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# Next Generation Immunotherapies – Emerging Strategies for Immune Modulation against Cancer, Infections, and Beyond

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Our special issue entitled "Next Generation Immunotherapies" focuses on emerging strategies for immune activation, modulation, and tolerance in the context of immunotherapy.

Cancer immunotherapy, including immune checkpoint blockers (ICBs) and chimeric antigen receptor (CAR) T-cell therapy, has revolutionized how we treat cancer patients. Cancer immunotherapy aims to establish anti-tumor immunity for eradication of cancer. Despite their wide success in the clinic, there are still many critical issues to address. "Cold" tumors that lack tumor-infiltrating T-cells do not respond well to ICBs. In addition, ICBs trigger immune-related adverse events in some patients with dose-limiting toxicities. While CAR T-cell therapy is potent against hematologic cancers, their efficacy is generally poor against solid tumors due to limited T-cell infiltration. In addition, production of CAR T-cells is not trivial, thus presenting additional challenges. Hence, new approaches are urgently needed to improve and expand our armamentarium against cancer.

Moreover, since late 2019, a novel coronavirus, termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has caused unprecedented havoc throughout the world. This triggered worldwide efforts to develop therapies and vaccines against SARS-COV-2, and fortunately, we now have several vaccines that are approved for emergency use. However, there is still more room for improvement as new SARS-COV-2 variants are threatening our progress toward normalcy. In this regard, next generation immunotherapies and vaccines are urgently needed to fight against SARS-COV-2 as well as against other emerging pathogens.

In this special issue of Next Generation Immunotherapy, we highlight emerging strategies and new opportunities to design and improve immunotherapies against various human pathologies. This special issue presents 21 outstanding contributions, which include 12 research articles and 9 review articles. Briefly, these articles report novel immunotherapies, vaccines, immunostimulatory agents, synthetic antigen-presenting cells, and engineered cytokines for improving the efficacy and safety of cancer immunotherapies. We also present how drug delivery platforms designed to either target lymphoid tissues or interact favorably with human blood proteins impact cellular and humoral immune responses. We also discuss new immunotherapies against SARS-COV-2, antibody engineering for neutralizing SARS-COV-2 variants, as well as innovative approaches for vaccination against high priority infectious pathogens.

First, emerging strategies for cancer immunotherapy and cancer vaccination are presented in the following series of research and review articles.

Prof. Yu-Kyoung Oh and co-workers from Seoul National University (article number 2000288) have developed a new nanoadjuvant based on imiquimod incorporated into amphiphilic peptide-based micelles. Using this system, they have demonstrated anti-tumor effects of external photosensitizer-free phototherapy combined with nanoadjuvant strategy against melanoma. Melanin-enriched B16F10 melanoma cells showed photothermal effects under irradiation of near-infrared wavelength light. The combination of photosensitizer-free phototherapy with a peptide micelle nanoadjuvant induced tumor-infiltrating $CD8^+$ T cell responses and prevented B16F10 lung metastasis, thus showing its potential as a melanoma vaccine.

Prof. Suzie Pun and co-workers from the University of Washington (2100005) have developed a cationic polymer-lytic peptide conjugate (VIPER), which is a polyplex subunit vaccine composed of conjugated peptide antigens and electrostatically complexed poly(I:C) nucleic acid adjuvant. VIPER delivered peptide antigens intracellularly, disrupted endosomes in antigen-presenting cells (APCs) in vitro, and generated strong antigen-specific cytotoxic T cell responses in melanoma-bearing mice. They have shown that VIPER's lytic bioactivity augments cellular immune responses by inducing local cell death.

Prof. In-San Kim and co-workers from Korea Institute of Science and Technology (2100025) have reported a new combination therapy that sensitized anti-programed cell death protein-1 (PD-1) antibody-resistant tumors to immune checkpoint blockade. They devised an oral delivery system to improve the bioavailability of simvastatin (SIMVA). A colloidal dispersion (CD) of SIMVA was prepared using N $\alpha$ -deoxycholyl-l-lysyl- methylester (DL) to enhance its solubility and permeation. The resulting SIMVA/DL-CD exhibited significantly increased oral absorption and oral

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bioavailability, compared to that of free SIMVA. SIMVA/DL-CD decreased CT26 tumor growth in mice, and the combination of oral SIMVA/DL-CD and oxaliplatin powder formulation exerted robust anti-tumor effect with strong $CD8^+$ T cell immunity and sensitized tumors to anti-PD-1 immune checkpoint blockade therapy.

Prof. Yong Taik Lim and co-workers from Sungkyunkwan University (2100026) have presented a review article that presents recent advances in cytokine-based cancer immunotherapy. They discussed exogenous cytokine delivery, focusing on new technologies that reduce systemic leakage and increase the local concentration of recombinant proteins. They were categorized as recombinant proteins delivered via targeting moiety-fused cytokines, cytokine-producing or backpacked engineered cells, or cytokines encapsulated or anchored on delivery systems. They have also presented technologies that promote endogenous cytokine production via the delivery of cytokine genes or cytokine-inducing immunomodulators.

Prof. Darrell Irvine, Prof. Paula Hammond, and co-workers from Massachusetts Institute of Technology (2100035) have presented a review article that covers the development and delivery of cytokines for cancer immunotherapy. A diverse array of cytokines has been studied in preclinical disease models since the 1950s, some of which became successful biopharmaceutical products with the advancement of recombinant protein technology in the 1980s. However, following these early approvals, clinical translation of these natural immune signaling molecules has been limited due to their pleiotropic action in many cell types, poor pharmacokinetics, and dose-limiting toxicities. They have focused on challenges that these immunomodulatory proteins present for clinical translation and engineering strategies designed to overcome these issues. The latter includes protein engineering, polymer conjugation, encapsulation of cytokines in polymeric matrices, and particle- mediated cytokine delivery. Further, they have discussed limitations of animal models such as the altered immune populations and receptor pharmacology that can lead to altered responses in humans.

Prof. Jian-Qing Gao and co-workers from Zhejiang University (2100030) have presented a review article that discussed applications of mesenchymal stem cells (MSCs) as the cellular carrier for tumor-targeting therapy. MSCs are one of the most studied stem cells in tissue repair and regeneration. Their tissue-homing capability and non-immunogenic properties make MSCs an attractive disease-targeted delivery platform. The authors have summarized the immunomodulating capability of MSCs and their influences on tumor progression and metastasis. They have also discussed the advantages and potential risks of MSCs focusing on their modulation of immune responses and factors determining their immune properties. This review provides a guidance for the rational design and proper application of MSCs-based targeting delivery system for cancer immunotherapy.

Prof. Gabriel Kwong and co-workers from Georgia Institute of Technology (2100034) have reported a new engineered platform for activation of antigen-specific T cells for adoptive cell therapy. In contrast to traditional methods that rely on non-specific activation of T cells by $\alpha$CD3/$\alpha$CD28, they have developed an approach that uses liposomes decorated with pMHC molecules, called synthetic antigen-presenting cells (synAPCs), for activation of antigen-specific T cells. They have demonstrated that synAPCs selectively targeted and activated antigen-specific T cells to levels similar to conventional protocols using $\alpha$CD3/$\alpha$CD28 without the need for co-stimulation. T cells treated with synAPCs produced effector cytokines and exhibited cytotoxic activity when co-cultured with tumor cells presenting target antigen in vitro. Following adoptive transfer into tumor-bearing mice, activated cells controlled tumor growth and improved overall survival, compared to untreated mice, thus showing the potential of synAPCs.

Prof. Betty Kim and co-workers from The University of Texas MD Anderson Cancer Center (2100046) have presented a review article that provides an overview on the current and emerging immunotherapies against glioblastoma (GBM). Developing an efficient immunotherapy for GBM requires understanding the glioblastoma immune microenvironment. The authors introduced the complex biology of GBM and outline challenges for developing effective therapeutics. They have summarized the current immunotherapy management for GBM and advanced studies, including checkpoint inhibitors, cell vaccines, and extracellular vesicle–based immunotherapy. They have also introduced GBM immunotherapy combined with other classical cancer therapies, especially chemo-immunotherapy, radiotherapy-immunotherapy, and the combination of gene therapy and immunotherapy and discussed the promise and challenges of developing effective immunotherapies against GBM.

Prof. Li Tang and co-workers from Ecole Polytechnique Federale de Lausanne (2100065) have reported the development of a new nanocluster for stimulation of the stimulator of interferon genes (STING) pathway. $Mn^{2+}$ ions have been recently discovered to directly activate the cyclic GMP-AMP (cGAMP) synthase (cGAS) and augment cGAMP-STING binding affinity. The authors have developed a PEGylated manganese(II) phosphate (MnP-PEG) nanocluster that potently stimulated the cGAS-STING pathway. Intratumoral administration of MnP-PEG nanoclusters markedly enhanced tumor infiltration as well as maturation of DCs and macrophages and promoted activation and cytotoxicity of T cells and natural killer cells in the tumor. MnP-PEG nanocluster in combination with anti-PD-1 immune checkpoint inhibitor led to significant tumor regression in the B16F10 murine melanoma model without any overt toxicities.

Prof. Jiahe Li and co-workers from Northeastern University (2100066) have reported the development of a new protein-based platform for the intracellular delivery of cyclic dinucleotides (CDNs), which are natural STING agonists. Compared with synthetic drug delivery vehicles, protein-based carriers represent an attractive delivery platform owing to their biocompatibility, amenability to genetic engineering, and intrinsic capacity to form well defined structures. Thus, the authors have focused on protein-based carriers and engineered a delivery system based on STING for intracellular delivery of CDNs. Formed by genetic fusion with a protein transduction domain, the recombinant STING platform penetrated cells and enhanced the delivery of CDNs in a mouse vaccination model and a syngeneic mouse melanoma model. Moreover, they have shown that the STING platform restored the STING signaling in a panel of lung and melanoma cell lines with impaired STING expression. This STING-based protein delivery platform may be useful for STING-silenced tumors and STING-based vaccine adjuvants.

Prof. Ashish Kulkarni and co-workers from University of Massachusetts at Amherst (2100086) have developed novel lipid nanoparticles (LNPs) for co-delivery of TLR7/8 agonists and SHP2 inhibitors to tumor-associated macrophages (TAMs). TAMs in tumors predominantly exhibit M2 tumor-aiding phenotype, and cancer cells overexpress CD47 that interacts with signal-regulating protein $\alpha$ (SIRP$\alpha$) expressed on macrophages and inhibit phagocytosis. To address these issues, they devised a LNP system co-loaded with amphiphilic R848-cholesterol (a TLR7/8 agonist) in its hydrophobic lipid bilayer and SHP099 (SHP2 inhibitor) in the hydrophilic core. In vitro studies showed that LNPs system repolarized M2 macrophages to M1 and enhanced their phagocytic potential. In vivo efficacy studies in 4T1 tumor-bearing mice showed that LNPs exhibited superior antitumor efficacy, compared with other control groups. Thus, their work showed that LNP-mediated co-delivery of TLR7/8 agonist and SHP2 inhibitor to TAMs may serve as a macrophage-targeted immunotherapy.

Prof. James Moon and co-workers from University of Michigan at Ann Arbor (2100093) have reported the development of a new nano-immunotherapy for personalized cancer immunotherapy. Photothermal therapy (PTT) and neoantigen cancer vaccine each offers minimally invasive and highly specific cancer therapy; however, they are not effective against large established tumors due to physical and biological barriers that attenuate thermal ablation and abolish anti-tumor immunity. The authors have shown that spiky gold nanoparticle-based PTT and synergistic dual adjuvant-based neoantigen cancer vaccine, each used as a single agent, efficiently regressed small tumors ($\approx$50 mm$^3$), but they were not effective against large tumors (>100 mm$^3$) due to limited internal heating and immunosuppressive tumor microenvironment. When PTT and neoantigen vaccination were combined, PTT sensitized tumors to neoantigen cancer vaccination by destroying and compromising the TME via thermally induced cellular and molecular damage, while neoantigen cancer vaccine reverted local immune suppression induced by PTT and shaped residual TME in favor of anti-tumor immunity. The combination therapy efficiently eradicated large local tumors and exerted strong abscopal effect against pre-established distant tumors with robust systemic anti-tumor immunity, thus showing the promise of PTT combined with neoantigen cancer vaccination.

The following series of research and review articles have focused on platform technologies that can target lymphoid tissues, interact with human blood proteins, or modulate the immune system for potential therapeutics against cancer, autoimmune diseases, and others.

Prof. Nicole Steinmetz and co-workers from University of California at San Diego (2100014) have reported the development of a single-dose vaccination platform targeting cholesterol checkpoint proteins. They have developed a trivalent vaccine candidate targeting proprotein convertase subtilisin/kexin-9 (PCSK9), apolipoprotein B (ApoB), and cholesteryl ester transfer protein (CETP). Vaccine candidates were developed using bacteriophage Q$\beta$-based virus-like particles (VLPs) displaying antigens of PCKS9, ApoB, and CETP, respectively. The delivery of the trivalent vaccine candidate via slow-release PLGA:VLP implants produced robust antibody response against the cholesterol checkpoint proteins, leading to the reduction in PCSK9 and ApoB levels in plasma, inhibition of CETP, and decrease in the total plasma cholesterol in mice.

Prof. Zhiping Zhang and co-workers from Huazhong University of Science and Technology (2100032) have presented a review article on the topic of nitric oxide (NO) based immunotherapies. NO is closely involved in the cardiovascular, immune, and central nervous system. NO not only participates in the proliferation, differentiation and activation of immune cells but also regulates the secretion and function of immune-related factors in the immune system. NO has a wide range of chemical activities and biological functions, and the pleiotropy of NO is affected by its production, concentration and duration, target cell types and subtypes, species, and pathogenesis of the immune-related diseases. Their review summarized the regulatory functions exerted by NO in immune response, and the pathological mechanism of tumors, autoimmune diseases and pathogenic infections in which NO is involved. The authors also discussed the application and trend of therapeutic strategies based on the direct regulation of NO in immunotherapy against cancer, autoimmune diseases, and infection.

Prof. Xun Sun and co-workers from Sichuan University (2100056) have presented a review article that provides an overview on lymphoid tissue-targeted immunotherapies for immune tolerance. Lymphoid tissues play integral roles in initiating immune activation and immunoregulation. Enormous efforts have driven the exploration of targeting delivery of tolerogenic agents to lymphoid tissues to reverse autoimmune disorders. The authors have introduced various tolerogenic therapies for autoimmune diseases, highlight the mechanisms of action from various lymphoid tissues to appropriate cell types by different administration routes, and discuss examples of lymphoid tissue-targeting strategies to improve tolerogenic therapy potency. They have summarized lymph nodes-targeting strategies for tolerance induction after interstitial or intra-lymph node injection and described liver and spleen-mediated tolerance after intravenous administration. They have also discussed oral tolerance-based therapies.

Prof. Evan Scott and co-workers from Northwestern University (2100062) have investigated and reported how nanocarrier morphology and surface chemistry affected adsorption of proteins from human blood on the surfaces of nanocarriers. They have synthesized a library of nine soft PEGylated nanocarriers that differed in their combination of morphology (spheres, vesicles, and cylinders) and surface chemistry (methoxy, hydroxyl, and phosphate). Their quantitative analyses based on label-free proteomic techniques revealed that specific combinations of surface chemistry and morphology adsorbed unique protein signatures from human blood, resulting in differential activation of complement and elicitation of distinct pro- inflammatory cytokine responses. Furthermore, the nanocarrier morphology was shown to primarily influence uptake and clearance by human monocytes, macrophages, and dendritic cells. This comprehensive article provides mechanistic insights into rational design choices that impact the immunological identity of nanocarriers in human blood, which can be leveraged to enhance drug delivery vehicles for precision medicine and immunotherapy.

Prof. Bruno de Geest and co-workers from Ghent University (2100079) have developed lipid-PEG (PEG: poly(ethyleneglycol)) amphiphiles as well-defined amphiphilic carriers for small

molecule TLR7/8 agonists and studied lymph node-targeted delivery and immune responses. They have reported that both the nature of the lipid as well as the alkyl chain length have a dramatic influence on cellular uptake and delivery to lymph nodes. Whereas shorter dioctyl lipids showed poor performance, larger dioctadecyl lipids were prone to solubility issues. Didodecyl lipids with intermediate length were on par with cholesteryl-PEG conjugates in vitro and in vivo, in terms of lymphatic delivery. Immunization with a model antigen combined cholesteryl-PEG-imidazoquinoline induced immune responses that were qualitatively different from Montanide-adjuvanted antigen, characterized by effector $CD8^+$ T cells with cytotoxic potential and robust $IgG_2$ antibody responses. These studies showed the crucial roles of adjuvants in vaccine applications.

Prof. Willem Mulder and co-workers from Technische Universiteit Eindhoven (2100083) have presented a comprehensive review on natural apolipoprotein A1 (apo-A1)-based immunotherapies. Apo-A1 is a helical, amphipathic macromolecule and the main constituent of high-density lipoprotein. ApoA1 interacts specifically with innate immune cells, such as monocytes and macrophages, to collect and transport fatty molecules throughout the body. Thus, apoA1 is a compelling elementary constituent of biocompatible self-assembled nanotherapeutics. The authors have introduced apoA1's properties and discussed how these can be exploited to generate libraries of A1-nanotherapeutics using advanced manufacturing approaches, such as microfluidics or continuous flow methods. The authors have also focused on high-throughput in vitro screening methods and in vivo imaging for identifying promising formulations. Moreover, the authors presented three distinct immunotherapy strategies, including vaccination, co-stimulation/checkpoint inhibition, and trained immunity, for treating a variety of diseases.

Lastly, the following series of research and review articles presented emerging immunotherapies and vaccines against infectious pathogens, including SARS-CoV-2.

Prof. Fangfang Zhou and co-workers from Soochow University (2100044) have presented a review article that introduces immunotherapies against Coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2. SARS-CoV-2 can induce uncontrolled inflammation and cause a lack of antiviral response, thereby aggravating the disease. Therefore, recovery of immune functions is key to COVID-19 treatment. Early administration of interferons may prevent COVID-19 exacerbation and/or promote recovery from the diseases. Inhibitors of inflammation can prevent cytokine storms and multi-organ damage. Convalescent plasma containing neutralizing antibodies played an important role in therapeutic options at the beginning of the pandemic owing to the lack of other effective methods. The authors have presented various immunotherapies against COVID-19, including treatment with interferons, inhibition of pro-inflammatory mechanisms, and the use of convalescent plasma.

Prof. Liangfang Zhang and co-workers from University of California at San Diego (2100072) have presented a review article that discussed the evaluation of nanoparticle-based vaccines as well as the development of nanotoxoids as vaccines against infectious pathogens. Cell membrane-coated nanoparticles are an emerging class of nanocarrier that have been utilized for a wide range of biomedical applications. Prepared by the complexation of toxins with cell membrane-coated nanoparticles, nanotoxoids can safely deliver virulent proteins in their native conformation, thus generating strong and high avidity immune responses. The synthesis of nanotoxoids leverages the natural affinities of virulence factors with cell membranes, enabling for the rapid development of multi-antigenic formulations from unknown mixtures of toxins. The authors have provided an overview on the development and application of nanotoxoids as vaccines against infectious agents. Continued research on nanotoxoids may lead to new and more effective vaccine formulations against high priority infectious diseases.

Prof. Pete Tessier and co-workers from University of Michigan at Ann Arbor (2100099) have presented a facile multivalent engineering approach that can achieve large synergistic improvements in the neutralizing activity of a SARS-CoV-2 cross-reactive nanobody (VHH-72) initially generated against SARS-CoV. The COVID-19 pandemic continues to be a severe threat to human health, especially due to current and emerging SARS-CoV-2 variants with potential to escape humoral immunity developed after vaccination or infection. The development of broadly neutralizing antibodies that engage evolutionarily conserved epitopes on coronavirus spike proteins represents a promising strategy to improve therapy and prophylaxis against SARS-CoV-2 and variants thereof. The authors have shown that VHH-72 nanobody, engineered as a hexavalent Fc-fusion construct, retained binding to spike proteins from multiple highly transmissible SARS-CoV-2 variants (B.1.1.7 and B.1.351) and potently neutralized them. Multivalent VHH-72 nanobodies also displayed drug-like biophysical properties, including high stability, high solubility, and low levels of non-specific binding. These studies showed that VHH-72 multivalent nanobodies are attractive therapeutics against SARS-CoV-2 variants.

Overall, this special issue presents the latest advances in immunotherapies, vaccines, and immune-modulatory strategies and their potential applications for the treatment of various diseases. We hope the work presented in these manuscripts can help the readers appreciate the most recent advances in immunotherapies. We also hope this special themed issue would provide excellent opportunities to facilitate communication and foster collaborations in our science community.

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**Bruno G. De Geest** is full professor at the Department of Pharmaceutics at Ghent University, Belgium. His lab operates at the interface between materials chemistry and immunology and has a strong interest in engineering the immune system via functional materials that can either modulate the pharmacokinetic and pharmacodynamic profile of immuno-modulatory stimuli and antigens. This is combined with developing strategies that modulate the interaction between immune cells and cancer cells. The lab's endeavors focus on engineering innate immune activation, cancer vaccines and T cell engineering. He graduated as chemical engineer in 2003 from Ghent University where he obtained his PhD in pharmaceutical sciences in 2006, after postdoctoral training at the University of Utrecht, The Netherlands, he was appointed as professor in 2012 at Ghent University. He is currently a recipient of an ERC Consolidator Grant.

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