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State of the Art in Nanomedicine

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1.1 Intractable Diseases and Development of the Related Novel Therapy and Medicines

Lots of people get sick now and then in their lives, reducing their quality of life and even threatening their lives. Many diseases not only cause great suffering for people themselves but also for their families and then of the whole society, particularly for those intractable diseases, such as tumors or cancers, rheumatoid arthritis, cerebrovascular diseases (e.g. stroke, cerebral thrombosis, myocardial infarction), neural diseases (e.g. Parkinson's, Alzheimer's, depression), recurrent and infectious skin diseases (particularly relating to blood, endocrine, and immunity), and highly contagious and lethal infectious diseases (e.g. HIV, COVID-19), and so on. Among them, cerebrovascular diseases are the first killers of people, particularly for those more than 50 years old. There were about 1.79 million people in 2016 who died of these kinds of diseases all over the world, about 31% of global causes of death, according to the statistics of the World Health Organization (WHO). Cardiovascular outpatients in China are already more than 0.29 billion (B). Nearly three million people die from cardiovascular and cerebrovascular diseases in China every year, about 51% of the whole causes of death. Cerebrovascular diseases are the fifth cause of death in 2016, with about 373 deaths per 1 M people. In addition, these kinds of diseases preserve features of high suddenness, high disability rate (about 75% of the surviving patients have varying degrees of loss of labor ability and 40% are severely disabled), high recurrence rate, and more multiple complications (e.g. coronary heart disease, myocardial infarct, vascular dementia, subarachnoid hemorrhage, respiratory tract infection, and sudden deafness). China has entered an aging society like other developed countries (e.g. Japan) even though China is still a developing

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country. The population of coronary artery disease (CAD) in China has increased from 2.27 M in 2016 to 2.53 M in 2020, with a compound annual growth rate of 2.7%. It was said that China's precision percutaneous coronary artery therapy (PCI) market has increased from ~$25.7 M in 2016 to ~101.4 M in 2020, with a compound annual growth rate of ~ 40.8%. The global market scale of PCI also shows a growth trend, which was $9.49 B in 2019 and was expected about $13.26 B in 2025 with a compound annual growth rate of ~ 5.4% (https://www.163.com/dy/article/HPGQBS2R051481OF.html; https://wenku.baidu.com/view/933bb2d7f624ccbff121dd36a32d7375a417c6c4.html?_wkts_=1711608332102&bdQuery=2023%E7%BB%8F%E7%9A%AE%E5%86%A0%E7%8A%B6%E5%8A%A8%E8%84%89%E6%B2%BB%E7%96%97%E5%85%A8%E7%90%83%E5%B8%82%E5%9C%BA%E8%A7%84%E6%A8%A1).

Although cancers are the second killers of people, next to cerebrovascular diseases, the pain and burden of patients caused by cancers far outweigh the former due to their characteristics of chronic redundant diseases. It is said that there were about 19.29 M new cases of cancers, among which there were 10.06 M male cases and 9.23 M female cases according to the statistics in 2020. There were about 9.96 M death cases, including 5.53 M male cases and 4.43 M female cases. It is expected that there will be more than 21 M new cases in 2030 [1–3]. There are about 4.82 M and 2.37 M new cases of cancers, and about 3.21 M and 0.64 death cases of cancers, in China and the United States, respectively [1]. Partially thanks to innovative drugs and therapies promoted by medical technology, the overall trend of death cases of cancers in the United States is accelerated down since 1991 [1]. It is predicted that the new cases and death cases in 2023 will be continuously reduced by 410 K and 30 K compared with those in 2022. However, in China, the new cases and death cases of cancers in 2022 increased by 250 K and 210 K compared with those two years earlier (2020), and the 2022 death/new incidence rate in China is far more than that in United States (67% versus 27%) [1]. The death cases of lung cancers is the first among all death cases in China, and then the summed death cases of liver and pancreatic cancers. Particularly, the cases of liver cancers in China are almost half of the cases in the world. For many cases, cancers were found to be mostly the terminal stage [4–6]. While, thanks to vigorous anti-smoking measures in the United States, the first death case is breast cancer, not lung cancer, in the United States. The survival rates for some special cancers in China are lower than those in the United States, particularly for breast cancers and colorectal cancers. It is also said that cancer prognosis in China is much worse than that in the United States. China needs to make more efforts to provide effective cancer treatment and improve universal health coverage. New medicine and therapy of high anti-cancer efficiency are extremely urgent currently, especially for China. At the same time, the global market of anti-tumor medicine has increased to $192.2 B in 2022 with a compound annual growth rate of ~12.7% while in China, the sales of anti-tumor drugs have been showing a steady growth trend in recent years. The market size of anti-tumor drugs reached $28.2 B in 2020 and will have an estimated compound annual growth rate of ~16.1% from 2020 to 2025.

As for hyperuricemia and gout, there were about 1.03 billion (B) outpatients all over the world and about 0.18 B in China in 2022. The global market for gout

medicine was about $3.0 B. The gout medicine market in China will grow rapidly in the future, which is expected to be about $1.54 B in 2030. China has been known as the country with the largest population of diabetes in the world. The total number of people related to diabetes exceeded 260 million in 2018, including 114.39 M diabetes outpatients and 148.70 M pre-diabetes population. It is forecasted that the number of diabetes people will reach 320 M, which will create a huge market for diabetes medicine, with a potential scale expected to reach $19.3 B.

There are four types of neural diseases: absence of symptoms, release of symptoms, irritation and shock, such as Parkinson's, Alzheimer's, Depression, and Huntington's diseases. There are more than 0.1 B people more than 15 years old with various mental disorders in China, among which there are about 16 M patients with severe mental disorders and most of the rest are people with mental or behavioral disorders such as depression or autism. These kinds of illnesses not only torture patients but also haunt their families for a long time. Particularly, they preserve some certain psychological infectivity (e.g. resulting in mass suicide groups), leading to great social harm. Developing these kinds of drugs for anti-mental disorders has to overcome the obstacle of passing the blood–brain barrier (BBB). It is more difficult for these drugs into nerve cells to break through the protective membranes of dendrites, myelin sheaths, axons, terminals, etc.

Clearly, fighting these intractable diseases is a long and arduous task for human beings, whose key is to develop diagnosis methods for early disease identification, innovative drugs, and subversive therapies. Starting from this century, medicine and health care entered into a rapid transformation period promoted by the interdisciplinary crossover of life science, biology, biophysics, biochemistry, nanotechnology, and information technology [7, 8]. As a result, many creative medicine or medical technologies sprout recently, such as personalized medicine, precise medicine, nanomedicine, and lots of innovative therapies, such as gene therapy (e.g. mRNA, DNA), targeting therapy (e.g. cell targeting, tumor microenvironment targeting), immunotherapy (e.g. PD1, PD-L1, vaccine, CAR-T,), new physical field ablation therapy (e.g. nanosecond pulsed electrical field (nsPEF) ablation), new physicochemical therapy (e.g. ferroptosis, cuproptosis, photothermal therapy, photodynamic therapy, magnetothermal therapy, magnetodynamic therapy), and heavy particle radiation therapy (e.g. proton beam radiation, neutron scattering radiation, boron neutron capture therapy) [7–21].

Particularly, nanomedicines, which are translated from some functional nanomaterials, deal with nanoscale matters that can be used in biomedicine or biomedical engineering as bioprobes for the detection of biomolecule, organelle, cells or tissues, or as biosensors for the diseases or pathological metabolism diagnosis, or special drugs for some disease treatment or life function regulation. Based on nanodrugs and nanomedical engineering, lots of disruptive solutions have been advanced for the treatment of intractable diseases, such as anti-tumor nanomedicines [22], nanodrugs for rheumatoid arthritis [23], efficient nanodrugs for nerve or brain diseases by overcoming brain–blood barriers [10, 24], and oral administration nanodrugs for anti-HIV at low dosage [25]. Why does nanomedicine have so many special and powerful functions in disease diagnosis and therapy?

1.2 Key Features of Nanomedicines

There are several critical features of nanomaterials for their translation into special and efficient drugs and therapies. First, as shown in Figure 1.1a,b, most nutrition molecules and key functional molecules in the cell microenvironment range from molecule size to nanoscale (e.g. H_2O, glucose, phospholipid, protein, antibody, antigen, DNA, RNA). The cross-membrane transportation sizes for small molecules are usually less than 10 nm, which are better if less than 6 nm, and the best ones for each component are 2–3 nm or less (Figure 1.1a, the red dotted circle) [26]. Nanoscale materials can be controlled and synthesized by matching to the nanoscale range of the key biological macromolecules (e.g. protein transport pathways, lysosome, centriole, ribosome) very well. Once their surfaces are modified similarly to those biomolecules (e.g. full of –OH ligands, amino acid side groups, glucose, lipids), they can preserve invisibility to the immune system and behave like zymogen during transportation. Second, sizes of organelles and majority of microstructures formed in cell membranes and organelles usually range from 30 nm (e.g. ribosome) to 10 μm (Figure 1.1a, the dotted pink circle, Figure 1.1c). Except that the nonmembrane structured ribosomes are 15–30 nm, the other organelles are generally ranging from 100 nm to 1.0 μm of mitochondria (the pink dotted circle in Figure 1.1a). As for the cells or bacteria for the motion space of nanomaterials and organelles, their sizes range from more than 1 μm for common bacterial to the smaller cells (i.e. red blood cells), and then at most up to less than 1 mm for the human eggs or frog cells (Figure 1.1a, the green dashed circle). The sizes for endocytosis and exocytosis for macromolecules, such as varieties of RNA, DNA, or proteins, range from several nanometers to several hundreds of nanometers or larger [27–29]. Three kinds of membrane transporting channels (i.e. voltage gates, ligand gates, and pressure activation channels) are all in the nanoscale [28, 29]. These size features of organelles and microstructures of cells provide enough free motion space for nanomaterials less than 10 nm and/or their aggregates less than 1.0 μm to exert their functions. As these nanomaterials are less than 10 nm, they can be surface-modified and functionalized easily by conjugating to some biomolecules and organelles, which facilitates their cross-membrane transportation and interaction with some certain organelles, and then targeting certain fine microstructures of organelles. Even they aggregate to several 10 nm or several 100 nm after biomolecule functionalization due to the strong interaction (e.g. coupling, crosslinking, salt bridges) or weak molecule interaction (e.g. van der Waals forces, hydrogen bonds), they can cross-membrane via endocytosis and exocytosis out or into cells and then lysis into nanometer or sub-nanometer effective components by special biomolecules or other cell microenvironment parameters (e.g. lysosomes, pH). Since they can be constructed with much similar surface properties and microstructures as those biomaterials in organisms, they can successfully avoid most of attacks from immunogenicity or autoimmunity, which can last their retention in organisms, leading to their unique enhanced permeability and retention (EPR) effect together with their high permeability [30–32].

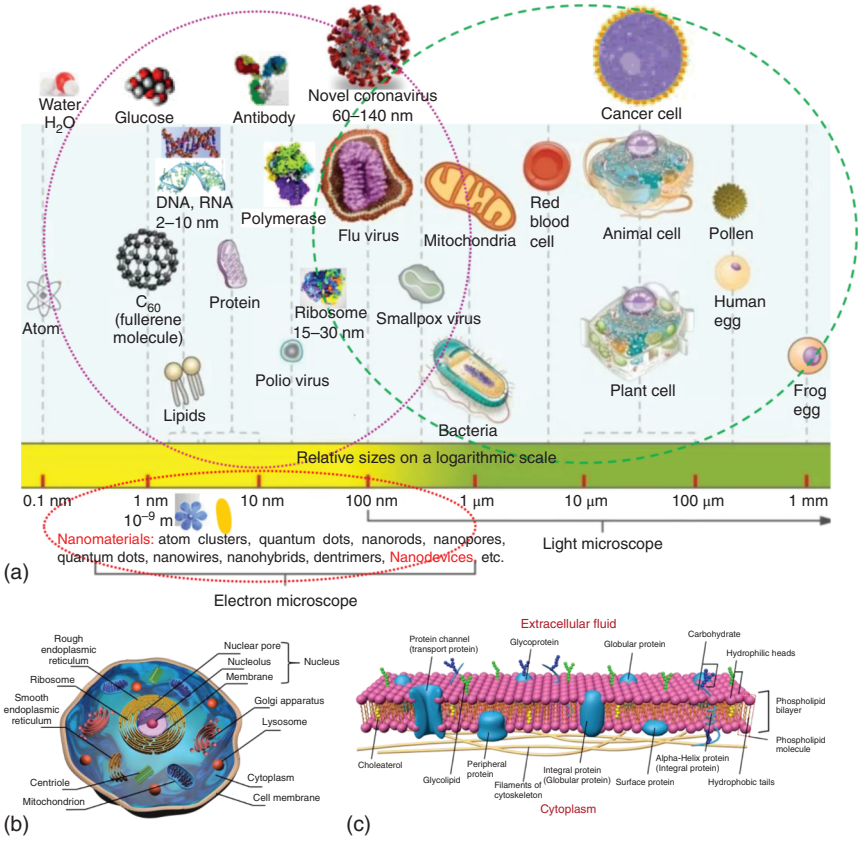

Figure 1.1 (a) Scale comparison of nanometer and some typical biomolecules and cells. Source: Adapted from Beijing Liuzhi Information Technology Co., Ltd./http://www.360doc.com/showweb/0/0/1102300150/last accessed December 28, 2023. The other small illustrations are original; (b) biomolecules and functional microstructures in cell membranes; and (c) organelles and microstructures in one single cell.

Clearly, due to their size effects and flexible surface modification and biomolecule functionalization, they show high biocompatibility and EPR effect as they interact with organs, tissues, cells, and organelles, which also benefit for them to overcome BBB for enhanced drug delivery to special focus in special organs or tissues and cells (e.g. brain or spinal, nerve cells, thrombus [preventing atherosclerosis]) [10, 33–35]. Particularly for those nanoparticles no more than 6 nm, better for 2–3 nm, they preserve much high bioactivity for efficiency-enhanced curative effect for treatment of tumors, cerebrovascular diseases, neural disease, etc. After they finish their bioactivity, they can be cleared via both urinary system and fecal system, which endows them high biosafety [10, 26, 36].

Nanomedicines can be constructed from organics, inorganics, or composites with single components or multi-hierarchy microstructures to realize some targeting functions: detection, and/or diagnosis, and/or therapy. Usually, their core parts can be ranged from 1.0 to 100 nm, which can be assembled into several hundreds

of nanometers or even into several micrometers. Broadly, those with nanometers or even micrometers by assembly of subnanometer components can be generally called nanomedicines. Recently, many multi-mode nanomedicines that preserve functions of detection, imaging, diagnosis, and therapy have been developed or called nanotheranostics and nanotherapeutics [8, 37, 38], which can be further developed as smart nanomedicine, or intelligent nanomedicine if some biomarkers for special molecules or cells are conjugated with these nanomedicines [39].

1.3 Nanotechnology Translational Nanomedicine: Emergence and Progress

Nanotechnology has developed rapidly over the past several decades and nanotechnology translational nanomedicine entered into a blowout development stage by coupling with other biomedical technology and artificial intelligence since 2015 [8, 10, 22, 27, 37, 40–58], as shown in Figure 1.2. Up to now, they have constructed many exciting contributions to the treatment of intractable diseases, such as cancers [7, 22, 114, 118], the rheumatoid arthritis [23], or the collagen-induced arthritis [107], nerve or brain diseases [10, 24], anti-HIV [25], cerebrovascular diseases [34, 42], and tissue regeneration (e.g. spinal cord regeneration [128]), skin diseases (e.g. diabetic wound healing) [129–133], as well as precise diagnosis of many special diseases and tracking some key biological processes as ultrasensitive visible bioprobes [83, 98, 134–149]. Figure 1.2 gives the historical timeline of major developments of nanomedicines, revealing a gradually developing process for nanoscale materials translational medicine since the first nanoscale medicine, or liposome nanostructures was published in 1964, which were constructed by phospholipids nanoemulsion used as drug carriers to encapsulate readymade low molecule medicine or drugs not compatible with body liquids [59, 114]. In 1964, silicon polymer of high biocompatibility was also developed into nanocarriers for prolonged drug lasting time [70]. The first organic nanodrug entering the technical level was Gris (Griseofulvin)-PEG (polyethylene glycol) oral tablet contenting submicro griseofulvin particles with ultra-high absorption rate, which was applied and issued in 1970 [150]. Langer and Folkman reported the first polymer nano-system for sustained controlled release of ionic molecules and macromolecules in 1976 [60]. In 1986, the EPR effect of nanoparticles was revealed, which is a unique vascular phenomenon for selective concentration of nanoscale agents in tumor or lesion tissues and can greatly increase the utilization efficiency of drugs [32, 61, 62]. For this goal, drugs with long retention time during circulation was desired. However, some negative effects during the drug circulation, such as destruction of immune response and cellular microenvironment factors (e.g. phagocytosis of macrophages, protein corona), have to be addressed by surface modification and using materials of high biocompatibility, hydrophilicity, and biodegradability [118, 151–153]. Therefore, long-circulating poly(lactic acid)-*co*-poly(ethylene glycol) (PLGA-PEG) copolymers-based drugs (e.g. PLGA-PEG encapsulating RNAi or genes) were developed in 1994 by Langer et al. since PLGA-PEG copolymers

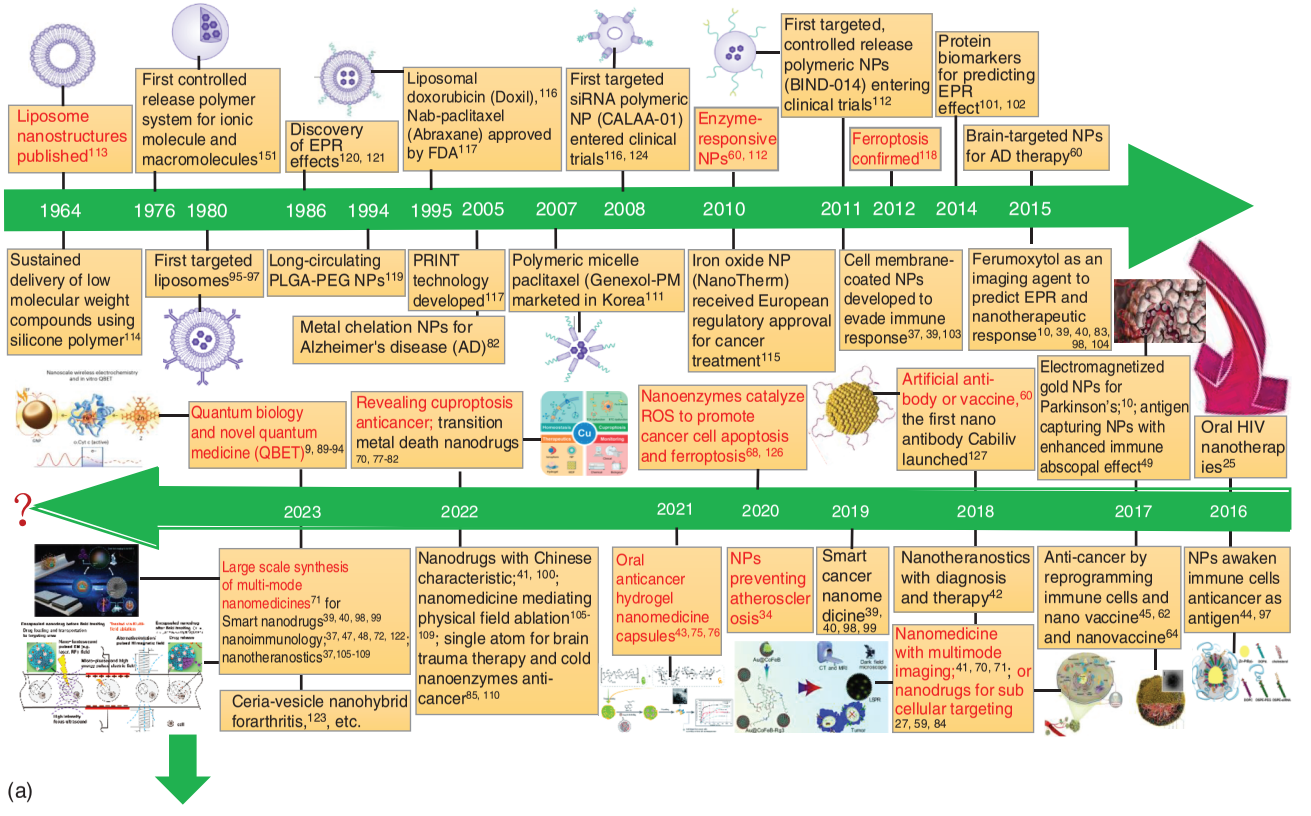

Figure 1.2 (a) Historical timeline of major developments of nanomedicine. EPR, enhanced permeability and retention; FDA, US Food and Drug Administration; nab, nanoparticle albumin-bound; NP, nanoparticle; PLGA-*b*-PEG: poly(D,L-lactic-*co*-glycolic acid)-*b*-poly(ethylene glycol); PRINT, particle replication in nonwetting template; siRNA, small interfering RNA; HIV, human immunodeficiency virus; QBET, quantum biological tunnelling for electron transfer. (Scheme modified from Figure 1 in the key reference [114] and literatures for the main progress published before 2015 and other related figures clipped from key references for the major progresses in nanoenzymes [66], ferroptosis [117], NPs awaken immune cells anti-cancer as antigen [56, 73], the immune abscopal effect [49], reprogramming immune cells [45, 115, 118], artificial anti-body or nanovaccines [46, 55, 116, 119–121], nanoenzymes catalyzing to produce ROS to promote cancer cell apoptosis [95, 122], nanomedicine with multi-mode imaging [41, 89, 98] or targeting and regulation of sub-cellular organelles [27] and tumor microenvironment [41, 101, 123, 124], oral anti-cancer hydrogel-encapsulating nanomedicines [43, 111, 112], cuproptosis anti-cancer mechanism reveal and other T (transition)-metallotosis therapy confirmed [76, 89–94], smart nanodrug systems confirmed [10, 39, 40, 55, 82, 101, 110, 113, 125–127] and quantum effects in biology confirmed and forming quantum medicine [9, 83–88], etc., montage with permitted copyright.)

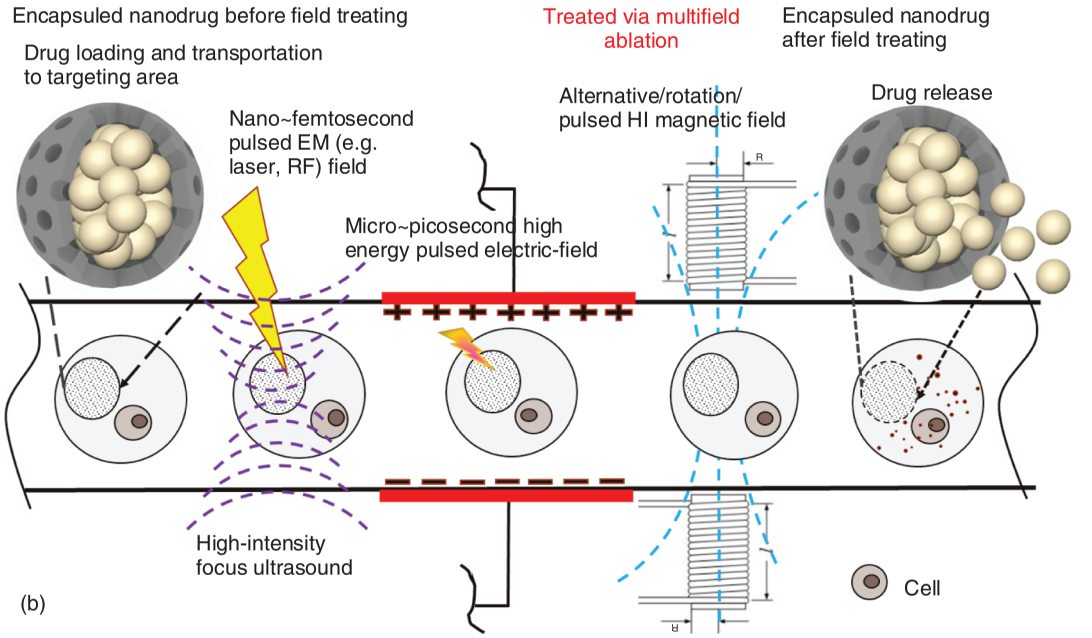

Figure 1.2 (*continued*). (b) The magnified scheme showing nanomedicine mediated multi-physical field (e.g. nanosecond–femtosecond electromagnetic (EM) field, high-intensity focus ultrasound (HIFU), microsecond–picosecond high-energy pulsed electric field, alternative/rotation/pulsed high-intensity magnetic field) ablation on single cells with controlled release and multi-modal therapeutical effects.

are of high biocompatibility and hydrophilicity [74]. However, active targeting is correspondingly of much importance when the tissue accumulation of drugs does not depend on EPR [154] or when the delivery of therapeutic agents requires active transcytosis of physiological barriers such as the intestinal mucosa or the BBB [155–157]. Therefore, many studies focused on the development of active targeting drugs and then the concept of active nanoparticle targeting was introduced in the 1970s [71, 72]. In 1976, some synthesized nanodrugs with active targeting functions made their way into clinical trials [30]. The liposomal doxorubicin (Doxil) nanodrugs of active targeting tumors were approved by FDA in 1995, showing greatly enhanced efficiency in cancer treatment [63, 114]. This is encouraging for the field of cancer nanomedicine. Then NP albumin-bound paclitaxel (nab-paclitaxel; Abraxane) became the second class of nanomedicines for long circulation to be approved by FDA in 2005 [63, 114] and commercialized, such as polymeric micelle paclitaxel (Genexol-PM) was successfully marketed in Korea in 2007 [158]. The nab platform enables formulation of hydrophobic drugs while largely mitigating the need to use toxic excipients, and then the regulatory filing for the approval of Vyxeos was projected in late 2016 [114]. Encouraged by the successful commercialization of Abraxane by addressing the enhanced retention time of drugs by the nanoscale strategy, the first targeted siRNA polymeric NPs (CALAA-01) were approved and entered into clinical trials in 2008 [50, 159]. Up to now, there are many targeting nanodrugs developed with controlled release including typical examples of targeted liposomes (for example, HER2) and single-chain variable fragment (scFv)-targeted liposome (MM-302) [160], the first targeted and controlled-release polymeric NP (BIND-014) [161], and the first targeted siRNA NPs (CALAA01) [50, 114, 162]. Simultaneously, the inorganic nanodrugs contenting 15 nm iron oxide particles were also approved as specific drugs for treatment of anemia in 1974 by FDA, following which many inorganic nanodrugs contenting gold, silver, iron oxides, silicon oxides, and titanium oxide nanoparticles have been gradually approved for clinical study by FDA [26, 114].

Besides the enhanced circulation time in body, the design and synthesis of nanodrugs have to consider how to avoid the immune response before they can be transported into the targeted lesion or cells. Many strategies were invented including the previous methods using materials of high biocompatibility, hydrophilicity, and biodegradability [118, 151]. Based on the direct bionics, cell membrane-coated NPs were further developed to construct cell membrane cloaking nanodrug aggregates by loading nanomedicine into the void cells (e.g. red blood cell) [79, 163, 164]. These cell membrane-coating nanodrug systems can efficiently evade immune response since surface characteristics of these cell membrane-coating nanodrugs are almost the same as those of healthy living cells [79, 163, 164]. During the development of nanodrugs, studies on EPR effects and the related biological mechanism of drugs continue to be paid attention to as their sizes are reduced to nanoscale. For these studies, many multi-mode nanobioprobes, such as protein biomarkers published in 2014 and the imaging agent of ferumoxytol in 2015, have been developed for predicting EPR effects and nanotherapeutic responses, which promotes the progress of nanodrugs with long retention time and active targeting. Together with the gradual realization

of multi-functions of nanodrugs, these studies finally led to the emergence of new fields of precise nanomedicine [8, 27], smart nanomedicine [40], and nanotheranostics [37, 42, 165]. According to the therapy effect analysis on nearly 350 kinds of new drugs, contenting nanomaterials submitted for FDA certification from 1970 to 2015 by FDA Drug Evaluation and Research Center in the United States (CDER), the application cases of nanodrugs increased gradually in the past 20 years, among which some have been used to serve people for the treatment of many intractable diseases (e.g. cancers, stroke, Parkinson's, Alzheimer's) [166].

With the progress of nanomedicine and their processing technologies (e.g. synthesis, structures and function characterization, multi-functionalization and clinical practice), the related biological mechanism on their fundamental biomedical effects has been deeply revealed, particularly the discovery of nonapoptotic forms of cell death of iron-based nanoparticles, termed as ferroptosis that can significantly promote death of tumor cells, by Dixon et al. [117]. Ferroptosis is dependent upon intracellular iron, but not other metals, and is morphologically, biochemically, and genetically distinct from apoptosis, necrosis, and autophagy. It has been confirmed that the small molecule ferrostatin-1 is a potent inhibitor of ferroptosis in cancer cells and glutamate-induced cell death in organotypic rat brain slices, suggesting similarities between these two processes like glutamate, erastin inhibits cystine uptake by the cystine/glutamate antiporter, creating a void in the antioxidant defenses of the cell and ultimately leading to iron-dependent, oxidative death. Thus, activation of ferroptosis results in the nonapoptotic destruction of certain cancer cells, whereas inhibition of this process may protect organisms from neurodegeneration for some neurodisease treatments [117]. Since 2016, a research frenzy on ferroptosis in tumor treatment was sparked and many transition metal-based medicines or nanomedicines have been found preserving similar nonapoptotic forms of cell death [94, 122, 167–169].

In 2022, the biological mechanism of copper-induced cell death was successfully revealed by Tsvetkov et al. [94]. Copper is an essential cofactor for all organisms, and yet it becomes toxic if concentrations exceed a threshold maintained by evolutionarily conserved homeostatic mechanisms. In human cells, copper-dependent, regulated cell death is distinct from known death mechanisms and is dependent on mitochondrial respiration. Tsvetkov et al. found that copper-dependent death occurred by means of direct binding of copper to lipoylated components of the tricarboxylic acid (TCA) cycle. This results in lipoylated protein aggregation and subsequent iron–sulfur cluster protein loss, which leads to proteotoxic stress and ultimately cell death. These findings may explain the need for ancient copper homeostatic mechanisms. Cell death is an essential, finely tuned process that is critical for the removal of damaged and superfluous cells. Multiple forms of programmed and nonprogrammed cell death have been identified, including apoptosis, ferroptosis, and necroptosis. Using genetically modified cells and a mouse model of a copper overload disorder, the researchers report that excess copper promotes the aggregation of lipoylated proteins and links mitochondrial metabolism to copper-dependent death. Lipoylation determines sensitivity to copper-induced cell

death. It can be proposed that Cu-based medicines preserve great promise in tumor cell treatment if they can be precisely targeted into the tumor cells.

With the investigation of the biomedical function of varieties of transition metal-based nanomedicines (e.g. Fe, Co, Cu, Au, Mn, and their compounds or alloys), their enzyme-like mechanism for disease treatment become more and more clear, which can catalyze many key molecule pathways (e.g. reactive ROS, glutamate) using nanomedicines based on the NPs of the transition metals, which can produce similar nonapoptotic forms of cell death in the cancer treatment as ferroptosis or cuproptosis [41, 94, 117, 170, 171], which can be reasonably termed as transition-metalloptosis [90]. Since almost all these nanomedicines preserve the enzyme-like catalyzing functions, which was further defined as a new research arena: nanoenzymes, recently [41, 58, 66, 95, 96, 104, 108–110, 172–175].

The research field of nanoenzymes has seen exponential growth over the past few years since the term was coined in 2016. This unique modality of cell death, driven by metal-dependent catalysis of some key biological reactions (e.g. ROS, glutamate, phospholipid peroxidation), is regulated by multiple cellular metabolic pathways, including produce of ROS; redox homeostasis; metal handling; mitochondrial activity; and metabolism of key amino acids, lipids, and sugars, in addition to various signaling pathways relevant to disease.

With the gradual reveal of the biomedical effects and their fundamental therapeutic mechanism, and the breakthrough in the controlled preparation methods and the administration technologies of nanomedicine, nanomedicine ushered in a blowout of development since 2016, as shown in Figure 1.2. Particularly, besides nanoenzyme functions [41, 58, 66, 95, 96, 104, 108–110, 172–175], many of them have played key roles in the overcome of multi-drug-resistant (MDR) during cancer treatment [176–180], in the development of innovative immunotherapy by the reveal of their immunological effects for nanoimmunotherapy [27, 37, 40, 47, 89, 110, 114, 118, 124, 168, 181–183].

MDR is a frequently encountered thorny issue as using traditional or even some innovative drugs to treat many diseases, particularly for some persistent infectious diseases and difficult miscellaneous diseases (e.g. cancers), which impedes the successful treatment of targeting diseases [176–180]. Developing novel long-circulating, self-assembled core–shell nanoscale coordination polymer (NCP) nanoparticles that efficiently deliver multiple therapeutics with different mechanisms of action to enhance synergistic therapeutic effects is an innovative strategy to overcome this multi-drug-resistant issue [179]. For example, Lin et al. invented NCPs code liver chemotherapeutics and siRNAs to eradicate tumors of cisplatin-resistant ovarian cancer in 2016 [178]. These NCP particles contain high payloads of chemotherapeutics cisplatin or cisplatin plus gemcitabine in the core and pooled siRNAs that target MDR genes in the shell. The NCP particles possess efficient endosomal escape via a novel carbon dioxide release mechanism without compromising the neutral surface charge required for long blood circulation and effectively downregulate MDR gene expression in vivo to enhance chemotherapeutic efficacy by several orders of magnitude. By silencing MDR genes in tumors,

self-assembled core–shell nanoparticles suggest a more effective chemotherapeutic treatment for many challenging cancers [178].

1.4 Interdisciplinary Features of Nanomedicines: Multi-mode and Multi-function Features Promoting Nanomedicine-mediated Immunotherapy and/or Physical Field Ablation Therapy for Subversive Therapy

Tumor immunotherapy has become one of the key innovative methods in tumor treatment and many immunotherapy drugs (e.g. PD-1, PD-L1, CTLA-4) and therapy. (e.g. cart-T) have been developed recently. However, existing cancer immunotherapy drugs work in only 20%–30% of patients [44, 73], particularly for those patients with solid tumor. In some cases, even when the checkpoint molecules are blocked, there are too few active T cells around to sound the immune alarm, says Jedd Wolchok, a cancer immunotherapy expert at the Memorial Sloan Kettering Cancer Center in New York City [73]. Additionally, the key reason is that tumors do not display enough of the T cell's targets, so-called tumor antigens, on their surface. However, nanoparticles and their functionalized species can behave similar to antigens as they enter into bodies [44, 73, 184]. The immunological mechanism of nanomedicine has been studied intensively for the treatment of tumors in the past decade. Results indicate that nanomedicines preserve intensive immune abscopal effects and have great potential as artificial antigens to activate and train immune cells [185], and they can even reprogram cancer cells or immune cells to reshape the tumor immune microenvironment [37, 46–49, 51, 184, 186, 187]. It is said that tumor cells are usually produced every day in our body due to a variety of causes, which will not lead to cancer if they can die through their routine apoptosis themselves or be cleaned by our immune system [47, 48, 113, 188–192]. However, tumor cells are smart and can escape from our immune system since they can disguise themselves by releasing some signals or chemicals to let immune cells confirm that they are healthy cells, and then suppress the secretion function of immune cells not to release the corresponding cytokines killing cancer cells [47, 48, 54, 113, 189, 190, 193]. The immunological abscopal effect of nanomedicines was revealed in 2017 by Min et al. [49]. One function of nanomedicines on the immune system is to activate or awaken immune cells, called an immune agonist [44, 73, 178, 194], such as using the paclitaxel nanoparticles to awaken immune system to fight against cancer studied by Tang et al. [194]. In 2016, Lin et al. developed self-assembled core–shell NCP nanoparticles that efficiently delivered multiple therapeutics with different mechanisms of action to enhance synergistic therapeutic effects for ovarian cancer treatment [44]. These NCP NPs contained high payloads of chemotherapeutics cisplatin or cisplatin plus gemcitabine in the core and pooled siRNAs that target multi-drug-resistant (MDR) genes in the shell [44].

Some physical therapies, such as radiation therapy [44, 73] and electrical field therapy [13–15, 195, 196], can break tumor cells to expose some antigens. Therefore, the self-immune systems of some patients can be activated to produce

immune response effects similar to immunotherapy after some physical therapy [15, 44, 178, 197, 198]. Based on this phenomenon, Lin et al. from the University of Chicago invented photosensitive ultra-small nanodrugs for tumor treatment, which can ignite the immune responses for some tumors insensitive to immunotherapy by coupling them with radiation therapy [44, 73]. The recent progress suggests that these nanodrug-mediating immunotherapies and/or physical field treatments are expected to enter into clinical trials, which have become the best partner in the field of immunotherapy [15, 37, 44, 73, 178, 197–199].

Another immunological function of nanomedicines was to reprogram immune cells to recognize cancer cells advanced in 2018 by Roth et al. [115] and Yang et al. [200], such as reprogramming the function and specificity of human T cells with nonviral genome targeting for anti-cancer therapy [115]. This strategy has been modified for the development of Parkinson's disease therapy using nanoparticles, such as electromagnetized gold nanoparticles mediating direct lineage reprogramming into induced dopamine neurons for the treatment of Parkinson's [10].

At the same time, some artificial antibodies (vaccines) have been developing, with active targeting functions [46]. In 2018, Cao and Wang developed a conformational engineering method to create an NP-based artificial antibody, denoted "Goldbody," through conformational reconstruction of the complementary-determining regions (CDRs) of natural antibodies on gold NPs (AuNPs) [46]. Upon anchoring both terminals of the free CDR loops on AuNPs, the "active" conformation of the CDR loops can be reconstructed by tuning the span between the two terminals, endowing these inorganic NPs the original specificity. Two Goldbodies have been created by this strategy to specifically bind with hen egg white lysozyme and epidermal growth factor receptor, with apparent affinities several orders of magnitude stronger than that of the original natural antibodies. As a result, it is possible to create protein-like functions on these much more stable metallic NPs in a protein-like way, namely by tuning flexible surface groups to the correct conformation, which will finally build up a category of Goldbodies that can target different antigens and thus be used as substitute for natural antibodies in various applications [46]. The first approved anti-body nanodrug Cabiliv (Caplacizumab-Yhdp) was approved by the European Union and then commercially launched for clinical use in 2018, which became the first nano anti-body drug for the treatment of allergic purpura (Henoch-Schonlein syndrome, HSS) in the world. (https://www.sohu.com/a/252250535_119250; https://www.vodjk.com/news/180904/1503187.shtml: Cabiliv™ [caplacizumab] approved in Europe for adults with acquired thrombotic thrombocytopenic purpura [aTTP]; Sanofi gets EU OK for Ablynx flagship drug Cabiliv). This drug was then approved by FDA in the United States in February 2019 (https://www.sohu.com/a/252250535_119250; https://www.drugs.com/history/cablivi.html). Since then, nanomedicines not only with more intimate to immunological effects but also with multiple-therapy functions and multi-targeting functions (e.g. cancer cells, organelles, or tumor microenvironment) have been developed forming a novel field of nano-immunotherapy up to now [47, 48, 51, 57, 101, 118, 182–184]. If readers need more details on the immunological effects of nanomedicines, Chapter 10 in this monograph can be referred to.

Simultaneously, coupling of nanomedicines to some advanced biophysical or biochemical methods for subversive combined therapies has been on the way for difficult miscellaneous diseases [27, 37, 40–42, 47, 58, 89, 110, 114, 118, 124, 165, 168, 181–183, 201–203]. Besides their biomedical functions (e.g. immunological effects, enzyme-like functions), nanomedicines constructed by nanohybrids conjugating to varieties of biochemical drugs or preparation, preserve unique physicochemical characteristics and can simultaneously interact with several physical fields (e.g. electrical field, magnetic field, optical field, ultrasound field, electric–magnetic field) [37, 46–48, 51, 186, 187]. Multi-mode therapy and diagnosis based on these multi-functional nanomedicine-mediated physical field ablation and/or the corresponding molecule imaging and bioprobe function have been developed recently into one novel medical methodology with both diagnosis and therapy functions, or nanotheranostics [37, 42]. Particularly, nanotheranostics as these multi-mode nanomedicines coupling with physical field ablation preserve greatly enhanced therapeutical effects and in situ precise diagnosis functions for treatment of varieties of intractable diseases by comparing with their counterparts (either physical field ablation or solely nanomedicine), many of which have been implemented in the clinical trial [37].

Besides the enhanced apparent therapeutic effect due to the synergistic effects among varieties of physical fields and nanomedicines, another amazing achievement of nanomedicine mediated physical field ablation in their biomedical application is the dramatically improved immunological effects that are far beyond that one single physical field or nanodrug can do. Table 1.1 summarizes the multi-mode of action, advantages and disadvantages, immunological effects, and clinical progress of multi-modal ablation by multiple physical field coupling.

Currently, there are five typical physical field coupling modes in the road of clinical trials as follows. (i) The coupling of high-intensity focused ultrasound (HIFU), with light irradiation (PR) or fluorescence excitation (CLE) produces dual mode and multi-modal therapeutic effects, such as tissue tearing, cell fragmentation, sonodynamic therapy (SDT), and photochemical kinetic ablation (PDT), leading to more active molecules (e.g. reactive oxygen species [ROS]) and high-temperature thermal effects [204–206]. These effects promote the release of tumor antigens to activate immune cells, induce inflammatory reactions, and enhance immune memory of related immune cells. To date, this kind of HIFU coupling to PP or CLE technology is still in preclinical stage. (ii) The coupling of HIFU and electrical field (EFT) produces dual mode or multi-modal effects, such as tissue tearing and cell fragmentation caused by ultrasound, and electrochemical (EDT) or electrostatic field polarization (ESFP) by electrical fields, and pore formation and polarization by nsPEF for cell internalization of drugs and surface electrical dipole regulation of sub-cellular structures [207], leading to ROS formation, reversible perforation, and electrical dipole interaction with organelles at the related microregions or biomolecules and their functional groups [208]. This kind of combined therapy by coupling HIFU and EFT preserves multi-immunological effects, such as immune cell activation, enhanced anti-tumor immune response, immune related gene expression regulation (pro-inflammatory and anti-inflammatory factors),

Table 1.1 Working modes of the currently developed multi-modal ablation therapy based on multi-physics coupling and their main features and key challenges, immunological effects, clinical progress, and main affiliation.

Modes of multi-physical field coupling	Energy/Momentum mode and/or biochemical reaction activation mode (modality)	Multi-functional modes at work	Main features	Key challenges	Immunological effects and mechanisms	Affiliation (company or institute)	Clinical stage	References
High-intensity focused ultrasound (HIFU), light irradiation (PR)/ fluorescence excitation (CL): dual mode multi-modal	Tissue tearing, cell fragmentation, sonochemical therapy (SDT), photochemical kinetic ablation (PDT)	Reactive oxygen species (ROS), high-temperature thermal effects	High tissue penetration, spatiotemporal control, and synergistic effects	Cause thermal damage to people	Promote the release of tumor antigens, activate immune cells, induce inflammatory reactions, and enhance immune memory	Ruiya biotechnology, Canada Covidien, United States	Preclinical	[204–206]
High-intensity focused ultrasound (HIFU) plus electrical field (EFT): dual mode multi-modal	Tissue tearing and cell fragmentation caused by ultrasound, electrochemical (EDT) or electrostatic field polarization, nanosecond pulsed electric field (nsPEF) pore formation and polarization	ROS, reversible perforation, and interaction with organelles or biomolecular microregions and functional groups	High tissue penetration, oxygen independence, and synergistic effects	Thermal damage to human body, embedded electrode	Immune cell activation, enhanced anti-tumor immune response, immune related gene expression regulation (pro-inflammatory and anti-inflammatory factors), angiogenesis inhibition, and immunosuppressive cell regulation	MIT and SonaCare Medical Co, Shenzhen Maiwei Medical Company, University of Science and Technology Beijing	Preclinical, Clinical Phase I, II	[207, 208]

(Continued)

Table 1.1 (Continued)

Modes of multi-physical field coupling	Energy/Momentum mode and/or biochemical reaction activation mode (modality)	Multi-functional modes at work	Main features	Key challenges	Immunological effects and mechanisms	Affiliation (company or institute)	Clinical stage	References
Photoenergy irradiation (PR)/ chemical fluorescence excitation (CLE) plus electric field (EFT): dual mode multi-modal	Photodynamic therapy (PDT), electrochemical therapy (EDT), or electrostatic field polarization	ROS, high-temperature thermal effects, etc.	Temporal and spatial control, oxygen independence, and synergistic effects	Thermal damage to human body, embedded electrode	Activation and proliferation of immune cells and immune regulatory cells, enhancement of anti-tumor immune response and expression of immune related factors (pro-inflammatory and anti-inflammatory)	Griffin Adam holiday, Norway Photocure ASA, Hangzhou Ruidi Biotechnology Co., Ltd	Preclinical	[209, 210]
Electromagnetic field/magnetic field plus temperature field ablation: dual/triple mode multi-mode	Radiofrequency ablation (RFA)/ alternating magnetic field heat plus cryoablation (CryoA) cold plus radiofrequency chemical dynamic ablation	High temperature thermal effect, low temperature freezing effect, cell rupture	High tissue penetration, targeted therapy, noninvasive, synergistic effects	Depth limitation, uneven heat distribution, damage to people	Immune cell activation and enhancement (tumor-specific T cells and NK cells), anti-tumor immune response enhancement, immune-related gene expression regulation, immune regulatory cell regulation	Medtronic, United States; Immodulon Therapeutics Ltd, United Kingdom; Shanghai Meijie Medical Technology Co., Ltd.; Beijing Haijieya Medical Device Co., Ltd, Magforce AG, Germany; Lodespin Labs, Canada	Preclinical, Clinical Phase I, II	[211–214]

Electrical field plus radio-frequency electromagnetic field plus plasma physics: dual/three-mode multi-mode	Radio frequency ablation (RFA) plus electro-chemical therapy (EDT) plus electrostatic field polarization or pulsed electric field (PEF) ablation, or plus low-temperature plasma (CAP)	ROS, thermal effect, alternating electrical or electrostatic field, and pulse electric field polarization and activation, plasma activation	Targeted therapy, synergistic effects, noninvasive, and low toxicity side effects	Improved accuracy, parameter and dose optimization, tumor type free	Stimulate the activation and proliferation of immune cells (natural killer cells [NK cells] and cytotoxic T lymphocytes [CTLs]), inflammatory response, tumor microenvironment regulation, and promote tumor antigen expression	Oncosec Medical Incorporated, Korea Adtec Healthcare	Preclinical, Clinical Phase I, II	[215]

Source: Son et al. [217]/Royal Society of Chemistry.

angiogenesis inhibition and immunosuppressive cell regulation. Most of these therapies are in preclinical stage and some of them have been into clinical Phase I or II stage. (iii) The coupling of photo irradiation (PR) and/or chemical fluorescence excitation (CL) with EFT can also produce multi-modal therapeutic effects, such as photodynamic effect therapy (PDT) mode, electrochemical reaction therapy (EDT) mode and ESFP mode, which can ignite activation and proliferation of immune cells and immune regulatory cells, can enhance anti-tumor immune response and the related expression of immune-related factors (e.g. pro-inflammatory and anti-inflammatory) [209, 210]. This kind of multi-mode therapy is currently in preclinical stage. (iv) The coupling of EM field and/or magnetic field with temperature field ablation, which can produce the combination of dual or triple mode therapy, such as the combination of two or more ablation modes of radiofrequency ablation (RFA), alternating magnetic field heat, cryoablation (CryoA) cold ablation, and radiofrequency chemical dynamic ablation [211–214]. These multi-mode therapies can preserve the below immunological effects: immune cell activation and function enhancement (tumor-specific T cells and NK cells), enhanced anti-tumor immune response, and regulation of immune-related gene expression and immune regulatory cell. (v) The coupling of electrical field ablation, radiofrequency EM field ablation and plasma ablation, which can result in the multi-modal combination of RFA, EDT, ESFP, PEF, and low-temperature plasma (CAP). Studies on these multi-modal combinations indicate that they can activate the immune cells, stimulate the proliferation of immune cells (e.g. natural killer cells [NK cells] and cytotoxic T lymphocytes [CTLs]), promote tumor antigen expression, enhance the inflammatory response by recruiting more immune cells into lesions, and regulate the tumor microenvironment. Generally, most research suggests that multi-physical field coupling ablation can produce much more improved therapeutical effects, particularly in the activation of immune cells, enhancement of immune responses and the regulation of immune system, more than one single physical field mode due to their multi-field synergistic effects [215]. However, recent progress suggests that these immunological effects are generally not as great as desired when they are entering into the clinical trial stages, and most of these multi-modal therapies by the combination of different physical fields need agonists or multi-mode responsive nanomedicines to amplify their immunological effects and the final therapeutical effects or overcome some shortcomings [11, 37, 38, 42, 165, 207].

Typical cases of nanomedicines mediated physical-field ablation therapies were summarized in Table 1.2, which shows a brief description of their working modes, therapeutical effects, and anti-tumor applications. These multi-mode nanomedicines could also behave like agonists or amplifiers for their immunological effect by improving tri-interactions among medicines, physical fields, and lesions.

There are generally about nine types of multi-mode therapies based on nanomedicines mediated physical field ablation. (i) Nanomedicines (e.g. liposome nanodrugs) mediated HIFU by Indian Sun Pharmaceutical and Taiwan Toyo Pharmaceutical can result in tissue tearing and cell fragmentation by sonodynamic therapy (SDT) that increase the nanodrug cell internalization, utilization, and safety via overcoming BBB, which has entered into the clinical phases I and

Table 1.2 Working modes, therapeutical characteristics, applications, representative institutions, and clinical progress of nanomedicine-mediated physical field ablation therapies.

Physical ablation mode		Energy/Momentum or biochemical activity or working mode	Nano drug carrier	Conjugating drugs	Imaging mode	Therapeutical characteristics	Application fields	Representative institutions	Clinical stage	References
High-intensity focused ultrasound (HIFU)		Tissue tearing and cell fragmentation Sonochemical therapy (SDT)	Liposomes	Small molecules–macromolecules–nanomedicines	Ultrasonic	Enhances delivery safety and efficiency of drug utilization administration	Blood brain barrier, etc.	Indian sun pharmaceutical	Phase I/II	[217–221]
				Drug vesicles	Ultrasonic	Trigger drug release	Blood brain barrier, etc.	Taiwan Toyo Pharmaceutical	Phase I/II	[222–224]
Traditional electro-magnetic field	RF ablation (RFA) RF-CDT	High frequency electromagnetic wave	Polymeric nanoparticle	Biomacro-molecules	–	Greatly inhibiting tumor recurrence and preventing tumor metastasis	Breast cancer, etc.	Soochow University; Macau University of Science and Technology	Phase I/II	[105]
	Microwave ablation (MWT)	Microwave hyperthermia (MTT) Microwave-dynamic (MDT)	Liposomes	Small molecule drugs	–	Enhance ablation effect and prevent recurrence	Liver cancer, etc.	The Fifth Medical Center of Chinese People's Liberation Army General Hospital	Preclinical	[102]

(Continued)

Table 1.2 (Continued)

Physical ablation mode		Energy/Momentum or biochemical activity or working mode	Nano drug carrier	Conjugating drugs	Imaging mode	Therapeutical characteristics	Application fields	Representative institutions	Clinical stage	References
New electro-magnetic field	Photo irradiation (PR) or CLE, pulsed laser photogenesis, photoshock	Photochemical photodynamic therapy (PDT)	Polymer, core–shell metal NPs	Macromolecular/small molecule drugs, porphyrins/saponins	Optical	Precision treatment	Breast cancer, liver cancer,-etc.	Fudan Zhangjiang Bio-pharmaceuticals	Clinical approval, preclinical	[225–230]
		Photothermal ablation (PTA)	Inorganic metal NPs	Iron oxide nanomedicine	MRI	The curative effect is clear, and combined with other ways to enhance its curative effect	Prostate-cancer, glioblastoma, etc.	AMAG Pharma-ceuticals; Advanced Magnetics, Inc	Phase I/II	[231–236]
		Chemilumine-scence biochemical reaction excitation (CL PDT)	Inorganic NPs, liposomes, etc.	Photosensitizers, small molecule drugs	–	High tumor targeting and high efficiency	Lung cancer,-etc.	Seoul National University; Shanghai Institute of Silicate, Chinese Academy of Sciences	Preclinical	[237–239]
	X ray	X-ray photochemical dynamics (PDT)	Inorganic metal nanoparticles	Sustained luminescent nanoparticles	–	Low dose X-ray can activate	Breast cancer	Memorial Sloan Kettering Cancer Center; Fuzhou University	Preclinical	[240, 241]
		Radionuclide mediating photochemical dynamic therapy (CR PDT)	Polymers, inorganic NPs	Photosensitizer	–	Expanding the application scope of radiation therapy	Breast cancer, liver cancer,-etc.	China Pharmaceutical University	Preclinical	[80, 237]

	High energy radiation therapy (HERT)	Isotope decay emits high energy γ or β Isoray	Inorganic metal NPs	Au, Bi, Gd, Cs^{131} complexes	MRI, CT, nuclear imaging	Enhance the curative effect of radiotherapy	Liver cancer, pancreatic cancer, esophageal cancer, nonsmall cell lung cancer, etc.	Johnson& Johnson's subsidiary Janssen Pharmaceuticals, nanobiotix, IsoRay company	Preclinical, Phase I/II	[238, 242–245]
Electrical field	Conventional electric field	Electrochemical (EDT) or electrostatic field polarization	Inorganic metal nanoparticles	Pt nanoparticles, etc.	–	Minimal invasion and uniform ablation covering the relatively large tumors wholly	Breast cancer, etc.	Zhejiang University	Preclinical	[199]
		Millisecond to microsecond pulsed electric field (ms–μsPEF)	Liposomes	Small molecule drugs	–	Improving the transport of nanomedicines	Colon cancer, etc.	Wroclaw Medical University	Phase I/II/III	[246]
	New electrical field ablation	Nanosecond to picosecond pulsed electrical field ablation (ns–psPEF)	Inorganic NPs multi-mode nanomedicine	Small molecule drugs	MRI, CT	Improve drug delivery while improving curative effect and preventing recurrence	Liver cancers, etc.	AngioDynamics; Shanghai Ruidao Medical; Hangzhou Ruidi Biotechnology Parade Company; USTB	Preclinical	[247]
Magnetic field (alternating or pulsed amt/pmt)		Magnetocaloric (MHT) or magneto-dynamics	Inorganic metal nanoparticles	Iron oxide nanoparticles	MRI	High tissue permeability	Nonsmall cell lung cancer, breast cancer	Xiamen University, National University of Singapore	Preclinical	[12]

RF, radiofrequency; CDT: chemodynamic therapy; CLE, chemical luminescent excitation; MRI, magnetic resonance imaging; CT, computed tomography; USTB, University of Science and Technology Beijing (USTB).
Source: Irvine and Dane [47] and Gawne et al. [37].

II [217–224]. (ii) Nanomedicines (e.g. some macromolecule or small molecule nanodrugs encapsulated by polymeric or liposome micelles) mediated traditional EM fields (e.g. RF ablation [RFA]: RF-CDT; microwave ablation [MWT]) can produce RF wave-induced chem-dynamic reaction and/or hyperthermia effects, which can greatly increase the ablation effects and prevent tumor recurrence or metastasis [102, 105]. Both Soochow University; Macau University of Science and Technology, and the fifth medical center of the Chinese People's Liberation Army General Hospital in China have invested in this method, and have paved this combination therapy into the clinical phase I or II in the treatment of breast cancers and the preclinical stage for liver cancers [102, 105]. (iii) There are many new combined therapies developed by new types of nanomedicines mediated new EM field ablation. One of these new therapies is polymeric or metal-based core–shell nanoparticles mediated photoirradiation (photochemical or photodynamics) therapy (PDT), photothermal ablation (PTA), chemiluminescence biochemical reaction excitation (CLE), and pulsed laser photogenesis or photoshock ablation, developed by Fudan Zhangjiang Biopharmaceuticals, AMAG Pharmaceuticals, Advanced Magnetics Inc, Seoul National University, Shanghai Institute of Silicate, Chinese Academy of Sciences, and so on [225–239]. Among them, iron oxide nanomedicines mediated PTA has been in the clinical phase I or II in the treatment of prostate cancer and glioblastoma, showing enhanced curative effects [234, 235]. The second combination is the inorganic or polymeric nanomedicines with sustained luminescence or photosensitivity mediated X-ray radiation for enhanced X-ray photochemical dynamics (PDT) or the radionuclide mediated photochemical dynamic therapy (CR PDT), which can greatly reduce the essential activation dosage of X-ray [240, 241] and/or expand the application scope of X-ray radiation therapy [80, 237]. X-ray radiation-based therapies have been used in the preclinical treatment of breast cancers and/or liver cancers by Memorial Sloan Kettering Cancer Center; Fuzhou University [240, 241] and China Pharmaceutical University [80, 237]. The third combination is the inorganic metal NPs (e.g. Au, Bi, Gd complexes or Cs^{131} like isotope NPs that also preserve MRI, CT, and nuclear resonance multi-mode imaging functions) mediated high energy radiation therapy (HERT: e.g. γ-rays or β-rays emitted by isotopes' decay), which have been in preclinical stage or in the clinical phase I/II stage for the treatment of liver cancer, pancreatic cancer, esophageal cancer, nonsmall cell lung cancer, etc., developed by Johnson&Johnson's subsidiary Janssen Pharmaceuticals, Nanobiotix Company, and Isoray Company [238, 242–245]. (iv) The inorganic NPs, liposome encapsulating small molecules, or inorganic and organic complicated NPs mediated electrical field ablation therapies have been developed from the conventional electrochemical (EDT), ESFP or high-energy millisecond to microsecond PEF (ms–μsPEF)-based therapies to ultrafast high-energy PEF (ns–psPEF). Among them, nanomedicines mediated ms–μsPEF therapies have been developed by Wroclaw Medical University entering into the clinical phase I/II/III for the treatment of colon cancers, which can improve the liposome-encapsulating small molecule nanodrugs [246]. Since ns–psPEF ablation therapies have no obvious thermal effect but significant electrical polarization effect, dramatically enhanced cell internalization and lesion

penetration depth of drugs, their combination therapies have been paid much more attention rapidly recently, which have been used in the preclinical study for the treatment of varieties of cancers (e.g. liver cancers) by many company and institutes, such as AngioDynamics in the United States, Shanghai Ruidao Medical Company in China, Hangzhou Ruidi Biotechnology Company in China, Parade Company in China, and University of Science and Technology Beijing in China [207, 247]. (v) The inorganic NPs (e.g. iron oxide NPs) mediated magnetic fields (alternating or pulsed magnetic field) therapies can result in high tissue permeability of drugs due to the magnetocaloric (MHT) or magnetodynamic effects, which have been used in the treatment of nonsmall cell lung cancer and breast cancer by Xiamen University, National University of Singapore and currently in the preclinical stage [12].

Most of these multi-modal therapies by nanomedicines mediated a certain physical field ablation therapy are still in preclinical stages and some of them have been in clinical trials funded by some companies or institutes. Some of them have also shown greatly enhanced immunological effects during their treatment of intractable diseases (e.g. tumors) by comparing with the counterparts of the pure field ablation and/or only nanomedicines. Table 1.3 summarizes ten typical achievements obtained by the main leading institutes or companies in the physical field ablation and/or nanomedicine mediated physical field ablation to date, including types of multi-mode, their therapeutical characteristics and immunological effects, and their current clinical stages. Institutes or companies mainly include Covidien Company [72, 205–207, 217], Massachusetts Institute of Technology [209, 217] and Griffin Adam Holiday Company [88, 210, 211, 217] from the United States; King Saud University from Saudi Arabia [211–214, 216, 251, 252]; Immodulon Therapeutics Ltd. from the United Kingdom [88, 212–215, 217]; Shenzhen Maiwei Medical Company [254, 255], Hangzhou Ruidi Biotechnology Co. Ltd. (HZRD) [256–261], Shanghai Meijie Medical Technology Co. Ltd. [212, 213, 262], USTB and Zhenzhou Tianzhao Biomedical Company [41, 71, 89, 108, 111], and Peking University [268, 269] from China. Types of multi-mode are mostly focusing on the coupling effects from HIFU, electric fields, radiation by varieties of EM waves with broad wavelengths, radiofrequency radiation, temperature gradient induced thermal ablation and nanomedicine enhanced field ablation effects. All these combined therapies based on nanomedicines mediated multi-mode field ablation exhibit significant immunological effects (e.g. inducing inflammatory reactions and promoting the release of tumor antigens to recruit more immune cells and kill cancer cells; activating immune cells and immune regulatory cells; intensifying immune memory; enhancing anti-tumor immune response, and improving expression of related immune factors and immune genes) and the synergistic therapeutical effects (e.g. enhanced ablation effects to kill tumor cells; high penetration depth into lesion and cell internationalization of drugs to enhance the utilization of drugs; intensified dissolution and apoptosis of tumor cells). Particularly, the combined therapies by nanomedicines mediated ns–psPEF developed by USTB and HZRD have shown subversive therapeutic effects using HCC as the pathological model, which can co-stimulate the activation and proliferation of immune cells and promote the inflammatory response and expression of anti-tumor

antigen [41, 89, 108, 123, 207, 270]. Based on the nanomedicine mediated ns–psPEF and high spatiotemporal resolution magneto-optic detection system, Song's group from USTB currently focuses on the development of series of nanomedicine mediated multi-physical-field ablation instruments by coupling rotating or pulsed magnetic fields, laser irradiation and pulsed ultrafast laser, HIFU and ultrafast high intensity PEF [271]. As investigation of the therapeutical effects via multi-physics coupling to nanomedicines using this edge tool, the synergistic immunological effects under different combination modes between multi-physics and multi-mode nanomedicines will be investigated systematically and comprehensively, such as activation of immune cells and immune regulatory cells, enhanced immune memory, reprogram of tumor immune microenvironment, regulation of immune factors and immune gene and their expression-related cytokines and chemokines, as well as the corresponding cell signal and biomolecule pathways.

It can be deduced that the future developed smart nanomedicines with multi-mode imaging, self-targeting transportation and controlled release can not only be used both in visible molecule imaging for the disease precise diagnosis (e.g. tumor classification and phase confirmation) and lesion localization but also their lesion tissue penetration depth and cell internalization can be improved dramatically by the empowerment from the coupling to the corresponding multi-physical fields, leading to smart nanotheranostics [37, 41, 47, 89, 94, 104, 108, 110, 123, 170]. Particularly, for the treatment of cancers, the synergistic effects between the nanomedicines and physical field ablation can endow the corresponding therapy with more flexible intelligent regulation ability on the TIME [127], and consequently, the elimination of cancers and prevention of their recurrence and metastasis will be possibly realized [104, 109, 272, 273].

If readers need more details on the nanomedicines mediated physical field ablation therapy, Chapters 11–15 ("Nanomedicine medicating ultrasound therapy," "Nanomedicine mediated photodynamic and/or photothermal therapy," "Nanomedicine mediated pulsed electric field therapy," "Nanomedicine mediated magneto-dynamic and/or magneto-thermal therapy," "Nanomedicine mediated radiofrequency or nuclear radiation therapy") in this monograph can be referred to.

In addition, there are lots of molecule-like features or discrete energy and momentum emerging in some nanomaterials with sizes less than 2 nm (i.e. superatom cluster with atom numbers from one to no more than several hundreds, such as $Au_{25}L_{18}$, $Ag_{44}L_{30}$, etc., where "L" denotes the ligand, such as thiolate) [274–277]. As they are translated into drugs, they can be called superatom cluster drugs or quantum drugs, which can also include the assemble of these superatom cluster drugs or even some assembly of single atom catalysts of special biological functions (e.g. molecule surgery) [53, 98, 174, 274, 277]. Together with many quantum effects that have also been found in many molecule signal pathways of biological processes (e.g. photosynthesis of chlorophyll) [9, 85, 88, 278]. These superatom cluster drugs will not only promote the progress of nanoenzymes and nanoimmunotherapy but also lead to a new arena in subversive medicines: quantum medicines or quantum drugs (Figure 1.2: one of the major breakthroughs or progresses in 2023) [9, 83–88], for the treatment of many complicated diseases precisely and efficiently, which

Table 1.3 Achievements of multiple physical field ablation and/or multi-mode nanomedicines mediated physical field ablation and the related instrumentation and clinical stages, obtained by 10 currently leading institutes or companies.

Institutes/ Company	Multi-modal therapy via multi-physical field coupling	Typical therapeutical characteristics	Immunological effects	Clinical stage	References
Covidien, United States	High-intensity focused ultrasound, light irradiation/ fluorescence excitation	High organizational penetration, spatiotemporal control, synergistic effects, and ROS generation	Promote the release of tumor antigens, activate immune cells, induce inflammatory reactions, and enhance immune memory	Preclinical, Clinical Phase I, II	[204–206, 216, 248]
Massachusetts Inst of Technology, United States	High-intensity focused ultrasound, electric field heating/polarization/perforation/ molecular microstructure	High tissue penetration, oxygen independence, and synergistic effects	Dissolution and apoptosis of tumor cells, activation of immune cells, induction of immune memory, and regulation of immune suppression	Preclinical, Clinical Phase I, II	[208, 216, 249]
Griffin Adam Holiday, United States	Light energy irradiation/ chemical fluorescence excitation, electric field heating/polarization/perforation/ molecular microstructure	Spatiotemporal resolution control, oxygen independence, synergistic effects	Activation and proliferation of immune cells and immune regulatory cells, enhanced anti-tumor immune response, and improved expression of related immune factors	Preclinical	[209, 210, 216, 250]
King Saud University, Saudi Arabia	Nanomagnetic induction hyperthermia; magnetothermal/magnetodynamics; radiotherapy and chemotherapy	Enhancing sensitivity to radiotherapy or chemotherapy	Tissue penetration, targeted therapy, noninvasive, with synergistic effects of nanomedicines	Preclinical, Clinical Phase I, II	[211–214, 216, 251, 252]
Immodulon Therapeutics Ltd., United Kingdom	Low-temperature freezing, radio frequency high-temperature thermal/biochemical reaction activation	High temperature thermal effect, low temperature freezing effect, and cell rupture	Multiple immune cell activation and enhancement, enhanced anti-tumor immune response, and regulation of immune-related gene expression	Preclinical, Clinical Phase I, II	[211–214, 216, 253]

(Continued)

Table 1.3 (Continued)

Institutes/ Company	Multi-modal therapy via multi-physical field coupling	Typical therapeutical characteristics	Immunological effects	Clinical stage	References
Shenzhen Maiwei Medical Company	Low-temperature freezing, pulsed electric field heating/polarization/perforation/molecular microstructure, RF high-temperature thermal/biochemical reaction activation	Efficient, minimally invasive, precise lesion localization, multi-modal ablation	Antigen release, immune cell activation, inflammatory response, immune memory	Preclinical, clinical phase I, II	[254, 255]
Hangzhou Ruidi Biotechnology Co., Ltd	Low temperature freezing, pulsed electric field heating/polarization/perforation/molecular microstructure, RF high-temperature thermal/biochemical reaction activation	Efficient, minimally invasive, precise lesion localization, and no heat sink	Stimulation of immune cell activation and proliferation, inflammatory response, regulation of tumor microenvironment, and promotion of tumor antigen expression	Preclinical, Clinical Phase I, II	[256–261]
Shanghai Meijie Medical Technology Co., Ltd	Low temperature freezing, radio frequency high temperature thermal/biochemical reaction activation	Accurate treatment of lesions	Multiple immune cell activation and enhancement, enhanced anti-tumor immune response	Clinical trial	[212, 213, 262]
University of Science and Technology Beijing, Zhenzhou Tianzhao Biomedical Company	Multi-mode nanomedicine mediated ns–psPEF ablation, alternating magnetic field, and laser irradiation; PEF or laser radiation producing thermal/polarization/perforation effects; magnetothermal/magnetodynamics; multi-function activation of nanomedicines	Noninvasive visible targeting lesion at nanoscale; overcoming multi-drug resistance; preventing recurrence and metastasis; regulating tumor cell microenvironment and cytokines; activating immune cells and their proliferation	Nanodrugs coupling multi-field ablation synergistically enhances the activation and the proliferation of immune cells, the inflammatory response, the regulation of tumor microenvironment, and the promotion of tumor antigen expression	Preclinical	[41, 89, 108, 111, 123, 247, 263–267]
Peking University	Light irradiation/fluorescence excitation; magnetothermal/magnetodynamics; radiotherapy and chemotherapy	Noninvasive, precise treatment of lesions, adjustable treatment effect	Heat stress effect, immune cell activation, inflammatory response, immune memory	Preclinical	[268, 269]

have been paid attention again recently [84, 85, 109, 174, 279]. These quantum medicines may also show great potential in mediating multi-physical field ablation for subversive therapies for intractable diseases.

1.5 Future Development of Nanomedicines by Coupling Advanced Biomedicines (Including Biochemistry and Biophysics), Modern Physicochemical Technologies, and Artificial Intelligence Technology

However, there are still many questions in the development of nanomedicines and the corresponding multi-mode therapies. One of the current key issues is how to obtain these smart nanomedicines with a desired multi-mode imaging function, zymogen-like immune stealth transport ability and special cell or cell microenvironment targeting releasing function. Usually, addressing all of these abilities or functions by one kind of materials is extremely difficult but varieties of materials are coupled together. Fortunately, Song et al. from USTB have developed a novel nanomedicine design strategy as below. The inorganic–organic nanocomposites are constructed based on onion-like nanohybrids of metal alloy or core–shell metal compounds as inorganic cores with multi-mode imaging functions, which can be surface modified and stabilized by organic shells forming inorganic and organic nanocomposites and then conjugating to some organic drugs (e.g. ginsenoside, Paclitaxel, Doxorubicin, antibodies [e.g. PD-L1], etc.) to construct high biocompatible nanomedicine [41, 89, 108]. Finally, these nanomedicines can be encapsulated by hydrogel (e.g. PEG-*g*-chitosan) or amphiphilic polymers (e.g. PEG-*b*-PLGA) forming nanocapsules or nanovesicles that can be simultaneously surface-linked with some cell or microenvironment specific targeting biomolecules [111]. Recently, Song's group invented a pilot microfluidic process (Figure 1.2) [98], an ultrasonic atomization coupling pyrolysis process, [258, 259, 280] a facile freeze-annealing process [281] and a solid-phase sintering and vapor–liquid–solid growth (SS–VLS-like) method [282] for the large-scale synthesis of these multi-mode nanoparticles, single atom clusters, N, P-doping 3D graphene nanodots supporting quantum dots and as precursors with flexible size (from single atoms to tens of nanometers), shape, composition, and supporter control as well as desired physicochemical properties (e.g. magnetic, optical, electronic, sonic, or mechanical properties) [41, 89, 98, 281, 283–290], for this kind of novel smart nanomedicines or nanoenzymes according to this strategy.

Another key issue is to develop varieties of multi-modal theranostics instruments based on the optimized coupling modes among varieties of nanomedicines and multi-modal ablation determined by varieties of multi-physical-field coupling ablation. Particularly, how to design the key devices or units realizing multi-mode nanomedicines mediated the suitable multi-physical-field coupling ablation instrument with high spatial-temporal resolution (for space time resolution: from μs to ns or even to fs; for space resolution: from micrometer to nanometer and

even single molecule or ligand level) is crucial to optimize their synergistic effects to develop subversive smart therapies by comprehensively regulating the key immune systems (e.g. immune cells, immunological gene expression, and cell microenvironment) [37, 216, 291, 292]. Overcoming this issue is also essential to fulfill the clinical trials of nanomedicines themselves and these combination therapies [37, 41, 108, 187, 189]. However, this project is not a trivial work. Fortunately, many groups in institutes and companies have been on this road. For example, Song's group in USTB also developed the combined therapy by their multi-mode nanomedicines mediated the nsPEF, which has shown excellent therapeutic effects and great potential in the immune system regulation in the HCC treatment [207]. A novel type of instrument with many subversive features has been on the agenda based on multi-functional nanomedicines mediated multi-physical field coupling ablation by conjugating the rotating or pulsed magnetic fields, laser irradiation and ultrafast pulsed laser, HIFU to their instrument of multi-mode nanomedicines mediated nsPEF ablation [271].

In addition, the recently developed artificial intelligence generation content (AIGC) or machine learning technologies have been successfully used in the rapid and accurate development of varieties of novel specific drugs and therapy designs for intractable diseases [22, 293–296]. AIGC can be further used in the design and microstructure optimization of multi-mode nanomedicines and the assembly of multi-physical fields, as well as the optimization of the coupling modes and key devices, accelerating the instrument development of nanomedicines mediated multi-physical field coupling ablation and the key devices (e.g. tubing type microprobes assembling several physical fields for diagnosis and therapy of diseases). In the following 5–10 years, fundamental research and the key clinical trials of nanomedicines mediated multi-physical field coupling ablation may focus on the following aspects by the combination of progresses in fundamental biomedicines, modern physicochemical technologies, and artificial intelligence: (i) high-throughput design and synthesis methods (e.g. microfluidic processes for drug design and screening) for multi-functional nanohybrids and smart nanomedicines; (ii) basic studies on the interactions between nanomedicines and single cell, sub-cellular units, colossal cells and tissues (particularly for tumor cells and stem cells and neurocytes), the T-cells and B-cells or other immune cells for intractable disease therapy, and the related gene expression and molecular or signal pathway; (iii) nanomedicine database built-up and development assisted by AIGC; (iv) precise and personalized nanomedicine development assisted by AIGC. (v) database building of multi-physical field coupling ablation and their therapeutical effects and immunological effects assisted by AIGC; (vi) subversive therapy development and fundamental biomedical mechanism investigation of nanomedicines mediated multi-physical field ablation assisted by AIGC; and (vii) quantum medicine development for intractable diseases and their therapeutical mechanism study assisted by AIGC. For details on AIGC-assisted design of nanomedicines and combined therapies, readers can refer to Chapters 16 "Nanomedicine conjugating with AI Technology and Genomics for Precise and Personalized Therapy" and 17 "Microfluidic Conjugating AI Platform for High Throughput Nanomedicine Screening" of this monograph.

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