crystals

MDPI

Review

Targeted Chemotherapy Delivery via Gold Nanoparticles: A Scoping Review of In Vivo Studies

Ștefan Morărașu [1], Ștefan Iacob [1,*], Ionuț Tudorancea [2], Sorinel Luncă [1,3] and Mihail-Gabriel Dimofte [1,3]

[1] The Second Surgical Oncology Department, The Regional Institute of Oncology, 2-4 General Henri Mathias Berthelot, 700483 Iași, Romania; stefan.v.morarasu@d.umfiasi.ro (Ș.M.); sorinel.lunca@umfiasi.ro (S.L.); mihail.dimofte@umfiasi.ro (M.-G.D.)
[2] Department of Physiology, Grigore T. Popa University of Medicine and Pharmacy, 16 University Street, 700115 Iași, Romania; ionut.tudorancea@umfiasi.ro
[3] Department of Surgery, Grigore T. Popa University of Medicine and Pharmacy, 16 University Street, 700115 Iași, Romania
* Correspondence: dr.iacobstefan@yahoo.com or stefan-iacob@email.umfiasi.ro; Tel.: +40-746944932

Abstract: In the field of oncology, a lot of improvements in nanotechnology creates support for better diagnosis and therapeutic opportunities, and due to their physical and chemical properties, gold nanoparticles are highly applicable. We performed a literature review on the studies engaging the usage of gold nanoparticles on murine models with a focus on the type of the carrier, the chemotherapy drug, the target tumoral tissue and outcomes. We identified fifteen studies that fulfilled our search criteria, in which we analyzed the synthesis methods, the most used chemotherapy conjugates of gold nanoparticles in experimental cancer treatment, as well as the improved impact on tumor size and system toxicity. Due to their intrinsic traits, we conclude that chemotherapy conjugates of gold nanoparticles are promising in experimental cancer treatment and may prove to be a safer and improved therapy option than current alternatives.

Keywords: nanoparticles; chemotherapy; animal model; targeted delivery

Citation: Morarașu, Ș.; Iacob, Ș.; Tudorancea, I.; Luncă, S.; Dimofte, M.-G. Targeted Chemotherapy Delivery via Gold Nanoparticles: A Scoping Review of In Vivo Studies. *Crystals* **2021**, *11*, 1169. https://doi.org/10.3390/cryst11101169

Academic Editor: Shujun Zhang

Received: 25 August 2021
Accepted: 24 September 2021
Published: 26 September 2021

1. Introduction

Due to the advancements made in nanotechnology, especially in the last ten years, nanoparticles (NPs) have been increasingly proposed as a targeted diagnostic and therapeutic agent. The hype created around NPs originates from their stable, high carrier capacity. NPs are defined as nano-sized particles, ranging from 1 to 100 nm at least in one dimension. Depending on their structure and chemical composition, NPs can be classified as organic polymers (carbon-based, lipid-based), with an important advantage of being biodegradable, and as inorganic (such as gold, silver, iron oxide) [1]. In addition to their small size, they have a large surface area, thus, they can act as carriers for a wide range of peptides, antibodies, pharmaceuticals or contrast agents [1–3]. Due to their properties, NPs have a high accumulation rate especially in dysfunctional tissues, such as tumors, where the wide gaps in endothelium and disrupted lymphatic vessels leave room for NPs to migrate and congregate. Large NPs (~100 nm) can also accumulate via the macrophage pathway in lymph nodes, spleen or liver [4]. Based on these characteristics NPs are also studied in the field of nano-imaging. Apart from their diagnostic role, NPs have recently emerged as therapeutic carriers leading their way into theranostics research. In the field of nano-oncology, NPs are used for the targeted accumulation of chemotherapeutic agents into the tumor site. Gold NPs (AuNPs) are one of the main choices in such scenarios, due to their high biocompatibility, non-immunogenicity and stable carrier capacity [1,2]. Moreover, AuNPs have unique optical characteristics due to the interaction of light with the free electrons on their metal

surface. When facing an optical beam, the free electrons oscillate in a coherent fashion creating a resonance in the visible spectrum. This unique property is termed surface plasmon resonance (SPR) and through this AuNPs can be successfully used as contrast agents. Even under the dark field monochromatic light illumination, the high-scattering surface resonance of AuNPs can be easily visualized, thus enabling their use as optical biomarkers in cancer theranostics [5], with another great examples in this field represented by the iron oxide NPs, which have proven to be a valuable asset in the field of targeted MRI imaging [6,7]. In addition, due to their high atomic number, AuNPs are able to absorb X-rays and they may be used as focusing points for radiotherapy waves [8]. A plethora of studies have reported various ways to link AuNPs to chemotherapeutics, but due to high heterogeneity, it seems there is no clear-cut plan for which protocol to use and how to translate in vivo experiments into clinical studies. Animal models are at the core of NPs research through their ability to create a physiological environment where the tissue accumulation, receptor binding and, more importantly, the toxicity of NPs (which can determine unique changes within the living organism) [9] can be observed. For these reasons, in this structured review we aimed to analyze the advances in current models of in vivo AuNP-chemotherapeutic conjugates (AuNPCC) and address technical traits used to increase the applicability of AuNPCC. Further, we critically reviewed the results of AuNPCC in targeting malignant cells and how nanotechnology can be streamlined towards clinical translation.

The use of animal as in vivo models is based, among other reasons, on the fact that human diseases affect other animal species and cancer is one of the very common conditions encountered, with pathology mechanism being almost similar. Laboratory inbred mice strains (with homogenous genetic composition) is the most frequently used animal cancer model in cancer research mostly due to its adaptability to different environments, genetic variability and physiological similarities to humans [9]. The clinical translation process requires a mandatory and adequate understanding of the interaction of nanoparticles with different cells and tissues in an in vivo setting. There is a wide variety of events that we can observe in an in vivo experiment such as NPs interactions with neutrophils, monocytes/macrophages (with the Tumor-associated macrophages type) and Kupfer cells with cancer cells [10–14]. An interesting approach to in vivo studies of NPs is with its interaction with tumor endothelial cells, which could be an important niche in targeted antineoplastic therapy [15].

2. Materials and Methods

2.1. Literature Search and Study Selection

A systematic search of PubMed and EMBASE databases was performed for all published studies relating to animal models of chemotherapy delivery via AuNPs using the following search algorithm: gold AND nanoparticles AND chemotherapy AND cancer AND animal AND/OR model. The systematic search was done by adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines which were adapted to experimental studies. The PRISMA checklist was followed to conduct the methodology (Figure 1). Inclusion criteria were used according to the Problem/Population, Intervention, Comparison and Outcome (PICO) formula. All studies published in English from the earliest time point to 30 July 2021 describing chemotherapy delivery via AuNPs conjugates in animal models were selected for full-text review. The experimental lot (population) consisted of experimental murine models (BALB/c mice).

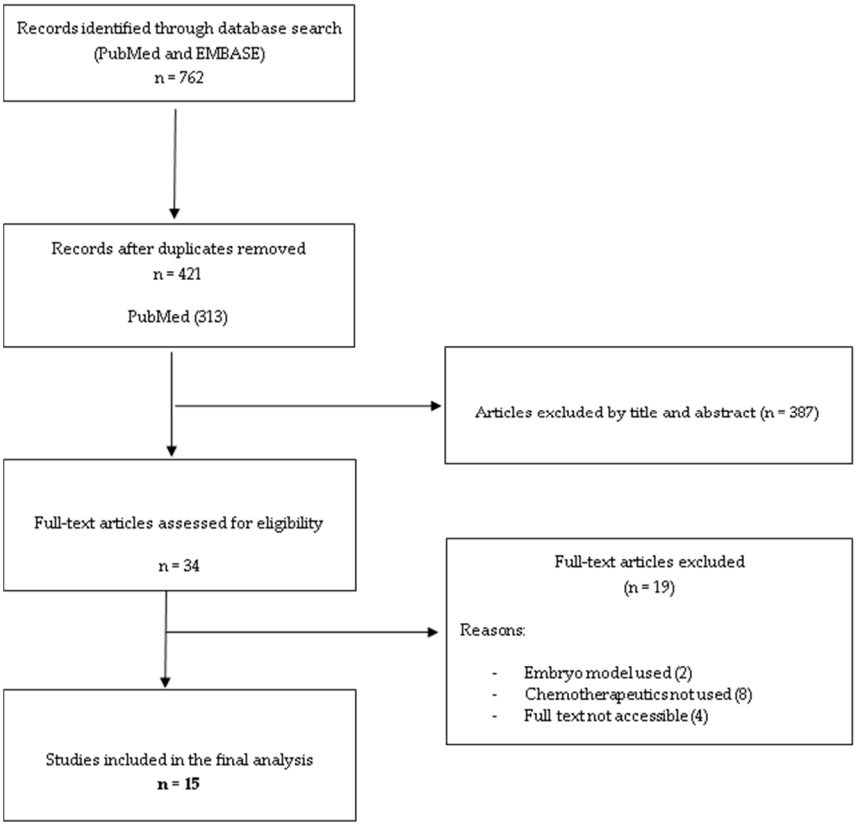

Figure 1. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow-chart.

Embryos, cell cultures, in vitro or human studies were excluded. The intervention was defined as the administration of AuNPCC in tumor bearing mice. Comparison criteria were further selected from subgroups of the included studies. Control tumor-bearing mice were studied in comparison to tumor bearing mice who received AuNPCC. Primary outcomes were tumor regression, AuNPCC synthesis and biodistribution studies.

2.2. Data Analysis

The following data information regarding each included study was extracted: the author's name, the year of publication, the type of AuNPCC, the chemotherapy used, the animal model used, the tumor cell line used, the protocol of tumor implantation, the protocol of AuNPCC synthesis, the method of delivery, the follow-up of outcomes including the immunohistochemistry studies, the haematological studies and the histology analysis.

2.3. Quality Assessment

Two authors (S.M. and S.I.) independently examined the title and abstract of citations, and the full texts of potentially eligible studies were obtained; disagreements were resolved by discussion. The ARRIVE guidelines were used to quantify the quality of included studies (Figure 2) [16]. Each study was marked for each ARRIVE item with 0 if data was lacking, 1 if data was incomplete and 2 if data was complete. The reference lists of retrieved papers were further screened for additional eligible publications. Only studies that had an ARRIVE score above 20 were included, thus ensuring streamlined quality of research among included papers.

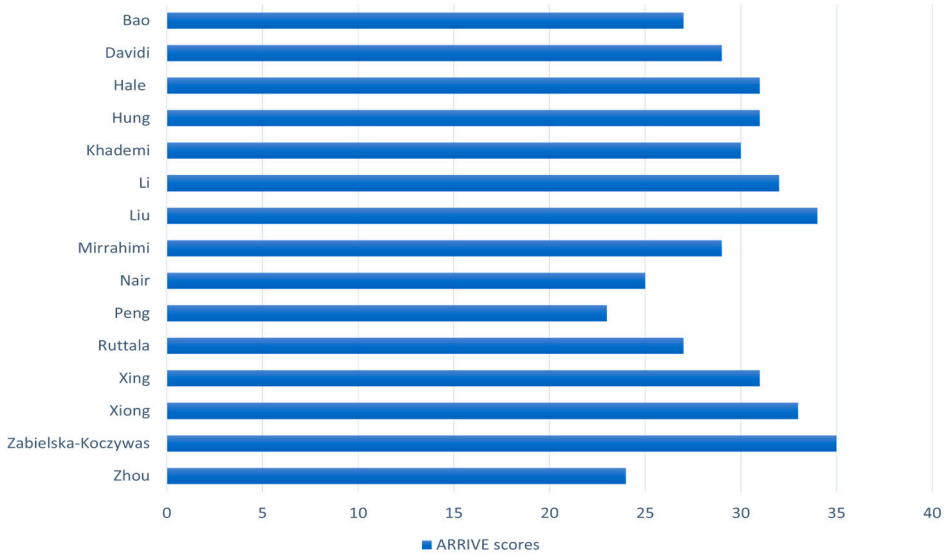

Figure 2. ARRIVE (Animal Research Reporting of In Vivo Experiments) scores.

3. Results

3.1. Literature Review and Design of Eligible Studies

Fifteen studies were selected based on the inclusion criteria [17–31]. After removing duplicates, the initial search found 421 studies. Thirty-four full-text manuscripts were assessed for eligibility. All manuscripts were published between 2014 to 2020. Each study brought its own contribution to the better understanding of the field nanotechnology. The novelty information offered by each included study is presented in Table 1.

Table 1. Novelty information offered by the included studies.

First Author	Novelty
Bao [17]	Using hybrid liposome with AuNPs to investigate the therapeutic outcome
Davidi [18]	AuNPs acting as drug carrier, radiosensitizer and contrast agent
Hale [19]	Using 2nm AuNPs conjugated with maytansine analogue for the treatment of hepatocellular carcinoma
Hung [20]	Significant inhibition of mutated KRAS gene in colorectal cancer cells using AuNPs with Doxurubicin (DXR)
Khademi [21]	Efficient usage of Chitosan coated-AuNPs with aptamers and DXR on cancer cell lines
Li [22]	Reducing toxicity and improving tumoral tissue destruction with heated-hallow AuNPs with thermosensitive liposomal DXR carriers
Liu [23]	Using chemo-photothermal synergic cancer therapy through AuNPs-Paclitaxel (PTX) and a hydrogel with gold nanorods with phototermal properties
Mirrahimi [24]	Using a novel nanocomplex comprising alginate nanogel co-loaded with Cisplatin (CIS) and AuNPs for chemo-phothermal therapy
Nair [25]	Enhanced anti-tumor effect and diminished system toxicity through chemo-photothermal therapy with hallow AuNPs and docetaxel
Peng [26]	The use of renal-clearable AuNPs with DXR
Ruttala [27]	Usage of a nanoplatform created from AuNPs-Lonidamine-Albumin-Aptamer for targeted tumoral destruction
Xing [28]	Using AuNPs-DXRcomplex co-encapsulated withing a liposome for targeted chemo-photothermal therapy
Xiong [29]	Prevention of tumoral CIS-induced chemoresistence throught AuNPs

	pretreatment
Zabielska-Koczywas [30]	The biodistribution and the effect of Glutathione-stabilized AuNPs with DXR in Feline Injection Site Sarcoma
Zhou [31]	The diagnostics and therapeutic effect of fluorescent gold nanoclusters conjugated with CIS and folic acid

3.2. Synthesis and Delivery of AuNPs-Chemotherapy Conjugates (AuNPCC)

Chemical methods are the preferred option for AuNPs synthesis (Table 2) mostly due to cost-effectiveness and due to the good production rate. All included papers used variations from Brust–Schiffrin, Turkevich or citrate reduction of $HAuCl_4$. To facilitate the coupling of nanoparticles with chemotherapeutics and to improve tumor targeting, it is imperative to use a surface ligand, usually a biopolymer which can bind drugs and has an affinity towards malignant cells. Polyethylene Glycol (PEG) or Folic Acid (FA) were commonly used to create nanoparticle conjugates (Table 3), the interaction with the specific receptor (e.g., the folate receptor) facilitating cellular internalization of the AuNPCC and intracellular delivery of the chemotherapeutic. Tumor penetrability has been improved in some studies by using carrier systems, namely liposomes [17,22,28]. Liposomes are probably the golden standard in targeted nanodrug transport as they can safely store, transport and release drugs at a specific location, increasing retention at the tumor site and reducing systemic toxicity [32,33].

Table 2. Overview of included studies.

First Author	Year of Publication	Type of AuNPCC	Chemotherapeutic	Type of Tumor	Route of Administration
Bao [17]	2014	loaded in liposomes	PTX	Hepatic carcinoma	intravascular
Davidi [18]	2017	simple conjugates	CIS	A431 (squamous carcinoma)	intravascular
Hale [19]	2018	maytansine conjugated AuNPs (Brust–Schiffrin synthesis)	Maytansine	HCC	intravascular
Hung [20]	2019	biopolymer composite	DXR	DLD-1 (colorectal adenocarcinoma)	intraperitoneal
Khademi [21]	2020	biopolymer composite	DXR	4T1 (breast carcinoma)	intravascular
Li [22]	2018	loaded in liposomes	DXR	MCF-7 (breast carcinoma)	intravascular
Liu [23]	2019	biopolymer composite	PTX	4T1 (breast carcinoma)	subcutaneous
Mirrahimi [24]	2019	nanogel construct	CIS	CT26 (colon adenocarcinoma)	intraperitoneal
Nair [25]	2020	folate-calix construct	DXR	HeLa (cervical cancer); A549 (lung	intraperitoneal

				adenocarcinoma)	
Peng [26]	2019	simple conjugates	DXR	4T1 (breast carcinoma)	intravascular
Ruttala [27]	2020	simple conjugates	Ionidamine	DU-145 (prostate cancer)	intravascular
Xing [28]	2018	loaded in liposomes	DXR	U14 (cervical carcinoma)	intravascular
Xiong [29]	2014	simple conjugates	CIS	SK-OV-3 (ovarian carcinoma)	intraperitoneal
Zabielska-Koczywas [30]	2018	glutathione conjugates	DXR	FFS1 (feline fibrosarcoma)	intravascular
Zhou [31]	2016	folate-calix construct	CIS	4T1 (breast carcinoma)	intravascular

In our analysis, all the studies performed chemical synthesis of NPs, but only three of them [17,22,28] used liposomes as carriers for AuNPCC (Table 3). Synthesis of Liposome AuNPCC was done by using the thin hydration film method. Briefly, soy lecithin, cholesterol and AuNPCC are dissolved and centrifuged. Once the solvent is removed, the thin layer consists of liposomes that incorporate the NPCC. Bao et al. [17] showed important benefits in using liposomes for drug delivery. In their in vivo experiment, liposome loaded AuNPCC (AuNPs-PEG- PTX) showed improved biodistribution compared to simple AuNPCC. The delivery capacity of liposome-loaded AuNPCC showed two key differences [17,22,28]. The release of AuNPCC within the bloodstream was slower, reducing systemic loss of the drug and subsequent toxicity, while the penetrability of AuNPCC within the liver increased significantly quicker than the other groups. The improved biodistribution of liposomes would seem an ideal option for targeted chemotherapy delivery, but the delivery timing and rate cannot be controlled; this is where AuNPs play an important role. Loaded into liposomes, AuNPCCs are better distributed at the tumor site, and upon reaching the tumor, release of AuNPCCs can be remotely triggered through near-infrared laser illumination. Moreover, by adding AuNPs, the tumoral release and distribution of these complexes can be visualized via fluorescent imaging [18,28,31].

Table 3. Chemical synthesis of NPs.

First Author	Type of Surface Ligand	Type of Carrier
Bao [17]	PEG	liposomes
Davidi [18]	PEG7	not used
Hale [19]	direct surface bond	not used
Hung [20]	PEG; PEI	not used
Khademi [21]	chitosan	not used
Li [22]	direct surface bond	liposomes
Liu [23]	direct surface bond	nanogel
Mirrahimi [24]	direct surface bond	nanogel
Nair [25]	Folic Acid	not used
Peng [26]	PEG; MBA	not used
Ruttala [27]	aptamer AS1411	not used
Xing [28]	direct surface bond	liposomes

Xiong [29]	direct surface bond	not used
Zabielska-Koczywas [30]	glutathione	not used
Zhou [31]	Folic Acid	not used

Abbreviations: PEG (polyethylene glycol); PEG7 (heptaethylene glycol); PEI (polyethylenimine); MBA (4-mercaptobenzoic acid).

3.3. Selection of Chemotherapeutics

DXR, CIS and PTX were the chemotherapeutics used to load into AuNPs (Table 2). DXR is a highly efficient chemotherapy agent, used in many malignancies including leukaemia, breast carcinoma, ovarian carcinoma or gastric carcinoma [34,35]. However, it has a safety issue with its dose-dependent cardiotoxicity [36–38] and, for this reason, it is commonly used in experimental studies in an attempt to reduce its systemic side effects. Another clear reason why DXR is usually used in experimental research is that it has an intrinsic fluorescence making it easy to visualize its internalization into tumor cells, thus offering precise mapping of its biodistribution [39,40]. In seven studies DXR was loaded on AuNPs [20–22,25,26–28,30]. Hung et al. [20] linked DXR to a biopolymeric AuNP composite through π-stacking. The interaction proved to be stable in the blood flow, and the drug was released only after the linkage broke down in the acidic environment of the tumor. More recently, Nair et al. [19] used a tumor-homing compound bound to DXR to target malignant cells reducing cardiotoxicity and DXR resistance. This complex was incubated with hollow AuNPs forming AuNPCC. The DXR complex was targeted against cervical cancer cell lines (HeLa) and lung adenocarcinoma cell lines (A549) and released from the AuNPCC upon laser irradiation, thus obtaining a significant reduction in the tumor mass with marked survival benefits.

PTX is a truly potent chemotherapeutic but with the price of high toxicity. In its Taxol® formulation (dissolved in Kolliphor EL and ethanol) it presents the fastest elimination (the plasma concentration at 36 h after injection being 18.8 times lower compared with AuNP-PTX-liposome complexes) along with the shortest half-life (6.85 h, compared with its hybrid liposomes-bound form of 14.69 h) [17]. Bound to a copolymer, the AuNPs-PTX complex proved to be efficient in delivering synergic chemo-photothermal by releasing the PTX when the containing hydrogel was heated in vitro to about 50 °C through irradiation [23].

CIS is well known for its ability to crosslink DNA and alter the structure in addition to activating apoptosis through various signal transduction pathways, but it fails to have acceptable toxicity properties. However, when bound to AuNPs conjugated with glucose, increased uptake by tumor tissue was identified [18]. Moreover, when bound to fluorodeoxyglucose (18F), it also offered real-time imaging of the treated tumor [24].

3.4. In Vivo Antitumoral Activity of AuNPCC

The antitumor activity of NPCC has been tested for a wide range of cell lines. In all studies, tumor cell lines were injected into the animal's flank, while the AuNPCC, in most cases, were injected intravascular into the animal's tail vein (Table 2). The 4T1 breast carcinoma cell line is the most frequently used, being a highly carcinogenic line. The 4T1 cells are capable of distant spread and independent metastasis growth, similar to humans. In four papers AuNPCC were against 4T1 tumors [21,23,26,31]. In the form of biopolymers or simple chemotherapy-AuNPs conjugates, they were effective in reducing tumor diameter and weight. Studies showed a 40 to 60% tumor reduction in the AuNPCC group alongside a more focused biodistribution at the tumor site, with less systemic spillage of chemotherapy drugs. Similarly in colonic adenocarcinoma models, AuNPCC showed improved tumor targeting and prolonged local chemotherapy release resulting in 30% more tumor regression than control groups [20]. AuNPCC also proved efficacious in ovarian [29], cervical [28], hepatic [19] and sarcoma cell lines [30] (Table 2).

3.5. Systemic Biodistribution of AuNPCC

The in vivo distribution of clear nanoparticles is variable depending on their size, diameter, weight and, more importantly, adsorption of various blood proteins on their surface which changes their physical properties (usually increasing their weight and diameter) and subsequently changing the cellular internalization of nanoparticles [41,42]. To overcome this, in the studies analyzed in the current review, authors have prefabricated AuNPs with vitamins (e.g., Folic Acid) to improve the specificity towards malignant cells. Route of administration also influences nanoparticle distribution and should take into account the size of the AuNPs (for the transdermal administration), the shielding of the NPs to prevent enzymatic degradation (oral delivery), the aerodynamic diameter of the NPs (through the inhalation route) and the eluding of the reticuloendothelial system (for the intravascular system) [43]. In most studies (n = 10), authors used an intravascular approach via the animal's dorsal vein. Others (n = 5) used the intraperitoneal route, while Liu et al. [23] injected the AuNPCC subcutaneously, adjacent to the tumor site. Six studies analyzed the systemic distribution of AuNPCC, most commonly via an ex vivo analysis of the major organs using near-infrared fluorescence imaging, chromatography or atomic absorption spectroscopy. In vivo distribution of AuNPCC was mapped in two studies using fluorescent imaging techniques (e.g., NIR—near infrared spectroscopy [22], FOBI—fluorescence in vivo imaging system [27]) (Table 4).

Table 4. Biodistribution of AuNPCC.

First Author	Route of Administration	Ex Vivo/In Vivo Analysis	Imaging Evaluation
Bao [17]	intravascular	ex vivo	chromatography
Davidi [18]	intravascular	ex vivo	atomic absorption spectroscopy
Khademi [21]	intravascular	ex vivo	NIR fluorescence
Li [22]	intravascular	in vivo	NIR fluorescence
Nair [25]	intraperitoneal	ex vivo	ICP-MS
Ruttala [27]	intravascular	in vivo	FOBI fluorescence imaging

Abbreviation: ICP-MS (inductively coupled plasma mass spectrometry).

4. Discussion

4.1. Literature Overview

Metallic nanoparticles are in high demand in nanomedicine, and gold is one of the common choices due to its chemical, electrical and physical stability [44–47]. AuNPs have a fluctuation of positive and negative electrons at their surface creating a variable charge which resonates differently depending on the size, shape and medium in which the NPs are introduced [44]. Developing stable AuNPs is usually done through either a top-down approach by subtracting nanoscale objects from large materials or a bottom-up approach in which molecules are condensed to create clusters of molecules linked together using energy. The bottom-up chemical synthesis of nanoparticles is the norm among researchers in this field and this review emphasized this [48]. A technique described by Turkevich et al. [49] and commonly used in the selected studies involves the chemical reduction of hydrogen tetrachloroaurate ($H[AuCl_4]$) by sodium citrate ($Na_3C_6H_5O_7$) in boiling water. A highly appreciated technique for AuNPs synthesis is the Brust–Schiffrin method [50,51], which uses tetraoctylammonium bromide ($[CH_3(CH_2)_7]_4NBr$) to transfer gold salt to the toluene phase and then uses sodium borohydride ($NaBH_4$) in the second phase to reduce the solution. The resulting thiol protected AuNPs are highly stable and can be redispersed in other solutions to create complexes without fears of aggregation. The preferred synthesis method depends on the size of the future nanoparticle, the Turkevich technique being suitable for the synthesis on AuNPs 10–50 nm in size, while the Brust–Schiffrin method is best used for the synthesis of AuNPs of under 10 nm in size

[52]. Some studies reported using microorganisms to create AuNPs [53–55] in which positively charged gold molecules interact with the negatively charged bacterial wall and through endocytosis the gold molecules are transported inside the cell where bacterial enzymes can induce the synthesis of AuNPs [56].

AuNPs have made their way into cancer treatment research as efficient carriers that can change the pharmacokinetics of drugs, improving their cancer-targeting capabilities [27] and reducing systemic side effects [22,25]. Focusing drug delivery only to malignant cells by the use of nanoparticles and in a safe manner may be a real paradigm shift in cancer treatment. Drug-loaded NPs can penetrate more easily through the distorted tumor tissue. This effect, known as "the enhanced penetration and retention effect", is at the core of NPs beneficial characteristics in cancer research.

In our analysis, liposomes were commonly identified as AuNPCC carriers. The ability of amphiphilic lipid molecules to arrange into various 3D structures incorporating AuNPCC was commonly used by researchers to deliver AuNPs at the tumor site [57]. Chitchrani et al. [58] showed a 1000-fold increase in cellular endocytosis when AuNPs were incorporated into liposomes. In other studies, researchers created pH, thermic or even oxygen-sensitive liposomes which can release the cargo in the hypoxic environment of tumors. Li et al. [22] used thermo-sensitive liposomes loaded with Doxorubicin and AuNPs which were released when exposed to near-infrared heat causing ablation of tumors via thermal stress and release of DXR. A similar approach was used by Xing et al. [28] who compared the in vivo antitumor activity between AuNPCC loaded liposomes irradiated with near-infrared laser, AuNPCC loaded liposomes and free DXR. The first group showed a 30% increase in tumor suppression compared to the second group and 50% compared to free chemotherapy. The authors also evaluated the tissue toxicity of AuNPCC loaded liposomes by harvesting treated and normal tissue samples of the heart, the liver, the spleen, the lung and of the kidney. No histological marks of toxicity could be found in either sample, emphasizing the safety of delivering such compounds.

The BALB/c mice act as optimal animal models for NPs studies in experimental cancer treatment on account of their acquisition, their maintenance and experimental costs, their easy tumor xenograft inoculation (commonly in the flank), the chemotherapy administration by body weight and biodistribution monitoring through the lateral veins of the tail. In some studies these models undergo simple radiotherapy and combined it with either free chemotherapy or AuNPCC [18] which allows a better understanding of this potential novel cancer treatment.

In contrast with the abundance of animal model studies of NPs, some obstacles prevent the presence of clinical treatments based on this promising medical technology. Firstly, the current challenges can be related to the costs, techniques and infrastructure required for NPs synthesis, in addition to the lack of quality control methods. Secondly, there is a wide gap in the understanding of the NPs interactions with cells and tissues and the toxic effect it might present, which would imply that there is an imperious need to develop more specialized toxicology studies. Lastly, nanomedicine requires clear regulatory guidelines regarding its use and a simplified method of patent approval and intellectual property [10,59]. Solving these issues will allow to further move into clinical trials.

4.2. Limitations of the Study

The inclusion criteria used in this review were restricted to studies that used only AuNPCCs to deliver targeted therapy at animal models of experimental tumors. This resulted in a limited number of eligible manuscripts limiting the addressability of this review. However, by using a specific search algorithm, this scoping review included only studies with matching methodologies thus increasing comparability between experiments and reducing confounding biases between inter-study results. By narrowing the inclusion criteria and subsequent number of analyzed manuscripts, researchers can have a clear overview of outcomes in this specific research question, aiding them to find

gaps in research and, more importantly, by grouping outcomes of similar experiments, the research power increases and translation of results will be easier. Moreover, only studies that had an ARRIVE score of at least 20 were included, thus ensuring only quality research was analyzed.

5. Conclusions

In the in vivo studies analyzed in this manuscript, AuNPs were efficacious not only as drug carriers, but also as imaging, through the surface-enhanced Raman scattering (SERS effect) and thermal therapy agents. Through surface ligands, usually PEG, authors have attached chemotherapy drugs to the surface of NPs generation thus AuNPCC. Tumoral tissue concentration of AuNPCC was improved when compared with control groups in all experiments. Results from the studies identified and discussed in this paper clearly show the beneficial effect of AuNPCC in theranostics of cancer and pave the way for the development of new therapeutic strategies.

Author Contributions: Conceptualization, M.-G.D. and S.L.; methodology, Ș.M., I.T.; investigation, Ș.M. and Ș.I.; resources, Ș.M., Ș.I.; data curation, I.T.; writing—original draft preparation, Ș.M., Ș.I., I.T.; writing—review and editing, Ș.I., M.-G.D.; supervision, M.-G.D., S.L.; project administration, M.-G.D., S.L. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded through the Iceland Liechtenstein Norway Grant EEA-RO-NO-2018-0246.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: Computer and internet access—2nd Surgical Oncology Department, Regional Oncology Institute Iasi Romania.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Najahi-Missaoui, W.; Arnold, R.D.; Cummings, B.S. Safe nanoparticles: Are we there yet? *Int. J. Mol. Sci.* **2021**, *22*, 385, doi:10.3390/ijms22010385.
2. Baetke, S.C.; Lammers, T.; Kiessling, F. Applications of nanoparticles for diagnosis and therapy of cancer. *Br. J. Radiol.* **2015**, *88*, 20150207, doi:10.1259/bjr.20150207.
3. Wolfram, J.; Zhu, M.; Yang, Y.; Shen, J.; Gentile, E.; Paolino, D.; Fresta, M.; Nie, G.; Chen, C.; Shen, H.; et al. Safety of nanoparticles in medicine. *Curr. Drug Targets* **2015**, *16*, 1671–1681; doi:10.2174/1389450115666140804124808.
4. Gustafson, H.H.; Casper, D.H.; Grainger, D.W.; Ghandehari, H. Nanoparticle uptake: The phagocyte problem. *Nano Today* **2015**, *10*, 487–510, doi:10.1016/j.nantod.2015.06.006.
5. Kang, M.S.; Lee, S.Y.; Kim, K.S.; Han, D.W. State of the art biocompatible gold nanoparticles for cancer theragnosis. *Pharmaceutics* **2020**, *12*, 701, doi:10.3390/pharmaceutics12080701.
6. Zhu, L.; Zhou, Z.; Mao, H.; Yang, L. Magnetic nanoparticles for precision oncology: Theranostic magnetic iron oxide nanoparticles for image-guided and targeted cancer therapy. *Nanomedicine* **2017**, *12*, 73–87, doi:10.2217/nnm-2016-0316.
7. Zhu, W.; Xu, Y.; Jin, R.; Wu, C.; Ai, H. MRI tracking of dendritic cells loaded with superparamagnetic iron oxide nanoparticles. *Methods Mol. Biol.* **2020**, *2126*, 107–116, doi:10.1007/978-1-0716-0364-2_10.
8. Exbrayat, J.M.; Moudilou, E.N.; Lapied, E. Harmful effect of nanoparticles on animals. *J. Nanotechnol.* **2015**, 861092, doi:10.1155/2015/861092.
9. Onaciu, A.; Munteanu, R.; Munteanu, V.C.; Gulei, D.; Raduly, L.; Feder, R.; Pirlog, R.; Atanasov, A.G.; Korban, S.S.; Irimie, A.; Berindan-Neagoe, I. Spontaneous and Induced Animal Models for Cancer Research. *Diagnostics* **2020**, *10*, 660, doi:10.3390/diagnostics10090660.
10. Hua, S.; De Matos, M.B.; Metselaar, J.M.; Storm, G. Current Trends and Challenges in the Clinical Translation of Nanoparticulate Nanomedicines: Pathways for Translational Development and Commercialization. *Front. Pharmacol.* **2018**, *9*, 790, doi:10.3389/fphar.2018.00790.
11. Naumenko, V.; Nikitin, A.; Melnikov, A.G.P.; Vodopyanov, S.; Kapitanova, K.; Potashnikova, D.; Vishnevskiy, D.; Alieva, I.; Ilyasov, A.; Eletskaya, B.Z.; et al. Neutrophil-mediated transport is crucial for delivery of short-circulating magnetic nanoparticles to tumors. *Acta Biomater.* **2020**, *104*, 176–187.

12. Weissleder, R.; Nahrendorf, M.; Pittet, M.J. Imaging macrophages with nanoparticles. *Nat. Mater*. **2014**, *13*, 125–138.
13. Krenkel, O.; Tacke, F. Liver macrophages in tissue homeostasis and disease. *Nat. Rev. Immunol.* **2017**, *17*, 306–321.
14. Kapitanova, K.S. Advances and challenges of nanoparticle-based macrophage reprogramming for cancer immunotherapy. *Biochemistry* **2019**, *84*, 729–745.
15. Lin, Q.; Fathi, P.; Chen, X.Nanoparticle delivery in vivo: A fresh look from intravital imaging. *EBioMedicine* **2020**, *59*, 102958, doi:10.1016/j.ebiom.2020.102958.
16. Percie du Sert, N.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*. **2020**, *18*, e3000410, doi:10.1371/journal.pbio.3000410.
17. Bao, Q.Y.; Zhang, N.; Geng, D.D.; Xue, J.W.; Merritt, M.; Zhang, C.; Ding, Y.The enhanced longevity and liver targetability of Paclitaxel by hybrid liposomes encapsulating Paclitaxel-conjugated gold nanoparticles. *Int. J. Pharm.* **2014**, *477*, 408–415, doi:10.1016/j.ijpharm.2014.10.040.
18. Davidi, E.S.; Sert, N.P.d.; Hurst, V.; Ahluwalia, A.; Alam, S.; Avey, M.T.; Baker, M.; Browne, W.J.; Clark, A.; Cuthill, I.C.; et al. Cisplatin-conjugated gold nanoparticles as a theranostic agent for head and neck cancer. *Head Neck* **2018**, *40*, 70–78, doi:10.1002/hed.24935.
19. Sarah, J.M.; Perrins, R.D.; García, C.E.; Pace, A.; Peral, U.; Patel, K.R.; Robinson, A.; Williams, P.; Ding, Y.; Saito, G.; et al. DM1 Loaded Ultrasmall Gold Nanoparticles Display Significant Efficacy and Improved Tolerability in Murine Models of Hepatocellular Carcinoma. *Bioconjug. Chem.* **2019**, *30*, 703–713, doi:10.1021/acs.bioconjchem.8b00873.
20. Hung, W.H.; Zheng, J.H.; Lee, K.C.; Cho, E.C. Doxorubicin conjugated AuNP/biopolymer composites facilitate cell cycle regulation and exhibit superior tumor suppression potential in KRAS mutant colorectal cancer. *J. Biotechnol.* **2019**, *306*, 149–158, doi:10.1016/j.jbiotec.2019.09.015.
21. Khademi, Z.; Lavaee, P.; Ramezani, M. Co-delivery of doxorubicin and aptamer against Forkhead box M1 using chitosan-gold nanoparticles coated with nucleolin aptamer for synergistic treatment of cancer cells. *Carbohydr. Polym.* **2020**, *248*, 116735, doi:10.1016/j.carbpol.2020.116735.
22. Li, Y.; He, D.; Tu, J.; Wang, R.; Zu, C.; Chen, Y.; Yang, W.; Shi, D.; Webster, J.T.; Shen, Y. The comparative effect of wrapping solid gold nanoparticles and hollow gold nanoparticles with doxorubicin-loaded thermosensitive liposomes for cancer thermo-chemotherapy. *Nanoscale* **2018**, *10*, 8628–8641, doi:10.1039/c7nr09083h.
23. Liu, M.; Huang, P.; Wang, W.; Feng, Z.; Zhang, J.; Deng, L.; Dong, A. An injectable nanocomposite hydrogel co-constructed with gold nanorods and paclitaxel-loaded nanoparticles for local chemo-photothermal synergetic cancer therapy. *J. Mater. Chem. B* **2019**, *7*, 2667–2677, doi:10.1039/c9tb00120d.
24. Mirrahimi, M.; Abed, Z.; Beik, J.; Shiri, I.; Dezfuli, A.S.; Mahabadi, V.P.; Kamrava, S.K.; Ghaznavi, H.; Shakeri-Zadeh, A. A thermo-responsive alginate nanogel platform co-loaded with gold nanoparticles and cisplatin for combined cancer chemo-photothermal therapy. *Pharmacol. Res.* **2019**, *143*, 178–185, doi:10.1016/j.phrs.2019.01.005.
25. Nair, J.B.; Joseph, M.M.; Arya, J.S.; Sreedevi, P.; Sujai, P.T.; Maiti, K.K. Elucidating a Thermoresponsive Multimodal Photo-Chemotherapeutic Nanodelivery Vehicle to Overcome the Barriers of Doxorubicin Therapy. *ACS Appl. Mater. Interfaces* **2020**, *12*, 43365–43379, doi:10.1021/acsami.0c08762.
26. Peng, C.; Xu, J.; Yu, M.; Ning, X.; Huang, Y.; Du, B.; Hernandez, E.; Kapur, P.; Hsieh, J.; Zheng, J. Tuning the In Vivo Transport of Anticancer Drugs Using Renal-Clearable Gold Nanoparticles. *Angew. Chem. Int. Ed. Engl.* **2019**, *58*, 8479–8483, doi:10.1002/anie.201903256.
27. Ruttala, H.B.; Ramasamy, T.; Poudel, B.K.; Ruttala, R.R.T.; Jin, S.G.; Choi, H.G.; Ku, S.K.; Yong, C.S.; Kim, J.O. Multi-responsive albumin-lonidamine conjugated hybridized gold nanoparticle as a combined photothermal-chemotherapy for synergistic tumor ablation. *Acta Biomater.* **2020**, *101*, 531–543, doi:10.1016/j.actbio.2019.11.003.
28. Xing, S.; Zhang, X.; Luo, L.; Cao, W.; Li, L.; He, Y.; An, J.; Gao, D. Doxorubicin/gold nanoparticles coated with liposomes for chemo-photothermal synergetic antitumor therapy. *Nanotechnology* **2018**, *29*, 405101, doi:10.1088/1361-6528/aad358.
29. Xiong, X.; Arvizo, R.R.; Saha, S.; Robertson, D.J.; McMeekin, S.; Bhattacharya, R.; Mukherjee, P. Sensitization of ovarian cancer cells to cisplatin by gold nanoparticles. *Oncotarget* **2014**, *5*, 6453–6465, doi:10.18632/oncotarget.2203.
30. Zabielska-Koczywąs, K.; Wojtalewicz, A.; Użarowska, E.; Klejman, A.; Wojtkowska, A.; Dolka, I.; Wojnicki, M.; Sobczak, K.; Wójcik, M.; Shen, H.; et al. Distribution of Glutathione-Stabilized Gold Nanoparticles in Feline Fibrosarcomas and Their Role as a Drug Delivery System for Doxorubicin-Preclinical Studies in a Murine Model. *Int. J. Mol. Sci.* **2018**, *19*, 1021, doi:10.3390/ijms19041021.
31. Zhou, F.; Feng, B.; Yu, H.; Wang, D.; Wang, T.; Liu, J.; Meng, Q.; Wang, S.; Zhang, P.; Zhang, Z.; et al. Cisplatin Prodrug-Conjugated Gold Nanocluster for Fluorescence Imaging and Targeted Therapy of the Breast Cancer. *Theranostics* **2016**, *6*, 679–687, doi:10.7150/thno.14556.
32. ud Din, F.; Aman, W.; Ullah, I.; Qureshi, O.S.; Mustapha, O.; Shafique, S.; Zeb, A.Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *Int. J. Nanomed.* **2017**, *12*, 7291–7309, doi:10.2147/IJN.S146315.
33. Yingchoncharoen, P.; Kalinowski, D.S.; Richardson, D.R. Lipid-Based Drug Delivery Systems in Cancer Therapy: What Is Available and What Is Yet to Come. *Pharmacol. Rev*. **2016**, *68*, 701–787, doi:10.1124/pr.115.012070.
34. Prados, J.; Melguizo, C.; Ortiz, R.; Velez, C.; Alvarez, P.J.; Arias, J.L.; Ruiz, M.A.; Gallardo, V.; Aranega, A. Doxorubicin-loaded nanoparticles: New advances in breast cancer therapy. *Anticancer Agents Med. Chem.* **2012**, *12*, 1058–1070, doi:10.2174/187152012803529646.

35. Norouzi, M.; Yathindranath, V.; Thliveris, J.A.; Kopec, B.M.; Siahaan, T.J.; Miller, D.W. Doxorubicin-loaded iron oxide nanoparticles for glioblastoma therapy: A combinational approach for enhanced delivery of nanoparticles. *Sci. Rep.* **2020**, *10*, 11292, doi:10.1038/s41598-020-68017-y.
36. Songbo, M.; Lang, H.; Xinyong, C.; Bin, X.; Ping, Z.; Liang, S. Oxidative stress injury in doxorubicin-induced cardiotoxicity. *Toxicol. Lett.* **2019**, *307*, 41–48, doi:10.1016/j.toxlet.2019.02.013.
37. Koleini, N.; Nickel, B.E.; Edel, A.L.; Fandrich, R.R.; Ravandi, A.; Kardami, E. Oxidized phospholipids in Doxorubicin-induced cardiotoxicity. *Chem. Biol. Interact.* **2019**, *303*, 35–39, doi:10.1016/j.cbi.2019.01.032.
38. Zhang, Y.Y.; Yi, M.; Huang, Y.P. Oxymatrine Ameliorates Doxorubicin-Induced Cardiotoxicity in Rats. *Cell Physiol. Biochem.* **2017**, *43*, 626–635, doi:10.1159/000480471.
39. Uyar, T.B.; Wu, K.; He, M.; Khan, I.; Royzen, M.; Yigit, M.V. Switchable Fluorescence of Doxorubicin for Label-Free Imaging of Bioorthogonal Drug Release. *Chem. Med. Chem.* **2020**, *15*, 988–994, doi:10.1002/cmdc.202000065.
40. Dong, X.; Yang, A.; Bai, Y.; Kong, D.; Lv, F. Dual fluorescence imaging-guided programmed delivery of doxorubicin and CpG nanoparticles to modulate tumor microenvironment for effective chemo-immunotherapy. *Biomaterials* **2020**, *230*, 119659, doi:10.1016/j.biomaterials.2019.119659.
41. Charbgoo, F.; Nejabat, M.; Abnous, K.; Soltani, F.; Taghdisi, S.M.; Alibolandi, M.; Shier, W.T.; Steele, T.W.J.; Ramezani, M. Gold nanoparticle should understand protein corona for being a clinical nanomaterial. *J. Control. Release* **2018**, *272*, 39–53, doi:10.1016/j.jconrel.2018.01.002.
42. Bai, X.; Wang, J.; Mu, Q.; Su, G. In vivo Protein Corona Formation: Characterizations, Effects on Engineered Nanoparticles' Biobehaviors, and Applications. *Front. Bioeng. Biotechnol*. **2021**, *9*, 263, doi:10.3389/fbioe.2021.646708.
43. Chenthamara, D.; Subramaniam, S.; Ramakrishnan, S.G.; Krishnaswamy, S.; Essa, M.M.; Lin, F.H.; Qoronfleh, M.W.Therapeutic efficacy of nanoparticles and routes of administration. *Biomater. Res.* **2019**, *23*, 20, doi:10.1186/s40824-019-0166-x.
44. Amina, S.J.; Guo, B. A review on the synthesis and functionalization of gold nanoparticles as a drug delivery vehicle. *Int. J. Nanomed.* **2020**, *7*, 9823–9857, doi:10.2147/IJN.S279094.
45. Jennings, T.; Strouse, G. Past, present and future of gold nanoparticles. *Adv. Exp. Med. Biol.* **2007**, *620*, 34, doi:10.1007/978-0-387-76713-0_3.
46. Bracamonte, M.V.; Bollo, S.; Labbe, P.; Rivas, G.A.; Ferreyra, N.F. Quaternized chitosan as support for the assembly of gold nanoparticles and glucose oxidase. Physiochemical characterization of the platform and evaluation of its biocatalytic activity. *Electrochim. Acta* **2011**, *56*, 1316–1322, doi:10.1016/j.electacta.2010.10.022.
47. Aillon, K.L.; Xie, Y.; El-Gendy, N.; Berkland, C.J.; Forrest, M.L. Nanomaterials physiochemical properties on in vivo toxicity. *Adv. Drug. Deliv. Rev.* **2009**, *61*, 457–466, doi:10.1016/j.addr.2009.03.010.
48. Iravani, S.; Korbekandi, H.; Mirmohammadi, S.V.; Zolfaghari, B. Synthesis of silver nanoparticles: Chemical, physical and biological methods. *Res. Pharm. Sci.* **2014**, *9*, 385–406.
49. Turkevich, J.; Stevenson, P.C.; Hillier, J. Nucleation and growth process in the synthesis of colloidal gold. *Discuss Faraday Soc.* **1951**, *11*, 55–75.
50. Perala, S.R.; Kumar, S. On the mechanism of metal nanoparticle synthesis in the Brust-Schiffrin method. *Langmuir* **2013**, *29*, 9863–9873, doi:10.1021/la401604q.
51. Uehara, A.; Booth, S.G.; Chang, S.Y.; Schroeder, S.L.; Imai, T.; Hashimoto, T.; Mosselmans, J.F.; Dryfe, R.A. Electrochemical Insight into the Brust-Schiffrin Synthesis of Au Nanoparticles. *J. Am. Chem. Soc.* **2015**, *137*, 15135–15144, doi:10.1021/jacs.5b07825.
52. Zhao, P.; Li, N.; Astruc, D. State of the art in gold nanoparticle synthesis. *Coord. Chem. Rev.* **2013**, *257*, 638–665, doi:10.1016/j.ccr.2012.09.002.
53. 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.
54. Elahi, N.; Kamali, M.; Baghersad, M.H. Recent biomedical applications of gold nanoparticles: A review. *Talanta* **2018**, *184*, 537–556, doi:10.1016/j.talanta.2018.02.088.
55. Yeh, Y.C.; Creran, B.; Rotello, V.M. Gold nanoparticles: Preparation, properties, and applications in bionanotechnology. *Nanoscale* **2012**, *4*, 1871–1880, doi:10.1039/c1nr11188d.
56. Hu, X.; Zhang, Y.; Ding, T.; Liu, J.; Zhao, H. Multifunctional gold nanoparticles: A novel nanomaterial for various medical applications and biological activities. *Front. Bioeng. Biotechnol*. **2020**, *8*, 990, doi:10.3389/fbioe.2020.00990.
57. Musielak, M.; Potoczny, J.; Boś-Liedke, A.; Kozak, M. The Combination of Liposomes and Metallic Nanoparticles as Multifunctional Nanostructures in the Therapy and Medical Imaging-A Review. *Int. J. Mol. Sci.* **2021**, *22*, 6229, doi:10.3390/ijms22126229.
58. Chithrani, D.B.; Dunne, M.; Stewart, J.; Allen, C.; Jaffray, D.A. Cellular uptake and transport of gold nanoparticles incorporated in a liposomal carrier. *Nanomedicine* **2010**, *6*, 161–169, doi:10.1016/j.nano.2009.04.009.
59. Cheng, Z.; Li, M.; Dey, R.; Chen, Y. Nanomaterials for cancer therapy: Current progress and perspectives. *J. Hematol. Oncol*. **2021**, *14*, 85, doi:10.1186/s13045-021-01096-0.