



Potential of mRNA vaccines to become versatile cancer vaccines

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Abstract

For centuries, therapeutic cancer vaccines have been developed and tried clinically. Way back in the late 19th century, the Father of Immunotherapy, William Coley had discovered that bacterial toxins were effective for inoperable sarcomas. In the 1970s, the Bacillus Calmette-Guérin (BCG) vaccine was repurposed, *e.g.*, for advanced melanomas. Then, therapeutic cancer vaccines based on tumor-associated antigens (found on the surfaces of cancer cells) were tried clinically but apparently have not made a really significant clinical impact. For repurposed pathogen vaccines, only the BCG vaccine was approved in 1989 for local application to treat nonmuscle-invasive bladder cancers. Although the mildly toxic vaccine adjuvants deliberately added to conventional pathogen vaccines are appropriate for seasonal applications, when repurposed for continual oncology usage, toxicity may be problematic. In 2010, even with the approval of sipuleucel-T as the very first cancer vaccine (dendritic cell) developed for designated prostate cancers, it has also not made a really significant clinical impact. Perhaps more "user friendly" cancer vaccines should be explored. As from approximately 30 years ago, the safety and effectiveness of mRNA vaccination for oncology had already been studied, the current coronavirus disease 2019 pandemic, though disastrous, has given such progressively advancing technology a kickstart. For oncology, other virtues of mRNA vaccines seem advantageous, *e.g.*, rapid and versatile development, convenient modular design, and entirely cell-free synthesis, are being progressively recognized. Moreover, mRNAs encoding various oncology antigens for vaccination may also be tested with the combination of relatively non-toxic modalities of oncology treatments, *e.g.*, metformin or metronomic (low-dose, prolonged administration) chemotherapy. Admittedly, robust clinical data obtained through good quality clinical trials are mandatory.

Key Words: Cancer vaccine; Cyclophosphamide; Metformin; Metronomic chemotherapy; mRNA vaccine; Myocarditis; Tumor microenvironment

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Core Tip: Although vaccines are effective for pathogen prevention and cancers, hitherto, oncology vaccines have not yet made a very significant clinical impact. Currently, as mRNA vaccines already have a proven safety profile, it is highly appropriate to further develop the decades-old mRNA technology for oncology. Compared to other approved cancer vaccines, oncology mRNA vaccines may be more versatile, pragmatic, affordable, and effective. To combat the notoriously resistant tumor microenvironment, the probable mutual enhancement effects with, *e.g.*, metronomic chemotherapy should be fully explored, especially as no significant added toxicity is anticipated. Clearly, undertaking much more research work (especially clinical) is mandatory.

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INTRODUCTION

The term "cancer vaccine" includes vaccines, pathogen, or otherwise that induces the innate and adaptive immunities for specific purposes; as such, it does not include items like oncolytic viruses. Although cancer immunotherapy is now well recognized to be a significant modality of treatment, interestingly, way back in the 1890s, an American orthopedic surgeon, William Coley had already documented an unexpected regression of a sarcoma when a surgical wound failed to close due to wound infections. Coley hypothesized that the tumor regression may be related to the patient's febrile erysipelas infection (caused by *Streptococcus pyogenes* bacteria). Eventually, he had developed the very first cancer vaccine containing toxins from killed *Streptococcus pyogenes* and *Serratia marcescens* bacteria [1]. However, with variable successes across other patients, Coley's approach unfortunately waned in popularity especially upon the advent of radiotherapy and the then very novel cancer chemotherapy. Nevertheless, he had most remarkably pioneered the concept of bacterial toxins inducing immunity that was also effective for eradicating cancer cells. For this, he was subsequently honored as the "Father of Immunotherapy". Rather unfortunately, cancer vaccines had not subsequently made very significant clinical impacts since then. Actually, specific cellular vaccines have been made to induce satisfactory immune responses against cancer cells, for instance, autologous cell-based cancer vaccines, *e.g.*, for hematological and other cancers[2,3]. However, these may be less versatile, too time consuming to process, and too costly to exert a significant impact on a good number of cancer patients. Apparently, as the very first cancer vaccine [dendritic cell (DC)] developed for specific prostate cancers (sipuleucel-T) has also not made a really significant clinical impact, it may be appropriate to explore other more "user friendly" cancer vaccines.

TUMOR-ASSOCIATED ANTIGENS

Remarkably, cancer immunotherapy in the form of cancer therapy vaccines (that followed William Coley's discoveries) waxed and waned probably because both radiotherapy and chemotherapy were developing steadily by then. Yet, by the 1970s, the Bacillus Calmette-Guérin (BCG) vaccine had actually been repurposed for cancer therapy and tried clinically, *e.g.*, for melanoma[4]. Another form of immunotherapy involved stimulating the cancer patient's own innate and adaptive immunities using the cancer's tumor-associated antigens (TAAs) for developing cancer vaccines. Basically, TAAs are related to antigen molecules present on tumor cell surfaces, *e.g.*, embryonic proteins and glycoprotein antigens. These have been exploited to develop TAA cancer vaccines[4]. However, even though most TAAs are being overexpressed on cancer cells, they are actually not specific enough as these antigens are also expressed in normal tissues[5]. Thus, as TAAs may arise, *e.g.*, as oncofetal antigens, a peripheral tolerance may have already developed to these antigens and would thus preclude a satisfactory immune response to TAAs. Admittedly, despite the encouraging evaluation of numerous vaccine strategies targeting various tumors, the efficacy of therapeutic cancer vaccines has not been clearly demonstrated through robust clinical trials[6]. Notably, most of the tumor antigens employed for cancer vaccines were non-mutated, overexpressed self-antigens, eliciting mostly T cells having low-affinity T cell receptors (TCRs) that were deemed the most appropriate to mediate an effective anti-tumor response. Taken together, TAA cancer vaccines have not yet made a significant clinical impact on cancer control[6,7].

REPURPOSING PATHOGEN VACCINES

Repurposing pathogen vaccines for oncology has also been proposed as a feasible modality of cancer immunotherapy. Actually, even in the 1970s, the BCG vaccine had already been tried clinically, *e.g.*, for melanoma[4]. It was felt that, despite some demonstrable effect due to BCG, it did not seem to influence significantly the course of the advanced melanoma. Subsequently, pre-clinical studies of some other pathogen vaccines seemed to be more encouraging. However, it was found that possibly, one of the best applications was intratumoral administrations of pathogen vaccines to turn "cold" tumors into "hot" ones, *i.e.*, having more abundant immune cells (see Section "Tumor Microenvironment"). Admittedly, although this may be very helpful, low-dose cyclophosphamide injected more conveniently through the intravenous route, would also have a similar effect[8]. Currently, of all the pathogen vaccines, only BCG is approved in 1989 for local treatment of nonmuscle-invasive bladder cancers, even though the exact mechanism is still controversial[9,10].

Importantly, although the mildly toxic vaccine adjuvants deliberately added to conventional pathogen vaccines to boost the immune response was appropriate for seasonal application, continual oncology usage may be controversial[11]. Even though aluminum salts are the commonest vaccine adjuvants, in extreme cases, heavy metal poisoning may occur especially if very frequent administrations of these repurposed pathogen vaccines are given. Currently, the potential toxicity of aluminum is increasingly recognized[12]. Perhaps intratumoral administrations would be most appropriate, except for the fact that the mode of administration is technically more complicated[13,14]. Taken together, with repurposing of pathogen vaccines for oncology, the frequency of administration should be noted well. For instance, a study administering a weekly combination of several repurposed pathogen vaccines for lung cancer [NCT02333474] might have problems related to vaccine adjuvant toxicity.

MRNA VACCINES

Remarkably, during this coronavirus disease 2019 (COVID-19) pandemic, mRNA vaccinations have demonstrated their remarkable success and good safety profile. mRNA for incorporation into vaccines is synthesized *in vitro* to mimic the host mRNA in order to increase mRNA stability and translation efficiency[15]. Moreover, unlike conventional pathogen vaccines, mRNA vaccines are devoid of any cellular or animal components. Additionally, some mRNA vaccines do not require any adjuvants to boost their immune effectiveness[16]. As booster pathogen mRNA vaccines are often given at intervals of 5 mo or less for healthy subjects, when applied for cancer patients, repeated applications would most probably be feasible even at shorter intervals. This is especially so for those mRNA vaccines that have no added adjuvants. Of course, more robust data upon further clinical studies are required for confirmation.

DEVELOPMENT OF MRNA VACCINES

Currently, three major types of mRNA vaccines are available: (1) Conventional, non-amplifying mRNA molecules; (2) Base-modified, non-amplifying mRNA molecules incorporating chemically modified nucleotides; and (3) Self-amplifying mRNAs (saRNAs) that maintain the auto-replicative activity derived from an RNA virus vector. Thus, saRNAs would encode both the antigen and the viral replication machinery which enables intracellular RNA amplification and ample protein expression[17]. saRNAs may thus be advantageous as they maintain all the advantages of mRNA vaccines (rapid development, convenient modular design, and entirely cell-free synthesis), let alone a significantly lower dose of mRNA is now feasible, due to the self-replicating properties[17].

Admittedly, despite much work on TAA vaccines, there are still no very significant clinical impacts. On the other hand, mRNA vaccines may generate potent and protective immune responses of both cellular and humoral types. Basically, mRNA is an intermediate between the translation of protein-encoding DNA and protein production[16]. Notably, unlike pathogen vaccines, adjuvants to enhance vaccine immunity are no longer essential and so, repeated administration for oncology therapy would unlikely be problematic. Moreover, through billions of administrations of mRNA vaccines, the safety profile can be better confirmed. Lastly, it is also most unlikely to have any chance of incorporation into potential oncogenic sites within the genome[15].

REFINING MRNA VACCINES

Although pioneer mRNA vaccines (for oncology) were naked, *e.g.*, the version employed by a German group, subsequent work had appropriately enabled encapsulation in a lipid nanoparticle (LNP)[18,19]. This effectively limits detection by the innate immune system, enhances the cellular uptake of the

mRNA, and prolongs as well as enhances protein expressions. Moreover, the ionizable cationic lipid can also improve the release of mRNA from the endosome to the cytoplasm and markedly prolong protein expressions[16]. Importantly, encapsulation may also serve as a self-adjuvant purpose (see below).

For administration, they can be injected subcutaneously, intradermally, or directly into lymph nodes or tumors. Notably, the production of mRNA vaccines is potentially faster, more flexible, and less expensive and can even be used for precise and individualized therapies. During this pandemic, the rapid and safe vaccine production was clearly shown[16]. Vaccine adjuvants are usually not required as the LNP already induces an innate immune response – a self-adjuvant. With continual mRNA vaccine development, the structured 5' as well as 3' termini and the double-stranded RNA replication intermediates of saRNA vaccines would be recognized, leading to a type I interferon (IFN1) response (see below). Remarkably, this immune stimulation would serve as a self-adjuvant to increase vaccine immunogenicity[15].

Lastly, vaccine quality may be improved by nucleoside modification or complexed mRNAs, and further shaped or influenced by the choice of the delivery routes and formats, *e.g.*, LNP vaccines. It was also found that the introduction of noninflammatory modified nucleosides into the mRNA was advantageous as they induce potent T follicular helper and also germinal center B cell responses[20].

TWO MRNA VACCINES FOR COVID-19

By December 2020, two mRNA vaccines from BioNTech/Pfizer and Moderna were approved against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, representing the very first approval for any mRNA vaccines. For development, BioNTech/Pfizer had previously compared several RNA-based COVID-19 pandemic vaccine candidates in clinical studies in Germany and the US. Despite incomplete data publication of all technical details then, mRNA vaccines were known to be LNP-formulated and nucleoside-modified. Eventually, two most promising vaccine candidates were selected: BNT162b1 (encoding the SARS-CoV-2 receptor-binding domain and BNT162b2 (encoding a modified version of the SARS-CoV-2 full-length spike protein)[21]. As BNT162b2 was found to exhibit a good balance of efficacy and safety even at a low dose of 30 µg, it attained the international phase 2–3 clinical trials[22]. Approximately 44000 adults were subjected to two intramuscular injections of 30 µg of BNT162b2 (21 d apart) (NCT04368728). That regimen could confer 95% protection against SARS-CoV-2. Moreover, the titers of SARS-CoV-2-neutralizing antibodies either resembled or exceeded those found in patients who had recovered from COVID-19[16].

SAFETY ASPECTS OF MRNA FOR COVID-19

Before 2020, no mRNA vaccine had ever been approved. During this pandemic, with the authorization and approval of mRNA vaccines against SARS-CoV-2, the safety profile become better recognized. As mRNA vaccines are devoid of any cellular or animal components, they have since been shown to be generally safe and well-tolerated. Integration into the subjects' genome is deemed not possible. With storage at very low temperatures, microbial contaminations are also extremely unlikely. Serious adverse effects were few, although local pain and redness at the injection sites may occur. Rarely, systemic allergic reactions may also ensue. Besides systemic inflammatory reactions, a theoretical risk of inflammation and autoimmunity may occur, likely due to the induced IFN1 response[23]. In patients with systemic lupus erythematosus or other similar autoimmune diseases, anti-self RNA antibodies may develop and could worsen their autoimmunity[24]. Moreover, toxic side effects related to the delivery compounds or complexing agents and potentially, to inserted nucleotides may occur[16]. Although rare, a serious adverse event of myocarditis and/or pericarditis may occur especially in younger adults and adolescents, predominantly in males (12.6 cases/million doses of the second-dose mRNA vaccine) – a few days after the dose, chest pain may occur, with ECG changes, raised serum cardiac troponin levels, and myocarditis features on MRI. Although the mechanism is unclear, it mostly resolves spontaneously [25]. Obviously, many more long-term clinical studies on a wider population spectrum are mandatory.

EFFECT OF EXOGENEOUS MRNA ON IMMUNITY

Upon confirmation that exogenous mRNA is being processed as for any endogenous mRNA, for more efficient T cell activation, a costimulatory signal was found to be helpful for inducing a better immune response with consecutive cytokine production[26]. Naturally, DCs would express these costimulatory signals (such as B7 molecules) after sensing pathogen-associated molecular patterns (PAMPs) that indicate microbial infection or imminent danger. Pharmacologically, this can be achieved by exploiting toll-like receptor (TLR) ligands[27]. TLRs are related to the innate immune system's ability to detect PAMPs. Induction of IFN1 by viruses or other pathogens is indispensable for innate immune responses

and would thus confer anti-microbial activities[28]. Upon sensing PAMPs, an immediate innate inflammatory response (including IFN1 induction) is initiated. Exogenous mRNAs are likewise sensed by TLRs and double-strand RNAs can induce a strong IFN1 response. Eventually, clonal expansion of antigen-specific B and T cells results in target cell elimination. Although this may be less complicated for infection prophylaxis, significant problems abound for effective control of advanced cancers. Conceptually, resistant cancers often have immunosuppressive tumor microenvironments (TMEs) through recruiting myeloid-derived suppressor cells (MDSCs), regulatory T cells, macrophages, *etc.*, to the TME, let alone the production of immunosuppressive cytokines. Hence, very much more robust modalities of treatments than infection prophylaxis are called for (see Section "Tumor Microenvironment").

PIONEERING MRNA TECHNOLOGY FOR ONCOLOGY

Remarkably, even as early as 1995, the feasibility of using mRNA technology for oncology was noted [29], with mRNA transcripts encoding luciferase and human carcinoembryonic antigen (CEA) then. Subsequently, DCs were transfected with either mRNA encoding TAAs[4] or mRNA technology was employed for *in vivo* induction of T cell immunity[30]. Notably, DCs could utilize mRNA encoded TAAs for the induction of anti-cancer immunity[31]. Throughout the decades, more and more research work had validated the feasibility and possible efficacy of mRNA vaccination for immunotherapy against cancers.

Eventually, knowledge on mechanisms involved in innate and adaptive immune sensing ensued. Moreover, various novel approaches of mRNA delivery and complexing of the vaccine could be implemented. Undoubtedly, these may have paved the way to current successful clinical trials on COVID-19. Even for oncology mRNA vaccines, these may be generated using *ex vivo* loaded or electroporated DCs, usually with a known carrier. DCs are then isolated and subsequently transfected with mRNA encoding, *e.g.*, TAA(s) before re-infusion into the patient. For instance, transfection by electroporation has been found to be safe for cancer patients[32]. DCs electroporated with mRNA encoding ovalbumin or tumor-derived mRNAs can actually generate robust tumor-specific immune responses in different murine melanoma models and even in patients undergoing vaccination trials (see Table 1).

EARLY CLINICAL TRIALS OF MRNA FOR ONCOLOGY

In 2002, oncology mRNA vaccines for enhancing immunity were reported to be useful for a patient with a carcinoembryonic antigen-expressing adenocarcinoma[33]. As mRNA was known to be basically a copy of the coding genomic information, it was thus found to be useful for the expression of therapeutic proteins[18]. In mice, naked mRNA coding for tumor antigens was administered by injecting intradermally. Most interestingly, it resulted in protein expression as an immune response. Subsequently, the same protocol was applied to 15 melanoma patients in the first phase I/II trial and found to be safe[19]. Notably, some patients even had an increase in antitumor humoral immune response. After the injection of the mRNA cancer vaccine, the encoded protein was translated and presented to the immune system – closely resembling the natural course of a viral infection and its consecutive induction of a protective immune response. Importantly, upon entering to the cytoplasm, the exogenous mRNA had been found to be processed as for any endogenous mRNA[16].

THERAPEUTIC MRNA VACCINES: INJECTION SITES

Although pathogen mRNA vaccines are nearly always given intramuscularly, there are various other different routes for therapeutic mRNA vaccines. These other injection sites may impact on the induced immune response. As the human skin has many antigen-presenting cells (APCs), especially interstitial DCs in the dermis[34], after intradermal injection, exogenous mRNAs are taken up and locally expressed by ample APCs there. Despite scanty immune cells in muscles, circulating immune cells would eventually reach the injection site to process and present the antigen locally. This is just like the expected actions caused by traditional pathogen vaccine adjuvants. It is where the usual local inflammatory reaction at injection sites promotes significant immune cell activities[16]. Even as technical details are beyond the scope of this article, upon local injection of mRNA vaccines, mRNAs will eventually be processed by APCs reaching there and antigen-specific CD8+ T cells are induced.

IMMUNOGENIC CELL DEATH

It is increasingly recognized that the cancer cell killing by chemotherapy (ChT) is not just by direct

Table 1 Selected national registered clinical trials on combination mRNA oncology vaccines^a

mRNA vaccine	I.S.	Combo agent	Ph	Cancer	Oncol status	Yr	Country	Trial status	NCT number
mRNA-2752	i.t.	+ durva	1	Solid ca, lymph	R/R	2018	United States	Recruiting	03739931
BI 1361849	i.d.	+ durva +/- treme	1/2	NSCLC	Adv	2017	United States	Completed	03164772
mRNA-4157	i.m.	+/- pembro	1	Solid ca	Resected	2017	United States	Recruiting	3313778
mRNA-5671/V941	i.m.	+/- pembro	1	NSCLC/ CRC/ pancCA	Adv	2019	United States	Not yet recruiting	03948763
TriMix ^b	i.t.	Neoadj ChT +/- TriMix ^b	1	Breast	Early	2018	Belgium	Recruiting	03788083
W_ova1	i.v.	+ neoad + adj ChT	1	Ovarian ca	Early	2019	Nether-lands	Recruiting	04163094
W_pro1	i.v.	+/- cemip	1/2	mCRPC	Adv	2020	United States	Recruiting	04382898
Trivalent DCs ^c	i.d.	TMZ/RT +/- DCs	2/3	GBM	Post-op	2018	Norway	Recruiting	03548571
PSCT19 ^d	i.v.	allo-SCT +/- PSCT19 ^d	1/2	Hemat	Post- allo-SCT	2015	Nether-lands	Completed	02528682
WT1 DC	i.d.	adj TMZ +/- WT1 DC	1/2	GBM	Post-op	2016	Belgium	Recruiting	02649582
pp65 DC ^e	i.d.	adj TMZ +/- pp65 DC ^e	2	GBM	Post-op	2015	United States	Recruiting	02465268
pp65 DC ^f	i.d.	+/- varli	2	GBM	Post-op	2018	United States	Recruiting	03688178
RO7198457	i.v.	+/- pembro	2	Melanoma	Adv	2019	United States	Not yet recruiting	03815058
RO7198457	i.v.	+/- atezo	1	Solid tumors	Adv	2017	United States	Not yet recruiting	03289962

^aFor combinations with therapeutic mRNA vaccines, in principle, the best candidates are those without immune suppressive properties, *e.g.*, while maximum tolerated dose chemotherapy (ChT) may suppress immunity induced by mRNA vaccines, ironically, mChT could have the opposite effect of priming resistant tumors to be more responsive ones[8,63].

^bCD40L, CD70, and constitutively active toll-like receptor 4.

^cDendritic cells (DCs) transfected with mRNA of neoantigens (survivin, hTERT) and autologous tumor stem cells.

^dPSCT19: MiHA-loaded PD-L-silenced DC Vaccination.

^epp65-shLAMP mRNA (autologous) DCs with GM-CSF.

^fHuman CMV pp65-LAMP mRNA-pulsed autologous DCs. Adj: Adjuvant; Adv: Advanced; Allo-SCT: Allogeneic stem cell transplantation; Atezo: Atezolizumab; Ca: Cancer; Cemip: Cemiplimab; ChT: Chemotherapy; CRC: Colo-rectal cancer; Durva: Durvalumab; GBM: Glioblastoma multiforme; Hemat: Hematological malignancies; i.d.: Intradermal; I.S.: Injection site; i.t.: Intratumoral; Lympho: Lymphoma; mCRPC: Metastatic castration-resistant prostate cancer; MTD: Maximum tolerated dose; Neoadj: Neoadjuvant; NSCLC: Non-small-cell lung cancer; Oncol: Oncology; PancCA: Pancreatic cancer; Pembro: Pembrolizumab; Ph: Phase; Post-op: Post-operative; R/R: Relapsed/residual; RT: Radiotherapy; SCT: Stem cell transplant; TMZ: Temozolomide; Trema: Tremelimumab; Varli: Varlilumab.

cytotoxicity, but also by restoring immunity primed by the mechanism of immunogenic cell death (ICD). Intriguingly, dying cancer cells may be immunogenic provided that they emit a set of immunostimulatory signals inducing an activation of intracellular stress response pathways. As the phenomenon of ICD has already been described elsewhere, it is not repeated in this perspective article[35,36]. Briefly, ICD is characterized by cancer cell killing through cell-surface translocation of calreticulin (CRT), extracellular release of ATP and high mobility group box 1 (HMGB1), as well as stimulation of IFN1 responses. For ICD, emission of signals or "damage-associated molecular patterns" (DAMPs) is required. It is akin to a significant quantity of specific cancer cell death debris that may induce strong immune effects. Although ICD is a very attractive oncology phenomenon, maximum tolerated dose chemotherapy (MTD ChT) may have suppressed much of the immunity so induced, and metronomic chemotherapy (mChT) is preferred[37,38]. Moreover, certain mChT agents, *e.g.*, cyclophosphamide, also induce ICD itself (see Section "Combining mRNAs with Metronomic Chemotherapy"). Notably, the tumor infiltrating lymphocytes (TILs) are also modulated and would reactivate antitumor immunity within the notorious and immuno-suppressive TME.

TUMOR MICROENVIRONMENT

Most advanced cancers would deliberately produce a TME to disable and evade the body's immunity. The TME is now well recognized to be the main culprit for the vast majority of cancer resistance. With a serious lack of essential nutrients, *e.g.*, glucose, and oxygen, infiltrating immune cells are thus starved in this deliberately hostile environment[39]. Yet, cancer cells in the TME manage to survive readily through consuming minimal nutrients. Moreover, they also can manage by-products to their own advantage, *e.g.*, lactic acid which can reduce immune cell functions to the cancer cells' advantage. Taken together, the TME is most elusive and resilient and has various "plan Bs" and "plan Cs" to enable an almost intractable resistance to most conventional oncology treatments, especially immunotherapy, except perhaps, some immune checkpoint inhibitors (ICIs, see below). To tackle such TMEs, a multi-prong approach is most appropriate.

Notably, tumors having a robust TME may also be described as "cold" tumors, being unresponsive to most oncology treatments, whereas "hot" tumors are the exact opposite. Now, various innovative methods may be required to render such "cold" tumors into "hot" ones (having abundant immune cells). Notably, intratumoral mRNA vaccines might turn "cold" tumors into "hot" ones[40]; similarly, low-dose intravenous cyclophosphamide has also been found to act likewise[8]. This mechanism may be very useful for resistant tumors as there may be a much desired effect of "mutual enhancement" to tackle tumors which would go hand-in-hand with very evasive TMEs.

COMBINING MRNA VACCINES WITH IMMUNE CHECKPOINT INHIBITORS

In this era of cancer immunotherapy, ICIs have been widely applied for managing cancer patients. Although ICIs do not share similar toxicities with cancer ChTs, ICIs have their own disadvantages as has been discussed elsewhere[36]. Briefly, the response rates are too low and the adverse effects (mostly autoimmune related) may also be significant, so much so that patients with pre-existing autoimmune disorders are deprived of the benefits of ICIs. Realistically, the majority of cancer patients would not derive any benefit from ICIs. Moreover, the "one size fits all" dosage commonly approved for ICI prescriptions may be associated with higher adverse effect rates, especially in Asians who usually have smaller body builds than Caucasians. Although the combination with mRNA cancer vaccines might be beneficial [NCT03948763 (see Table 1)], *e.g.*, to raise the response rates, as both modalities of treatments are immune related, whether immune-related adverse effects might be even more common would require careful documentation, even as the higher cost of such combinations could be ignored.

COMBINING MRNAS WITH METRONOMIC CHEMOTHERAPY

Recently, the advantages of using mChT as one of the ways to patch up immunotherapy deficits have been detailed elsewhere[36]. Briefly, mChT agents not only act akin to targeted therapy agents but are also much less toxic than MTD ChTs so that they would not suppress immunity generated by combination cancer immunotherapy agents. Actually, as some ChT agents have the ICD phenomenon, immunity is enhanced (see Section "Immunogenic Cell Death"). Although MTD ChT has been designed to achieve maximum cancer cell killing, such very high dosages would likely suppress any immunity so generated, be it by mRNA vaccines or by the ICD phenomenon. Thus, as mChT usually does not suppress immunity, it is more appropriate for these combinatory purposes.

Intriguingly, mChT, *e.g.*, very short courses of intravenous low-dose cyclophosphamide, may ironically have a useful action of turning "cold" tumors "hot"[8]. For cyclophosphamide, the personal experience[36] and others[42] tally with such an action, even though the mechanism was entirely unknown decades ago. Importantly, the current evidence is on enhancing immunity mainly by modifying regulatory T cells (Tregs). mChT may even prime "cold" tumors into "hot" ones (see Section "Tumor Microenvironment"). Coincidentally, mRNA vaccines can also act likewise[40] so that there would now be a most desirable mutual enhancement effect. Such combinations are highly worth exploring further, especially as currently, mRNA vaccines may become a potential oncology breakthrough – thus, mChTs with ICD mechanisms[36] would work hand-in-hand with mRNA vaccines for the desired mutual enhancement effect. Although far too few clinical trials have been done on its combination, the remarkable safety profile of mChT is advantageous as no untoward toxicities are expected upon the combination.

COMBINING MRNA VACCINES WITH METFORMIN

Another similar agent deemed suitable for combination with mRNA vaccines is metformin. It has a similarly good safety profile as mChTs[43]. The details have already been reviewed elsewhere[41].

Briefly, despite its discovery around 100 years ago as an anti-diabetic, it is recently known as an agonist of the adenosine monophosphate-activated protein kinase (AMPK) that inhibits the mammalian target of rapamycin (mTOR), especially as mTOR is activated in cancer cells and would even convey drug resistance[44]. Metformin also has an ability of preferentially targeting cells that have abnormal or altered glycolysis, including cancer associated fibroblasts (CAFs). These cells may thus be rendered more susceptible than other cells to the action of cisplatin ChT[45]. This is valuable as CAFs play a vital role in the TME, currently deemed to be the worst culprit for cancer resistance.

Importantly, metformin can actually eradicate cancer stem cells, a pivotal aspect of cancer therapy, but conventional MTD ChT agents can hardly do so[46]. Moreover, MDSCs, being a main player of the TME[47], are also targeted by metformin[48,49]. For usually resistant cancers, *e.g.*, basal breast cancers, pre-clinical studies showed that a combination of metformin and a targeted therapy (erlotinib) could have encouraging results[50]. Thus, apart from observational and preclinical studies revealing metformin's activities on various cancers, it may now be worthwhile to undertake clinical trials (Figure 1). On the safety profile, despite being an anti-diabetic agent, hypoglycemia is hardly a significant problem, unlike most other anti-diabetics. Actually, over many decades, it has proven to be well tolerated and safe.

DISCUSSION

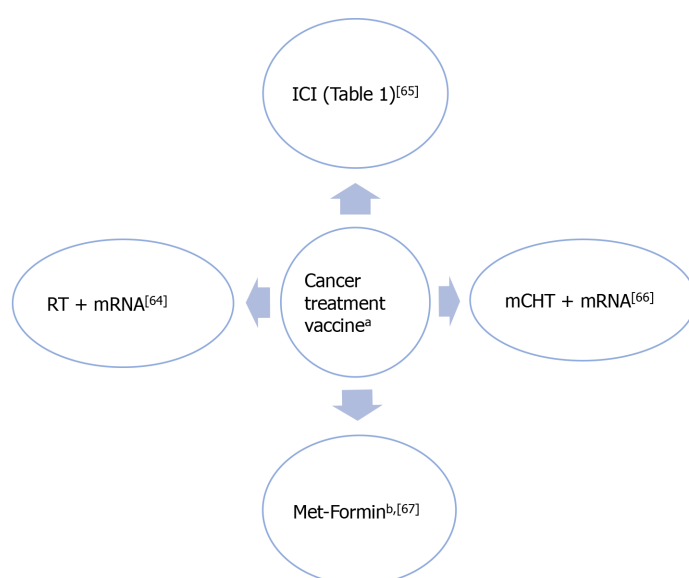
Although mRNA vaccines have already been tried clinically for oncology even two decades ago, the implementation for oncology has obviously been lagging behind. Actually, there has been significant technical advancements[51,52]. The current COVID-19 pandemic, though most disastrous, has ironically provided a good platform to highlight the safety profile of mRNA vaccines when the nucleoside-modified mRNA-LNP vaccines have a remarkable safety track record[53]. Actually, mRNA-LNPs can induce superior T follicular helper cell responses than that of an adjuvanted protein subunit vaccine even though the exact mechanism is still unclear. Moreover, although conventional pathogen vaccines usually require adjuvants to boost the much desired immunity, LNPs readily act as self-adjuvants[54]. Repeated oncology administrations would thus be facilitated, as there is hardly any issue of possible toxicity due to vaccine adjuvants typical of repurposed pathogen vaccines.

Importantly, mRNA vaccines represent a promising platform for the development of oncology vaccines as they can induce potent T cell responses and can also be readily modified[55]. Moreover, as mRNA vaccine design is highly flexible, it would enable the development of personalized neoantigen cancer vaccines, unless the cost becomes a significant concern, *e.g.*, during the current severe economic recession. As various aspects of novel developments, pivotal considerations, as well as current challenges for successful development of the self-amplifying RNA (saRNA) vaccines are already fully discussed elsewhere, suffice it to say that, even though the saRNA is very remarkable for enabling lower vaccine doses, the stability and manufacturing may still be challenging[17,56-59]. Encouragingly, it is now feasible to design very promptly a new saRNA vaccine for testing, as in the case of the Imperial College London[59]. The rapid and easy manufacture of saRNA vaccines could enable local productions so as to reduce logistical and cold-chain issues of current mRNA vaccines. Importantly, minimizing the required dose is highly desirable as it reduces side effects, *e.g.*, myocarditis and permits repeated usage for oncology practice.

Although testing of new modalities of oncology treatments often involve advanced cancers, the TME is actually very well known to be a major factor preventing successful testing of treatment options designed to cater for advanced cancers[60]. It would be more appropriate to test clinically these novel agents without the interference of the TME. For instance, for advanced melanomas, a recent randomized phase II clinical trial was on the efficacy of autologous DCs co-electroporated with mRNA coding for TriMix as well as mRNA encoding one of four TAAs linked to one HLA class II targeting signal (TriMixDC-MEL) (see Table 1)[61]. The randomization involved 41 patients (21 receiving TriMixDC-MEL; 20 had placebo). All patients had stage III/IV melanomas but no evidence of any residual disease (after resecting all macro-metastases). The vaccine was found to be tolerable and the 1-year disease free survival rate was 71% for the TriMixDC-MEL arm *vs* 35% of the placebo arm[61]. Admittedly, although not all melanoma metastases could likewise be resected, this trial would still demonstrate the vaccine's tolerability and probable effectiveness. This could not have been accomplished had the trial been performed on patients with significant TMEs.

CONCLUSION

The future development of mRNA vaccines for oncology is two pronged. On the one hand, as neoantigens of cancer cells are often dissimilar among individual patients, personalized vaccines are most appropriate, *e.g.*, the intranodal vaccine injection with free mRNA encoding 10 neoepitopes on 13 advanced melanoma patients could generate T cell immunity against multiple neoepitopes in all 13 patients[56,62]. Several personalized cancer vaccines using lipid nanoparticle-mRNA formulations have



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Figure 1 Selected combinations with cancer treatment vaccines: Immune checkpoint inhibitors, radiotherapy, metronomic chemotherapy, and metformin. ^aEspecially mRNA cancer vaccines: cell-free, rapid production, versatile and inherent adjuvant properties outperforming pathogen vaccines repurposed for oncology. Even balancing innate and adaptive immunities is feasible with mRNA. ^bMetformin's long standing safety track record, ready availability and eminent affordability may enable an ideal combination with mRNA cancer vaccines. ICI: Immune checkpoint inhibitors; mCHT: Metronomic chemotherapy; RT: Radiotherapy.

also entered clinical trials, *e.g.*, mRNA-4157 is being tried actively both as monotherapy and in combination with ICIs (see [Table 1](#)).

On the other hand, such most impressive personalized oncology treatments, though much more specific, probably effective, and now with reduced processing time than other personalized vaccines, may not be readily affordable for the vast majority of cancer patients especially at this very trying period of severe economic recession. Therefore, for priming tumors having highly evasive TMEs, combination chemotherapy, radiation, and vaccines may have better efficacy[63]. As there may even be a highly beneficial mutual enhancement effect of turning "cold" tumors into "hot" ones[8,40], it really pays to explore further by performing robust clinical trials to document if such combinations have the potential of being a more versatile approach.

FOOTNOTES

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