GOLD NANOPARTICLES AND THEIR CONJUGATES WITH ANTICANCER DRUGS: MODERN ERA OF CANCER TREATMENT

SUMAIRA AKRAM[1*], ASAD ULLAH[1], UMBREEN SHAHEEN[2], JAFFAR ALI[3], MUHAMMAD KASHIF ALI[4], MUHAMMAD ATIF ALI[5], NIMRA NAZ[1], AYESHA MASOOD[6], MUHAMMAD ZAHID MUSTAFA[1], SHAGUFTA FAHMID[7] AND FAIZAN ALI[2]

[1]Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Quetta, Pakistan
[2]Department of Zoology, University of Balochistan, Quetta, Pakistan
[3]Office of Agriculture Extension (Plant Protection), Barkhan, Balochistan, Pakistan
[4]Combined Military Hospital, Nowshera
[5]Office of Agriculture Extension (Soil Fertility), Barkhan, Balochistan, Pakistan
[6]Department of Botany, University of Balochistan, Quetta, Pakistan
[7]Department of Chemistry, Sardar Bahadur Khan Women's University, Quetta, Pakistan
Corresponding Author's E-mail: akramsumaira503@gmail.com

خلاصہ

کینسر ایک انتہائی خطرناک بیماری ہے جو پوری دنیا میں تیزی سے پھیل رہی ہے۔ کینسر کا علاج کرنا کافی مشکل ہے۔ اگرچہ بہت سے علاج دستیاب ہیں جیسے کیموتھراپی، ریڈیو تھراپی، سرجری وغیرہ، تاہم ان کے مضر اثرات ہوتے ہیں۔ نینو پارٹیکل کی اینٹی کینسر خصوصیات کی صلاحیت کی دریافت نے تحقیق کے اس میدان میں نئی راہیں کھول دی ہیں۔ مطالعات سے پتہ چلا ہے کہ جب اینٹی کینسر ادویات کو NPs کے ساتھ منسلک کیا جاتا ہے تو وہ کینسر کے خلیوں کے خلاف اپنی سرگرمی میں اضافہ کرتے ہیں جو صحت مند خلیوں پر غیر ارادی زہریلے اثرات میں نمایاں کمی دکھاتے ہیں۔ اینٹی کینسر کے علاج میں مختلف NPs قسم کے Au-NPs کا استعمال کیا جاسکتا ہے لیکن اس جائزے میں کینسر کے علاج میں استعمال ہونے والے Au-NPs کی کیمیائی غیر فعالیت کی وجہ سے Au-NPs کی خصوصیات پر توجہ مرکوز کی گئی ہے۔ ان Au-NPs کے ممکنہ امکانات جو انہیں کینسر تھراپی اور متعدد بائیومیڈیکل استعمال کے لئے ایک بہترین امیدوار بناتی ہیں۔ اس جائزے میں متعدد اینٹی کینسر ادویات کا احاطہ کیا گیا ہے جن کو Au-NPs کے ساتھ منسلک کیا جاسکتا ہے اور وٹرو میں کینسر کے مختلف خلیوں کی اقسام پر ان کنجوگیٹس کے کیا اثرات ہیں۔اس مطالعے میں شامل ویوو اور ان ویٹرو تحقیقات میں متعدد نے ظاہر کیا کہ Au-NPs پہلی نسل کے کینسر کی ادویات کو نشانہ بنانے کے لئے ایک امید افزا آپشن ہیں۔ اس کے علاوہ، ان ادویات کو Au-NPs کے ذریعہ براہ راست ہدف کے خلیوں تک پہنچا کر خوراک اور منفی ضمنی اثرات کو کم کیا جاسکتا ہے۔NPs conjugation

Abstract

Cancer is an extremely serious disease with increasing prevalence all over the world. It is quite challenging to treat cancer. Although many treatments options like chemotherapy, radiotherapy, surgery etc. are currently available; however, they have damaging side effects. The discovery of nanoparticle's potential to have anticancer properties has opened new avenues in this field of research. Studies have revealed that when anticancer drugs are conjugated with Nanoparticles (NPs), they enhance their activity against cancerous cells showing a remarkable reduction in unintentional toxic effects on healthy cells. A variety of NPs can be used in anticancer treatment, but this review focused on gold nanoparticles with the potential scope to be used in cancer treatment. Due to their chemical inertness, gold nanoparticles are a fantastic option for cancer treatment and a wide range of other biological applications. This review covers several anticancer drugs that can be conjugated with gold nanoparticles and discuss the effects of these conjugates on various cancer cell types *in vitro*. The numerous *in vivo* and *in vitro* investigations covered in this study demonstrated that gold nanoparticles are a promising option for first-generation cancer medication. Moreover, by delivering these medications straight to the target cells by gold nanoparticles conjugation, the dosage and negative side effects can be decreased.

Keywords: Cancer; Cancer treatments; Gold nanoparticles; Conjugates; Anticancer drug

Introduction

With considerable incidence and mortality rates in both industrialized and developing nations, cancer is the second most common cause of death worldwide and a serious public health concern. The onset of it happens when a cell begins to proliferate uncontrollably and deviates from the standard laws of cell division (Miller *et al*., 2022). Cancer is the result of aberrant cells proliferating out of control because of an inability to govern apoptosis, or programmed cell death. Uncontrollably growing and developing cells might result from disruptions in their programming (Giaquinto *et al*., 2022).

Cancer Prevalence: Cancer constitutes a huge burden on society and the event of cancer is expanding globally due to various factors. (Torre *et al*., 2015). Public health depends on knowing the cancer prevalence, which indicates the number of people who have the disease at any given time. By using this data, healthcare organizations may plan and distribute resources more effectively, resulting in an adequate supply of services for support, care, and treatment (Mao *et al*., 2022). Policymakers can more effectively prioritize financing and research to enhance patient outcomes and overall quality of life by knowing the prevalence of cancer. This all-encompassing perspective aids in decision-making for more efficient disease combat (Corso *et al*., 2023). According to GLOBOCAN 2018, there were 18 million new cases of malign tumors and approximately in 2018, 9.6 million human beings died from most cancers globally (Bray *et al*., 2018)**.** According to current data, it's far expected that during 2019, there may be more than 1.7 million new instances and 0.6 million deaths due to cancer within the United States only (Siegel *et al*., 2019). There were estimated to be instances of most cancers in 2020 (Sung *et al*., 2021). Similarly, in 2023, 1,958,310 new instances of different cancers and 609,820 deaths because of cancers are anticipated to arise within United States (Siegel *et al*., 2023). Moreover, predictions indicate that cancer is expected to cause 26 million new instances and 17 million deaths each year by 2030 (Thun *et al*., 2010). On comparing the incidence of most cancers in Pakistan with other neighboring countries such as India, Bangladesh and Afghanistan, Pakistan was ranked second, while the mortality rate and prevalence also showed similar results (Abbas *et al*., 2020).

Types of Cancer: There are over a hundred diverse forms of cancers. It is difficult to treat due to high rate of mutation and metastasis of the cancer cells (Chen and Zhao, 2018). Breast, lip, oral cavity, cervix, uterus, colorectal and bladder are the most common types of cancer in Pakistan (Sarwar and Saqib, 2017).

Conventional Treatments: Chemotherapy, radiation therapy, hormonal treatment, surgical treatments and a variety of anticancer drugs are common treatment alternatives for cancer (Wallington *et al*., 2016). Chemotherapy is one of the methods of treating this disease and the advances in anti-cancer drugs have improved patient care. Regrettably, the traditional chemical drugs also have negative side effects on healthy cells and tissues, including alopecia, nausea, vomiting, and restriction of bone marrow function (Baskar *et al*., 2014; Sak, 2012).

Radiotherapy (RT), also known as radiation therapy, is one of the most effective treatments for cancer providing survival benefits as well as palliative care (Liauw *et al*., 2013; Prise, 2006). RT uses high radiation dosages to kill the cancer cells and reduce size of tumors, however, high radiation doses can also damage the neighboring healthy cells. RT cures cancer by enhancing the resistant framework against the cancer cells. However, it frequently happens that the resistant framework acts against sound cells and tissues (Boisselier and Astruc, 2009).

Nanoparticles**:** Although the above treatments are not always successful and have some side effects, it's still the first-line clinical treatment for cancer. The progress in nanotechnology has resulted in the creation of novel nanomaterials possessing a multitude of appealing characteristics, presenting a hopeful avenue for the integration of diverse cancer therapies (Deng *et al*., 2018). The focus of study is on nanoparticles (NPs) as a novel platform for site-specific cancer therapy. Innovative instruments called nano carriers are used to deliver cancer therapies to precise target locations. Different nanoparticles (NPs) have led to the development of numerous distribution techniques over the ages. These include nano polymers, NP liposomes, nano dendrimers, nanorods, and nanotubes. Focused therapy of cancer makes use of these various kinds of nanoparticles (Beik *et al*., 2016). Scientists and researchers are creating novel instruments and fresh methods for basic research topics, even if extensive research is still in its early phases. These comprise toxicity reduction, target indications, medication synthesis, drug carriers, and tool optimization (Brown *et al*., 2010). Nanomaterials have the potential to enhance the effectiveness of medications in specifically targeting cancer cells while reducing the adverse effects on non-targeted cells (Muntimadugu *et al*., 2017). Because of the way these nanomaterials interact with the cytosolic glutathione, they have a tendency to collect within cells. Heavy-atom nanoparticles tend to accumulate more in tumors than in normal tissues because glutathione levels are much higher in cancer cells than in normal cells (Stobiecka *et al*., 2019). The NPs between 5 and 100 nm in size are more likely to survive renal clearance to have good circulation time to accumulate in tumors (Albanese *et al*., 2012). Because of their size advantage, scientists have concentrated on creating new NPs for use in diagnosis and on creating nanotechnology-based pharmaceutical devices for disease staging, response to treatment management, and stratification of disease (Aljaraba *et al*., 2022).

Gold Nanoparticles: Our study concentrated on Au-NPs among other NPs because of their unique qualities that could be used for various biological applications. For qualities like resistance to surface oxidation and chemical inertness, Au-NP is a great option for treating cancer (Eleraky *et al*., 2020). Use and exploration of gold has a long history dating back thousands of years. Indian, Chinese, and Arabic scientists who were studying colloidal

gold as early as the fifth or fourth century BCE left treatises marks the earliest evidence of the substance (Cabuzu *et al*., 2015). Medical research has turned its attention to gold nanoparticles (GNPs) with specific optical and geometrical features. Numerous domains, including biosensors, immunology, clinical trials, genomics, laser tumor therapy, drug delivery, and diagnostics, find use for them. By combining cutting-edge techniques for specific medicinal applications, GNPs provide sophisticated approaches for identifying, visualizing, and directing cancer cells and tissues (Jazayeri *et al*., 2016). Their potential as adjuvants is enhanced by their high surface-to-volume ratio, biocompatibility, inertness, and capacity to be functionalized with diverse groups. For medications and vaccines, they may lessen toxic effects, improve immunogenicity, and offer stability (Carabineiro, 2017).

Due to their effectiveness in cancer treatment and medication administration, GNPs, have become extremely popular. By skillfully employing functional groups, they have blazed their own route from discovery to therapeutic applications in contemporary medicine. They exhibit the ability to transport proteins, nucleic acids, amino acids, and gene therapies, addressing symptom management and in vivo treatment (Larm *et al*., 2018). There are numerous ways to synthesize Au-NPs. The chemical techniques rely on the reduction of chloroauric acid ($HAuCl_4$). In 1951, Turkevitch and his fellow researchers introduced the citrate reduction method, which produced Au-NPs with a diameter of about 20 nm (Turkevich *et al*., 1951). A novel method for producing 1.5–5 nm thiol-capped Au-NPs was introduced by Schiffrin and Brust in 1994. The process involved the use of sodium borohydride as a lowering agent while the preferred ligand i.e. thiol-terminated long-chain alkane was present. (Brust *et al*., 1994; Turkevich *et al*., 1995). The Au-NPs can be directly or indirectly linked to a variety of molecules such as drugs, nucleic acids (Deoxyribonucleic acids (DNAs) or Ribonucleic acids (RNAs)), proteins or peptides, antibodies and targeted ligands to attain perfect and diverse biological properties and medical purposes. Gold can be conjugated to the drug using agents such as thiolated polyethylene glycols (PEG) and indirect ligands as well (Cui *et al*., 2017).

Table-1: Synthesis of gold nanoparticles by chemical method.

Chemical methods	Procedure	References
Turkevich Method	Tannic acid, ascorbic acid, and citrate are examples of mild reducing agents that can decrease Au+3 ions. For the synthesis of Au-NPs during this process, it is important to manage factors including temperature, pH and concentration.	Hussain *et al*., 2020; Kimling *et al*., 2006; Larm *et al*., 2018
Brust-Schiffrin Method	Using tetraoctylammonium bromide (TOAB) as a phase-transfer and sodium bohydride (NaBH4) as a reduction agent, tetrachloroaurate (AuCl4) was transferred from an aqueous to toluene in the presence of dodecanethiol.The organic phase turns deep brown instead of orange when reducing chemicals are used.	Brust *et al*.,1994
Seeding Growth Method	It is possible to generate particles ranging from 5-40 nm in size by adjusting the seed to metal salts ratio. This process has benefits due to a cost-effective, simple and rapid.	Jana*et al*., 2001; Siti, *et al*., 2013

Gold Nanoparticles for Anticancer Application: The Au-NPs can be utilized as a drug delivery platform by combining the active drugs with the Au-NPs, typically using spacers like SH-PEG (Thiolated Polyethylene Glycol) that are covalently attached to the gold; The aim is to achieve plasmatic stabilization, selectivity and active drug release through metabolic pathways (Cui et al., 2017). To increase paclitaxel's solubility in aqueous solutions, Mirkin and associates created conjugates of oligonucleotide and gold nanoparticle. (Zhang *et al*., 2020). To achieve selectivity and stability properties, oligo- and Polyethylene glycol (PEG) are commonly used as spacers for covalently join AuNPs and drug molecules at the terminal (Aryal *et al*., 2009; Paciotti *et al*., 2006). 5-fluorouracil is another medication that has been conjugated with AuNPs and is an anticancer agent which prevents synthesis of DNA and RNA. (Longley *et al*., 2003).

Table-2: Some examples of bio functionalized GNPs.

Anticancer drug	Conjugation	Types of cancer cells	Outcomes	References
Naproxan	Naproxen-derived compounds-Gold nanoparticles (NDC-AuNP)	Ovarian cancer	Higher rates of apoptosis were induced by Gold Nanoparticles (AuNP) systems, providing a unique and easy approach to assess the efficacy of potential medications in drug discovery research.	Tunc *et al*., 2023
Doxorubicin	Acid-labile linkage Doxorubicin-Polyethylene glycol-Gold nanoparticles (DOX-PEG-GNP)	Breast cancer	More cytotoxicity and improved drug accumulation over free Doxorubicin (DOX).	Wang *et al*., 2011
5-Fluorouracil	5-Fluorouracil-glutamic acid-Gold nanoparticles (5-FU-glu-AuNPs (GNPs)	Colon and rectal cancerous cell lines	Greater cytotoxic impact on cancer cells in comparison to 5-Fluorouracil (5-FU) that is not conjugated.	Safwat *et a*l., 2016
Paclitaxel	Paclitaxel-Red blood cell membrane- Anti-epithelial cell adhesion molecule antibody- Gold nanoparticles (PTX-RBC-EpCam-GNP)	mouse breast and mammary gland cancerous cells	Possibly directed toward cancerous cells as compared to alone paclitaxel (PTX).	Zhu *et al*., 2018
Bleomycin	Bleomycin-Arginylglycylaspartic acid- Gold nanoparticles (BLM-RGD-GNP)	Breast cancer	It Reduced survival and more Desoxyribonucleic acid (DNA) damage compared to free Bleomycin (BLM)	Yang *et al*., 2016
Methotrexate	Methotrexate- Gold nanoparticles (MTX-GNP)	Lewis lung carcinoma cells;	In tumor cells, more tumor inhibition, more cytotoxicity, and greater accumulation in contrast to free Methotrexate (MTX).	Chen *et al*., 2007

Au-NPs had been covalently joined with doxorubicin for the remedy for breast cancer that resists drugs (Wang *et al*., 2011). Additionally, the anticancer drug conjugated to Au-NPs was the polypeptide drug (Hosta *et al*., 2009). Paclitaxel (PTX) can be targeted to cancer cells 4T1 *invitro* and emitted when exposed (NIR) compared to other combinations, the EpCam-RBC-PTX-GNP-NIR complex demonstrated more cytotoxicity and decreased cell survival. (Zhu *et al*., 2018). Methotrexate (MTX) was more cytotoxic and could accumulate more in tumor cells when conjugated with Au-NPs than its free from. Additionally, in mice with Lewis lung carcinoma (LL2) ascites tumors, MTX-AuNP conjugates outperformed free MTX in inhibiting tumor growth (Chen *et al*., 2007). An *in vitro* look confirmed that Bleomycin (BLM) conjugated to Au-NPs and RGD-peptide induced higher DNA harm and a higher rate of death compared to free BLM treated with Malondialdehyde (MDA) target MB231 cells (Yang *et al*., 2016).

When the concept of active targeting NPs to a known oncogene was initially created, the antibodies showed to be a perfect targeting molecule. Antibody AuNPs may have developed more quickly due to the emergence of antibodies as stand-alone, clinically authorized treatments. Treatment options for overexpressing human epidermal growth factor receptor 2 (HER2) breast tumors include the clinically approved antibodies cetuximab and trastuzumab. (McKeage and Perry, 2002) which have been employed to target tumors with Au-NPs for improved RP (Chattopadhyay *et al*., 2013; Popovtzer *et al*., 2016). Peptides are comparatively tiny molecules made up of amino acids, that can be synthesized chemically, and completely characterized according to considered specifications (Biscaglia *et al*., 2019). In the literature, several examples of peptides being used to target Au-NPs

to several cancers for imaging has been documented (Avvakumova *et al*., 2014). Arg-glycoprotein (RGD) and a cyclic derivative (c(RGD)) can be directed by AuNPs towards a broad spectrum of tumors because angiogenesis promotes the proliferation of endothelial cells that express the alpha subunit of integrin (alphavβ3) (Desgrosellier and Cheresh, 2010). The EGFR over expressing glioblastoma cells have been exposed to AuNPs containing the photosensitizer Pc4 through EGFR targeting peptide GE11 (YHWYGYTPQNVI) (Cheng *et al*., 2011). There are many aptamers that have been created for different purposes, but only a few have been used to deliver Au-NPs. AS1411 is the most widely studied aptamer for the delivery of Au-NPs. AS1411 is a 26- base guanine-rich aptamer for the targeting of nucleolin, an over expressed phosphoprotein in cancer cells (Berger *et al*., 2015).

Photothermal Action: In photothermal therapy (PTT), heat is produced using laser light in combination with light-absorbing materials (such dyes or nanoparticles). This heat is concentrated on tissues or cells, usually malignant ones, resulting in localized destruction and damage (Guan *et al*., 2021). Hyperthermia is the use of heat to ablate the cancer cell, increase tumor's drug delivery, enhance the chemotherapeutic response of the tumor, or enhance the body defenses against cancer by the immune system (Schildkopf *et al*., 2010). In PTT, nanoparticles are inserted into tumor to produce warmness in reaction to externally applied laser light. Research indicates that PTT is especially powerful in most cancers' treatment. As a combination, PPT is being utilized with other treatments such as gene regulation and chemotherapy (Riley and Day, 2017). Numerous preclinical research was accomplished to illustrate that each radio and chemotherapy may be stronger with the aid of using concurrent hyperthermic therapy (Peeken *et al*., 2017). In the realm of cancer treatment, PPT is a promising strategy that holds the promise of efficient, tailored therapy with fewer side effects and better patient outcomes (O'Neal *et al*., 2004). Hleb *et al*. used a rat model tumor as an *in vivo* targeting agent, in which Au-NPs were conjugated to C225 monoclonal antibodies. Compared to cells without Au-NPs, the laser influence threshold for bubble formation and the amount of cell injury was reduced 100-folds in GNP-containing cells (Hleb *et al*., 2008).

Conclusion

Study concluded that Au-NPs offer a good chance for treating different types of cancer. By delivering these medications straight to the target cells through Au-NPs conjugation, the dosage and negative side effects can be decreased. It can be utilized for diverse chemotherapeutic drugs, binding agents of distinctive structures and lengths, allows incorporating various terminal end groups, like dicarboxylates or even thiols, and combining them with a variety of release modulators, chemo sensitizing agents, and targeting agents. Due to the versatility of Au-NPs, a wide group of gold and platinum NPs can now be created to refine and accomplish the innovation.

Acknowledgement

The Centre for Advanced Studies in Vaccinology and Biotechnology (CASVAB) and University of Balochistan, Quetta is highly acknowledged for providing the resources and conductive environment for this research.

References

Abbas, G., Shah, S., Hanif, M., Asghar, A., Shafique, M. and Ashraf, K. (2020). Cancer prevalence, incidence and mortality rates in Pakistan in 2018. Bull. Cancer. 107(4), 517-518.

Albanese, A., Tang, P. S. and Chan, W. C. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. Annu. Rev. Biomed. Eng. 14, 1-16.

Aljarba, N. H., Imtiaz, S., Anwar, N., Alanazi, I. S. and Alkahtani, S. (2022). Anticancer and microbial activities of gold nanoparticles: A mechanistic review. J.King. Saud. Univ.-Sci. 34(4), 101907. doi.org/10.1016/j.jksus.2022.101907.

Aryal, S., Grailer, J. J., Pilla, S., Steeber, D. A. and Gong, S. (2009). Doxorubicin conjugated gold nanoparticles as water-soluble and pH-responsive anticancer drug nanocarriers. J. Mater. Chem. 19(42), 7879-7884.

Avvakumova, S., Galbiati, E., Pandolfi, L., Mazzucchelli, S., Cassani, M., Gori, A., Longhi, R. and Prosperi, D. 2014. Development of U11-functionalized gold nanoparticles for selective targeting of urokinase plasminogen activator receptor-positive breast cancer cells. Bioconjugate Chem. 25(8), 1381-1386.

Baskar, R., Dai, J., Wenlong, N., Yeo, R. and Yeoh, K-W. (2014). Biological response of cancer cells to radiation treatment. Front. Mol. Biosci. 1, 24. doi:10.3389/fmolb.2014.00024.

Beik, J., Abed, Z., Ghoreishi, F. S., Hosseini-Nami, S., Mehrzadi, S., Shakeri-Zadeh, A. and Kamrava, S. K. (2016). Nanotechnology in hyperthermia cancer therapy: From fundamental principles to advanced applications. J. Control. Release. 235, 205-221.

Berger, C. M., Gaume, X. and Bouvet, P. (2015). The roles of nucleolin subcellular localization in cancer. Biochimie. 113, 78-85.

Biscaglia, F., Ripani, G., Rajendran, S., Benna, C., Mocellin, S., Bocchinfuso, G., Meneghetti, M., Palleschi, A. and Gobbo, M. (2019). Gold nanoparticle aggregates functionalized with cyclic RGD peptides for targeting and imaging of colorectal cancer cells. ACS Appl. Nano Mater. 2(10), 6436-6444.

Boisselier, E. and Astruc, D. (2009). Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. Chem. Soc. Rev. 38(6), 1759-1782.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A. and Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 68(6), 394-424.

Brown, S. D., Nativo, P., Smith, J. A., Stirling, D., Edwards, P. R., Venugopal, B., Flint, D. J., Plumb, J. A., Graham, D. and Wheate, N. J. (2010). Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. J. Am. Chem. Soc. 132(13), 4678-4684.

Brust, M., Walker, M., Bethell, D., Schiffrin, D. J. and Whyman, R. (1994). Synthesis of thiol-derivatised gold nanoparticles in a two-phase Liquid–Liquid system. J. Chem. Soc., Chem. Commun. (7), 801-802.

Cabuzu, D., Cirja, A., Puiu, R. and Grumezescu A. M. (2015). Biomedical applications of gold nanoparticles. Curr. Top. Med. Chem. 15(16), 1605-1613.

Chattopadhyay, N., Cai, Z., Kwon, Y. L., Lechtman, E., Pignol, J-P. and Reilly, R. M. (2013). Molecularly targeted gold nanoparticles enhance the radiation response of breast cancer cells and tumor xenografts to X-radiation. Breast Cancer Res. Treat. 137(1), 81-91.

Chen, H. and Zhao, Y. (2018). Applications of light-responsive systems for cancer theranostics. ACS Appl. Mater. Interfaces. 10(25), 21021-21034.

Chen, Y. H., Tsai, C. Y., Huang, P. Y., Chang, M. Y., Cheng, P. C., Chou, C. H., Chen, D. H., Wang, C. R., Shiau, A. L. and Wu, C. L. (2007). Methotrexate conjugated to gold nanoparticles inhibits tumor growth in a syngeneic lung tumor model. Mol. Pharm. 4(5), 713-722.

Cheng, Y., Meyers, J. D., Agnes, R. S,, Doane, T. L., Kenney, M. E., Broome, A. M., Burda, C. and Basilion, J. P. (2011). Addressing brain tumors with targeted gold nanoparticles: a new gold standard for hydrophobic drug delivery? Small. 7(16), 2301-2306.

Corso, G., Janssens, J. P. and La Vecchia, C. (2023). Cancer prevention: innovative strategies in the role of the European Cancer Prevention Organization. Eur. J. Cancer Prev. 32(3), 203-206.

Cui, T, Liang, J. J., Chen, H., Geng, D. D., Jiao, L., Yang, J. Y., Qian, H., Zhang, C. and Ding, Y. (2017). Performance of doxorubicin-conjugated gold nanoparticles: regulation of drug location. ACS Appl. Mater. Interfaces. 9(10), 8569-8580.

Deng, J., Xun, X., Zheng, W., Su, Y., Zheng, L., Wang, C.and Su, M. (2018). Sequential delivery of bismuth nanoparticles and doxorubicin by injectable macroporous hydrogels for combined anticancer kilovoltage X-ray radio-and chemo-therapy. J. Mater. Chem. B. 6(47), 7966-7973.

Desgrosellier, J. S. and Cheresh, D. A. (2010). Integrins in cancer: biological implications and therapeutic opportunities. Nat. Rev. Cancer. 10(1), 9-22.

Eleraky, N. E., Allam, A., Hassan, S. B. and Omar, M. M. (2020). Nanomedicine fight against antibacterial resistance: an overview of the recent pharmaceutical innovations. Pharmaceutics. 12(2), 142. doi.org/10.2217/nnm-2023-0022.

Giaquinto, A. N., Sung, H., Miller, K. D., Kramer, J. L., Newman, L. A., Minihan, A., Jemal, A. and Siegel, R. L. (2022). Breast cancer statistics, 2022. CA Cancer J. Clin. 72(6), 524-541.

Guan, G., Win, K. Y., Yao, X., Yang, W. and Han, M. Y. (2021). Plasmonically modulated gold nanostructures for photothermal ablation of bacteria. Adv. Healthc. Mater. 10(3), 2001158.

Hleb, E. Y., Hafner, J. H., Myers, J. N., Hanna, E. Y., Rostro, B. C., Zhdanok, S. A. andLapotko, D. O. (2008). LANTCET: elimination of solid tumor cells with photothermal bubbles generated around clusters of gold nanoparticles. Nanomedicine (Lond). 3(5), 647-667.

Hosta, L., Pla-Roca, M., Arbiol, J., López-Iglesias, C., Samitier, J., Cruz, L. J., Kogan, M. J. and Albericio, F. (2009). Conjugation of Kahalalide F with gold nanoparticles to enhance in vitro antitumoral activity. Bioconjugate Chem. 20(1), 138-146.

Hussain, M. H., Abu Bakar, N. F., Mustapa, A. N., Low, K. F., Othman, N. H. and Adam, F. (2020). Synthesis of various size gold nanoparticles by chemical reduction method with different solvent polarity. Nanoscale Res. Lett. 15(1), 1-10.

Jana, N. R., Gearheart, L. and Murphy, C. J. (2001). Seeding growth for size control of 5− 40 nm diameter gold nanoparticles. Langmuir. 17(22), 6782-6786.

Jazayeri, M. H., Amani, H., Pourfatollah, A. A., Pazoki-Toroudi, H. and Sedighimoghaddam, B. (2016). Various methods of gold nanoparticles (GNPs) conjugation to antibodies. Sens Bio-Sens Res. 9, 17-22.

Kimling, J., Maier, M., Okenve, B., Kotaidis, V., Ballot, H. and Plech, A. (2006). Turkevich method for gold nanoparticle synthesis revisited. J. Phys. Chem. B. 110(32), 15700-15707.

Larm, N. E., Essner, J. B., Pokpas, K., Canon, J. A., Jahed, N., Iwuoha, E. I. and Baker, G. A. (2018). Room-temperature turkevich method: Formation of gold nanoparticles at the speed of mixing using cyclic oxocarbon reducing agents. J. Phys. Chem. C. 122(9), 5105-5118.

Liauw, S. L., Connell, P. P. and Weichselbaum, R. R. (2013). New paradigms and future challenges in radiation oncology: an update of biological targets and technology. Sci. Transl. Med. *5*(173), 173sr2.doi: 10.1126/scitranslmed.3005148.

Longley, D. B., Harkin, D. P. and Johnston, P. G. (2003). 5-fluorouracil: mechanisms of action and clinical strategies. Nat, Rev. Cancer. 3(5), 330-338.

Mao, J. J., Pillai, G. G., Andrade, C. J., Ligibel, J. A., Basu, P., Cohen, L., Khan, I. A., Mustian, K. M., Puthiyedath, R., Dhiman, K. S., Lao, L., Ghelman, R., Guido, P. C., Lopez, G., Gallego, D. F. and Salicrup, L. A. (2022). Integrative oncology: Addressing the global challenges of cancer prevention and treatment. CA Cancer J. Clin. 72(2), 144-164.

McKeage, K. and Perry, C. M. (2002). Trastuzumab: a review of its use in the treatment of metastatic breast cancer overexpressing HER2. Drugs. 62(1), 209-243.

Miller, K. D., Nogueira, L., Devasia, T., Mariotto, A. B., Yabroff, K. R., Jemal, A., Karmer, J. and Siegel, R. L. (2022). Cancer treatment and survivorship statistics, 2022. CA Cancer J. Clin. 72(5), 409-436.

Muntimadugu, E., Kommineni, N. and Khan, W. (2017). Exploring the potential of nanotherapeutics in targeting tumor microenvironment for cancer therapy. Pharmacol. Res. 126, 109-122.

O'Neal, D. P., Hirsch, L. R., Halas, N. J., Payne, J. D. and West, J. L. (2004). Photo-thermal tumor ablation in mice using near infrared-absorbing nanoparticles. Cancer lett. 209(2), 171-176.

Paciotti, G. F., Kingston, D. G. and Tamarkin, L. (2006). Colloidal gold nanoparticles: a novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors. Drug Dev. Res. 67(1), 47-54.

Peeken, J. C., Vaupel, P. and Combs, S. E. (2017). Integrating hyperthermia into modern radiation oncology: what evidence is necessary? Front. Oncol. 7, 132. doi: 10.3389/fonc.2017.00132.

Popovtzer, A., Mizrachi, A., Motiei, M., Bragilovski, D., Lubimov, L., Levi, M., Hilly, O., Ben-Aharon, I. and Popovtzer, R. (2016). Actively targeted gold nanoparticles as novel radiosensitizer agents: an in vivo head and neck cancer model. Nanoscale, 8(5), 2678-2685.

Prise, K. M. (2006). New advances in radiation biology. Occup. Med (Lond). 5*6*(3), 156-161.

Riley, R. S. and Day, E. S. (2017). Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment. Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 9(4): e1449. doi: 10.1002/wnan.1449.

Safwat, M. A., Soliman, G. M., Sayed, D. and Attia, M. A. (2016). Gold nanoparticles enhance 5-fluorouracil anticancer efficacy against colorectal cancer cells. Int. J. Pharm. 513(1-2), 648-658.

Sak, K. (2012). Chemotherapy and dietary phytochemical agents. Chemother. Res. Pract. 2012(7), 282570. doi: 10.1155/2012/282570.

Sarwar, M.R. and Saqib, A. (2017). Cancer prevalence, incidence and mortality rates in Pakistan in 2012. Cogent Med. 4(1), 1288773. doi.org/10.1080/2331205X.2017.1288773.

Schildkopf, P., Ott, O. J., Frey, B., Wadepohl, M., Sauer, R., Fietkau, R. andGaipl, U. S. (2010). Biological rationales and clinical applications of temperature controlled hyperthermia-implications for multimodal cancer treatments. Curr. Med. Chem. 17(27), 3045-3057.

Siegel, R. L., Miller, K. D. and Jemal, A. (2019). Cancer statistics, 2019. CA Cancer J. Clin. 69(1), 7-34.

Siegel, R. L., Miller, K. D., Wagle, N. S. and Jemal, A. (2023). Cancer statistics, 2023. CA Cancer J. Clin. 73(1), 17-48.

Siti, R. M., Khairunisak, A. R., Aziz, A. A. and Noordin, R. (2013). Green synthesis of 10 nm gold nanoparticles via seeded-growth method and its conjugation properties on lateral flow immunoassay. Adv. Mater. Res. 686, 8-12.

Stobiecka, M., Ratajczak, K. and Jakiela, S. (2019). Toward early cancer detection: Focus on biosensing systems and biosensors for an anti-apoptotic protein survivin and survivin mRNA. Biosens Bioelectron. 137, 58-71.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 71(3), 209-249.

Thun, M. J., DeLancey, J.O., Center, M. M., Jemal, A. and Ward, E. M. (2010). The global burden of cancer: priorities for prevention. Carcinogenesis, 31(1), 100-10.

Torre, L. A, Bray, F., Siegel, R. L, Ferlay, J., Lortet-Tieulent, J. and Jemal, A. (2015). Global cancer statistics, 2012. CA Cancer J. Clin. 65(2), 87-108.

Tunc, C. U., Kursunluoglu, G., Akdeniz, M., Kutlu, A. U., Han, M. I., Yerer, M. B.and Aydin, O. (2023). Investigation of Gold Nanoparticle Naproxen-Derived Conjugations in Ovarian Cancer. ACS Mater. Au. 3(5), 483-491.

Turkevich, J, Stevenson, P. C. and Hillier, J. (1951). A study of the nucleation and growth processes in the synthesis of colloidal gold. Discuss. Faraday Soc. 11, 55-75.

Wallington, M., Saxon, E. B., Bomb, M., Smittenaar, R., Wickenden, M., McPhail, S., Rashbass, J., Chao, D., Dewar, J., Talbot, D., Peake, M., Perren, T., Wilson, C. and Dodwell, D. (2016). 30-day mortality after systemic anticancer treatment for breast and lung cancer in England: a population-based, observational study. Lancet Oncol. 17(9), 1203-1216.

Wang, F., Wang, Y. C., Dou, S., Xiong, M. H., Sun, T. M. and Wang, J. (2011). Doxorubicin-tethered responsive gold nanoparticles facilitate intracellular drug delivery for overcoming multidrug resistance in cancer cells. ACS Nano. 5(5), 3679-3692.

Yang, C., Uertz, J. and Chithrani, D. B. (2016). Colloidal gold-mediated delivery of bleomycin for improved outcome in chemotherapy. Nanomaterials (Basel), 6(3), 48.

Zhang, X. Q., Xu, X., Lam, R., Giljohann, D., Ho, D. and Mirkin, C. A. (2020), Strategy for Increasing Drug Solubility and Efficacy through Covalent Attachment to Polyvalent DNA-Nanoparticle Conjugates. In Spherical Nucleic Acids, 451-473. Jenny Stanford Publishing.

Zhu, D. M., Xie, W., Xiao, Y. S., Suo, M., Zan, M. H., Liao, Q. Q., Hu, X. J., Chen, L. B., Chen, B., Wu, W. T., Ji, L. W., Huang, H. M., Guo, S. S., Zhao, X. Z., Liu, Q. Y. and Liu, W. (2018). Erythrocyte membrane-coated gold nanocages for targeted photothermal and chemical cancer therapy. Nanotechnology. 29(8), 084002.