flesis I, Vetrugno V, Pocchiari M, Sy MS, Biocca S (2005) Trapping prion protein in the endoplasmic reticulum impairs PrPC maturation and prevents PrPSc accumulation. J Biol Chem 280(1):685–694. https://doi.org/10.1074/jbc.M407360200

287. Zhang JF, Xiong HL, Cao JL, Wang SJ, Guo XR, Lin BY, Zhang Y, Zhao JH, Wang YB, Zhang TY, Yuan Q, Zhang J, Xia NS (2018) A cell-penetrating whole molecule antibody targeting intracellular HBx suppresses hepatitis B virus via TRIM21-dependent pathway. Theranostics 8(2):549–562. https://doi.org/10.7150/thno.20047

288. El-Sayed A, Futaki S, Harashima H (2009) Delivery of macromolecules using arginine-rich cell-penetrating peptides: ways to overcome endosomal entrapment. AAPS J 11(1):13–22. https://doi.org/10.1208/s12248-008-9071-2

Springer