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Active Targeting Gold Nanoparticle for Chemotherapy Drug Delivery: A Review

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Abstract

Active targeting strategy in chemotherapy drug delivery aims to improve the therapeutic outcomes and minimize the side effects of chemotherapeutics. This review discusses utilizing ligands attached to gold nanoparticles (AuNPs) along with several specific ligands attached to AuNPs for active targeting in chemotherapy drug delivery. Antibodies, peptides, vitamins, DNA, polysaccharides, aptamers, and hormones showed active-targeting abilities as ligands attached to AuNPs. Active-targeting AuNPs enhanced cellular uptake and cytotoxicity in a specific cancer cell *in vitro* while reducing tumor growth *in vivo* by improving the photothermal, photodynamic and chemotherapy effects. Active-targeting ligands increased the internalization of AuNPs loaded onto the specific tumor site and minimized the accumulation in the normal site. AuNPs with active-targeting ligands such as antibodies, peptides, vitamins, DNA polysaccharides, aptamers, and hormones can improve the therapeutic outcomes of chemotherapeutics and can attenuate the toxicity effect in normal cells. For further research and development, researchers should be addressing AuNP characterization, drug–ligand disposition, active-targeting AuNP quantification, and target-AuNPs pertinence concerning the desired therapeutic outcomes.

Introduction

Targeted-delivery chemotherapy utilizing nanotechnology is simpler, cheaper and less time-consuming than conventional chemotherapy. Nanoparticles (NPs) optimize the therapeutic effects and minimize the adverse effects of previously approved active pharmaceutical substances, including chemotherapeutics.[1] NPs can revamp the conventional drug delivery through passive and active targeting. Passive-targeting NPs exploit biological processes through the enhanced permeation and retention (EPR) effect that allowed small NPs and macromolecular drugs to accumulate more in tumors than in normal tissues, due to the larger pore size of neo-vasculatures and the poor lymphatic clearance of tumors.[2] Active-targeting or ligand-mediated delivery utilizes specific ligands on the surface of NPs for the retention and uptake of chemotherapeutics into targeted cells.[3] Ligand specific interaction with overexpressed receptors of cancer cells enhances uptake of drug delivery onto cancer cell target, increasing drug efficiency rather than passive targeting delivery and avoiding non targeted cells.[4] McDaid *et al.*[5] showed that cetuximab conjugation with camptothecin-polymeric NPs improved the NP binding and delivery of camptothecin against cetuximab-resistant cancer cell lines and reduced the tumor growth *in vivo*. A specific binding to an overexpressed receptor in the cancer cell leads to better uptake and internalization of the chemotherapeutic effect into the cancer cell.[6,7]

AuNPs with other metal NPs such as silver,[8] silica,[9] platinum[10] and iron oxide[11] had been explored for chemotherapy drug delivery uses.[12] Among other metal NPs, AuNPs and silver nanoparticles (AgNPs) have unique optical and surface plasmon resonance (SPR) properties.[13,14] *In-vitro* and *in-vivo* studies have indicated that AuNP were non-toxic, easy to obtain with various shapes and sizes, while AgNPs were more expensive in design and had significant potential toxicity to normal cells *in vitro*.[15]

AuNPs' surface modification abilities and controllable synthesis enable the AuNP surface to conjugate with another molecular compound. AuNPs have a cytotoxic effect due to the interaction of the Au atom with the intracellular protein and DNA. AuNPs also have the potential to enhance local radiation and radiation sensitivity.[16] AuNPs exhibit photoacoustic and photothermal properties because of their ability to absorb light at a specific wavelength. These properties contribute to AuNPs' phototherapy and radiotherapy functions. Thus, AuNPs can carry out the functions of chemotherapy, phototherapy and radiation altogether as drug delivery systems.

Bednarski *et al.*[17] reported that AuNPs were absorbed only in small amounts after oral administration. After intravenous administration, AuNPs are mainly accumulated in the liver, lungs and spleen, and only

slightly removed from the body in the urine and faeces. AuNPs physicochemical properties (shape, size and surface chemistry) may affect its pharmacokinetics and pharmacodynamics, including AUNPs toxicity.[18] Functionalizing the AuNPs surface with ligands would help regulate and detoxify the uptake of these AuNPs.[19]

AuNPs were produced from the reduction of Au in an oxidation state using chemical, physical and biological techniques. The most well-known chemical approach for producing AuNP was the citrate reduction method developed by Turkevich in 1951. The reaction was initiated thermally or through ultraviolet irradiation, and resulted in a wide range of sizes (9–120 nm), with the defined size distribution of AuNP.[20] Brust-Schiffrin synthesized AuNP in 1994 using tetraoktilamoniumbromida in toluene and natrium borohydride. This method could produce highly stable thiol-functionalized NPs and access to AuNP preparation in large amounts.[21] Producing AuNPs through the physical method can be achieved using laser ablation,[22] microwave,[23] or ultrasound.[24] AuNP can be synthesized through the biological method using a biological membrane or a microorganism such as *Enterococcus sp*, *Pseudomonas sp,* or *Streptomyces sp.*[25,26] The microbial synthesis of AuNPs using bacteria has already resulted in three patents.[27] Other than conventional methods, AuNPs can also be produced through sputtering deposition in ionic liquid (IL). This novel method promises simpler and purer results because of the minimal use of chemicals and stabilizers. The formation of AuNPs through sputtering deposition involves an electrical field of IL in a vacuum chamber that produces a potential difference between cathodes and anodes.[28,29]
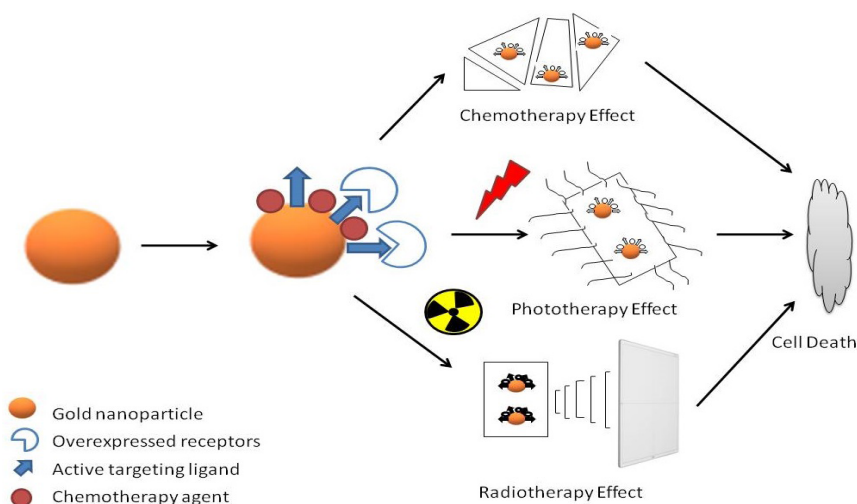
Gold Nanoparticle Conjugation in Chemotherapy Drug Delivery

The biodistribution of AuNPs as drug delivery systems, like any other nanosystem, is non-specific. Therefore, to enhance their efficacy and decrease their potential toxicity, a selective moiety was preferred to attach to the AuNP surfaces.[30] The advantages and disadvantages of active and passive targeting delivery of AuNPs were presented in Table 1.

A selective moiety assists AuNP in binding with a cancer cell overexpressed receptor and improving the therapeutic outcome. This review discusses previous studies regarding ligands attached to AuNPs as carriers of cytotoxic drugs: how the combination made, the *in-vivo* and *in-vitro* studies and the advantages and drawbacks of active-targeting AuNP chemotherapy delivery systems. The outlines of this review highlighted active targeting AuNPs based on the active-targeting ligand used: AuNPs with antibodies, AuNPs with peptides, AuNPs with vitamins and AuNPs with other ligands. Examples of active-targeting AuNPs are present in Table 2.

Table 1. Advantages and disadvantages of active and passive targeting delivery of AuNPs.

AuNPs Drug Delivery Strategies	Advantages	Disadvantages
Passive Targeting	-Easier control of obtained size and shape of AuNPs[31] -Non-immunogenic[32]	-Quantity of drug load in cell targeting not significant (less than 20%)[33]
Active Targeting	-Enhance uptake/internalization on targeted cell[34] -Reduce toxicity in normal cells[19,35] -Localization led to better photothermal, photodynamic, chemotherapy effects[36,37]	-Possibility of immunogenic response[38] -Increasing in particle size[39] -Certain ligands may carry toxicity[40] -More complex and costly in designing the systems than passive targeting system[4]

Figure 1. Effects of active-targeting gold nanoparticle.

Table 2. Examples of chemotherapeutics active-targeting gold nanoparticles.

| Active-targeting drug delivery	Ligand	Chemo-therapeutic	Target	Method of evaluation	Mechanism of action in the targeted site	Results
Antibodies-AuNP	Cetuximab	-	EGFR	*In vitro*	Radio-enhancing the effect of proton therapy	Enhanced proton irradiation in A431 cells[41]
	Rituximab	-	CD20	*In vitro*	Increasing the rituximab chemotherapy effect	Rituximab AuNP had better efficacy than rituximab alone[42]
	Cetuximab	ZnD	EGFR	*In vitro* and *in vivo*	Enhancing the chemotherapy effect of an original anticancer agent ([Zn(DION)2]Cl2)	Increased efficacy against DOX-resistant colorectal carcinoma; reduction of tumor growth[43]
	Cetuximab	-	EGFR	*In vitro* and *in vivo*	Improving radioimaging	Improved CT imaging by contrast; effect of relative binding more prominent rather than relative half of the time[44]
	Cetuximab		EGFR	*In vitro* and *in vivo*	Enhancing the chemotherapy effect of cetuximab	Suppressed cell lung cancer proliferation and accelerated apoptosis; reduced the tumor weight and volume[45]
	Trastuzumab	monomethyl auri-statin E (MMAE)	AntiHER2/ ERRB2	*In vitro*	Increasing the cellular internalization of trastuzumab	Increased uptake *in vitro* for the SKBR-3 cell line and no increase in uptake for the MCF-7 and MDA-MB-231 cell lines[46]
	Anti-PD-L1 antibody	Doxorubicin	PD-L1	*In vitro*	Increasing the photothermal (NIR irradiation) and chemotherapy effects	Increased apoptosis[47]
	Anti-VEGF anti-body	-	VEGF	*In vitro* and *in vivo*	-	Unsuccessful uptake of AuNP alone and anti-VEGF-conjugated-AuNP through GL261 brain tumor xenograft[48]
	Rituximab	-	CD20	*In vitro*	Enhancing the photothermal effect, increasing the chemotherapy effect	Reduction of anti-apoptosis/BCl-2 protein; carrying and released cytotoxicity upon laser irradiation[49]
	CD133 antibody	Docetaxel	CD133	*In vitro* and *in vivo*	Photothermal + photodynamic + chemotherapy	Multimodal imaging with photodynamic/photothermal chemotherapy activity[50]
Peptide-AuNP	RVG peptide & Lamp2 exosome	-	αvβ3 integrins	*In vitro* and *in vivo*	Chemotherapy effect accumulation	Binding to the brain cell and blood-brain barrier[51]
	RGD peptide	Bleomycin	αvβ3 integrins	*In vitro*	Enhancing radiation therapy + chemotherapy	Internalized cell, improved cytotoxicity and radiotherapy[52]
	cRGD peptide	-	αvβ3 integrins	*In vitro*	Radiotherapy, radioimaging, chemotherapy	Targeting activity present when cRGD was linked to a short olygolysine spacer[53]
	P4 peptide	Chlorambucil, malphalan, bendamustin	HL-60 and NB-4 human cells	*In vitro*	Enhancing the chemotherapeutic effects	Active targeting of P4 depending on the receptor cell surface density and the mechanism of internalization[54]
	CPP	Trastuzumab	Various	*In vitro*	Enhancing the chemotherapeutic effects	Improved the intracell internalization in SKBR3, DLD1, MDA MB 23 and MCF-7[55]
	Angiopep-2 peptide	-	LRP1	*In vitro* and *in vivo*	Enhancing the chemotherapeutic effects	Crossed the blood-brain barrier and did not produce a toxic effect in the normal cells[56]

Table 2. Continued.

Vitamin-AuNP	Folic acid	Methotrexate	Folate receptors	*In vitro* and *in vivo*	Enhancing the chemotherapeutic effects	Threefold-improved cytotoxicity in the brain cell; tenfold-improved cytotoxicity in the cervical HeLa cells[57]
	Folic acid	Doxorubicin	Folate receptors	*In vitro*	Enhancing the chemotherapeutic and photothermal effects	Increased efficacy of active-targeting-involved upregulation of the pro-apoptotic protein p53 and downregulation of the anti-apoptotic protein Bcl-2[58]
	Biotin	Paclitaxel		*In vitro*	Enhancing the chemotherapeutic effect	High affinity for the HeLA, A549 and MC63 cancer cell lines; low affinity to the NIH3T3 normal cell line[59]
Protein	Angiopep 2	Doxorubicin	LRP1	*In vitro* and *in vivo*	Enhancing the chemotherapeutic effect	Distributed at a higher intensity into the glioma than the free doxorubicin or AuNP without a ligand[60]
	Endostatin	Doxorubicin	Carbonic anhydrase protein IX	*In vitro*	Enhancing the chemotherapeutic effect	Enhanced the selective delivery of doxorubicin up to 2.5 times, and minimized the chemoresistance[61]
Polysaccharide	Hyaluronic acid	-	CD44	*In vitro*	Enhancing the chemotherapeutic effect	Prolonged blood circulation, improved cellular uptake, enhanced solubility and improved selectivity[62]
Aptamer & DNA	AS1411 aptamer	Ionidamine	Nucleolin receptor	*In vitro* and *in vivo*	Enhancing the chemotherapeutic effect	Showed a positive effect in tumor tissues, without evident damages to the surrounding healthy tissues[36]

Active targeting purposes with medium-large size and minimum weight loading led to better exposure to tumor cells and reduce exposure to normal cells.[63] Ligand structure also influences cellular uptake. The more stable (lower entropy) of ligand-AuNP would lead to better uptake.[64] Cellular uptake of active targeting AuNP or internalization may involve receptor-mediated endocytosis or energy independent diffusion across the cell membrane through pores or opening lipid bilayer by ligand-AuNP.[65] Modifying AuNP can affect its photothermal activity, although the effect may be from active-targeting localisation rather than the direct effect of surface modifying to AuNP's SPR properties. Increasing near-infrared (NIR) irradiation and SPR temperature of ligand-AuNP enhance localization. Localization led to accumulation onto targeted cells, increasing the AuNPs conjugate's phototherapy effect and increasing the probability to destroy cancer cells. Figure 1 shows that the functionalized active-targeting ligand conjugated with AuNP for chemotherapy drug delivery had photothermal, photodynamic and chemotherapeutic effects on cancer cells.

We classify the conjugation of AuNP into two categories: that using an additional linker and that without one. The conjugation of a ligand with AuNP by a thiol-Au covalent bond was created by adding PEG-NHS, PEG-COOH or PEG-SH to AuNP. The ligand-AuNPs conjugate went through filtration, centrifugation, dialysis or chromatography to purify the conjugate from excess materials. The construction of an active-targeting AuNP was illustrated in Figure 2.

AuNPs conjugated with a linker has a stronger bond than those conjugated without a linker. This bond should cleave once it reaches its target site. Podsiadlo reported the successful conjugation of 6-mercaptopurine with AuNP by the thiol group in 6-mercaptopurine-9-β-D-ribofuranoside.[66] The conjugate enhanced the antiproliferative effect against K-562 leukimia cells compared to 6-mercaptopurine-9-β-D-ribofuranoside in free form, and showed excellent stability over one year, without loss of inhibitory activity. The cleavage mechanism of the linker was delivered at a lower pH (4.1) by severing the S–Au bond.[46,67] Cruz and Keyser synthesized spherical AuNPs surface-functionalized with trastuzumab and drug conjugate (ADC). The ADC was produced through the chemical attachment of monomethyl auristatin E (MMAE) to trastuzumab through a cathepsin-cleavable valine-citrulline linker and further reacted with a sulfhydryl-containing linker for surface conjugation with AuNP.[46] Brown *et al.*[68] functionalized carboxylate group-
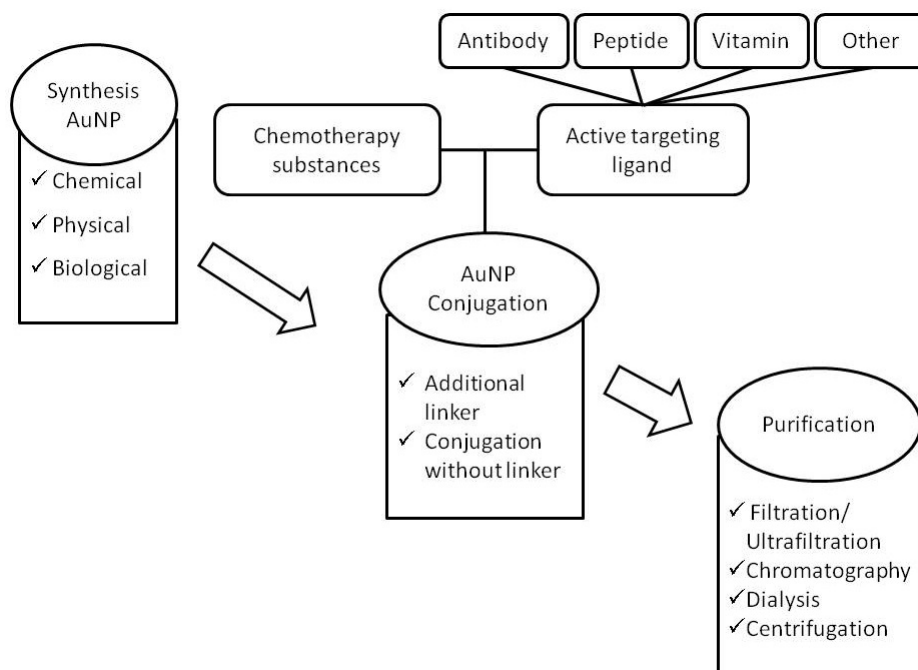
Figure 2. Conventional steps in constructing active-targeting AuNPs.

capped-AuNPs with thiolated polyethene glycol (PEG) monolayer and active component of oxaliplatin. *In vitro* study of AuNP-oxaliplatin's active-component complex in the A549 lung epithelial cancer cell line showed AuNP-oxaliplatin's active-component complex had better cytotoxicity than oxaliplatin alone and could penetrate lung cancer cells.[68] However, the stronger bond between an AuNP and a chemotherapeutic means that it has become more difficult to sever the bond. Ligand-AuNPs conjugation bonds lead to several questions about the nature of AuNP conjugation for drug delivery, such as the cleavage mechanism of the chemical bond and release mechanism of the chemotherapeutics from the AuNP complex related to their therapeutic effect. *In vivo* studies should yet be conducted regarding this mechanism.[47]

Yahyaei and Pourali[69] reported that one-step conjugation of biologically produced AuNP with capecitabine, tamoxifen and paclitaxel without using additional linkers could be done by directly dropping the chemotherapeutics in liquid AuNP. The conjugation was possible due to the presence of the capping protein surface of biologically produced AuNP. However, capecitabine and tamoxifen did not show a toxic effect against the MCF-7 cell lines. The difference between the average sizes of the AuNP and the conjugated AuNP probably contributed to this cytotoxicity result. AuNP had an average size of 54.27 ± 3.1 nm, capecitabine-conjugated AuNP has an average particle size of 83.51 ± 5.5 nm, and paclitaxel-conjugated AuNP has an average particle size of 61.37 ± 5.1 nm. The differences in drug loading, also could lead to a different pharmacological effect. A study by Chu *et al.*[70] involving docetaxel-loaded NP in two similar average particle sizes resulted in different pharmacokinetic profiles and efficacy levels. The 9% weight loading of docetaxel in NP had a superior pharmacokinetic profile and enhanced efficacy in a murine cancer model compared to a higher docetaxel weight loading in NP (20% NP). The 9% NP docetaxel increased the plasma and tumor docetaxel exposure and reduced the liver, spleen and lung exposure compared to 20% NP docetaxel. The particle sizes of these two different docetaxel-loaded NPs were similar: 216 ± 2 nm for the 20% docetaxel-loaded NP and 216 ± 1 nm for the 9% docetaxel-loaded NP. In 2020, Mirrahmi *et al.*[71] designed a nanoplatform from alginate hydrogel, cisplatin and AuNP to deliver the functions of chemotherapy, phototherapy and radiation therapy. The nanoplatform increased tumor heating and damaged tumor cells *in vivo* and *in vitro*.[71,72] The use of AuNP for phototherapy can cause hyperthermia, which leads to the apoptosis of cancer cells. Thermal stress also results in the radiosensitization of the cancer site, thus assisting radiotherapy.[73]

Gold Nanoparticles with Antibodies as Ligands
Antibodies have chemotherapeutic properties and also have potential use for specific-targeting drug delivery. Tumor antigens such as epidermal growth factor receptor (EGFR), erythroblastic oncogene B2, vascular endothelial growth factor (VEGF), cytotoxic T-lymphocyte-associated antigen-4 (CTLA4), CD20, CD30 and CD52 could exploit as antibody targets. For specific targeting, the target antigen should be abundant, accessible and preferably expressed on the surfaces of cancer cells homogeneously, consistently and exclusively.[74] Antibodies as ligands were the most researched active-targeting ligands attached to AuNPs for chemotherapy drug delivery.

Antibodies are commonly conjugated with AuNPs using a PEG linker, although antibody–AuNP conjugation without a linker was also possible. Boca *et al.*[42] synthesized

rituximab-conjugated AuNP by mixing a rituximab solution with AuNP. The formation of an intracellular vesicle on the membrane cell surfaces accomplished the internalization of rituximab AuNP in the chronic lymphocytic leukemia cells CLL-AAT and HS 505. The AuNP surface-functionalized with trastuzumab and MMAE drug conjugate (ADC) increased uptake *in vitro* for the SKBR-3 cell line (HER2-targeting cell line) and did not affect the uptake for the MCF-7 and MDA-MB-231 cell lines, which are not subject to HER-2 regulation.[67] However, the growth rate inhibition for the SKBR3 and SKOV 3 cell lines was lower in ADC than in MMAE alone. This unexpected result of ADC cytotoxicity might be due to its cleavage mechanism to release MMAE. The functionalization of the AuNP surface with the Au–S bond between AuNP and an antibody can help resolve this release issue.[45] Pedrosa *et al.*[75] showed that multifunctional AuNPs with cetuximab ligands effectively inhibited cell proliferation and triggered the death of the doxorubicin-resistant colorectal carcinoma cell.[43] Cetuximab attached to AuNP suppressed the proliferation and migration of EGFR highly expressed in tumor cells and accelerated tumor apoptosis. The active-targeting group had greater tumor accumulation in tumor-inducing mice than the group without a targeting ligand, although the cetuximab attached to AuNP had a shorter residence time in the blood than the non-targeting AuNP and the antibody NP. The tumor accumulation might have been more affected by the relative binding affinity of ligands than by the circulation half-life of ligand attached to the AuNP.[44]

Antibody AuNP conjugation has a role in photothermal and photodynamic therapy. The conjugation may improve the therapeutic outcome of photothermal therapy, photodynamic therapy and chemotherapy altogether. The Au nanosystem might also control the release of ligand-AuNP through irradiation. Emami *et al.*[76] conjugated AuNP with doxorubicin and the anti-PD-L1 antibody. Adding PD-L1 antibody to AuNP increased the photothermal effect of AuNP exposed to NIR irradiation from room temperature (27^0C) to a temperature that could destroy tumor cells (45^0C). Although the photothermal conversion efficiency of AuNP has a higher value ($49.6 \pm 3.8\%$) than that of PD-L1-AuNP-DOX ($45.6 \pm 3.1\%$), PD-L1-AuNP-DOX has a successful photothermal effect. The SPR and NIR irradiation of PD-L1-AuNP-DOX increased apoptosis (66%) of the CT-26 cells. Cetuximab-conjugated AuNP, investigated by Li *et al.*[41] showed enhanced irradiation in the EGFR-overexpressing A431 cell, potentiated its radiotherapy effect and showed prominent specific targeting. The multifunctional nanoplatform GNS@IR820/DTX-CD133 and AuNP coated with PEG increased the SPR temperature to 66 °C and the *in-vivo* NIR irradiation to 39.9 °C whereas destroying most of the cancer cells in the group of animal trials. *In-vivo* studies revealed no major organ damage (heart, lung, liver, kidney, spleen) after GNS@IR820/DTX-CD133 therapy; thus, the conjugate was biodistributed mainly at the tumor site. Tan *et al.*[50] also reported the elevation of docetaxel release under NIR irradiation from CD133 antibody-Au nanostar loading IR820.

Specific tumor targeting *in vivo*, however, may bring more challenges across some biological barriers. Khongkow *et al.*[51] showed that the targeting properties of the short-peptide rabies virus glycoprotein (RVG) and Lamp2 protein exosome attached to AuNP could bind AuNP to the brain cells and enhance the transport across the blood-brain barrier. RVG-Lamp2 exosome AuNPs accumulated in the mouse brain after intravenous injection. However, the conjugation of AuNP and anti-VEGF IG had not changed glioblastoma multiform tumor progression in induced mice. Another study by Betzer *et al.*[77] showed that active targeting AuNP improved radiotherapy in mice with glioblastoma multiform and glucose-coated AuNP accumulated in the brain by the GLUT-1 transporter aided permeation through the blood-brain barrier. The unsuccessful uptake of AuNP alone and anti-VEGF conjugated with AuNP through GL261 brain tumor xenograft was more reasonable because the amount of AuNPs was not enough for them to be able to cross the GL261 plasma cell membrane and blood-brain barrier rather than because of the psychochemical characteristics of AuNP.[48,77,78]

Gold Nanoparticles with Peptide as a Ligand
Peptide attached to AuNP did not manifest any drawback from antibody-AuNP conjugation, such as handling difficulty during synthesis and high study cost. A peptide can interact with a specific receptor in the cancer cell, and the peptide sequence can be modified. Modifying the peptide sequence can contribute to peptide-AuNP synthesis without other additional linkers.[79] It had been reported that peptide-AuNP conjugates enhance the macrophage responses compared with sole peptide or AuNP.[80]

Overexpression receptor αβ3 integrin was detected in the melanoma, glioblastoma and tumor vasculature.[52] Domain peptide RGD binds to αβ3 integrin and can be exploited as the target domain of active-targeting nanoplatforms. Yang *et al.*[53] attached bleomycin to AuNP surfaces through thiol bonding, using sequential conjugation of an RGD domain peptide (CALNN peptide). The surface of AuNP was modified with a peptide sequence (CKKKKKKGGRGDMFG) containing the RGD domain and bleomycin without PEG as a linker. The efficacy of bleomycin-AuNP was measured by visualizing DNA breaking into the human breast cancer cells (MDA-MB-231). The 16 h irradiation treatment showed that the human breast cancer cells (MDA-MB-231) internalized with the complex had a $32 \pm 9\%$ cell survival decrease and statistically significant damage enhancement compared to the control cells (irradiated without AuNPs) after receiving a radiation dose of 2 Gy with 6 MV photons.[53] However, an *in-vitro* study on the colorectal cancer cells Caco2 and SW620 showed that the cyclic RGD attached to AuNP and

linked to a PEG chain lost its targeting ability. The targeting activity of cRGD-AuNP proved to be present when cRGD was linked to PEG and a short oligolysine spacer. The fact that cRGD-AuNP was unreachable by a membrane receptor could be because the PEG layer hindered active-targeting ligands and caused loss of the active-targeting ability.[54]

Kalimuthu et al.[55] investigated the PEG/PDC P4 peptide and chemotherapeutic (PDC) conjugation with AuNP on A20 cells. PDC was attached to the PEG-coated AuNP and conjugated to a chemotherapy drug (chlorambucil, melphalan, bendamustine) to stabilize and improve the bioavailability. The A20-like leukemia cells internalize P4 peptide but not P6, a negative control. The P4 peptide was bound to the NB4 cells (10.3%) and HL-60 cells (13.8%). Meanwhile, the P6 was not bound to the NB4 cells and bound only 6.9% to the HL-60 cells. Compared to the free drugs and free PDCs, which reduced the cytotoxicity after 24 h, PDC P4-AuNP had prolonged activity 48 h after incubation. However, PDC-AuNP reduces the cytotoxicity of chlorambucil and melphalan towards the A20 cell but improves the cytotoxicity of bendamustine. This difference in cytotoxicity effect due to the active-targeting PDC-AuNP depends on the receptor cell surface density and the mechanism of internalization.

Cruz and Kayser attached trastuzumab-AuNP (ADC) to a cell-penetrating peptide to enhance intracellular delivery. The incorporation of the HIV-TAT cell-penetrating peptide (CPP) enhanced the intracellular internalization in four different cancer lines (SKBR-3, DLD-1, MDA-MB-231 and MCF-7). Uptake into cells heavily influenced by surface charge and a peptide linker had a more pronounced impact than the addition of an antibody-targeting agent for changing the surface charge.[56,67] Tapia-Arellano et al.[57] functionalized PEG/angiopep-2 peptide (TFFYGGSRGKRNNFKTEEY) to improve the delivery of Au nanoprisms to the central nervous system. The evaluation of AuNP-PEG-ang2 showed improved delivery through BBB in zebrafish larvae by utilizing the presence of red signal fluorescence as an indicator. An in-vivo study on zebrafish larvae through immersion revealed that although the nanoplatform crossed the blood-brain barrier, it did not produce a toxic effect and was completely removed after 24 hours. Neither did the in-vitro cell viability produce a cytotoxic effect on SH-SH5Y neuroblastoma. In conclusion, even if AuNP-PEG-ang2 was able to cross the blood-brain barrier, it did not have a toxic effect on the normal cells.

Gold Nanoparticles with Vitamins as Ligands
Vitamins were compelling as active-targeting ligands because they had relatively small molecular sizes, are easy to handle and are cost-effective.[58] Active targeting system may exploit the overexpression of the folate, biotin and vitamin B12 receptors in cancer cells. Yucel et al.[81] synthesized glutathione-coated folic acid attached to AuNP and methotrexate loaded to glutathione-FA-AuNP. The in-

vitro study showed that the cytotoxicity of glutathione-FA-AuNP increased threefold in the brain cells and tenfold in the cervical HeLa cells. No significant effect on lung carcinoma, prostate carcinoma and healthy kidney cells were found from in vivo evaluation.

Folic acid-targeting also has potential use in photothermal drug delivery. Banu et al.[82] study the combination of folic acid and doxorubicin AuNP for photothermal therapy in the breast cancer lines (MCF-7 cell line). The increased efficacy of this active-targeting nanoplatform was the collective effect from active targeting along with the mechanism of cell death involving the upregulation of the pro-apoptotic protein p53 and the downregulation of the anti-apoptotic protein Bcl-2. A polyethyleneimine-capped AuNP-based non-viral delivery system to deliver si-RNA into prostate cancer cells using folic acid as a targeting ligand (AuNPs-PEI-FA)showed internalization of AuNPs-PEI-FA to the LNCap cells (prostate-cancer-overexpressing folate) while no internalization in the prostate cell line that was not expressing a folate substrate.[83]

Biotin is essential for cancer cells for rapid proliferation. Heo et al.[59] functionalized the AuNP surface with PEG, biotin, paclitaxel and β-cyclodextrin. This nanoplatform has a high affinity to the HeLa, A549 and MC63 cancer cell lines and low affinity to the NIH3T3 normal cell. The affinity of the nanoplatform to cancer cells was threefold higher than to normal cells. The biotin-conjugated Au nanoplatform induced cytotoxicity fivefold in HeLa and below onefold in NIH3T3 compared to paclitaxel alone. The in-vitro study of the copper complex, AuNP and biotin nanoplatform against the HeLa and HaCaT cells showed no uptake and activity difference with the biotin-attached nanoplatforms and without biotin. However, an in-vivo study showed that the biotin-attached AuNP nanoplatforms reduced tumor growth 3.8-fold and the nanoplatforms without biotin reduced tumor growth 2.3-fold.[60] Biotin attached nanoplatform may not improve cellular uptake of nanoplatforms in vitro but has significant targeting ability.

Gold Nanoparticles with Proteins as Ligands
Aside from antibodies, other proteins could act as an active targeting ligand. Ruan et al.[61] loaded AuNP with doxorubicin through hydrazine and functionalized it with angiopep-2, a ligand of receptor-related lipoprotein 1 (An-PEG-DOX-AuNP) to deliver the drug to the blood-brain barrier. The study of doxorubicin release from systems showed that the doxorubicin from the An-AuNP-loaded DOX was released more quickly at a lower pH. The in-vivo study with glioma-bearing mice showed that An-PEG- AuNP-loaded DOX had higher intracellular uptake intensity and distributed at a higher intensity into glioma than the free doxorubicin or the AuNP without a ligand. In vivo study in mice found that the nanosystem-treated mice had a longer median survival time than the doxorubicin-treated mice. Combination therapy endostatin-AuNP with 5-fluorouracil is more effective than 5-fluorouracil

monotherapy. *In vivo*, recombinant human endostatin-AuNP increased the pericyte expression while inhibiting the vascular endothelial growth factor receptor-2 and inhibiting the anterior gradient-2 expression in metastatic colorectal cancer xenografts. *In vitro*, recombinant human endostatin-AuNP reduced cell migration and tube formation induced by anterior gradient 2 in human umbilical vein endothelial cells. Treatment with recombinant human endostatin-AuNP attenuated the anterior gradient 2-mediated activation of MMP2, cMyc, VE-cadherin, phosphorylation of p38 and extracellular signal-regulated protein kinases 1 and 2 in the human umbilical vein endothelial cells. AuNP targeting carbonic anhydrase IX (CA IX) epitope protein, an overexpressed receptor in hypoxic tumor cells, was constructed using low-molecular-weight carbonic anhydrase inhibitor ligands and doxorubicin. The nanoplatforms killed HT-29 tumor cells under hypoxic conditions. Besides their efficacy during the hypoxic condition, the complexes also enhanced the selective delivery of doxorubicin up to 2.5 folds, and minimized chemoresistance, showing better tumor drug penetration than free-drug treatment.[84]

Transferrin (TF), a glycoprotein, is a ligand to transferrin receptor overexpressed in malignant cells. Conjugation TF-AuNP by Yang *et al.*[85] showed cellular uptake of TF-AuNP on the surface of living cells through endocytosis. TF-AUNP internalized into a cellular plasma of NPC cells by TF receptor interaction, while AuNP alone did not.[65] TF-AuNP conjugated with photodynamic pro drug PC-4 increase cellular uptake compared to untargeted AuNP and had a more significant cytotoxicity effect than free PC4 in glioma cancer cells.

Gold Nanoparticles with Polysaccharides as Ligands
Polysaccharides such as hyaluronic acid exploit as a ligand for active-targeting CD44 receptors in the cancer cell. Jacinto *et al.*[62] functionalized Au core silica shell nanorods with hyaluronic acid and vitamin E PEG succinate (AuMSS-TPGS-HA) for cancer-targeted photothermal therapy that prolongs systemic circulation, improves cellular uptake, enhances solubility and improves the selectivity of nanorods. Functionalized Au core silica shell nanorods with hyaluronic acid and vitamin E PEG succinate did not affect the size or photothermal activity of Au core silica shell nanorods against HeLa cells but affected the surface charge.

Gold Nanoparticles with Aptamers as Ligands
Aptamers are short single-stranded DNA or RNA that selectively bind to the specific target. Ruttala attached ionidamine and aptamer AS1411 to AuNP. Conjugating AS1411 aptamer to the NP surface significantly improved the particle accumulation in cancer cells via affinity with the nucleolin receptors. An *in-vivo* study showed a positive effect in tumor tissues, without evident damages to the surrounding healthy tissues. The apoptosis effect was due to the increased reactive oxygen species production and the

cell migration inhibition.[36] The assembly of hybrid graphene oxide/AuNP (Au@GO NP)-based cancer-specific nucleic acid was conjugated for multimodal imaging and was combined with therapeutics, resulting in cancer detection and multimodal synergistic cancer therapy through the use of photothermal, genetic and chemotherapeutic strategies. Au@GO NP-NACs sensitively detect and effectively suppress cancer prosurvival genes.[86]

Gold Nanoparticles with Hormones as Ligands
Hormones such as ethinylestradiol (EE) and antiandrogen hormones also had been exploited as active targeting ligands. EE was selectively bound to estrogen receptors and targeted to intracellular receptors. EE conjugated with silica-coated gold nanoparticles and loaded with anticancer such as doxorubicin and quercetin. The release capacity of EE- AuNP-quercetin was 650 times higher than quercetin alone and the release capacity of EE- AuNP- doxorubicin was 2.5 times higher than doxorubicin *in vivo*. EE-AuNP also accumulated in tumor tissue whereby health cells were unaffected.[35]

Antiandrogen ligands (α-Bicalutamide and β-Bicalutamide) conjugated with AuNP via PEG -SH showed a reduction in cancer growth and progression. Antiandrogen conjugated AuNP selectively stimulated G-protein coupled receptor (GPRC6A) multivalent affinity and accumulated in hormone-insensitive and chemotherapy-resistant prostate cancer cells.[87]

Discussion and Future Recommendations
Active targeting AuNPs utilize a ligand attached to the AuNPs to improve the chemotherapeutic delivery. The attached ligand objective was to 'direct' the platform to the target cell. The term *directing* involves specific binding to an overexpressed receptor in the cancer cell that leads to better uptake and internalization of chemotherapeutic agents into the cancer cell, thus improving photothermal, photodynamic and chemotherapy effects of the platform towards the targeted cell while reducing effect towards normal cells.

Macromolecules and small molecules as active targeting ligands in AuNP conjugates exploit overexpressed receptors of the cancer cell. Thus, molecule selection as active targeting ligands must consider the specific overexpressed receptors of the cell target. Ligands-targeted receptor-bound would carry the desired targeting activity. Some targeted receptors showed in Table 3.

Macromolecules were better researched as active targeting ligands of AuNPs rather than small molecules. Small molecules such as vitamins are cost-effective and easy to handle but do not carry affinity and specificity as highly as macromolecules as ligands. Protein such as antibodies are the most well researched as ligand; they carry their cytotoxicity effect but are also immunogenic and complex in handling. Peptide sequences can modify ligand-AuNPs conjugate. Using peptides also overcomes the ligand antibody disadvantage. Proteins, including antibodies, can

Table 3. The targeted receptor of ligand AuNP.

| Overexpressed receptor on cancer cell	Ligands	Type of Cancer
EGFR	Cetuximab (Antibody)	Small Cell Lung cancer, Prostate cancer
CD 20	Rituximab (Antibody)	Lymphomas, colorectal cancer, thyroid cancer
ERRB2	Trastuzumab (Antibody)	Breast cancer, pancreatic cancer
VEGF	Anti-VEGF (Antibody)	Thyroid cancer, ovarian cancer, breast cancer
CD13	antiCD13 (Antibody)	Colon cancer, breast cancer
Avβ3 Integrin	PVC peptide, RGDpeptide	Prostate cancer
LRP1	Angiopep 2 (Protein)	Colon cancer, breast cancer
Carbonic anhydrase IX	Endostatin (Protein)	
PD-L1	AntiPD-L1 (Antibody)	Lung cancer, leukemia
CD44	Hyaluronic Acid (Polysaccharide)	Stem cancer, breast cancer
Folate Receptor	Folic Acid (vitamin)	Epithelial cancer (breast cancer, ovarian cancer, cervix cancer)
Transferrin Receptor	Transferrin (Protein)	Breast cancer
Estrogen Receptor	Ethinylestradiol/Estrogen (Hormones)	Breast cancer
Androgen Receptor	Antiandrogen (Hormones)	Prostate cancer

attach to AuNPs via polyvalent interactions, whereas small molecules such as vitamins could not obtain this property.

The attachment of active-targeting ligands could change the properties of an individual component, resulting in different pharmacokinetic and pharmacodynamic profiles. The previous *in-vivo* studies on active-targeting AuNP did not always support the active-targeting purposes, especially when it needed to attach to a more complex biological membrane (e.g. blood-brain barrier). The active-ligands attached to AuNPs nanoplatforms may contribute to the character of the AuNPs nanoplatforms, macrophage-induced response, the ligand-AuNP mechanism of cellular or intracellular attachment and internalization, thus affecting the delivery to the target. The inability to ensure the consistency of the quantity of substance attached to the AuNP surface, whether active ligands or chemotherapeutic agents, may result in varied pharmacokinetic and pharmacodynamic properties. The non-optimal drug release mechanism, e.g. because of the bond between ligand and AuNPs platform, can contribute

to unfavored active targeting. Another major concern was the lack of comparison between ligand-AuNPs interaction toward a similar target.

Current *in vivo* research of active targeting AuNP did not conduct on specific cancer organs. Most *in vivo* studies involve general tumor bearing mice when it was also necessary to study the effect of active targeting AuNP onto different and specific cancer cells *in vivo*. The affinity and binding capacity of ligand-AuNP in the same receptor/same cell target were not comparable between one another. Although some commercial Au, such as Aurimune™, auroshell® already in the clinical trial phase, ligand conjugated AuNPs were still yet to begin the clinical trial to support its commercial uses.

During AuNPs conjugate design, researchers should carefully address the correlation of characterization properties such as particle size, surface charge, loading efficiency and drug release mechanism with the therapeutic outcome. Further research and development should also consider drug disposition/internalization *in vivo* and
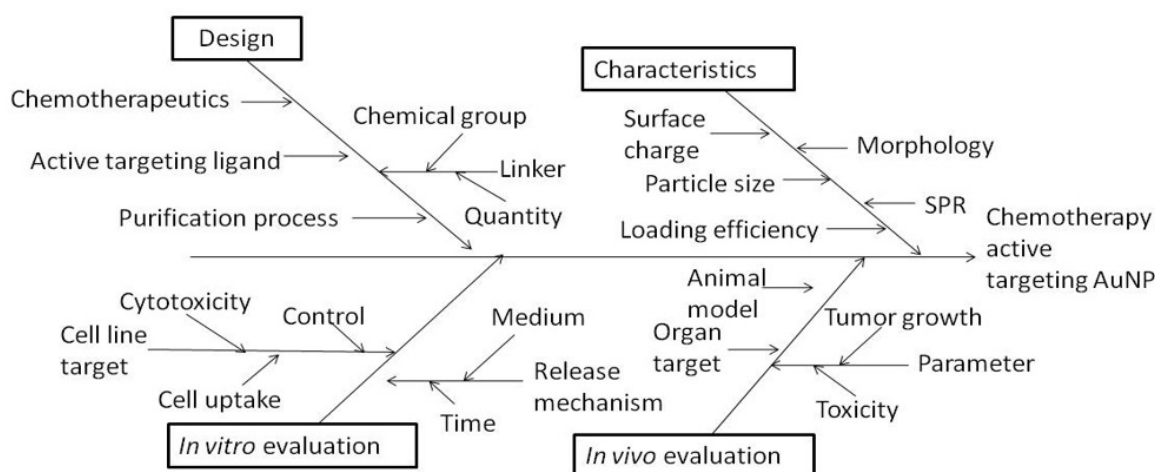
Figure 3. Fishbone diagram of the development of active-targeting gold nanoparticles.

quantification of the active-targeting AuNP delivery system to achieve the desired therapeutic outcome. Artificial intelligence may play a bigger role in active targeting AuNPs conjugate design for future development considering the vast amount of study. The optimized development of active-targeting chemotherapeutic AuNP is illustrated in Figure 3.

Conclusion

Active targeting AuNPs showed great potential in improving therapeutics outcomes and reducing toxicity in normal cells better than AuNP or conventional chemotherapeutics alone. Active targeting ligands such as antibodies, peptides, vitamins and small molecules, DNA with AuNPs enhance the photothermal, photodynamic and chemotherapy effects of AuNPs systems. More studies should be conducted to quantify the attached ligand to AuNPs nanoplatform and the relation between the quantity of attached ligand and its pharmaceutical effect. We also suggested conducting comparative studies between ligand-AuNPs interaction toward a similar target to optimize the active targeting AuNPs towards a specific target.

Author Contributions

We declare that this work was done by the author(s) named in this article and that all the liabilities from claims relating to the contents of this article will be borne by the authors. This study was written by AG, with input and correction from SS.

Conflict of Interest

The authors report no conflicts of interest.

References

1. Farokhzad OC, Langer R. Impact of nanotechnology on drug delivery. ACS Nano. 2007;3(1):16-20. doi:10.1021/nn900002m
2. Bennie LA, Helen OM, Jonathan AC. Enhanced nanoparticle delivery exploiting tumour-responsive formulations. Cancer Nanotechnol. 2018;9:10. doi:10.1186/s12645-018-0044-6
3. Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev. 2014;66:2-25. doi: 10.1016/j.addr.2013.11.009
4. Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol. 2019;7:1185-98. doi:10.1111/jphp.13098
5. McDaid WJ, Greene MK, Johnston MC, Pollheimer E, Smyth P, McLaughlin K, et al. Repurposing of Cetuximab in antibody-directed chemotherapy-loaded nanoparticles in EGFR therapy-resistant pancreatic tumours. Nanoscale. 2019;11(42):20261-73. doi:10.1039/c9nr07257h
6. Clemons TD, Singh R, Sorolla A, Chaudhari N, Hubbard A, Iyer KW. Distinction between active and passive targeting of nanoparticles dictate their overall therapeutic efficacy. Langmuir. 2018;34(50):15343–49. doi:10.1021/acs.langmuir.8b02946
7. Yameen B, Choi WI, Vilos C, Swami A, Shi J, Farokhzad OC. Insight into nanoparticle cellular uptake and intracellular targeting. J Control Release. 2014;190:485-99. doi:10.1016/j.conrel.2014.06.038
8. Karuppaiah A, Siram K, Selvaraj D, Ramasamy M, Babu D, Sankar V. Synergistic and enhanced anticancer effect of a facile surface modified non-cytotoxic silver nanoparticle conjugated with gemcitabine in metastatic breast cancer cells. Mater. Today Commun. 2020;23:10084. doi:10.1016/j.mtcomm.2019.100884
9. Li Y, Wang S, Song FX, Zhang L, Yang W, Wang HX, et al. A pH-sensitive drug delivery system based on folic acid-targeted HBP-modified mesoporous silica nanoparticles for cancer therapy. Colloids Surf. A. 2020;590:124470. doi:10.1016/j.colsurfa.2020.124470
10. Gurunathan S, Jeyaraj M, La H, Yoo H, Choi Y, Do JT, et al. Anisotropic platinum nanoparticle-induced cytotoxicity, apoptosis, inflammatory response, and transcriptomic and molecular pathways in human acute monocytic leukemia cells. Int J Mol Sci. 2020;21(2):440. doi:10.3390/ijms21020440
11. Ayyanaar S, Kesavan MP, Balachandran C, Rasala S, Rameshkumar P, Aoki S et al. Iron oxide nanoparticle core-shell magnetic microspheres: Applications toward targeted drug delivery. Nanomedicine. 2020;24:102134. doi:10213410.1016/j.nano.2019
12. Desai N, Momin M, Khan T, Gharat S, Ningthoujam RS, Omri A. Metallic nanoparticles as drug delivery system for the treatment of cancer. Expert Opin Drug Deliv. 2021;18(9):1261-90. doi:10.1080/17425247.2021.1912008
13. Liu X, Zhang Y, Wang S, Liu C, Wang T, Qiu Z et al. Performance comparison of surface plasmon resonance biosensors based on ultrasmall noble metal nanoparticles templated using bovine serum albumin. Microchem J. 2020;155:104737. doi:10.1016/j.microc.2020.104737
14. Huang X, El-Sayed MA. Gold nanoparticles: Optical properties and implementations in cancer diagnosis and photothermal therapy. J Adv Res. 2010;1(1):13-28. doi:10.1016/j.jare.2010.02.002
15. Ahamed M, Alsalhi MS, Siddiqui MKJ. Silver nanoparticle applications and human health. Clin Chim Acta 2010;411(23-24):1841-8. doi:10.1016/j.cca.2010.08.016.
16. Bai X, Wang Y, Song Z, Feng Y, Chen Y, Zhang D, et al. The basic properties of gold nanoparticles and their applications in tumor diagnosis and treatment. Int J

Mol Sci. 2020;21(7):2480. doi:10.3390/ijms21072480

17. Bednarski M, Dudek M, Knutelska J, Nowinski L, Sapa J, Zygmunt M, et al. The Influence of the route of administration of gold nanoparticles on their tissue distribution and basic biochemical parameters: In vivo studies. Pharmacol Rep. 2015;67(3):405-9. doi:10.1016/j.pharep.2014.10.019

18. Carnovale C, Bryant G, Shukla R, Bansal V. Identifying trends in gold nanoparticle toxicity and uptake: size, shape, capping ligand, and biological corona. ACS Omega. 2019;4(1):242-56. doi:10.1021/acsomega.8b03227

19. Sani A, Cao C, Cui D. Toxicity of gold nanoparticles (AuNPs): A review. Biochem Biophys Rep. 2021;26:100991. doi: 10.1016/j.bbrep.2021.100991

20. Kimling JM, Maier BO, Okenve B, Kotaidis V, Ballot H, Plech A. Turkevich method for gold nanoparticle synthesis revisited. J Phys Chem B. 2006;110(32):15700-7. doi:10.1021/jp061667w

21. Liz-Marzan, LM. Gold nanoparticle research before and after Brust Schiffrin method. Chem Comm. 2013;49:16-8. doi:10.1039/C2CC35720H

22. Correard F, Maximova K, Esteve MA, Villard C, Roy M, Al-Kattan A, et al. Gold nanoparticles prepared by laser ablation in aqueous biocompatible solutions: assessment of safety and biological identity for nanomedicine applications. Int J Nanomedicine. 2014;9:5415-30. doi:10.2147/IJN.S65817

23. Seol SK, Kim D, Jung S, Hwu Y. Microwave synthesis of gold nanoparticles: Effect of applied microwave power and solution pH. Mater Chem Phys. 2011;131(1-2):331-5. doi:10.1016/j.matchemphys.2011.09.050

24. Zou M, Li J, Zhang F, Jin Y. Ultrasonic synthesis of gold nanoparticles and its use in immunochromatographic assay for detection of kanamycin. Anal Lett. 2010;43(5):867-75. doi:10.1080/00032710903486336

25. Rónavári A, Igaz N, Adamecz DI, Szerencses B, Molnar C, Konya Z, et al. Green silver and gold: Biological synthesis approaches and potentials for biomedical applications. Molecules. 2021;26(4):844. doi:10.3390/molecules26040844

26. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.–mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res Int. 2020;27:8166-75. doi:10.1007/s11356-019-07511-x

27. Shedbalkar U, Singh R, Wadhwani S, Gaidhani S, Chopade BA. Microbial synthesis of gold nanoparticles: Current status and future prospects. Adv Colloid Interface Sci. 2014;209:40-8. doi:10.1016/j.cis.2013.12.011

28. Torimoto T, Okazaki KI, Kiyama T. Sputter deposition onto ionic liquids: Simple and clean synthesis of highly dispersed ultrafine metal nanoparticles. Appl Phys Lett. 2006;89(24):243117. doi:10.1063/1.2404975

29. Wender H, De Oliveira LF, Feil AF, Lissner E, Migowski P, Meneghetti MR, et al. Synthesis of gold nanoparticles in a biocompatible fluid from sputtering deposition onto castor oil. Chem Commun (Camb.). 2010;46(37):7019-21. doi:10.1039/c0cc01353f.

30. Almeida JP, Chen AL, Foster A, Drezek R. In vivo biodistribution of nanoparticles. Nanomedicine (Lond). 2011;6(5):815-35. doi:10.2217/nnm.11.79

31. Suchomel P, Kvitek L, Prucek R, Panacek A, Halder A, Vajda S, et al. Simple size-controlled synthesis of Au nanoparticles and their size-dependent catalytic activity. Sci Rep. 2018;8:4589. doi:10.1038/s41598-018-22976-5. doi:10.1038/s41598-018-22976-5

32. Tan J, Cho TJ, Tsai D, Liu J, Pettibone JM, You R, et al. Surface modification of cisplatin-complexed gold nanoparticles and its influence on colloidal stability, drug loading, and drug release. Langmuir. 2018;34(1):154-63. doi:10.1021/acs.langmuir.7b02354

33. Singh P, Pandit S, Mokkapati VRSS, Garg A, Ravikumar V, Mijakovic I. Gold Nanoparticles in diagnostics and therapeutics for human cancer. Int J Mol Sci. 2018; 19(7):1979. doi:10.3390/ijms19071979

34. Choi CHJ, Alabi CA, Webster P, Davis ME. Mechanism of active targeting in solid tumors with transferrin-containing gold nanoparticles. Proc Natl Acad Sci. 2010;107(3):1235-40. doi:10.1073/pnas.0914140107

35. Renner AM, Ilyas S, Schlößer H, Szymura A, Roitsch S, Wennhold K et al. Receptor-mediated in vivo targeting of breast cancer cells with 17α-Ethynylestradiol-conjugated silica-coated gold nanoparticles. Langmuir. 2020;36(48):14819-28. doi:10.1021/acs.langmuir.0c02820

36. Ruttala HB, Ramasamy T, Poudel BK, Ruttala RRT, Jin SG, Choi HG, et al. Multi-responsive albumin-lonidamine conjugated hybridized gold nanoparticle as a combined photothermal-chemotherapy for synergistic tumor ablation. Acta Biomater. 2019;101:531-4. doi:10.1016/j.actbio.2019.11.003

37. Eun JH, Dae GC, Min SS. Targeted and effective photodynamic therapy for cancer using functionalized nanomaterials. Acta Pharm Sin B. 2016;6(4):297-307. doi:10.1016/j.apsb.2016.01.007

38. Cheung WH, Chan VSF, Pang HW, Wong MK, Guo ZH, Tam PKH, et al. Conjugation of latent membrane protein (LMP)-2 epitope to gold nanoparticles as highly immunogenic multiple antigenic peptides for Induction of Epstein−Barr Virus-Specific Cytotoxic T-Lymphocyte responses in vitro. Bioconjug Chem. 2009;20 (1):4-31. doi:10.1021/bc800167q

39. Sardar R, Shumaker-Parry JS. Spectroscopic and microscopic investigation of gold nanoparticle formation: Ligand and temperature effects on rate and particle size. J Am Chem Soc. 2011;133(21):8179-90. doi:10.1021/ja107934h

40. Arvizo R, Bhattacharya R, Mukherjee P. Gold nanoparticles: Oppurtunities and challenges in nanomedicine. Expert Opin Drug Deliv. 2010;7(6):753-63. doi:10.1517/17425241003777010

41. Li S, Bouchy S, Penninckx S, Marega R, Fichera O, Gallez B, et al. Antibody-functionalized gold nanoparticles as tumor-targeting radiosensitizers for proton therapy. Nanomedicine (Lond). 2019:14(3):317-33. doi:10.2217/nnm-2018-0161

42. Boca S, Lucan C, Frinc I, Petrushev B, Simon T, Berce C, et al. Gold nanoparticles conjugated with rituximab for the treatment of chronic lymphocytic leukaemia. Farmacia. 2016;64:688-98

43. Pedrosa P, Lu M, Ferreira-silva M, Martins P, Carvalheiro MC, Costa PM, et al. Targeting Cancer Resistance via Multifunctional Gold Nanoparticles. Int J Mol Sci. 2019;20(21):5510. doi:10.3390/ijms20215510

44. Ashton JR, Gottlin EB, Patz Jr EF, West JL, Badea CT. A comparative analysis of EGFR-targeting antibodies for gold nanoparticle CT imaging of lung cancer. PloS One. 2018;13(11):e0206950. doi:10.1371/journal.pone.0206950

45. Mahato K, Nagpal S, Shah MA, Srivastava A, Maurya PK, Roy S, et al. Gold nanoparticle surface engineering strategies and their applications in biomedicine and diagnostics. 3 Biotech. 2019;9(2):57. doi:10.1007/s13205-019-1577-z

46. Adewale OB, Davids H, Cairncross L, Roux S. Toxicological behavior of gold nanoparticles on various models: Influence of physicochemical properties and other factors. Int J Toxicol. 2019;38(5):357-84. doi:10.1177/1091581819863130

47. Xiong C, Lu W, Zhou M, Wen X, Li C. Cisplatin-loaded hollow gold nanoparticles for laser-triggered release. Cancer Nano. 2018;9:6. doi:10.1186/s12645-018-0041-9

48. Da Silva VC, O Silva RN, Colli LG, De Carvalho MHC, Rodrigues SF. Gold nanoparticles carrying or not anti-VEGF antibody do not change glioblastoma multiforme tumor progression in mice. Heliyon. 2020;6(11):e05591. doi:10.1016/j.heliyon.2020.e05591.

49. Bisker G, Yeheskely-Hayon D, Minai L, Yelin D. Controlled release of Rituximab from gold nanoparticles for phototherapy of malignant cells. J Control Release. 2012;162(2):303-9. doi:10.1016/j.jconrel.2012.06.030

50. Tan H, Hou N, Liu Y, Liu B, Cao W, Zheng D, et al. CD133 antibody targeted delivery of gold nanostars loading IR820 and docetaxel for multimodal imaging and near-infrared photodynamic/ photothermal / chemotherapy against castration resistant prostate cancer. Nanomedicine. 2020;27:102192. doi:10.1016/j.nano.2020.102192

51. Khongkow M, Yata T, Boonrungsiman, Boonrungsiman S, Ruktanonchai UR, Graham D, et al. Surface modification of gold nanoparticles with neuron-targeted exosome for enhanced blood-brain barrier penetration. Sci Rep. 2019;9:8278. doi:10.1038/s41598-019-44569-6

52. Bianconi D, Unseld M, Prager GW. Integrins in the spotlight of cancer. Int J Mol Sci. 2016;17(12):2037. doi:10.3390/ijms17122037

53. Yang C, Uertz J, Chitrani DB. Colloidal gold-mediated delivery of bleomycin for improved outcome in chemotherapy. Nanomaterials. 2016;6(3):48. doi:10.3390/nano6030048.

54. Biscaglia F, Ripani G, Rajendran S, Benna C, Mocellin S, Bocchinfuso G, et al. Gold nanoparticle aggregates functionalized with cyclic RGD peptides for targeting and imaging of colorectal cancer cells. ACS Appl. Nano Mater. 2019;2(10):6436-44. doi:10.1021/acsanm.9b01392.

55. Kalimuthu K, Lubin BC, Bazylevich A, Gellerman G, Shpilberg O, Luboshits G, et al. Gold nanoparticles stabilize peptide-drug-conjugates for sustained targeted drug delivery to cancer cells. J Nanobiotechnology. 2018;16(1):34. doi:10.1186/s12951-018-0362-1

56. Ruseska I, Zimmer A. Internalization mechanisms of cell-penetrating peptides. Beilstein J Nanotechnol. 2020;11:101-23. doi:10.3762/bjnano.11.10

57. Tapia-Arellano A, Gallardo-Toledo E, Ortiz C, Henriquez J, Feijoo CG, Araya E et al. Functionalization with PEG/Angioppep-2 peptide to improve the delivery of gold nanoprisms to central nervous system: in vitro and in vivo studies. Mater Sci Eng C Mater Biol Appl. 2021;121:111785. doi:10.1016/j.msec.2020.111785

58. Darguzyte M, Drude N, Lammers T, Kiessling F. Riboflavin-targeted drug delivery. Cancers (Basel). 2020;2(2):295. doi:10.3390/cancers12020295

59. Heo DN, Yang DH, Moon HJ, Lee JB, Bae MS, Lee SC, et al. Biomaterials Gold nanoparticles surface-functionalized with paclitaxel drug and biotin receptor as theranostic agents for cancer therapy. Biomaterials. 2012;33(3):856-66. doi:10.1016/j.biomaterials.2011.09.064

60. Pramanik AK, Palanimuthu D, Somasundaram K, Samuelson AG. Biotin decorated gold nanoparticles for targeted delivery of a smart-linked anticancer active copper complex: in vitro and in vivo studies. Bioconjug Chem. 2016;27(12):2874-85. doi:10.1021/acs.bioconjchem.6b00537

61. Ruan S, Yuan M, Zhang L, Hu G, Chen J, Cun X, et al. Tumor microenvironment sensitive doxorubicin delivery and release to glioma using Angiopep-2 decorated gold nanoparticles. Biomaterials. 2015;37:425-35. doi:10.1016/j.biomaterials.2014.10.007

62. Jacinto TA, Rodrigues CF, Moreira AF, Miguel SP, Costa EC, Ferreira P, et al. Hyaluronic acid and vitamin E polyethylene glycol succinate functionalized gold-core silica shell nanorods for cancer targeted photothermal therapy. Colloids Surf B Biointerfaces. 2020;188:110778. doi:10.1016/j.colsurfb.2020.110778

63. Chakraborty A, Das A, Raha S, Barui A. Size-dependent apoptotic activity of gold nanoparticles on osteosarcoma cells correlated with SERS signal. J Photochem Photobiol B. 2020;203:111778. doi:10.1016/j.jphotobiol.2020.111778

64. Lee KH, Ytreberg FM. Effect of gold nanoparticle conjugation on peptide dynamics and structure.

Entropy. 2012;14(4):630-41. doi:10.3390/e14040630

65. Yang PH, Sun X, Chiu JF, Sun H, HE QY. Transferrin-mediated gold nanoparticle cellular uptake. Bioconjug Chem. 2005;16(3):494-6. doi:10.1021/bc049775d

66. Podsiadlo P, Sinani VA, Bahng JH, Kam NWS, Lee J, Kotov NA. Gold nanoparticles enhance the anti-leukemia action of a 6-Mercaptopurine chemotherapeutic agent. Langmuir. 2008;24(2):568-74. doi:10.1021/la702782k

67. Cruz E, Kayser V. Synthesis and enhanced cellular uptake in vitro of anti-HER2 multifunctional gold nanoparticles. Cancers (Baasel). 2019;11:870. doi:10.3390/cancers11060870

68. Brown SD, Nativo P, Smith JA, Stirling D, Edwards PR, Venugopal B, et al. Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. J Am Chem Soc. 2010;132 (13):4678-84. doi:10.1021/ja908117a

69. Yahyaei B, Pourali P. One step conjugation of some chemotherapeutic drugs to the biologically produced gold nanoparticles and assessment of their anticancer effects. Sci Rep. 2019;9(11):10242-15. doi: 10.1038/s41598-019-46602-0

70. Chu KS, Schorzman AN, Finniss MC, Bowerman CJ, Peng L, Luft JC, et al. Nanoparticle drug loading as a design parameter to improve docetaxel pharmacokinetics and efficacy. Biomaterials. 2013;34(33):8424-9. doi:10.1016/j.biomaterials.2013.07.038

71. Mirrahimi M, Beik J, Mirrahimi M, Alamzadeh Z, Teymouri S, Mahabadi VP, et al. Triple combination of heat, drug and radiation using alginate hydrogel co-loaded with gold nanoparticles and cisplatin for locally synergistic cancer therapy. Int J Biol Macromol. 2020;158:617-26. doi:10.1016/j.ijbiomac.2020.04.272.

72. Shi Z, Li Q, Mei L. pH-Sensitive nanoscale materials as robust drug delivery systems for cancer therapy. Chinese Chemical Letter. 2020;31(6):1345-1356. doi:10.1016/j.cclet.2020.03.001

73. Vines JB, Yoon JH, Ryu NE, Lim DJ, Park H. Gold nanoparticles for photothermal cancer therapy. Front Chem. 2019;7:167. doi:10.3389/fchem.2019.00167

74. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. Nat Rev Cancer. 2012;12(4):278-87. doi:10.1038/nrc3236

75. Qian Y, Qiu M, Wu Q, Tian Y, Zhang Y, Gu N, et al. Enhanced cytotoxic activity of cetuximab in EGFR-positive lung cancer by conjugating with gold nanoparticles. Sci Rep. 2014;4:7490. doi:10.1038/srep07490

76. Emami F, Banstola A, Vatanara A, Lee S, Kim JO, Jeong JH, et al. Doxorubicin and anti-PD-L1 antibody conjugated gold nanoparticles for colorectal cancer photochemotherapy. Mol Pharm. 2019;16(3):1184-99. doi:10.1021/acs.molpharmaceut.8b01157

77. Betzer O, Perets N, Angel A, Motiei M, Sadan T, Yadid G, et al. In vivo neuroimaging of exosomes using gold nanoparticles. ACS Nano. 2017;11(11):10883-93. doi:10.1021/acsnano.7b04495

78. Sousa F, Mandal S, Garrovo C, Astolfo A, Bonifacio A, Latawiec D, et al. Functionalized gold nanoparticles: a detailed in vivo multimodal microscopic brain distribution study. Nanoscale. 2010;2(12):2826-34. doi:10.1039/c0nr00345j

79. Jia Y, Yan X, Guo X, Zhou G, Liu P, Li Z. One step preparation of gold nanoparticles with tunable size. Materials (Basel). 2019;12(13):2107. doi:10.3390/ma12132107

80. Bastus NG, Sanchez-Tillo E, Pujals S, Farrera C, Lopez C, Giralt E, et al. Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophages response. ACS Nano. 2009;3(6):1335-44. doi:10.1021/nn8008273

81. Yucel O, Sengelen O, Emik S, Onay-Ucar E, Arda N, Gurdag G. Folic acid-modified methothrexat-conjugated gold nanoparticles as nanosized trojan for drug delivery to folate receptor-positive cancer cell. Nanotechnology. 2020;31(35):355101. doi:10.1088/1361-6528/ab9395

82. Banu H, Sethi DK, Edgar A, Sheriff A, Rayees N, Renuka N, et al. Doxorubicin loaded polymeric gold nanoparticles targeted to human folate receptor upon laser photothermal therapy potentiates chemotherapy in breast cancer cell lines. J Photochem Photobiol B. 2015;149:116-28. doi:10.1016/j.jphotobiol.2015.05.008

83. Rahme K, Guo J, Holmes JD. Bioconjugated gold nanoparticles enhance siRNA delivery in prostate cancer cells. Methods Mol Biol. 2019;1974:291-301. doi:10.1007/978-1-4939-9220-1

84. Shabana A, Mondal UK, Alam MR, Spoon T, Ross CA, Madesh M et al. pH-Sensitive multiligand gold nanoplatform targeting carbonic anhydrase IX enhances the delivery of doxorubicin to hypoxic tumor spheroids and overcomes the hypoxia-induced chemoresistance. ACS Appl Mater Interfaces. 2018;10(21):17792-808. doi:10.1021/acsami.8b05607

85. Yang L, Kim TH, Cho HY, Luo J, Lee JM, Chueng STD, et al. Hybrid graphene-gold nanoparticle-based nucleic acis conjugate for cancer-specific multimodal imaging and combined therapeutics. Adv Funct Mater. 2021;31(5):2006918. doi:10.1002/adfm.202006918

86. Dixit S, Novak T, Miller K, Zhu Y, Kenney ME, Broome AM. Transferrin receptor-targeted theranostic gold nanoparticles for photosensitizer delivery in brain tumors. Nanoscale. 2015;7(7):1782-90. doi:10.1039/c4nr04853a

87. Dreaden EC, Gryder BE, Austin LA, Defo BAT, Hayden SC, Pi M et al. Antiandrogen gold nanoparticles dual-target and overcome treatment resistance in hormone-insensitive prostate cancer cells. Bioconjug Chem. 2012;23(8):1507-12. doi:10.1021/bc300158k