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mRNA

From a chemical blueprint for protein production to an off-the-shelf therapeutic

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Two decades ago, mRNA became the focus of research in molecular medicine and was proposed as an active pharmaceutical ingredient for the therapy of cancer. In this regard, mRNA has been mainly used for ex vivo modification of antigen-presenting cells (APCs), such as dendritic cells (DCs). This vaccination strategy has proven to be safe, well tolerated and capable of inducing tumor antigen-specific immune responses. Recently, the direct application of mRNA for in situ modification of APCs, hence immunization was shown to be feasible and at least as effective as DC-based immunization in pre-clinical models. It is believed that application of mRNA as an off-the-shelf vaccine represents an important step in the development of future cancer immunotherapeutic strategies. Here, we will discuss the use of ex vivo mRNA-modified DCs and "naked mRNA" for cancer immunotherapy focusing on parameters such as the employed DC subtype, DC activation stimulus and route of immunization. In addition, we will provide an overview on the clinical trials published so far, trying to link their outcome to the aforementioned parameters.

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Introduction

In the past two decades, mRNA has gained growing attention in medical research, in particular as a vehicle to deliver tumor antigens as well as activation stimuli for the induction of immune responses against cancer.[1-8]

The growing interest in mRNA as an active pharmaceutical ingredient in anticancer immunotherapy can be explained by its versatility and the many advantages it offers. When compared with viral vectors or plasmid DNA, mRNA is safe, as it lacks genomic integration capacity and only results in transient expression of the encoded protein. Moreover, virtually any cell type can be modified with mRNA, including hard-to-modify cells, such as dendritic cells (DCs).[9] The latter are critical in the priming of effective immune responses.[10] Therefore, it is not surprising that mRNA has been extensively exploited to deliver tumor antigens to these professional antigen-presenting cells (APCs).[11-17] The use of mRNA to deliver tumor antigens offers a number of advantages. First, mRNA is characterized by a certain degree of flexibility in the sense that all proteins of interest can be generated and that enhanced protein expression hence presentation of antigenic peptides can be obtained by structural modification of the mRNA molecule.[6,18,19] Second, mRNA encodes the entire tumor antigen thus enabling presentation of all epitopes contained within the encoded protein in the context of MHC class I molecules to $CD8^+$ T cells.[20,21] In addition, presentation of antigenic epitopes in the context of MHC class II molecules to $CD4^+$ T cells can be achieved by fusion of the tumor antigen encoding sequence to class II targeting signals.[22,23] The latter is important, since a potent anti-tumor immune response requires activation of $CD4^+$ T helper 1 (T_H1) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs). Finally, mRNA can be easily produced at high quantity and purity without the use of problematic materials such as animal-derived proteins, resulting in batch-to-batch reproducibility

and the ability to scale up the production to meet world-wide demand.[1,3] The first description of an mRNA-based vaccination strategy was published already in the early nineties[24] at approximately the same time as DNA-based vaccination.[25] Although vaccination with mRNA resulted in the induction of adaptive B and T cell-mediated immune responses, DNA-based vaccines dominated the following ten years. Today, several strategies have been developed to deliver mRNA to DCs. These include methods to transfect DCs in vitro, including passive pulsing,[11,26-28] lipofection,[29] electroporation[12,13] and sonoporation[30] to name a few. As in vivo modification of APCs has gained interest over the years, it was also explored whether mRNA can be used for this purpose. The use of ex vivo mRNA-modified DCs and "naked mRNA" for cancer immunotherapy will be discussed in this review.

Immunization with Ex Vivo mRNA-Modified Dendritic Cells

Currently, the transfer of ex vivo mRNA-modified DCs is the method of choice for vaccination. In 1997, the FDA approved the first clinical trial based on the use of ex vivo mRNA-transfected DCs to induce an immune response in cancer patients.[26,31] Over the past decades, vaccines consisting of autologous DCs loaded with tumor antigen mRNA have proven to be safe, well tolerated and capable of inducing tumor antigen-specific immune responses in a substantial number of vaccinated patients.[20,21,32,33] Thus far several clinical trials using ex vivo mRNA-modified DCs have been conducted in different types of cancers (**Table 1**). Although objective tumor regression was not always observed, these clinical trials demonstrated the potential of mRNA-modified DCs for anti-cancer vaccination. To further optimize this approach several parameters can be scrutinized, including the exploited DC subtype and DC maturation stimulus as well as route, dose and frequency of vaccine administration.[34,35]

The use of monocyte-derived vs. plasmacytoid dendritic cells in anti-cancer therapy. DCs are a heterogeneous population of cells that can originate from both lymphoid and myeloid progenitors, giving rise to distinct DC subsets.[36] In humans, DCs are broadly divided into myeloid DCs (myDCs) and plasmacytoid DCs (pDCs). Most vaccination studies today use monocyte-derived DCs (moDCs), which are thought to resemble myDCs, as they can be obtained in vitro at sufficient high numbers starting from $CD34^+$ or $CD14^+$ progenitor cells. Moreover, these moDCs can be modified with mRNA at high efficiency.[37] In contrast, pDCs have not been extensively studied in the context of anti-cancer vaccination. Human pDCs are renowned for their ability to secrete large amounts of type I interferons (IFNs) upon virus encounter. This IFN production aids the maturation of myDCs and activation of T cells (T_H1 and CTLs) as well as cytolytic natural killer (NK) cells.[38-40] Today, it is recognized that pDC-derived type I IFNs also contribute to anti-cancer immunity.[41,42] Salio et al.[43] demonstrated in vitro that pDCs loaded with melanoma peptide and activated via CD40 ligand (CD40L) were able to activate naive $CD8^+$ T cells into melanoma-specific CTLs. In addition, similar to myDCs, pDCs were shown to infiltrate tumors, where they are trapped in an immature state.[43-45] Importantly, tumor-residing pDCs can be reverted into pDCs with tumoricidal activity when strong activation stimuli are applied.[46,47] These studies highlight the potential of exploiting pDCs similar to the use of myDCs for anti-tumor therapy.

Activation of dendritic cells. It is generally accepted that the maturation status of the DCs determines the outcome of vaccination.[59-64] In 1997, Jonuleit et al.[65] proposed an inflammatory cytokine cocktail (CC), consisting of IL-1β, IL-6, TNF-α and PGE_2 as a potent maturation stimulus. This CC has been extensively used for the maturation of mRNA-modified DCs. However, several drawbacks have been associated to the use of this CC.[66-68] Therefore, alternative strategies to mature DCs have been explored. In this regard, the use of Toll-like receptor (TLR) ligands popped up as a strategy to induce maturation of DCs. Activation of DCs via TLRs was shown to have several advantages, including hyperactivation of CTLs and inhibition of the suppressive activity of regulatory T cells (Tregs).[62,69-71] In addition, much effort has been put in the development of a one-step-procedure to simultaneously load DCs with tumor antigen mRNA and activate them. Examples hereof are co-electroporation of DCs with tumor antigen mRNA and Ampligen®, a dsRNA polyI:C analog[72] or TriMix mRNA, a mix of three mRNA molecules encoding CD40L, CD70 and caTLR4.[73,74] The combination of CD40L and a constitutive active form of TLR4 (caTLR4) mimics CD40 ligation[75] and TLR4 signaling on DCs[76] resulting in strong activation of the DCs. The third molecule, CD70 provides a co-stimulatory signal to CD27 expressing T cells resulting in their survival and proliferation.[8,73,77] In vitro studies demonstrated that DCs activated with Ampligen® or TriMix mRNA were superior to CC-matured DCs with regard to induction of tumor antigen-specific T cells.[72-74] In addition, the first clinical trials with the so-called TriMix-DCs, demonstrated the potential of these DCs for anti-cancer vaccination.[20,56,78,79]

Route of vaccine delivery. mRNA engineered DCs need to reach the lymph nodes to induce a potent immune response. Different routes of vaccination such as i.d., s.c., i.n. and i.v. administration have been tested during clinical trials and were well tolerated.[26,27,31,48-50,52-54] Two parameters have been considered when evaluating the best route for DC vaccination, i.e. the ability of the administered DCs to migrate to the lymph nodes and their ability to evoke immunological and/or clinical responses. Labeling of DCs with $Indium^{111}$ and subsequent imaging of their migration has demonstrated that s.c. administered DCs rarely reach regional lymph nodes. Similarly, it was shown that i.v. injection of DCs does not result in their accumulation in lymph nodes[80] but in an initial accumulation in the lungs and subsequent redistribution to the liver, spleen and bone marrow.[81] In contrast, it has been described that about 4% of ex vivo mRNA-modified DCs can be recovered from lymph nodes when administered i.d.[82] Finally, mRNA-transfected DCs were shown to accumulate in draining lymph nodes after intralymphatic administration.[83] Importantly, Lesterhuis et al.[84] compared i.n. and i.d. administration of

Table 1. Published clinical trials using ex vivo mRNA-modified DCs

Author	Year	Type of cancer	Administration route	Antigen mRNA	DC maturation and/or additional therapy	Immunological response	Clinical response
Rains et al.[48]	2001	colorectal cancer	i.v.	total tumor mRNA	inclusion of keyhole limpet hemocyanin (KLH)	induction of DTH reaction (11/13)	no clinical response
Heiser et al.[26]	2002	metastatic prostate cancer	i.v./i.d.	PSA mRNA		detection of PSA-specific T cell responses	decreased serum PSA levels (6/7) and undetectable circulating tumor cells (3/7)
Su et al.[27]	2003	renal cell carcinoma	i.v./i.d.	total tumor mRNA		induction of tumor antigen-specific T cell responses	8/10 patient received additional therapy, 7/15 SD
Morse et al.[31]	2003	metastatic CEA expressing cancers (lung, breast, colon cancer)	i.v./i.d.	CEA mRNA		induction of tumor-specific T cell responses	1/24 CR, 2/24 PR, 3/24 SD, 18/24 PD
Caruso et al.[49]	2004	brain cancer	i.d./i.v.	total tumor mRNA		induction of tumor-specific immune responses	4/7 SD, 1/7 PR, 2/7 PD
Caruso et al.[50]	2005	neuroblastoma	i.v./i.d.	total tumor mRNA		increased proliferation of tumor-specific T cells and induction of tumor-specific humoral response	1/11 SD, 10/11 no clinical response
Su et al.[51]	2005	prostate cancer	i.d.	mRNA encoding hTERT linked to LAMP1	IL-6, IL-1β, TNF-α, PGE_2 (20 h)	induction of CD4/CD8 T cell responses	no clinical response, reduction in circulating tumor cells
Dannull et al.[52]	2005	renal cell carcinoma/ ovarian cancer	i.d.	total tumor mRNA	regulatory T cell depletion IL-6, IL-1β, TNF-α, PGE_2 (20 h)	increase in tumor-specific T cell frequencies	8/11 PD
Mu et al.[53]	2005	androgen-resistant prostate cancer	i.d./i.n.	total mRNA of 3 allogeneic tumor cell lines	IL-6, IL-1β, TNF-α, PGE_2 (48 h)	DTH reaction, anti-vaccine specific response	i.d.: 3/9 PD, 6/9 SD i.n.: 5/10 PD, 5/10 SD
Kyte et al.[54]	2006	metastatic melanoma	i.d./i.n.	total tumor mRNA	IL-6, IL-1β, TNF-α, PGE_2 (48 h)	measurable T cell response in a number of patients	i.d.: 1/8 SD, 7/8 PD i.n.: 1/12 SD, 11/12 PD
Kyte et al.[55]	2007	metastatic melanoma	i.d.	total tumor mRNA	IL-6, IL-1β, TNF-α, PGE_2 (48 h)	CD4/CD8 T cell responses against multiple antigens	2/2 PD
Van Tendeloo et al.[33]	2010	acute myeloid leukemia	i.d.	WT-1 mRNA	PGE_2, TNF-α, KLH	CD8 T cell responses against multiple epitopes of WT-1	3/10 CR
Wilgenhof et al.[56]	2011	advanced melanoma	i.d.	4 TAAs: Mage-A3, Mage-C2, Tyrosinase, gp100 linked to DC-LAMP	TriMix mRNA, combination with IFNα-2b	induction of vaccinal antigen-specific CD8 T cells in 12/21 patients	1/17 PR, 5/17 SD

Abbreviations: i.d., intradermal; i.n., intranodal; i.v., intravenous; KLH, keyhole limpet hemocyanin; DTH, delayed type hypersensitivity; hTERT, human telomerase reverse transcriptase; PSA, prostate specific antigen; CEA, carcinoembryonic antigen; SD, stable disease; CR, complete response; PD, progressive disease; LAMP-1, lysosome-associated membrane protein-1; PGE_2, prostaglandin E_2; IL, interleukin; TNF, tumor necrosis factor; WT-1, Wilms' tumor-1; TAA, tumor-associated antigen; HLA, human leukocyte antigen; IFN, interferon; TriMix mRNA, a mix of 3 mRNA molecules encoding CD70, CD40L and a constitutive active form of toll-like receptor 4 (caTLR4); SKILs, skin infiltrating lymphocytes; MCM, monocyte-conditioned medium.

Table 1. Published clinical trials using ex vivo mRNA-modified DCs (continued)

Author	Year	Type of cancer	Administration route	Antigen mRNA	DC maturation and/or additional therapy	Immunological response	Clinical response
Van Nuffel et al.[57]	2011	advanced melanoma	i.d./i.v.	4 TAAs: Mage-A3, Mage-C2, Tyrosinase, gp100 linked to DC-LAMP	TriMix mRNA	enhanced CD4/CD8 T cell responses	1/1 PR
Aarntzen et al.[58]	2012	Advanced melanoma	i.n.	mRNA encoding gp100 and tyrosinase	KLH + matured by autologous MCM, PGE2, recombinant TNFα	Induction of TAA-specific $CD4^+$ and $CD8^+$ T cell responses in SKILs able to recognize multiple epitopes	stage III: 1/26 PD, 12/26 ongoing remission stage IV: 5/19 SD, 1/19 PR, 1/19 mixed response

Abbreviations: i.d., intradermal; i.n., intranodal; i.v., intravenous; KLH, keyhole limpet hemocyanin; DTH, delayed type hypersensitivity; hTERT, human telomerase reverse transcriptase; PSA, prostate specific antigen; CEA, carcinoembryonic antigen; SD, stable disease; CR, complete response; PD, progressive disease; LAMP-1, lysosome-associated membrane protein-1; PGE_2, prostaglandin E_2; IL, interleukin; TNF, tumor necrosis factor; WT-1, Wilms' tumor-1; TAA, tumor-associated antigen; HLA, human leukocyte antigen; IFN, interferon; TriMix mRNA, a mix of 3 mRNA molecules encoding CD70, CD40L and a constitutive active form of toll-like receptor 4 (caTLR4); SKILs, skin infiltrating lymphocytes; MCM, monocyte-conditioned medium.

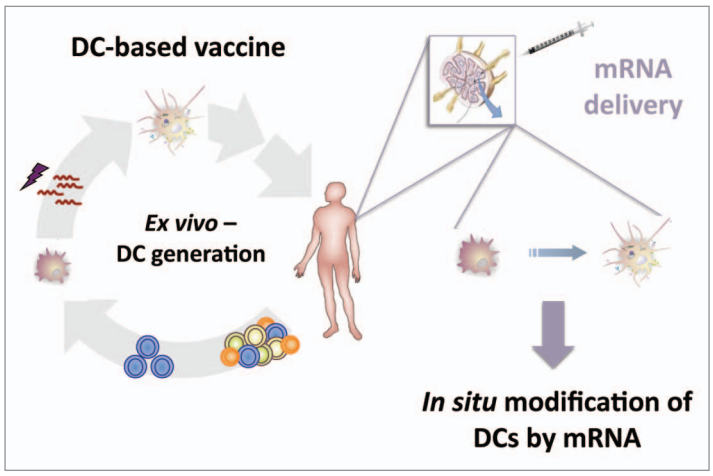

Figure 1. From an ex vivo generated DC-based vaccine toward the direct application of naked mRNA for modification of DCs in order to induce in vivo a potent immune response.

ex vivo modified DCs in patients with advanced melanoma, evaluating both migration and induction of tumor antigen-specific immune responses. Despite the higher number of DCs in lymph nodes after i.n. immunization, i.d. vaccination proved to be superior in inducing functional tumor antigen-specific T cells.

Immunization with mRNA: In Situ Modification of Antigen-presenting Cells

The direct application of "naked mRNA" for anti-tumor vaccination was instigated after Wolff et al.[85] described that injection of mRNA in the muscle of mice resulted in protein expression. It was Conry et al.,[86] who first exploited mRNA for anti-cancer therapy, demonstrating induction of protective anti-tumor immunity in mice after intramuscular injection of CEA mRNA. Since then, several papers reported on the induction of anti-tumor immune responses upon "naked mRNA" delivery in a variety of mouse models (**Fig. 1**; **Table 2**).

Dendritic cells instigate antigen-specific immune response after mRNA uptake. In the studies described in **Table 2**, the mRNA is often delivered in the dermis or lymph node. Although no direct evidence was provided, it is assumed that i.d. delivery of mRNA results in its uptake by Langerhans' cells and dermal DCs at the injection site for transport to draining lymph node. It is moreover assumed that these DCs transfer their antigenic cargo to lymph node-resident $CD8\alpha^+$ DCs when they arrive in the draining lymph nodes.[87-91] In this regard Harshyne et al.[92] demonstrated that antigens could be transferred between live DCs in vitro. In addition, Kleindienst et al.[93] demonstrated that viable vaccinal DCs that migrate to lymph nodes transfer their antigens to lymph node-resident DCs by direct DC-DC contact. Subsequently, these lymph node-resident DCs aid the initiation of antigen-specific T cell responses. Thus cross-presentation of antigenic peptides by endogenous DCs can be seen as a critical event in the effective expansion of functional CTLs. It has been demonstrated that i.n. delivery of mRNA results in the direct modification of lymph node-resident $CD11c^+$ cells and the induction of effective antigen-specific T cell responses.[94,95] Whether mRNA modified lymph node-resident DCs transfer their antigenic cargo to non-mRNA modified DCs for cross-presentation has thus far not been studied. Nonetheless, it is clear that DCs play a major role in the uptake and translation of mRNA. Therefore, several strategies have been evaluated to enhance the number of DCs

Table 2. Overview of pre-clinical trials using mRNA for in vivo modification of APCs

Author	Year	Route of Administration	Antigen mRNA	Additional Information	Outcome
Conry et al.[86]	1995	i.m.	CEA mRNA	globin UTR stabilized mRNA	induction of protective anti-tumor immune response
Hoerr et al.[99]	2000	i.d.	mRNA encoding defined tumor antigens or an antigen library	globin UTR stabilized mRNA or protamine stabilized mRNA	triggering of antibody and CTL responses
Granstein et al.[100]	2000	i.d.	total tumor mRNA		induction of protective anti-tumor immune response
Carralot et al.[101]	2004	i.d.	β –galactosidase mRNA	globin UTR stabilized mRNA, GM-CSF	priming of T_H2 (no GM-CSF) and T_H1 (GM-CSF) responses
Scheel et al.[102]	2006	i.t.	β-galactosidase mRNA	protamin-stabilized	induction of therapeutic anti-tumor immune response
Kreiter et al.[94]	2010	i.n.	HA-RNA or SIINFEKL mRNA		induction of therapeutic anti-tumor immune response
Fotin-Mleczek et al.[103]	2011	i.d.	OVA mRNA or PSMA mRNA	2-component vaccine: free and protamin-complexed mRNA	induction of therapeutic anti-tumor immune response
Kreiter et al.[104]	2011	i.n.	SIINFEKL mRNA	i.p. pre-treatment with Fsm-like tyrosinase kinase 3 ligand (Flt3L)	induction of therapeutic anti-tumor immune response
Van Lint et al.[95]	2012	i.n.	OVA, Trp2, WT-1 or tyrosinase mRNA	co-delivery of TriMix mRNA	induction of therapeutic anti-tumor immune response

Abbreviations: i.m., intramuscular; i.t., intratumoral; CAT, chloramphenicol acetyltransferase; UTR, untranslated region; HA, hemagglutinin; OVA, ovalbumin; PSMA, prostate-specific membrane antigen.

before administration of the mRNA, including pre-treatment with granulocyte macrophage-colony stimulating factor (GM-CSF)[96,97] and Fms-like tyrosinase kinase 3 (FLT3) ligand.[98] These strategies proved to enhance the mRNA-induced immune response.

Activation of dendritic cells. As with DC vaccination, it is of utmost importance that in situ modified DCs are equipped to stimulate effector T cells. This can only be achieved when these DCs are fully activated. Although it has been described that "naked mRNA" can trigger several pathogen recognition receptors (PRRs) as such providing immune-stimulating capacities,[3,94,105-111] much effort is put into the identification of applicable adjuvants to further activate DCs. The study performed by Diken et al.[112] highlights that the maturation stimulus and/or timing of its delivery have to be selected carefully as the uptake of mRNA is dependent on macropinocytosis, a function of immature DCs that is lost upon DC maturation. Consequently, co-delivery of classical maturation stimuli, such as lipopolysaccharide (LPS), with tumor antigen mRNA has a negative impact on the bioavailability of the antigen, a parameter which co-determines the induction of antigen-specific T cell responses.[95,112] To date two different strategies have been explored to simultaneously load the DCs with tumor antigen mRNA and activate them in vivo. Fotin-Mleczek et al.[103] described a two-component system containing free- and protamin-complexed mRNA, providing an antigen source for adaptive immunity together with enhanced triggering of the pathogen recognition receptor, TLR7. This immunization strategy resulted in the induction of a strong anti-tumor immune response and in sustained memory responses, which is important as memory T cells should avoid tumor re-appearance. We have evaluated the use of TriMix mRNA, which was initially described for activation of ex vivo generated DCs, to mature DCs in vivo. We demonstrated that co-delivery of TriMix mRNA did not hamper the uptake and translation of antigen mRNA. Importantly, TriMix mRNA induced a T cell attracting and stimulatory environment, resulting in enhanced induction of antigen-specific T cells. Importantly, mRNA vaccination was shown to be as efficient in induction of CTLs and in therapy as vaccination with mRNA electroporated DCs in several mouse tumor models, stressing the applicability of mRNA as an off-the-shelf therapeutic.[95]

Route of vaccine delivery. Similar to immunization with DCs, an important factor affecting the immunological outcome of mRNA-based immunization is the route of vaccine delivery. Thus far, i.d. administration of mRNA is most frequently used, allowing uptake of the mRNA by Langerhans' cells and dermal DCs after which transport to the draining lymph nodes is assumed. More recently, i.n. administration was

Table 3. Overview of published clinical trials using mRNA for immunization

Author	Year	Type of Cancer	Administration Route	Antigen mRNA	Maturation	immunological response	clinical response
Weide et al.[124]	2008	metastatic melanoma	i.d.	total tumor mRNA	GM-CSF	antibody and tumor specific T cell responses	no clinical response
Weide et al.[125]	2009	metastatic melanoma	i.d.	Melan-A, tyrosinase, gp100, MAGE-A1, MAGE-A3, survivin mRNA	protamin-protected mRNA, GM-CSF either without or with KLH	decrease in Tregs (KLH arm), decrease in MDSCs (non-KLH arm), induction of T cell responses	1/7 CR
Schmidt et al.[127]	2008	renal cell carcinoma	i.d.	MUC1, CEA, Her-2/neu, telomerase, survivin, MAGE-A1 mRNA at 20 μg or 50 μg/TAA	GM-CSF	induction of $CD4^+$ (3/7) and $CD8^+$ T cell responses (8/9)	20 μg/TAA: 1/14 PR and 6/14 SD 50 μg/TAA: 9/16 SD
Rittig et al.[126]	2010	renal cell carcinoma	i.d.	MUC1, Her-2/neu, telomerase, survivin, MAGE-A1 mRNA	GM-CSF	CD4/CD8 T cell responses against multiple antigens	1/30 PR, 15/30 SD, 14/30 PD
Curevac[128]	ongoing	non small cell lung cancer	i.d.	mRNA of 5 different TAAs of which 3 are cancer testis antigens	self-adjuvanted full length mRNA (RNActive®)	immune response rate of 84%, response against multiple antigens in 2/3 patients	ongoing
Curevac[129]	ongoing	Advanced castration resistant prostate cancer	i.d.	PSA, PSCA, PSMA, STEAP1 mRNA	self-adjuvanted full length mRNA (RNActive®)	immune response rate of 79%, in 58% of the patients these are against multiple antigens	prolonged stabilization of PSA levels, one patient had a drop of over 85% in PSA level

Abbreviations: MDSCs, myeloid-derived suppressor cells; MUC-1, mucin-1; PSCA, prostate stem cell antigen; STEAP1, six transmembrane epithelial antigen of the prostate 1.

proposed as the optimal route of mRNA delivery, since lymph nodes are at the center of our immune system harboring a relatively high number of DCs that are in close contact with T cells. When compared with i.d. administration of antigen mRNA, i.n. administration of the mRNA was shown to be superior with regard to induction of antigen-specific T cell responses.[94,95]

The first report on intralymphatic vaccination was already published in 1977 by Juillard et al.[113] They described the induction of enhanced tumor-specific responses in dogs after intralymphatic administration of the anti-cancer vaccine. Furthermore, it was reported that vaccination by i.n. vaccine delivery required far less antigen when compared with other vaccination routes.[114,115] Moreover, biodistribution studies in mice revealed that 100-fold higher antigen doses reached the lymph nodes after direct i.n. injection compared with s.c. injection near the draining lymph node.[116] In addition, Maloy et al.[117] showed that i.n. immunization with a plasmid DNA vaccine is 100- to 1,000-fold more efficient than immunization via conventional routes. Taken together, the use of i.n. vaccination results in a remarkable reduction of vaccine dose.[118] Today, intralymphatic administration is performed in various fields where conventional routes of administration produced insufficient results or where the goal was to maximize the immune response, such as in cancer vaccines. Nowadays, several clinical trials using i.n. immunization are ongoing.[58,84,119-123]

Clinical application of vaccination with "naked mRNA". Although the above studies highlight the increasing interest in mRNA as an active, off-the-shelf pharmaceutical ingredient, only a few clinical trials based on the use of "naked mRNA" have been described (**Table 3**). Weide et al.[124,125] performed small-scale clinical trials in which total tumor mRNA or stabilized tumor antigen mRNA was delivered i.d. in addition to GM-CSF. These studies described a positive impact on the anti-tumor immune response with even a complete clinical response in one out of seven patients. More recently, Rittig et al.[126] showed that i.d. administration of mRNA encoding different tumor antigens together with GM-CSF resulted in both $CD4^+$ and $CD8^+$ responses against multiple antigens and moreover induced clinical benefit in a number of patients.

Summary

Since decades the main goal of tumor immunologists has been to increase the capacity of the immune system to mediate tumor regression. The identification of on the one hand DCs and on the other hand tumor antigens paved the way for the development of potent immunotherapeutic strategies for cancer. The use of ex vivo mRNA-modified DCs resulted

in promising immunological and clinical responses. Therefore, immunotherapy based on this strategy can be seen as a fourth cancer treatment modality in addition to surgery, chemotherapy and radiotherapy. Furthermore i.n. delivery of tumor antigen mRNA in combination with the appropriate adjuvant for instance by co-delivery of mRNA encoding immune modulating molecules such as TriMix mRNA, represents a promising vaccination strategy. Moreover, the use of mRNA and its application as an off-the-shelf vaccine represent an important step in the development of future anti-cancer immunotherapeutic strategies.

Disclosure of Potential Conflicts of Interest

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