The application of carbon dots in tumor immunotherapy: researches and prospects

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Abstract

Since the rapid development of nanomedicine in oncotherapy, multiple nanomaterials are adopted to regulate the immune system in cancer individuals. Tumor immunotherapy enhances the immune function of patients to achieve the purpose of killing tumor cells by utilizing the organism immune mechanism. As emerging inorganic carbon nanoparticles, carbon dots (CDs) have been found as photosensitizers, vaccines, immunoadjuvants, and so on for cancer treatment due to their unique structure and property, such as effective platforms for drug delivery, immunomodulation, and phototherapy. In this review, we mainly discuss the recent application of CDs in tumor immunotherapy and the prospects of CDs in the field of immune medicine. By assessing the achievements and challenges of CDs in tumor immunotherapy, our review would provide mechanistic insights into the evolution of future nanomedicine.

The application of carbon dots in tumorimmunotherapy: researches and prospects

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ABSTRACT

Since the rapid development of nanomedicine in oncotherapy, multiple nanomaterials are adopted to regulate the immune system in cancer individuals. Tumor immunotherapy enhances the immune function of patients to achieve the purpose of killing tumor cells by utilizing the organism immune mechanism. As emerging inorganic carbon nanoparticles, carbon dots (CDs) have been found as photosensitizers, vaccines, immunoadjuvants, and so on for cancer treatment due to their unique structure and property, such as effective platforms for drug delivery, immunomodulation, and phototherapy. In this review, we mainly discuss the recent application of CDs in tumor immunotherapy and the prospects of CDs in the field of immune medicine. By assessing the achievements and challenges of CDs in tumor immunotherapy, our review would provide mechanistic insights into the evolution of future nanomedicine.

KEYWORDS

Carbon dots; Immunotherapy; Photodynamic therapy; Tumor; Vaccine

INTRODUCTION

Cancer is one of the most serious diseases affecting public health globally because of its high incidence and fatality rate. The inability to target and direct medications to the cancer cells specifically limits the capabilities of present technologies for the diagnosis and treatment of tumors. It is urgently necessary to get over these biological constraints in cancer diagnosis and treatment by developing novel tumor-targeted techniques[1]. Tumor immunotherapy has attracted much attention owing to its efficient targeting and lasting curative effect. Unlike previous surgical excision, chemotherapy, and radiotherapy, tumor immunotherapy is developed to activate the immune system of patients and is hoped to utilize their own immune function to kill cancer cells[2]. In physiological conditions, the immune system identifies foreign substances or abnormal cells and then neutralizes these to protect metabolic homeostasis and health in humans. However, some pathological conditions, especially cancer, would disturb this homeostasis[3]. On the one hand, the surviving cancer cells exhibit lower immunogenicity after undergoing a long-term mutation, on the other hand, the cancer cells would construct a special tumor microenvironment that reduces the inflammatory response of immune cells and escape the immune surveillance[4, 5]. In immunotherapy, macrophages and dendritic cells (DCs) have important roles in the direct recognition of a mutant cell[6]. They phagocytose mutant cells and secrete a plethora of pro-inflammatory mediators that can initiate the inflammatory cascade and present antigens to T cells[6, 7]. This initiates the adaptive immune response and further stimulates T cell division, proliferation, and maturation. Some mature T cells differentiate into memory T cells that retain antigen memory and respond quickly to antigen re-entry, while the majority differentiate into effector cells with immune effects, such as regulatory T cells that produce cytokines to regulate immune response, natural killer (NK) T cells that target and kill foreign cells, and effector T cells that stimulate B-lymphocyte proliferation and antibody production[4, 8, 9]. Over the last few years, increasing biomedical research have indicated the potential of nanomaterials in modulating the immune response[8, 10]. Biomaterials alone, or conjugated with specific biomolecules, can be used to directly regulate the immune system, hence providing new approaches for the enhancement of tumor immunotherapy[4, 11].

Carbon dots (CDs) are fluorescent carbon nanomaterials with a size of less than 10 nm and are composed of graphitizedsp^2 carbon nuclei and shells containing amino, carboxyl, and hydroxyl groups on the surface[12]. In recent years, as a new type of carbon-based nanomaterial, CDs have drawn considerable scientific attention for their distinctive optical properties, low cost, eco-friendliness, the abundance of functional groups, high stability, and electron mobility[13]. Because of their good photoluminescence (PL), high quantum yield (QY), low toxicity, small size, and appreciable biocompatibility, they are used in a variety of fields, including the fields of biomedicine, catalysis, optoelectronics, and anticounterfeiting[14]. The fluorescence excitation and emission wavelength falling within the biological transparency window (650–900 nm) is always one of the major research directions in CDs[15]. There are two benefits to using the near-infrared (NIR) region as the excitation wavelength for fluorescence imaging or photochemical reactions[16]. First, compared to UV-visible light, NIR light can penetrate deeper and gather richer tissue information because it scatters and absorbs less when it comes into contacting with skin and tissue. Second, because the organism emits primarily blue or green fluorescence, NIR light can reduce the interference from spontaneous background fluorescence and enhance the imaging signal-to-noise ratio[15, 16]. So far, there have been recent cases of visible absorption and emission in the NIR range, making CDs attractive agents for biological applications[17]. Their unique fluorescent features, in particular, have fostered their widespread use in a variety of life science domains, including bio-sensing and bio-imaging[18]. In addition to their multicolored emission, tunable optical properties, and outstanding photostability, CDs can also be used as efficient tools for tumor therapy owing to their easy surface functionalization and excellent biocompatibility[14, 19]. On the one side, CDs can be employed as phototherapy agents, including photodynamic therapy (PDT) and photothermal therapy (PTT)[20, 21]. On the other side, the rational design would confer CDs the properties of nanoenzymes or chemotherapeutic agents involved in tumor chemodynamic therapy (CDT)[22]. However, each traditional treatment has its

limitations and drawbacks. With a view toward more accurate and effective cancer treatment, collaborative treatments with immunotherapy are becoming increasingly attractive in current CDs research[4].

Tumor immunotherapy mainly includes nonspecific immunotherapy, monoclonal antibody immunotherapy, adoptive immunotherapy, and vaccine therapy[3]. In this mini-review, we will congregate and systematically discuss the relevant research available presently, regarding the application of CDs in tumor immunotherapy. The review collects the related immunotherapy applications of CDs in PDT, PTT, CDT, vaccine, and immunoadjuvant in recent years (Scheme 1). And then, we conclude the current situation of CDs in tumor immunotherapy, together with some challenges and prospects that remain in immunotherapy.

IMMUNOTHERAPY APPLICATIONS OF CARBON DOTS

PDT

PDT has been studied as an immunogenic cell death (ICD) inducer for eliciting direct tumor-killing effects through the creation of tumor antigen pools with danger signals that promote cancer-specific immunity[20]. Photosensitizers (PS) can produce reactive oxygen species (ROS) in response to exposure to a certain wavelength, which can then kill surrounding cancer cells by necrotic or apoptotic cell death[20]. Owing to the highly enriched distinctive physical and chemical characteristics, CDs have been applied in the synergistic treatment of PDT and immunotherapy [21]. Kim*et al.* designed chlorin e6 (Ce6)-loaded pH-sensitive CDs (Ce6@IDCDs) to establish superior antitumor immunity[23]. At tumoral pH 6.5, Ce6 was released four times compared with the release at physiological pH 7.4. In the bilateral CT-26-bearing mice model, the Ce6@IDCDs elicited significant anti-tumor effects at laser-treated-primary tumor regions via ROS generation. Moreover, Ce6@IDCDs upon laser irradiation recruited a large amount of activated $CD8^+$ T cells, NK cells, and mature DCs into tumor tissue and inhibited tumor growth even at untreated sites[23]. The immune checkpoint inhibitors in particular programmed death ligand 1 (PD-L1) and its receptor PD-1 have been able to reactivate dysfunctional and worn-out T cells, producing retention effects in 50-80% of tumor patients[3, 9, 24]. To this end, Zhang*et al.* developed the nanoparticle γ-PGA@GOx@Mn,Cu-CDs, which showed a long retention time at the tumor microenvironment and could further target cancer cells[25]. It displayed both photothermal and photodynamic effects under laser irradiation at 730 nm. By synergistically combining check-point-blockade therapy, this nanoparticle could activate systematic anti-tumor immune response which ablated primary and distant tumors[25] (Figure 1a). Overall, synergistic PDT and immunotherapy will be the main development direction of CDs in the removal of tumors, the inhibition of distant cancers, and the prevention of tumor relapse.

PTT

PTT is a viable alternative to conventional cancer treatment since it is non-invasive, has high spatiotemporal controllability, and few unfavorable side effects[21]. NIR light-reacting photothermal agents (PTAs) are the best for performing PTT on cancer. The development of novel PTAs for tumor treatment and eradication is currently undergoing significant research[14]. Lu*et al.* developed multifunctional nanoparticles based on polydopamine (PDA) for combining immunoadjuvant R848 to achieve FL and multispectral optoacoustic tomography (MSOT) dual-mode imaging for diagnostic purposes[26]. PDA-PEG-R848-CDs can be employed for NIR-induced PTT to eradicate distant/metastatic cancers by directly eliminating original tumor cells and inducing immune responses[26]. Further, PDA-PEG-R848-CD-based PTT combined with PD-L1 blockers would create a long-term immunological memory effect that could be used to prevent tumor recurrences[26]. Qian *et al.* effectively used a hydrogen bond/electrostatic-assisted co-assembly approach to consistently incorporate polymer-coated CDs into the ordered framework of mesoporous silica nanoparticles (CD@MSNs)[27]. The obtained CD@MSN was capable of accumulating dispersive CDs with enhanced photothermal effect and elevated targeting accumulation, which allowed for photothermal imaging-guided PTT both *in vitro* and *in vivo* [27]. It is interesting to note that CD@MSN-mediated PTT, which takes advantage of the biodegraded debris, has been shown to synergistically achieve immune-mediated inhibition of tumor metastasis by promoting the proliferation and activation of macrophages and NK cells while also increasing the secretion of relevant cytokines[27] (Figure 1b). Lu *et al.* reported a kind of Z-scheme

CDs-based PTAs consisting of two-dimensional (2D) ultrathin nonmetallic BxC/C Janus quantum sheets (BxC/C JQSs) which have a high photothermal conversion of 60% in NIR-II[28]. In addition, these new Z-scheme BxC/C-polyethylene glycol JQSs display effective tumor elimination outcomes both *in vitro* and *in vivo* through the synergistic photothermal-immunotherapy in the NIR-II with undetectable harm to normal tissues[28]. To sum up, these studies showed the potential of combining PDA-based PTT with cancer immunotherapy to achieve a remarkable synergistic therapeutic result in tumor treatment.

CDT

One of the most important aspects of CDT is the application of Fenton reaction to the tumor microenvironment (TME) for cancer therapy[22]. In tumors, H_2O_2 overexpression and mild acidity produce more cytotoxic hydroxyl radicals via Fenton and Fenton-like reactions. CDT mediated by ROS has demonstrated a significant anti-cancer effect without external stimulation or drug resistance and is currently viewed as a promising treatment for cancer[22, 29]. Numerous chemodynamic drugs, including nanomaterials based on Fe^{2+}, Cu^{+}, Mn^{2+}, Mo^{4+}, and Ti^{3+}, have been developed over time with improved CDT efficacy[29]. However, the inability of a single CDT approach to completely eradicate tumors paved the way for research into fresh system designs for multimodal therapy and improved CDT. Hou *et al.*designed a transformable honeycomb-Like nano-assemblies of CDs, which delivered doxorubicin, immunotherapeutic enhancer (Fe ions), and tumor microenvironment modifier losartan[30] (Figure 2a). The drug-loaded nano-assemblies firstly disassociated into individual CDs to release losartan to mitigate stroma and hypoxia. And then, the individual CDs carrying doxorubicin and Fe ion efficiently penetrated deep into tumor to trigger intensified immune responses, including effective T cell infiltration, tumor growth inhibition, and lung metastasis prevention[30]. A carbon quantum dots (CQDs)-based biocompatible nanozyme made from chlorogenic acid (ChA), a significant bioactive natural component from coffee, was reported by Yao*et al* [31]. They discovered that ChA CQDs had blatant GSH oxidase-like behaviors and consequently encouraged ferroptosis in cancer cells by interfering with GPX4-catalyzed lipid repair mechanisms[31]. ChA CQDs significantly reduced the tumor growth in HepG2-tumor-bearing mice *in vivo* and attracted large numbers of immune cells that infiltrated the tumor, such as T cells, NK cells, and macrophages, turning "cold tumors" into "hot tumors" that triggered systemic anti-cancer immune responses[31] (Figure 2b). Moreover, He *et al.* used a DA-CQD@Pd single atom nanozyme (SAN) and immune adjuvant CpGODN to create a bioadhesive injectable hydrogel for localized immunomodulation and catalysis-augmented immunotherapy[32]. The SAN, which has high water dispersibility, was made by adding Pd single atoms to a DA-CQD support[32]. Due to a dual catalytic mechanism, the DA-CQD@Pd SAN displayed excellent catalytic activity. The inherent catalytic activity of a single Pd atom, which can catalyze the conversion of H_2O_2/APS to hydroxy radicals (*OH), is one aspect. The other is the catechol-quinone redox pairs on the DA-CQD that catalyze the production of *OH from H_2O_2/APS as well[32] (Figure 2c). Noteworthily, the SAN converted H_2O_2 into hydroxyl radicals, causing immunogenic cell death (ICD) in tumors and producing tumor-associated antigens in the tumor lysate, which triggered an immune response against the tumor[32]. In addition, solvothermal-produced photoactivatable Pt(IV)-coordinated CDs (Pt-CDs) and their bovine serum albumin (BSA) complex (Pt-CDs@BSA) were created by Guo *et al* [33] (Figure 2d). In comparison to pure Pt-CDs, Pt-CDs@BSA exhibit expanded particle sizes of 50–120 nm, which have significantly increased cellular absorption and tumor accumulation. Under orange light, these materials effectively reduce Pt(IV) to Pt(II) and encourage the generation of *OH from water. Due to effective cytotoxic Pt(II) species release, *OH formation, and intracellular acidification, this novel approach with ultra-strong cancer cell killing capacities produced substantially stronger *in vivo* ICD than cisplatin at the same Pt dose. Pt-CDs@BSA treatment not only destroyed the main tumor but also inhibited distant tumor growth and lung metastasis, showing improved antitumor and antimetastatic activity[33]. Together, these studies indicate that the synergistic use of CDT and immunotherapy become one of the most popular cancer treatments in recent years.

Vaccine

Tumor vaccine therapy is designed by introducing tumor antigens into the patient to stimulate a specific anti-tumor immune response and improve the immune microenvironment[34, 35]. Due to its advantages of

tumor specificity and long maintenance time in vivo, vaccine therapy has become a popular research field in cancer therapy[24, 34]. The vaccine can be divided into therapeutic vaccines and preventive vaccines[36]. Therapeutic vaccines have significantly different characteristics from traditional preventive vaccines[36]. It needs to be designed and engineered to gain the ability for tumor-specific treatment[24]. Luo *et al.*synthesized a CD with citric acid and PEG-1500 as the vaccine adjuvants to be combined with the tumor protein antigen model ovalbumin (OVA)[37]. The combination of CDs and OVA (CDs-OVA) could accelerate antigen uptake and maturation of dendritic cells (DCs). After CDs-OVA treatment, the expression of costimulatory molecules CD80 and CD86 of DCs was increased, which subsequently stimulated splenocyte proliferation and the production of interferon-gamma (IFN-γ). In vivo, CDs-OVA also induced strong antigen-specific cellular immune responses to inhibit the growth of B16-OVA melanoma cancer in C57BL/6 mice[37]. Different chiral precursors may produce CDs with different properties. Another study by Liu *et al.* reported a chiral CD that was synthesized from citric acid and L/D glutamic acid and then bound to antigen model OVA[38] (Figure 3a). Compared to the L-OVA, D-OVA could be effectively internalized by DCs, boost DC maturation, cross-present antigen to T cells, and suppress the growth of B16-OVA melanoma[38] (Figure 3b). In conclusion, these works exhibit the high potential of CDs as the vaccine for tumor inhibition and immunotherapy.

Immunoadjuvant

Unlike vaccines, immunoadjuvants are not antigenic, which is used to increase tumor immunogenicity or enhance the immune response of immune cells in cancer[34]. Thus, immunoadjuvants are often used in conjunction with some immunotherapies, such as PD-1/PD-L1 blockers[34]. CDs have been increasingly applicated in immunoadjuvants in recent years[39]. Li*et al.* reported a nanoparticle prepared by the supramolecular assembling of CDs and Ricin toxin binding subunit B (RTB)[40]. The formed CDs-RTB can protect RTB against enzymatic hydrolysis, promote macrophage proliferation, and increase inflammatory cytokines secretion in macrophages[40]. CDs themselves also have the ability to play as immunoadjuvants. Cow manure-derived CDs were reported can induce many necrocytosis and inflammatory infiltrates in tumors, which suggested the potential of CDs as an immune therapy adjuvant[41]. In addition, Arezki*et al.* used citric acid and branched polyethyleneimine to synthesize a kind of cationic CDs that can induce inflammasome-dependent pyroptosis via lysosomal dysfunction[42]. It is worth noting that pyroptosis could induce the release of tumor cell antigens and recruit a large number of immune cells[42, 43]. Further, as the immunoadjuvants, the target of CDs is not only cancer cells. Zhou*et al.* reported that mannose-derived CDs (named as Man-CDs) could effectively capture several "danger signals" after microwave ablation treatment and then deliver these signals specifically to dendritic cells (DCs)[44]. In vivo, intratumoral injection of Man-CDs stimulated a potent tumor-specific immune response and suppressed both primary and distant tumors[44]. All of these studies demonstrated that CDs could be effective adjuvants that enhance tumor-specific immunotherapy.

CONCLUSION AND PERSPECTIVE

Immunotherapy is an essential approach to cancer treatment that has high specificity, long-term efficacy, reduces a large intake of drugs, and provides less invasive treatment than traditional therapies. Due to special properties or conjugation with different adjuvants, CDs are developed to inspire immune response which can be a novel approach to clinical application in tumor immunotherapy. In this review, we summarize and discuss the current application of CDs in tumor immunotherapy, including vaccines, immunoadjuvants, and synergistic therapy with PDT/PTT/CDT. In general, depending on the recent progress of CD good absorption in NIR and high ROS generation caused by CDs surface electronic transitions under light irradiation, it is rational for CDs to act as important tools in targeted tumor PDT/PTT. Further, smaller particle size, variable surface structure, and easy cell internalization make CDs become great vehicles for the delivery of chemotherapeutics, such as doxorubicin and platinum drugs. Nevertheless, it is often difficult to get the ideal long-term therapeutic effect with monotherapy. To address this, current studies on tumor therapy by using CDs are moving toward the synergistic use of multiple treatments, especially combined with immunotherapy. By ingenious design, CDs are able to elicit an anti-tumor immune response while performing PDT/PTT/CDT. On the other side, CDs, as presented, are directly used in tumor immunotherapy, namely, as vaccines or immunoadjuvants. Although the application of CDs in vaccines and immunoadjuvants

is still in its infancy, high biocompatibility and various functional surface groups help CDs to stimulate the tumor immune response and transform the low immunogenic "cold tumors" into the high immunogenic "hot tumors". In addition, CDs are explored to combine with tumor antigen models, such as OVA, to perform the dual role of immunoadjuvant and vaccine in tumor therapy.

However, there are also many challenges and clinical translation concerns of CDs in tumor immunotherapy. Firstly, the pharmacokinetic changes of CDs *in vivo* , especially the structure and functional group changes of CDs are unclear. Clinical application is often holding a cautious attitude to unclear medicines. Secondly, although most CDs have lower toxicity and better clearance efficiency *in vivo* , the information related to long-term toxicity remains missing since cancer treatment is a long and cumulative process. Thirdly, the current efficiency of CDs in immunotherapy has yet to be improved. Enhancing targeting and reducing side effects are long-term questions of CDs in immunotherapy. Last but not least, the applications of CDs in tumor immunotherapy are still in the earlier stages and the therapeutic experiments are mainly conducted in rodents. Further studies are needed to translate the results obtained in animal models into human applications. Collectively, CDs have made remarkable progress in tumor immunotherapy. The applications of CDs so far are very promising in tumor immunotherapy. CDs can play a role in directing immune response, but further investigation is essential to explore their full potential and revolutionize the future of medicine.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this review as no new data were created or analyzed in this article.

REFERENCES

[1] C. Roma-Rodrigues, R. Mendes, P. V. Baptista, A. R. Fernandes,*Int. J. Mol. Sci.* **2019** , 20.

[2] Y. Zhang, Z. Zhang, *Cell. Mol. Immunol.* **2020** , 17, 807.

[3] D. S. Chen, I. Mellman, *Nature* **2017** , 541, 321.

[4] S. Azevedo, R. Costa-Almeida, S. G. Santos, F. D. Magalhães, A. M. Pinto, *Applied Materials Today* **2022** , 27.

[5] Q. Muhammad, Y. Jang, S. H. Kang, J. Moon, W. J. Kim, H. Park,*Biomater Sci* **2020** , 8, 1490.

[6] N. R. Anderson, N. G. Minutolo, S. Gill, M. Klichinsky,*Cancer Res.* **2021** , 81, 1201.

[7] A. Gardner, B. Ruffell, *Trends Immunol.* **2016** , 37, 855.

[8] Y. Jiang, M. Fan, Z. Yang, X. Liu, Z. Xu, S. Liu, G. Feng, S. Tang, Z. Li, Y. Zhang, S. Chen, C. Yang, W. C. Law, B. Dong, G. Xu, K. T. Yong, *Biomater Sci* **2022** , 10, 6862.

[9] J. Cao, Q. Yan, *Trends in cancer* **2020** , 6, 580.

[10] J. Saleem, L. Wang, C. Chen, *Adv Healthc Mater***2018** , 7, e1800525.

[11] C. Yang, Z. I. Lin, J. A. Chen, Z. Xu, J. Gu, W. C. Law, J. H. C. Yang, C. K. Chen, *Macromol. Biosci.* **2022** , 22, e2100349.

[12] L. Ethordevic, F. Arcudi, M. Cacioppo, M. Prato, *Nat Nanotechnol* **2022** , 17, 112.

[13] J. J. Liu, R. Li, B. Yang, *ACS CENTRAL SCIENCE***2020** , 6, 2179.

[14] B. Wang, H. Cai, G. I. N. Waterhouse, X. Qu, B. Yang, S. Lu,*Small Science* **2022** , 2.

[15] Y. Q. Wang, X. C. Li, S. J. Zhao, B. H. Wang, X. Z. Song, J. F. Xiao, M. H. Lan, *Coord. Chem. Rev.* **2022** , 470.

[16] B. D. Mansuriya, Z. Altintas, *Nanomaterials (Basel)***2021** , 11.

[17] Z. Hallaji, Z. Bagheri, M. Oroujlo, M. Nemati, Z. Tavassoli, B. Ranjbar, *Mikrochim. Acta* **2022** , 189, 190.

[18] T. S. Atabaev, *NANOMATERIALS* **2018** , 8.

[19] J. Liao, Y. Yao, C.-H. Lee, Y. Wu, P. Li, *Pharmaceutics***2021** , 13.

[20] Z. Yan, C. Qin, C. Zhao, Z. Fang, *J. Nanopart. Res.***2020** , 22.

[21] D. Gao, X. Guo, X. Zhang, S. Chen, Y. Wang, T. Chen, G. Huang, Y. Gao, Z. Tian, Z. Yang, *Mater Today Bio* **2020** , 5, 100035.

[22] C. Jia, Y. Guo, F. G. Wu, *Small* **2022** , 18, e2103868.

[23] D. H. Kim, J. Seo, K. Na, *Mol. Pharm.* **2020** , 17, 2532.

[24] K. L. Hess, I. L. Medintz, C. M. Jewell, *Nano Today***2019** , 27, 73.

[25] M. Zhang, W. Wang, F. Wu, T. Zheng, J. Ashley, M. Mohammadniaei, Q. Zhang, M. Wang, L. Li, J. Shen, Y. Sun,*Biomaterials* **2020** , 252, 120106.

[26] Q. Lu, S. Qi, P. Li, L. Yang, S. Yang, Y. Wang, Y. Cheng, Y. Song, S. Wang, F. Tan, N. Li, *J Mater Chem B* **2019** , 7, 2499.

[27] M. Qian, L. Chen, Y. Du, H. Jiang, T. Huo, Y. Yang, W. Guo, Y. Wang, R. Huang, *Nano Lett.* **2019** , 19, 8409.

[28] S. Y. Lu, J. Wang, X. Wang, W. Yang, M. Jin, L. Xu, H. Yang, X. Ge, C. Shang, Y. Chao, L. Zhou, K. Yin, Q. Zhang, L. Gu, Y. Cao, H. Ran, S. Guo, H. Liu, *Small Methods* **2022** , 6, e2101551.

[29] C. Y. Cao, X. R. Wang, N. Yang, X. J. Song, X. C. Dong,*CHEMICAL SCIENCE* **2022** , 13, 863.

[30] L. Hou, D. Chen, R. Wang, R. Wang, H. Zhang, Z. Zhang, Z. Nie, S. Lu, *Angew. Chem. Int. Ed. Engl.* **2021** , 60, 6581.

[31] L. Yao, M.-M. Zhao, Q.-W. Luo, Y.-C. Zhang, T.-T. Liu, Z. Yang, M. Liao, P. Tu, K.-W. Zeng, *ACS nano* **2022** , 16, 9228.

[32] H. He, Z. Fei, T. Guo, Y. Hou, D. Li, K. Wang, F. Ren, K. Fan, D. Zhou, C. Xie, C. Wang, X. Lu, *Biomaterials* **2022** , 280, 121272.

[33] D. Guo, J. H. Lei, D. Rong, T. Zhang, B. Zhang, Z. Tang, H. M. Shen, C. X. Deng, S. Qu, *Adv Sci (Weinh)* **2022** , 9, e2205106.

[34] Z. Liao, J. Huang, P. C. Lo, J. F. Lovell, H. Jin, K. Yang,*J Nanobiotechnology* **2022** , 20, 345.

[35] M. Peng, Y. Mo, Y. Wang, P. Wu, Y. Zhang, F. Xiong, C. Guo, X. Wu, Y. Li, X. Li, G. Li, W. Xiong, Z. Zeng, *Mol. Cancer***2019** , 18, 128.

[36] H. Wu, X. Fu, Y. Zhai, S. Gao, X. Yang, G. Zhai, *Adv Healthc Mater* **2021** , 10, e2100299.

[37] L. Luo, C. Liu, T. He, L. Zeng, J. Xing, Y. Xia, Y. Pan, C. Gong, A. Wu, *Nanoscale* **2018** , 10, 22035.

[38] H. Liu, Z. Xie, M. Zheng, *ACS Appl Mater Interfaces***2022** , 14, 39858.

[39] L. Shabani, M. Abbasi, M. Amini, A. M. Amani, A. Vaez, *J. Neurol. Sci.* **2022** , 440, 120316.

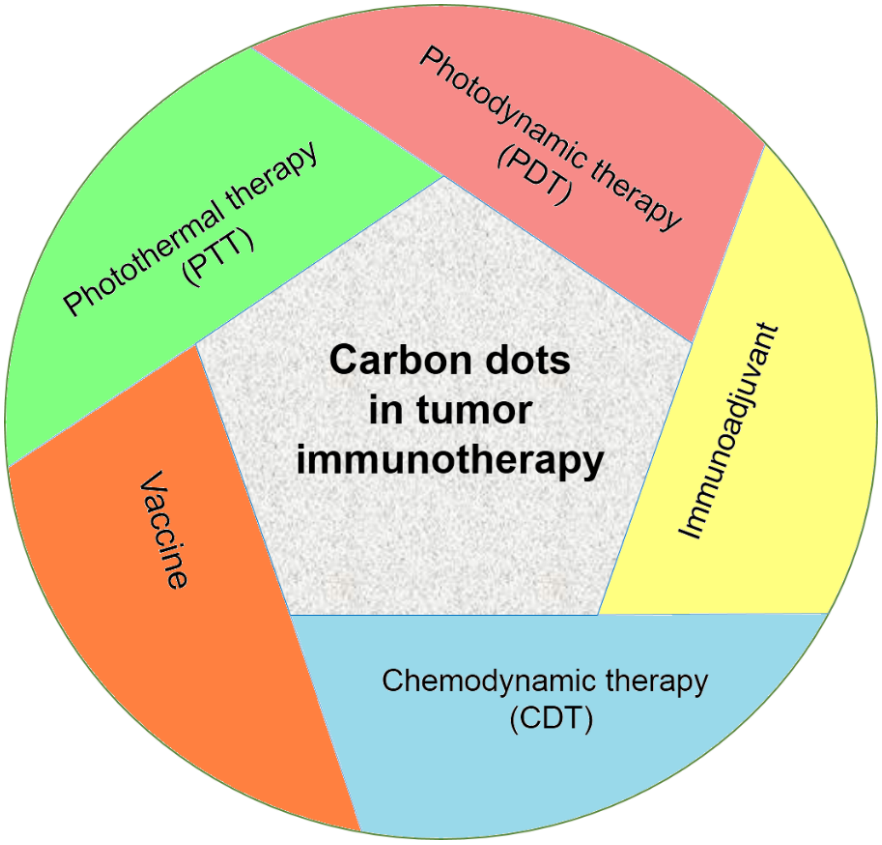
[40] Y. Li, W. Liu, C. Sun, M. Zheng, J. Zhang, B. Liu, Y. Wang, Z. Xie, N. Xu, *J. Colloid Interface Sci.* **2018** , 523, 226.

[41] F. H. Horst, C. Rodrigues, P. Carvalho, A. M. Leite, R. B. Azevedo, B. A. D. Neto, J. R. Correa, M. P. Garcia, S. Alotaibi, M. Henini, S. B. Chaves, M. O. Rodrigues, *RSC Adv* **2021** , 11, 6346.

[42] Y. Arezki, M. Rapp, L. Lebeau, C. Ronzani, F. Pons, *Front Toxicol* **2022** , 4, 925399.

[43] Z. Zhang, Y. Zhou, S. Zhao, L. Ding, B. Chen, Y. Chen,*Adv Sci (Weinh)* **2022** , 9, e2203583.

[44] Q. Zhou, N. Gong, D. Zhang, J. Li, X. Han, J. Dou, J. Huang, K. Zhu, P. Liang, X. J. Liang, J. Yu, *ACS nano* **2021** , 15, 2920.



Scheme 1 The application of carbon dots (CDs) in tumor immunotherapy, including PDT, PTT, CDT, vaccine, and immunoadjuvant.

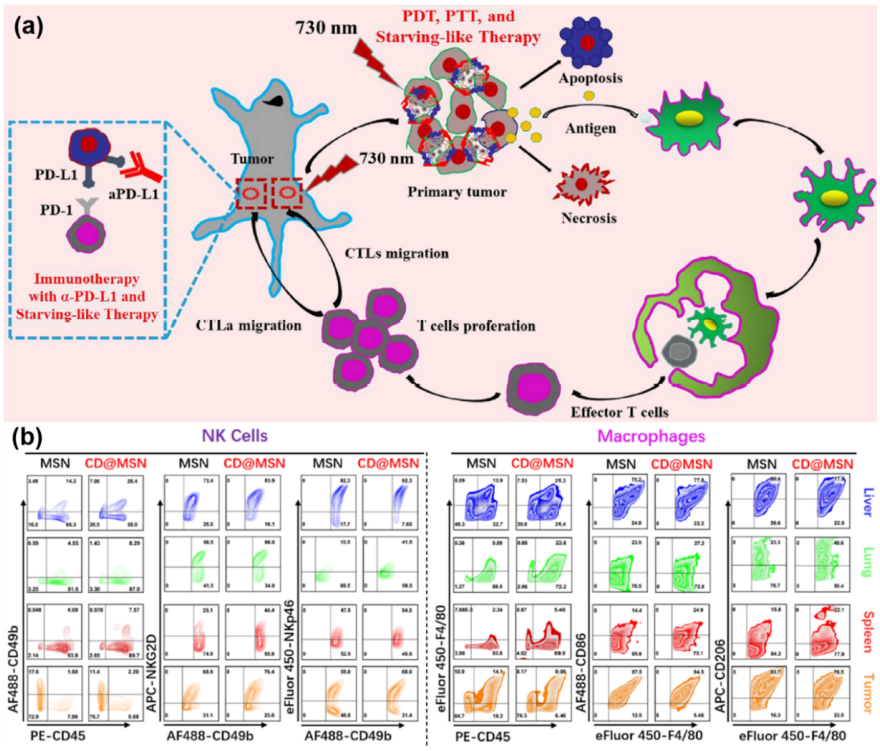


Figure 1 (a) Schematic illustration of phototherapy and immunotherapy mediated by γ-PGA@GOx@Mn,Cu-CDs NPs. Reproduced from Ref. [25] with permission of *Elsevier* . (b) Representative flow cytometric analysis images of NK cells and macrophages gating on CD45+ cells harvested from different organs. Reproduced from Ref. [27] with permission of *American Chemical Society* .

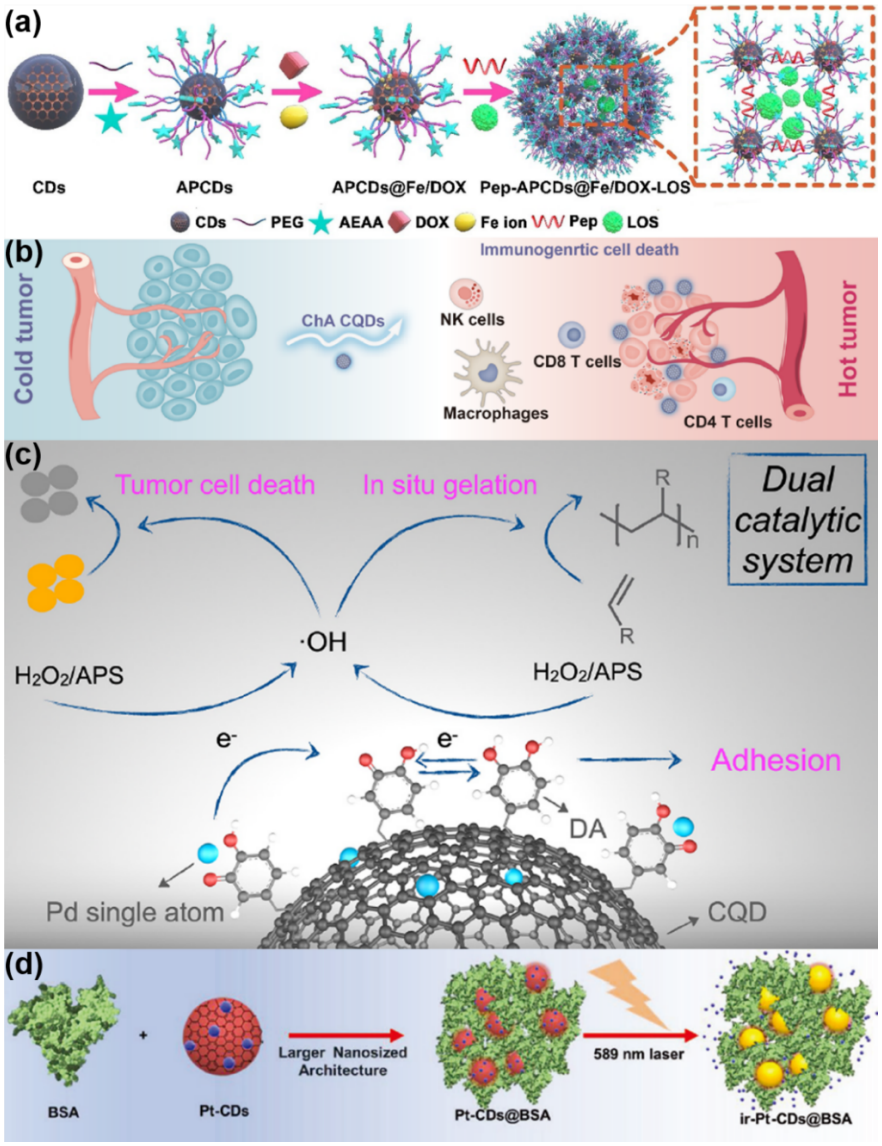


Figure 2 (a) Schematic illustration for the preparation of drug-loaded nano-assemblies. Reproduced from Ref. [30] with permission of *Wiley* . (b) Schematic diagram of the proposed mechanism for converting immunologically cold into hot tumors by ChA CQDs. Reproduced from Ref. [31] with permission of *American Chemical Society* . (c) The dual catalytic mechanism of DA-CQD@Pd SAN. Reproduced from Ref. [32] with permission of *Elsevier* . (d) Schematic illustration of the BSA composite Pt-CDs. Reproduced from Ref. [33] with permission of *Wiley* .

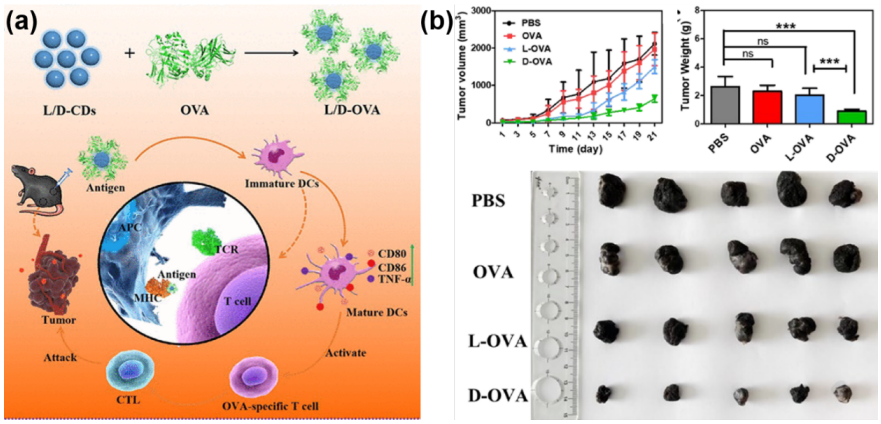


Figure 3 (a) Schematic illustration for the preparation of L/D-OVA and mechanism for tumor immunotherapy. Reproduced from Ref. [38] with permission of *American Chemical Society* . (b) Pictures and quantitative data of tumors in L/D-OVA treated mice. Reproduced from Ref. [38] with permission of *American Chemical Society* .