A SYSTEMATIC REVIEW OF TARGETED TITANIUM DIOXIDE NANOPARTICLES FOR CANCER THERAPY

BY

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Science in Medical Research . It is not to be sent to any other universities. With that, this research might be used for consultation and can be photocopied as reference.

Nur Hazirah binti Mohd Azlan

P-IPM0018/17

for Mak,
Ayah,
Kak Long,
Adik,
Raihan
Abu Dujanah

who help hold up my universe

&
are the world to me

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LIST OF UNITS

Gy	Gray
nm	Nanometer

LIST OF ABBREVATION

APTS	Amino-propyl trimethoxy silane
A549	Human alveolar basal epithelial adenocarcinoma
BEAS-2b	Human bronchoepithelial cell
CaCo-2	Human epithelial colorectal adenocarcinoma
CHO	Chinese hamster ovary
CMD	Carboxy-methyl dextran
DOX	Doxorubicin
dsDNA	Double strand dexoy-ribonuclei acid
DU145	Human prostatic carcinoma cell line
FA	Folic acid
HaCaT	Human keratinocyte cell line
HeLa	Human cervica epithelial cell line
HSC-1	Human oral squamous cell
HT29	Human colorectal carcinoma
MIAPaCa-2	Human pancreatic carcinoma cell line
MKN-45	Human gastric carinoma cell line
MWCNTs	Multi-walled carbon nanotubes
Nano-TiO_2	Titanium dioxide nanoparticles
NHF	Normal human fibroblast
NO	Nitric oxide
NIH3T3	Mouse embryonic fibroblast
OSCC	Human orla squamous cell carcinoma

PAA	Poly acrylic acid
PEG	Polyethylene glycol
PEGDA	Polyethylene glycol double acrylate
RES	Reticular endothelial system
ROS	Reactive oxygen species
SCC7	Mouse squamosu cell carcinoma
SHSY5Y	Human neuroblastoma cell line
SNB-18	Human glioblastoma cell line
T98G	Human glioma
UCNPs	Up-conversion nanoparticles
UV	Ultraviolet
ZnPc	Zinc pthalocyannine

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ABSTRAK

Partikel nano titanium dioksida (nano-TiO_2) mempunyai potensi untuk digunakan sebagai bahan terapeutik yang menyasarkan kanser. Kefahaman lanjut berkenaan garis panduan ketoksikan dan sifat-sifat fisio-kimia ideal amat penting untuk aplikasi klinikal yang cekap. Kajian ini menyediakan ulasan terkini ke atas nano-TiO_2 bahan terapeutik nano yang tertumpu kepada pengubahsuaian sifat-sifat fisio-kimia, laluan berpotensi pemberian terapi, biodistribusi, pembersihan dan profil ketoksikan. Ulasan ini dijalankan berdasarkan protokol PRISMA-P. Ulasan dimulakan dengan pencarian kepustakaan daripada pangkalan data terpilih; PubMed, Springer Link, Science Direct dan carian umum menggunakan enjin carian Google Scholar berdasarkan kata kunci yang ditetapkan (titanium dioxide nanoparticles, targeted drug delivery, targeted cancer therapy, surface modification, delivery route, route of administration). Pencarian menghasilkan jumpaan 873 manuskrip. Kesemua kajian pra-klinikal dan klinikal, artikel asli dan dalam cetakan yang diterbitkan sepanjang enam tahun kebelakangan (2013 sehingga 2018). Kajian melibatkan penggunaan partikel nano organik dan kajian yang diterbitkan di dalam jurnal pemangsa atau jurnal tersenarai hitam tidak disertakan untuk kajian ini. Kajian yang terpilih dinilai secara menyeluruh dan mendapati 61 artikel yang menepati soalan kajian dan memenuhi kriteria inklusi dan pengecualian. Hasil kajian mencadangkan nano-TiO_2 boleh dianggap calon ideal sebagai bahan terapeutik nano untuk kanser. Pengubahsuaian permukaan nano-TiO_2 dengan ligan yang berlainan mempertingkatkan keberkesanan rawatan dan meminimumkan kesan buruk yang mungkin diakibatkan oleh rawatan kanser

semasa. Penyampaian nano-TiO_2 di dalam badan manusia sebagai terapi kanser disasarkan melalui laluan berlainan menunjukkan potensi dan memerlukan pemeriksaan lanjut dan kajian berkenaan isu ini. Ulasan ini meringkaskan biodistribusi, pembersihan dan risiko ketoksikan nano-TiO_2 hendaklah ditangani dengan memodulasi sifat-sifat fisio-kimianya. Kajian akan datang harus diarahkan ke arah penemuan potensi sebenar nano-TiO_2 untuk bahan terapeutik kanser yang akan menggalakkan aplikasi klinikal di masa akan datang.

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ABSTRACT

Titanium dioxide nanoparticles (nano-TiO_2) provides promising application as targeted cancer therapeutics. Further understanding on the toxicity guidelines and ideal physio-chemical properties are crucial for efficient clinical application. This study provides an updated review on nano-TiO_2 as nanotherapeutics focusing on the modification of physio-chemical properties, potential routes of administration, biodistribution, clearance and toxicity profile. The review conducted is based on PRISMA-P protocol. It began with literature searching from selected databases; PubMed, Springer Link, Science Direct and general search engine Google Scholar based on the pre-determined keywords (titanium dioxide nanoparticles, targeted drug delivery, targeted cancer therapy, surface modification, delivery route, route of administration). The search generated 873 manuscripts. We included all pre-clinical and clinical studies, original and in-press articles published within the past 6 years (2013 till 2018). Studies involving usage of organic nanoparticles, and those in predatory or blacklisted journals were excluded. Selected studies underwent thorough appraisal yielded 61 articles that answered the research questions and met the inclusion and exclusion criteria. Results suggested that nano-TiO_2 can be considered an ideal candidate for cancer nanotherapuetics. Surface modification of nano-TiO_2 with different ligands enhanced the treatment efficacy and minimize the adverse effects that current cancer treatment might cause. Delivery of nano-TiO_2 in human as targeted cancer therapy through different routes seems promising and require further exploration and research on this issue. Review summarized that,

biodistribution, cleranace and toxicity risk of nano-TiO_2 can be tackeld by modulating their physio-chemical properties. Future studies should be directed towards discovering actual potential of nano-TiO_2 for cancer therapeutics which may indeed encourage its clinical application in near future.

CHAPTER 1
INTRODUCTION

1.1 CURRENT CANCER TREATMENT

Contemporary cancer treatment including surgery, chemotherapy and radiotherapy have been the main modality to treat cancer to date worldwide (Baskar et al., 2012, Bahrami et al., 2017). However, few studies demonstrated that there are limitations in their applicability and efficiency. Surgical procedure can only be done in solid tumours. Eventhough removal of a large bulk of tumour can relieve mass-effect which alliviate symptoms instantly, it is unable to completely kills the microscopic remnants around tumour margin consequently lead to recurrence (Kubota, 2011, Winship Cancer Institute, Taccone et al., 2009).

Chemotherapy has become a fundamental component of cancer treatment for most cancers. Despite years of effort on oncology research and discovery, conventional chemotherapeutic regimes still exhibit poor specificity by working non-selectively collaterally destroying the healthy normal cells. Besides, it has poor accessibility to tumour site making indispensable usage of higher dose drugs, narrow therapeutic window, and high intolerable toxicity (Vasir and Labhasetwar, 2005, Bardin et al., 2014).

In addition to the mentioned limitations, public health currently demands for high efficiency therapies to combat this world-wide health burden. Researchers in pharmaceutical sector have paved the ways towards achieving the goal by reformulating current effective established drugs in new delivery systems which can aggrandise their therapeutic activity.

1.2 NANOTECHNOLOGY FOR CANCER THERAPY

Nanotechnology has emerged in biotechnology and medical fields offering enormous potential in research and innovation. Recently, new advances have been developed and utilized in detection, diagnosis, imaging, monitoring and management of diseases (Mukherjee et al., 2015). Past few years have demonstrated a strong focus on utilization of nanotechnology for cancer therapeutics. Cancer nanotechnology has brought about a significant breakthrough in cancer management (Bertrand et al., 2014, Ferrari, 2005). It goes beyond just target-specific drug therapy by providing early diagnosis (*in vitro* and *in vivo*) of the cancer (Varvara and Stergios, 2015) accurate disease prognosis (Misra et al., 2010) added to an unprecedented growth in the number of highly effective therapeutic and diagnostic agents.

The use of nanobiomaterials also promises the development of tailor-made drug-delivery devices which be apt to carry large doses of chemotherapeutic drugs or therapeutic genes precisely into cancerous cells sparing normal healthy cells unaffected (Bae et al., 2011, Zhang et al., 2013a). These nanomaterials would greatly minimise or vanish the adverse side effects that often co-occur with conventional cancer therapies.

1.2.1 Development of nanoparticles

Nanoparticles are matters engineered in size scale from 1 to 100 nm in at least one dimension. They can be made using wide variety of materials such as polymers, lipids, inorganic and biological materials (Alexis et al., 2008b). During the past few years, a number of studies proposing on how nanoparticles were smartly designed for cancer diagnostic and treatment. Due to the minute size of nanoparticles, they have emerged as

an ideal candidates for nanotherapuetics platform providing selective or targeted intracellular drug transporter towards specific tissue or organ (Alf, 2009, Bahrami et al., 2017, Haley and Frenkel, 2008).

The first nanoparticles designed for drug carrier were the liposomes. Liposomes were nanoscale sized and compose of a lipid bilayer encompassing a water core confining the desired drug (Harris et al., 2001). It was developed to improve pharmacokinetics and biodistribution of the chemotherapeutics drug doxorubicin which induced cardiotoxicity limiting the administered dose. Liposomal-encapsulated doxorubicin was in clinical trial in the mid-1980s and available in market in 1995 (Allen and Cullis, 2013, Malam et al., 2009). Since then, scientists continue to develop numerous new nanoparticles in nanotherapeutics technology system in cancer treatment.

1.2.2 Nanoparticles as drug-delivery vehicles

Due to the compelling features of nanoparticles, currently many research are focusing in investigating and development of these nanoparticles as effective transporters in drug-delivery system. These include their ability to improve solubility of hydrophobic drugs, prolonging bioavailability, preventing uneviable adverse side effects, allowing for target specific and able to cross biological barriers which is limited in conventional drugs (Alf, 2009, Mudshinge et al., 2011, Gelperina et al., 2005, Singh and Lillard, 2009, Dikmen et al., 2011). Besides being a drug-vehicle, nanoparticles can simultaneously be engineered as an imaging probe (Pu et al., 2014) and designed to specifically target molecules of diseased tissues (Roney et al., 2005). The developmet of sustained-release drug delivery system in the future, may provide better efficacy and minimizing toxicity

risk (Rizvi and Saleh, 2018) of cancer drugs for treatment of both primary and secondary metastatic tumours.

1.3 METAL-BASED NANOPARTICLES

Metal-based nanoparticles are now being densely utilized in engineering and biomedical sciences. They have been described to play a promising and robust role in cancer therapy providing photocatalytic advantages and improved cancer cells targeting (Zhang et al., 2007, Schröfel et al., 2014, Grass et al., 2007, Lee et al., 2006, Wang et al., 2012). Currently, these nanomaterials can be customized with sundry of chemical functional groups which enable them to be linked with any ligands thus providing multi-potential applications including magnetic separation, targeted drug transporter, gene-delivery system and diagnostic imaging which revamp cancer management (Huang et al., 2006, Peer et al., 2007, Rajh et al., 2002, Zheng et al., 2006). Among the well-known metal-based nanoparticles include gold, silver, titanium dioxide, iron, graphene and copper nanoparticles.

1.4 TITANIUM DIOXIDE NANOPARTICLES

Titanium dioxide nanoparticles (nano-TiO_2) are inert material which have been widely used in many health-related sectors including dental, cosmetics, personal care, anti-microbial agents and pharmaceutical (Chen et al., 2014, Weir et al., 2012, Mahmoud et al., 2017). In view of few promising physio-chemical properties they exhibited, including low toxicity level, high chemical and physical stability, excellent photo catalyst liberating free-oxygen radicals and potent anti-microbial effects, nano-TiO_2 can be considered an ideal material for targeted cancer therapy compared to other nanoparticles

(Shah et al., 2017, Rezaei and Mosaddeghi, 2009, Ranjan et al., 2016, Chen and Mao, 2007).

1.4.1 Photocatalytic activity of TiO_2 nanoparticles

Nano-TiO_2 are well known for their unique feature; photocatalytic property. They have been employed in various photocatalytic applications including ultraviolet (UV) protection in sunscreen product and 'built-in' air purifier cement (Atta ur et al., 2018, Cerro-Prada Elena et al., 2016). Recently, due this to remarkable property, they are used in several treatment modalities of different cancers and other diseases. Induction of nano-TiO_2 photocatalytic activity are made possible by different irradiation methods with the aim to raise the cellular oxidative stress level. This is due to liberation of free radicals including generation of intracellular reactive oxygen species (ROS), increased hydrogen peroxide level, decrease glutathione peroxidase and reduced glutathione level which lead to decrease the mitochondrial membrane potential. (Lan et al., 2013, You et al., 2016b). The ROS consequently kill the cancerous cells via the mechanism of apoptosis or necrosis (Circu and Aw, 2010, Booth et al., 2011). Due to targeted killing mechanism, this concept lowering systemic toxicity thus taking the upper hand as compared to conventional cancer treatment.

1.4.2 Potentials of TiO_2 nanoparticles in cancer therapy

Currently, pristine or modified nano-TiO_2 conjugated with different ligands or drugs are being widely investigated in combatting cancer in pre-clinical studies. Study by Rezaei and Mosaddeghi (2009) demonstrated that combining targeted nano-TiO_2 with gamma-ray radiotherapy in treating breast cancer MCF-7 cell line has enhanced the killing effects on the cancer cells. Rezaei and Mosaddeghi claimed that cells received

both gamma-ray and nano-TiO_2 were found less viable than those received only gamma-ray. Besides, You et al. (2016b) mentioned that liver cell tumour were found supressed by ultrasound-activated nano-TiO_2 as they liberated free radical; ROS.

As a drug carrier, nano-TiO_2 can be used to augment the effects of immunotherapy and chemotherapy drugs. The therapeutic drugs are incorporated into the nano-TiO_2 either by entrapment, surface attachment, or encapsulation. Nano-TiO_2 were conjugated with monoclonal antibody against HER-2 to selectively load camtotechin to the targeted breast cancer cells (Yin et al., 2013).

In clinical practice, patient receiving chemotherapy will be prescribed few drugs regime for supplementation such as vitamin C, vitamin B-complexes, vitamin D, iron and folic acid. Based on Haghshenas-Lari et al. (2017), TiO_2 nanoparticles is used as a coating materials for vitamin C tablet to enhance its gut absorption and halting rapid gastric degradation especially in cancer patients.

In the past few years, application of nanotechnology-based TiO_2 drug carriers seem to yield more medical benefits compared to conventional cancer therapy and promise to have an ardent impact on healthcare. This review discusses the use of nano-TiO_2 as one of nanotherapeutic agent for treatment of cancer and future directions and perspectives of this cutting-edge technology.

1.5 PROBLEM STATEMENT AND STUDY RATIONALE

Eventhough the usage of nano-TiO_2 as targeted cancer therapy seems promising, there is a need for a more comprehend target analysis on its actual potential as targeted cancer therapeutics. Thus, the purpose of this study is to provide a better understanding of nano-TiO_2 surface modification technology, potential route of administration,

biodistribution, clearance profile and toxicity profile which suggest its potential application for targeted cancer therapy and eventually for the development of future cancer therapy.

1.6 OBJECTIVES OF THE STUDY

The objectives of this study are:

i. To scrutinise the method of nano-TiO_2 surface modification technology
ii. To study the potential route in nano-TiO_2 target administration
iii. To comprehend nano-TiO_2 biodistribution and clearance profiles
iv. To analyse the toxicity profile of nano-TiO_2 from pre-clinical studies

1.7 RESEARCH HYPOTHESIS

Alternative Hypothesis (H_A) – Based on the nano-TiO_2 surface modification technology, route of administration, biodistribution, clearance profile and toxicity profile there is potential application of nano-TiO_2 as targeted cancer therapy

Null Hypothesis (H_O) - Based on the nano-TiO_2 surface modification technology, route of administration, biodistribution and clearance profile and toxicity profile there is no potential application of nano-TiO_2 as targeted cancer therapy

CHAPTER 2
METHODOLOGY

2.1 SEARCH STRATEGY

The review conducted is based on PRISMA-P protocol. It began with comprehensive literature searching from selected electronic databases; Science Direct, PubMed, and Springer Link. A general search engine, Google Scholar was additionally used to obtain the research articles. No language restrictions were imposed. The following keyword; titanium dioxide nanoparticles, targeted drug delivery, targeted cancer therapy, surface modification, clinical route of administration, pharmacodynamic, pharmacokinetics, cytotoxicity and genotoxicity were used during the literature search. The keywords were combined interchangeably either with addition of Boolena operators 'and' or 'or' with the aim to broaden the search results. Titles and abstracts of the potential articles were screnned independently by the author to retrieve relevant articles from the mentioned full-text electronic journal databases.

In addition, the bibliographies of the selected articles were systematically screened by non-automated manual search to obtain other potentially relevant articles. Every steps taken in this process were meticulously documented to ensure transparency, replicability and feasibility to reanalyse. To avoid duplication of works, Endnote software version X7 was used as a reference manager to merge results of all extracted studies. Full text of each identified study was retrieved by author.

2.2 SELECTION CRITERIA

Both research and review articles including *in vitro, in vivo* and *ex vivo* studies were selected and included in this study based on the pre-determined inclusion and exclusion criteria regardless the results to avoid selection bias. There were no pre-clinical and clinical trial conducted fulfilling this research objectives to date.

2.2.1 Inclusion criteria

Inclusion criteria for retrieved studies were:

1) Titanium dioxide nanoparticles as targeted cancer therapy
2) Biodistribution and clearance of titanium dioxide nanoparticles
3) Pharmacodynamic and pharmacokinetics of titanium dioxide nanoparticles
4) Preclinical or clinical toxicity studies
5) Recent study 2013-2018 (including in-press articles)

2.2.2 Exclusion criteria

All searched literatures retrieved having any of these criteria were excluded:

1) Organic nanoparticles
2) Journal in predatory journals or blacklisted journals (refer to Beall's list of predatory journal)

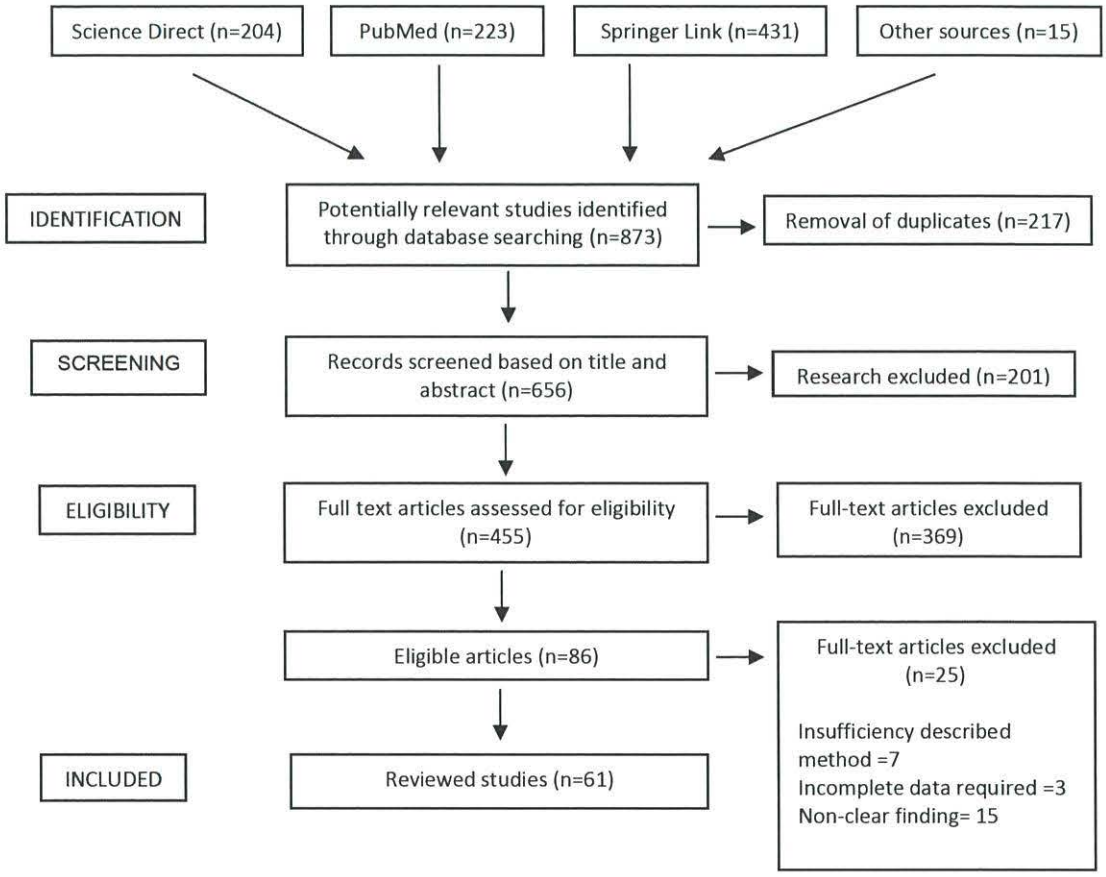

Figure 2.1 **Searching process flow-chart.** The flow chart above is adopted from the PRISMA-P protocol which describes the results from the searching process in all mentioned electronic databases on nano-TiO_2 as targeted cancer therapy.

2.2.3 Quality assesment of reviewed studies

Titles and abstracts for all 656 articles were screened with only 455 articles related to the research question. The quality assessment of studies included in this review was carried out based on the following criteria adapted from 27-point checklist of the PRISMA-P statement (Moher et al., 2015):

i. Adequate description of the aim of the research
ii. Adequate, clear and appropriate description of research method
iii. Duration of exposure clearly stated
iv. Adequate information on ethics approval by appropriate body
v. Clear explanation of the data collection methods and steps taken to increase rigor in the data collected
vi. Use of evidence-based assessment of validity, clarity of results and conclusion derived based evidence
vii. Results and evidence are clearly stated, credible and give a meaningful clarification of the studied subject
viii. Adequate and clear description of study limitations and weaknesses

The above study criteria were used aiming at providing quality evidence and eliminating biases. Below are the guidelines and checklists used in proposing the study criteria:

i. Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 checklist
ii. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) scoring system

2.3 DATA EXTRACTION

Data on the surface modification, physio-chemical properties (primary particle size, modified size, zeta potential, surface area), potential administration route, biodistribution, clearance profile and toxicity risk of nano-TiO_2 were independently extracted and analysed by the author. The studies were thoroughly reviewed qualitatively and quantitatively according to the objectives of the review.

2.4 DATA ANALYSIS

The data collected from the studies were sorted, concluded respectively and compared to ascertain the strength of each study. Nano-TiO_2 surface modification, potential routes of nano-TiO_2 administration and their bioavailability, clearance profile and toxicity profile were analysed descriptively. Primary particle size used in studies discussing toxicology profile of nano-TiO_2 were pooled, sorted, trial using statistical Minitab version 6.0 software to descriptively analysed the common particle size used. The quality of evidence for outcomes were determined using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) approach.

CHAPTER 3
RESULTS

3.1 SEARCH RESULTS

This systematic review preliminary search results using Science Direct, PubMed and Springer Link databases started on February 2018 yielded 858 articles. Google Scholar, a general search engine was simultaneously used for complementing the searching purposes based on pre-determined keywords generated 15 articles. All citations were then imported and merged in reference manager software, EndNote X7. 217 duplicates were removed left 656 articles for thorough screening. A total of 201 articles were excluded after title and abstract screening due to not meeting the research questions made a remaining of 455 articles listed as potentially eligible articles. Data extraction were carried out based on each research objectives and the specified keywords.

3.2 SURFACE MODIFICATION OF TiO_2 NANOPARTICLES FOR TARGETED CANCER THERAPY

A total of 224 papers were retrieved based on the pre-defined keywords aiming on answering this research question. Removal of duplicates yielded 125 papers which the screened through the title and abstract yielded 20 articles which were unrelated to the research questions. A remaining of 105 articles were found potentially eligible. Further appraisal of the full text articles narrowed down to only 28 eligible studies. There are 6 studies described the conjugation of nano-TiO_2 with chemotherapeutic drugs (Table 3.1) as it enhances their effectiveness and delivery. Besides, 6 studies report the involvement of nano-TiO_2 with radiotherapy as it able to produce radical oxygen species (ROS) which contribute to the killing mechanism of the cancer cells (Table 3.2). Finally, 15 selected studies elucidate the utilization of photodynamic advantages of nano-TiO_2 which can be

used alone to target cancer cells upon activation by different irradiation methods; UV light, visible light, near infra-red light and ultrasound (Table 3.3).

Based on the reviewed studies as in Table 3.1, nano-TiO_2 were used to enhance the chemotherapeutics drugs delivery at the tumour site preventing them to spread systemically destroying the healthy normal cells and empowering therapeutic activity of the drugs by releasing free radicals with liberation of reactive oxygen species (ROS). Nano-TiO_2 were surface modified with different ligands to achieve various goals. They were conjugated with polyethylene glycol (PEG) or folic acid (FA) as they enable nano-TiO_2 to selectively target the tumour cells by binding to specific receptors. Folate recpetor was found abundant on epithelial cancer cells made targeting with folic acid possible. Study conducted by Nakayama et al. (2016) showed that nano-TiO_2 be conjugated with poly-acrylic acid (PAA) to prevent aggregation of bare TiO_2 which hinder the physiological condition. All studies included showed that conjugation of nano-TiO_2 with chemotherapeutics drugs resulted in improvement of killing effect of the drugs which manifested by further decrease in cell viability.

Table 3.2 shows nano-TiO_2 being utilized to enhance radiotherapeutics for cancer treatment. They acted either as delivery vehicle for radio-isotopes or radio-sensitiser which due to the photodynamic advantages of nano-TiO_2. Anatase nano-TiO_2 demonstarted to take the upper hand as a photosensitiser compared to other form of nano-TiO_2; rutile, brookite or mixture of them. Radiosensitisation ability of nano-TiO_2 is largely determined by the zeta-potential which lead to the liberation of free radicals.

As a potential photo-sensitizer, nano-TiO_2 exhibits minimal dark cytotoxicity and excellent UV light triggered cytotoxicity. However, due to UV light potential adverse

teratogenicity and limited penetration power, more researches were diverted to study new ways to induce the photoactivity of nanoparticles by substitution with different irradiation source (photo-catalyst) including visible light, near infra-red (NIR) light and ultrasound. NIR radiation has remarkable deeper tissue penetration and is expected to cause minimal photo-damage to the biological specimen involved as compared to visible and UV light. It was then being converted by a transducer into visible or UV light to activate the photo-activity. While, irradiation of ultrasound to trigger the photo-activity provides higher penetration power which may catered the deep-seated tumour and enhanced cancer cell selectivity.

Review revealed that studies also used TiO_2 coated upconverted nanoparticles nanocomposite (UCNPs@TiO_2), due to its ability to absorb NIR light and convert it into high-energy photons in a very broad range from the UV to the NIR region efficiently that matches well with the absorption of TiO_2 shells. Cancer cells then will endocytose the UCNPs@TiO_2 liberating the ROS. Review also showed that conjunction of nano-TiO_2 augmented the radioactivity of ionizing radiation by liberation of free radicals including release of intracellular radical oxygen species (ROS) and aided in radionuclide delivery and retainment at target site in radio-therapeutics as shown by 6 studies (Cedrowska et al., 2018, Rezaei-Tavirani Mostafa et al., 2013, Mirjolet et al., 2013, Ouyang et al., 2016, Youkhana et al., 2017, Nakayama et al., 2016). An ideal nano-TiO_2 photocatalyst should yield highly stable free radical including ROS and have a vast degree of selectivity towards cancerous cell (Li et al., 2015, Ansari et al., 2016, Reddy et al., 2016, Thong et al., 2017)

Table 3.1 TiO_2 nanoparticles as chemotherapeutic drug-carrier

METHOD	AIM	DRUG CARRIED	TARGET	FUNCTION TARGETED	REFERENCES
ZnS QD	– Red shift in the UV emission band	5-Fluorouracil	CHO cells	– Not significant decrease cellular viability (dose dependent manner)	(Faria and de Queiroz, 2015)
Fe_3O_4	– Exhibit pH-dependent release and high loading capacity – Combined chemo-sonodynamic therapy	DOX	MCF-7	– Significant decrease cell viability	(Shen et al., 2015)
FA	– Exhibited a superior anticancer effect in osteosarcoma	Folic acid	Human Osteosarcoma MG63	– Significant decrease cell viability	(Ai et al., 2017)
PEG FA	– PEG- ability to evade the RES and extend the circulation time – FA- bind to cell surface and intracellular compartments effectively	Paclitaxel	HepG2	– Significant decrease cellular viability	(Venkatasubbu et al., 2013)
PEGDA	– Ability to evade the RES and extend the circulation time	DOX	MCF-7	– Photocatalytic activity	(Zhang et al., 2015)
dsDNA	– Safety release	DOX	MCF-7/ ADR cells	– Decrease mitochondrial membrane potential	(Shi et al., 2018)
Fe_3O_4	– Exhibit pH-dependent release and high loading capacity – Combined chemo-sonodynamic therapy	DOX	Mice with mouse ascites cell S180	– Marked coagulative necrosis	(Shen et al., 2015
PEGDA MWCNTs	– PEGDA- less toxicity and higher DOX accumulation in tumour – MWCNTs substrate - improve NIR absorption and enhance suppression of electron/hole recombination	DOX	Xenograft tumour bearing mouse model	– Significant decrease tumour size	(Zhang et al., 2015)
dsDNA	– Safety release	DOX	MCF-7/ADR tumour-bearing mice	– Significant decrease tumour size	(Shi et al., 2018)
PEG FA	– PEG- ability to evade the RES and extend the circulation time – FA- bind to cell surface and intracellular compartments effectively	Paclitaxel	Wintsar Albino Rats	– High anticancer activity with biochemical and haematological parameters	(Venkatasubbu et al., 2013)

ZnS QD = Zinc sulphide quantum dots, FA= folic acid, CHO= Chinese hamster ovary, DOX=doxorubicin, PEG=poly ethylene glycol, PEGDA=poly ethylene glycol double acrylate, MWCNTs= Multi-walled carbon nanotubes, S180=sarcoma

Table 3.2 TiO_2 nanoparticles as radiosensitising agent

	MODIFIACTION	IONIZING RADIATION DOSE	TARGET	FUNCTION TARGETED	REFERENCES
RADIO-DELIVERY	– Silane-PEG-NHS Targets NK1 receptors on the glioma cells	– ^{25} Ac Radioisotopes (α-emitter)	T98G	– Radio-sensitised – Deliver and retain radionuclide at target site – Further decrease cell viability	(Cedrowska et al., 2018)
RADIO-SENSITISER	– Free	2Gy	MCF-7 MKN-45	– Radio-sensitised – Significant reduced cell viability	(Rezaei-Tavirani Mostafa et al., 2013)
	– Free	0.1-10 Gy	SNB-19 U87MG	– Radio-sensitised – Decrease in DNA repair efficacy	(Mirjolet et al., 2013)
	– Free	2Gy	A549	– Radio-sensitised – Significantly increased cell killing during radiotherapy	(Ouyang et al., 2016)
	– PEG- allow dispersion in halocarbons – APTS - allow aqueous dispersion for cell studied	0-8 Gy	HaCaT DU145	– Radio-sensitised – Decrease cell viability at high concentration	(Youkhana et al., 2016)
	– PAA- ability to produce hydroxyl radicals in response to X-ray (dose and time-dependent)	10/30 Gy	MIAPaCa-2	– Radio-sensitised – Enhanced DNA damage → higher cytotoxicity	(Nakayama et al., 2016)
	– PAA- ability to produce hydroxyl radicals in response to X-ray (dose and time-dependent)	10/30 Gy	Male C nude mice	– Stronger tumour growth Inhibition	(Nakayama et al., 2016)

PEG= poly ethylene glycol, APTS= Aminopropyl trimethoxysilane, PAA=polyacrylic acid, T98G cells= human glioblastoma, MKN-45=human gastric cell line, A549= human alveolar basal epithelial cells adenocarcinoma, HaCaT= human keratinocyte cell line, MIAPaCa-2= Human pancreatic cell line, DU145=human prostatic carcinoma cell line, SNB-18= human glioblastoma, T98G= Glioma cells

Table 3.3 TiO_2 nanoparticles photocatalytic advantages

	MODIFICATION	SIZE (nm)	ZETA POTENTIAL (mV)	TARGET	KILLING MECHANISM	REFERENCES
ULTRA-VIOLET	Bare – Anatase – Rutile	6 10-70	*NA*	– LL2 mouse cell line	– Significant cytotoxicity (2 folds higher) – Significant apoptosis (dose-dependent) – High PD activity	Fujiwara et al. (2015)
	SiO_2- – Reduced the photocatalytic reactivity – Improved cytocompatibility	25	*NA*	– L929 mouse	– High PD reactivity – Further decrease cell viability (time-dependent) – Improved cell compatibility and killing effectiveness	Feng et al. (2013)
	Bare – Anatase – Anatase-Rutile	111.3 138.5	-16.7 ± 9	– MCF-7 – MDA-MB-468	– Increase apoptosis – Increase DNA fragmentation – Pure anatase exhibit superior effect	Lagopati et al. (2014)
	Reduced graphene oxide – Superior adsorption and killing efficiency	15.49	*NA*	– HepG2	– Increased apoptosis – Marked decrease mitochondrial potential – Increased oxidative stress	Shang et al. (2017)
	Bare – Anatase – Rutile	6 10-70	*NA*	– BALB/c mice – Fisher 344 rats (CT26 inoculated subcutaneous)	– Tumour growth inhibition (dose-dependent)	Fujiwara et al. (2015)
VISIBLE LIGHT	ZnPc- high photodynamic potential FA - targeting moieties	4-8	-16.60	– HeLa – NHF cell	– High PD reactivity – Selectively target – DOX-loaded significantly more cytotoxic	Flak et al. (2017)
	Reduced graphene oxide – Superior adsorption and killing efficiency	14.88	NA	– HepG2	– Increased apoptosis – Marked decrease mitochondrial potential – Increased oxidative stress	Shang et al. (2017)

	MODIFICATION	SIZE (nm)	ZETA POTENTIAL (mV)	TARGET	KILLING MECHANISM	REFERENCES
NEAR INFRA-RED LIGHT	– UCNPs- generate a higher energy light from a lower energy light	25	-20.2 ± 0.3	MCF-7	– Significant cell death	Yu et al. (2015)
	– Lysosome- markedly contribute to the programmed cell death sensitivity cancer cells – NO- tumoricidal – FA- targeting moieties	4	*NA*	MCF-7	– Enhanced cancer cell selectivity	Xiang et al. (2016)
	– UCNPs - generate a higher energy light from a lower energy light – Load DOX	35	–19.81 ± 2.01	HeLa	– Increase apoptosis – Fast uptake and high cytotoxicity	Tong et al. (2017)
	– UCNPs - generate a higher energy light from a lower energy light	25	*NA*	HeLa	– Increase apoptosis	Hou et al. (2015)
	– Black TiO_2	25	*NA*	Human bladder cell T24	– High PD reactivity – Enhanced biocompatibility	Ni et al. (2017)
	– UCNPs- generate a higher energy light from a lower energy light – Mal-PEG- ability to evade the RES and extend the circulation time	50	-25.5 ± 6.8	-Human OSCC cell -Mouse leukemic monocyte macrophage cell line -NHF cells	– NIR-triggered deep-tissue PDT	Lucky et al. (2015)
	– UCNPs- generate a higher energy light from a lower energy light – Hyaluronic acid- controlled release and targeted ability toward cluster determinant 44 overexpressed cancer cells – Load DOX	30	-24.0	MDA-MB-231 cells	– Increase apoptosis	Yin et al. (2014)

	– UCNPs- generate a higher energy light from a lower energy light	25	*NA*	HeLa tumour bearing mouse model	– Effective tumour growth suppression	Hou et al. (2015)
	– UCNPs- generate a higher energy light from a lower energy light	25	-20.2±0.3	Mouse model	– Tumour elimination in 6 days – HPE: Wide range of tissue damage – No abnormal changes; no toxicity	Yu et al. (2015)

	MODIFICATION	SIZE (nm)	ZETA POTENTIAL (mV)	TARGET	KILLING MECHANISM	REFERENCES
SONODYNAMIC (ULTRASOUND)	Avidin protein – Preferential uptake by cancerous cells	100	*NA*	MCF-7	– No significant decrease cell injury	Ninomiya et al. (2014)
	Hydrophilized TiO_2 (CMD) – Prolonged systemic circulating	25	−15.8	SCC7 NIH3T3	– Increase ROS generation – Significant decrease cell viability	You et al. (2016a)
	Silica – Sonocatalyst	10-30	*NA*	HSC-2	– Significant decrease cell viability	Moosavi Nejad et al. (2016)
	Silica – Sonocatalyst	10-30	*NA*	BALB/c athymic nude mice	– Significant necrosis and tissue damage	Moosavi Nejad et al. (2016)
	Hydrophilized TiO_2 (CMD) – Prolonged systemic circulating	25	−15.8	C3H/HeN mice (SCC7 bearing/	– High tumour targetability – Effective tumour growth suppression – Markedly increase proinflammatory cytokines – Intense vascular damage	You et al. (2016a)

SiO_2= silica oxide, LL2= mouse lung cancer cell line, L929= mouse fibroblast cell, HepG2= human hepatocellular carcinoma, CT26= undifferentiated carcinoma cell line, PD=photodynamic, MCF7=Human breast epithelial cell line, MDA-MB-468= Human breast adenocarcinoma, HeLa= Human cervical carcinoma cells, DOX=doxorubicin, ZnPc= zinc phthalocyanine, FA= Folic acid, NHF= Normal human fibroblast, UCNP= upconverting nanoparticles, DOX=doxorubicin, NHF= normal human fibroblast, MDA-MB-231= Human breast adenocarcinoma, OSCC= Oral squamous cell carcinoma, FA= Folic acid, PEG= polyethylene glycol, NO= nitric oxide, mal-PEG= Maleimide poly-ethylene glycol, SCC7= Mouse squamous carcinoma cell, NIH3T3= Mouse embryonic fibroblast, HSC-2= Human oral squamous cell, CMD= carboxymethyl dextran, ROS= Reactive oxygen species

3.3 TiO_2 NANOPARTICLES POTENTIAL ROUTE OF ADMINISTRATION

A total of 184 papers were retrieved based on the pre-defined keywords aiming on answering this research question. 18 duplicates were removed left another 166 papers for thorough screening. Title and abstract screening of articles yielded 57 articles which were unrelated to the research questions. From 109 papers, 100 of them were further excluded. After critically appraised the papers, another 3 papers were excluded left 6 (Table 3.4) in this study due to the following reasons:

1. Discussing on environmental exposure instead of as therapeutics agent
2. Other metal nanoparticles such as silica and zinc oxide nanoparticles
3. Studies conducted in years excluded from inclusion criteria

Table 3.4 Potential route of administration of TiO_2 nanoparticles in cancer therapy

ROUTE	DISCUSSION	REFERENCES
Dermal	Large surface area. Ability for UV absorption, antimicrobial properties, ability to penetrate skin easily.	Niska et al. (2017)
	Provides wide range of protection against UVA and UVB (No chemical composition upon UV irradiation) 10-50 nm= penetration, 20nm= no penetration (ability to across the stratum corneum deeper skin layers)	Labouta and Schneider (2013)
	Size manipulation → alter ability to reflect the UV light and visible light	Goyal et al. (2016a)
Intravenous	Absence of toxicological effects after TiO2 intravenous injection in lungs, blood, liver, spleen and kidneys	Elgrabli et al. (2015b)
Subcutaneous	Significant pathological injury and liver enzymes are significantly high (dose dependent)	Shakeel et al. (2016c)
Oral	No significant liver translocation and excreted faeces	MacNicoll et al. (2015)

Most articles retrieved based on the listed keywords discussed the toxicity developed after chronic exposure of nnao-TiO_2 through different routes instead of potential routes for targeted cancer therapy. Despite the adverse effect listed, it should be noted that the effectiveness and toxicity of nanoparticles are largely dependent on their physio-chemical properties including size, shape, zeta potential, polydispersity index and surface functionalization (Cai et al., 2017, Gao et al., 2018, Simon et al., 2017) .

Dermal has been the most common route applied in delivering of nano-TiO_2. As stated in Table 3.4, due to the unique properties of nano-TiO_2 which includes high anti-microbial activity, power of deep skin penetration and ability to reflect UV light made it a good choice in sunscreen products. This information are crucial and constructive in development of nano-TiO_2 as targeted cancer therapy via dermal route in future research.

Based on studies conducted by (Shakeel et al., 2016b, Elgrabli et al., 2015b), delivery of nano-TiO_2 via injection either intravenous or subcutaneous demonstrated that it is able to be excreted and cleared completely from the body thus not lead to toxicity. This is influenced by the physio-chemical properties of this nanoparticles including size, zeta potential, administration dose and period of exposure. An ideal cancer therapeutics must not only be effective but also need to be safe for human use.

The articles listed above are not precisely describing the potential route for nano-TiO_2 in cancer therapy but gave a prospective for future study. To the best our knowledge, there is no study yet discuss the clinical route of administration for this nanoparticle for cancer patient to date.

3.4 BODISTRIBUTION AND CLEARANCE PROFILE OF TiO_2 NANOPARTICLES

A total of 213 papers were retrieved based on the pre-defined keywords aiming on answering this research question. 50 duplicates were removed left another 163 papers for thorough screening. Title and abstract screening of articles yielded 71 articles which were unrelated to the research questions. A remaining of 92 articles were found potentially eligible. This current study collected 16 studies discussing on the biodistribution and clearance of nano-TiO_2 (Table 3.5).

Biodistribution and clearance of nano-TiO_2 are largely dependent on the delivery route and physio-chemical properties of the nanoparticles. Accumulation of nanoparticles in the body can affect the orangs they deposited and can induced toxicity. Review summarized that lungs have been the main organ found to deposit nano-TiO_2 through different routes of administration. 5 studies reported highest deposition of nano-TiO_2 in lung especially on the upper pole of the lung max at 48h in dose and time-dependent manner (Pujalte et al., 2017, Shinohara et al., 2015, Relier et al., 2017, Zhang et al., 2013, Landsiedel et al., 2014). Study has shown that nanosized TiO_2 was less effectively phagocytosed and cleared by alveolar macrophages than fine TiO_2 particles and thus could enter the alveolar interstitium. Upon reaching the alveolar walls, it can potentially enter pulmonary capillaries and further translocate to systemic.

Gastro-intestinal tract is another site that tend to accumulate the nanoparticles. However, >90% were egested in faeces within 12h (Doyle et al., 2015). Study shows exposure to 200 mg/kg for 30 days in rats with TEM images showed that some particles (60–200 nm) were located the stomach and small intestine mucosa but no significant