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FEATURE ARTICLE

Targeting carbon nanotubes against cancer

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The use of carbon nanotubes (CNTs) as polyvalent tools for cancer treatment is progressing at a very fast pace. The most promising approach is the targeted delivery of drugs, designed to selectively direct the therapeutic treatment towards the tumours. CNTs may offer several advantages to overcome one of the main limitations of most existing anticancer therapies, namely the lack of selectivity. Herein, an account of the existing literature on CNT-based nanomedicine for cancer treatment is given. The most significant results obtained so far in the field of drug delivery are presented for many anticancer chemotherapeutics (doxorubicin, methotrexate, taxanes, platinum analogues, camptothecine and gemcitabine), but also for immunotherapeutics and nucleic acids. Moreover, the alternative anticancer therapies based on thermal ablation and radiotherapy are discussed. The attention throughout the review is focused on the different targeting strategies proposed so far, mainly based on antibodies, but also on other specifically recognised molecules or on the application of an external magnetic field.

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[c] *CNRS, Institut de Biologie Moléculaire et Cellulaire, Laboratoire d'Immunologie et Chimie Thérapeutiques, Strasbourg, 67000, France. E-mail: a.bianco@ibmc-cnrs.unistra.fr*

1. Introduction

Despite significant advances, anticancer therapy strategies still suffer from severe drawbacks. One of the main limitations of most treatments is the lack of selectivity for the tumour tissues resulting in severe side effects for patients that limit their compliance.

Another major problem related to anti-neoplastic chemotherapy is multi-drug resistance (MDR).[1] This phenomenon can often be ascribed to the activity of P-glycoprotein (P-gp), an efflux pump, able to recognise the drug and transport it out

Chiara Fabbro

Chiara Fabbro obtained her M.Sc. degree in Medicinal Chemistry in 2007 from the University of Trieste (Italy) and her PhD in 2011, working on the functionalisation of carbon nanotubes for biomedical applications, under the supervision of Prof. Maurizio Prato and Dr Tatiana Da Ros. After a short internship at Novartis, in Vienna (Austria), she was a visiting researcher at the University of Castilla La Mancha in Ciudad Real (Spain), and at CNRS in Strasbourg (France). She is currently a postdoctoral fellow at the University of Namur (Belgium), working with Prof. Davide Bonifazi on the synthesis of modules for H-bonding driven supramolecular self-assemblies.

Hanene Ali-Boucetta

Hanene Ali-Boucetta graduated with a first-class (Honors) MPharm degree from The School of Pharmacy, University of London. In October 2006, Hanene joined the Nanomedicine Lab for a PhD under the supervision of Prof. Kostarelos. Her research focused on studying the important and critical parameters in carbon nanotubes pharmacokinetics and toxicology and establishing effective CNT nanovectors for cancer therapeutics. Upon completing her PhD, Hanene was awarded a fellowship from the University of London and rejoined the Nanomedicine Lab as a C W Maplethorpe Research and Teaching Fellow.

of the cells once it has been internalised, thus preventing it from exerting its cytotoxic action.[2] Importantly P-gp mediated MDR is often characteristic of residual tumour cells, limiting the efficacy of various chemotherapeutic agents.

Nanotechnology can help to overcome such limitations, by targeting cancer cells, either in non-specific, passive ways or *via* specific targeting ligand–receptor interactions. The passive targeting is mediated by the so-called enhanced permeability and retention (EPR) effect (Fig. 1).[3,4] Cancer tissue is usually characterised by rapid and abrupt angiogenesis processes, resulting in leaky and defective blood vessels with large fenestrations, due to extensive endothelial cell disorganisation. Also, the smooth-muscle layer is frequently absent or abnormal in the vascular wall, leading to passive dilatation of vessels. The consequence is the enhanced extravasation of macromolecules in the tumour tissue, in contrast to low molecular weight molecules that undergo rapid renal clearance.

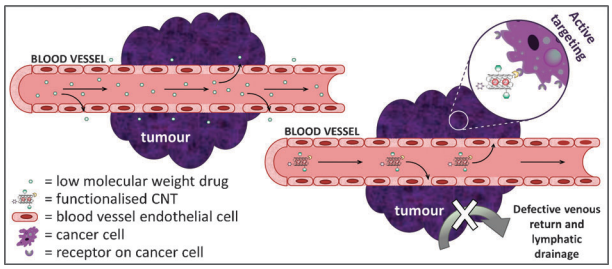
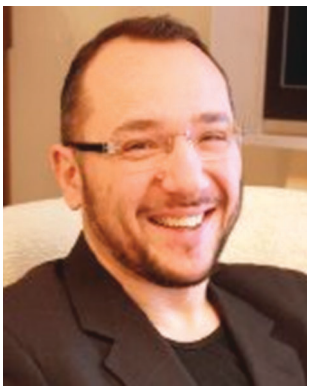


Fig. 1 Schematic representation of the enhanced permeability and retention (EPR) effect.

Moreover, slower venous return and poor lymphatic drainage can lead to retention of accumulated macromolecules in the tumour. For these reasons, drug delivery using nanoscale systems would be more effective compared to the free drug. Moreover, this effect can be further enhanced by attaching

Tatiana Da Ros

Tatiana Da Ros received her PhD in 1999 from the University of Milano (Italy). She worked at UCLA (1999), at Sussex University (2000) and at the Museum Nationale d'Historie Naturelle, Paris, in 2001. She is assistant professor of Medicinal Chemistry at the University of Trieste. Her scientific interests focus on the chemistry and the biological properties of carbon nanostructures. She is the co-author of more than 90 articles and book chapters, co-editor of the book "Medicinal Chemistry and Pharmacological Potential of Fullerenes and Carbon Nanotubes", and co-organizer of the symposium on biological applications of carbon nanostructures of the Electrochemical Society.

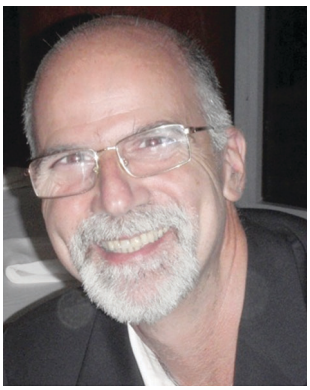


Kostas Kostarelos

Professor Kostarelos is the Chair of Nanomedicine & Head of the Centre for Drug Delivery Research at UCL School of Pharmacy, University College London. In 2010 he was awarded the Japanese Society for the Promotion of Science Professorial Invitation Fellowship. He has been invited Fellow of the Royal Society of Medicine, Fellow of the Institute of Nanotechnology and Fellow of the Royal Society of Arts all in the United Kingdom. Previous appointments include: Assistant Professor Cornell University Weill Medical College, NY, USA; Research Associate, University of California at San Francisco (UCSF) and Memorial Sloan-Kettering Cancer Center in New York, USA.

Alberto Bianco

Alberto Bianco received his PhD in 1995 from the University of Padova (Italy). As a visiting scientist, he worked at the University of Lausanne in 1992, at the University of Tübingen in 1996–1997 (as an Alexander von Humboldt fellow) and at the University of Padova in 1997–1998. He is currently a Research Director at the CNRS in Strasbourg (France). His research interests focus on the design and functionalisation of carbon-based nanomaterials and their use for therapeutic, diagnostic and imaging applications; the development of functionalised carbon nanotubes in nanomedicine and their impact on health and environment. He is Editor of Carbon, and in the Advisory Board of Nanomedicine, the Journal of Peptide Science, and Nanotechnology Reviews.



Maurizio Prato

Maurizio Prato graduated in Padova, where he was appointed Assistant Professor in 1983. Moved to Trieste as an Associate Professor in 1992, where he was promoted Full Professor in 2000. He spent sabbatical terms at Yale University (1986–1987), the University of California, Santa Barbara (1991–1992). He was Visiting Professor at the Ecole Normale Supérieure de Paris (2001) and at the University of Namur, Belgium (2010). He was the recipient of an ERC Advanced Research Grant, European Research Council, in 2008 and has become a Member of the National Academy of Sciences (Accademia Nazionale dei Lincei) in 2010.

targeting ligands to the nanocarriers such as antibodies or other targeting agents, able to recognise specific tumour markers.

At the same time, the delivery of a drug through a carrier could involve different metabolic and cellular pathways, thus offering a way to elude MDR.[5,6]

Carbon nanotubes (CNTs) are among the most interesting nanovectors currently under investigation. Functionalised CNTs have shown great promise as novel delivery systems based on their ability to cross biological barriers. In fact, even though the specific mechanism of internalisation (endocytosis or needle like penetration) is still not fully elucidated, it is generally recognised that CNTs are able to enter cells, independent of cell type and functional groups at their surface.[7,8] In addition, their high surface area provides multiple attachment sites for molecules, allowing for polyvalent derivatisation. Moreover, multiple *in vitro* and *in vivo* studies by different groups have shown, so far, that many types of chemically functionalised CNTs are biocompatible with the biological milieu, highlighting how the *in vivo* behaviour of this material could be modulated by the degree and type of functionalisation, both critical aspects that need to be accurately controlled.[9–15]

Generally, in cancer treatment, most modalities currently used clinically aim at the elimination of cancer cells. In that context, different types of severely cytotoxic agents (*e.g.* small molecules, radiation) are utilised as 'biologically active'. Since considerable interest lately has surrounded the determination of the safety profile of CNT, particularly in terms of unintentional environmental exposure, a clear distinction in purpose needs to be comprehended. In cancer therapy it is important to differentiate between the intended cytotoxicity from the therapeutic modality designed and the redundant possible toxicity from the carrier system itself, in this case the nanotube vector. While the former is required to achieve therapy, the latter needs to be minimised to avoid complications from therapy and side-effects. There is emerging understanding that the physicochemical characteristics of CNTs play a critical role in their ensuing toxicological profile.[16,17] For instance, the length[16] and diameter[17] of the CNTs have shown to be key players impacting their inflammogenic character. Shorter and thicker CNTs have been shown to be safer than their longer and thinner counterparts. Although such studies have only been performed with non-chemically functionalised (pristine) CNTs that are not explored therapeutically, we can begin to learn lessons and determine design parameters to be implemented in the development of CNT constructs for cancer therapy. Moreover, it is also becoming apparent that chemically functionalised CNTs and those suspensions exhibiting best aqueous dispersibility and stability in physiological environments can allow development in biomedical applications.[11,18–20] Another important factor considered to improve the overall safety profile of intentionally administered CNTs concerns their long term tissue accumulation. Design of biodegradable CNTs, given the recent findings about their degradation *in vitro*[21] and *in vivo*,[22] has become imperative. All the above suggest that, as with any other material developed with pharmacological intent (*e.g.* liposomes, polymeric micelles, dendrimers), knowledge of the critical parameters and understanding of their biological implications are unavoidable steps to allow the utilisation of CNTs in cancer therapy.

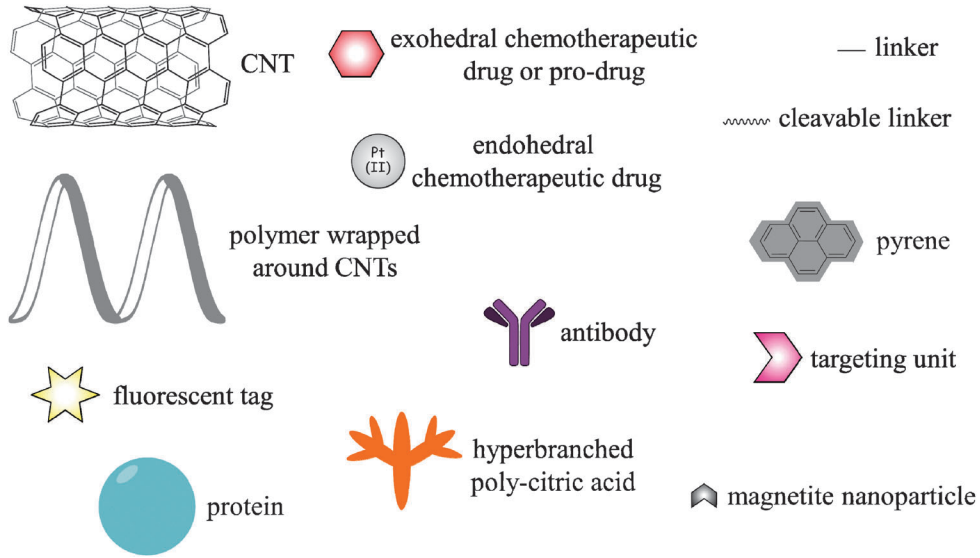
In this review, we analyse the most significant results reported so far in the field of drug delivery for cancer treatment. Conjugation of CNTs with many anticancer chemotherapeutics (doxorubicin, methotrexate, taxanes, platinum analogues, camptothecine and gemcitabine), along with immunotherapeutics and nucleic acids, is considered. Moreover, alternative anticancer therapies based on thermal ablation and radiotherapy are also discussed.

2. Delivery of chemotherapeutics

2.1 CNT–doxorubicin complexes

One of the advantages of using CNTs in cancer therapy is the capacity of their backbone to form supramolecular complexes with polycyclic aromatic molecules through π–π stacking. The most investigated anticancer drug in this context is the anthracycline doxorubicin (Dox). The earliest investigations in this regard were carried out initially with polyethylene glycol (PEG) functionalised single-walled CNTs (SWCNTs) or copolymer-coated multi-walled CNTs (MWCNTs) forming a supramolecular complex with doxorubicin (Table 1).[23,24] Ali-Boucetta *et al.*[23] found increased cell death with the MWCNT–Dox complex compared to Dox alone, when tested *in vitro* on human breast cancer cells (MCF7). Liu *et al.*[24] used either SWCNT non-covalently functionalised with a phospholipidic-PEG surfactant or oxidised SWCNT covalently PEGylated through amidation of the carboxylic moieties. It was reported that the interaction between the SWCNT and doxorubicin is pH-dependent, which meant that the loading was performed at pH 9, when doxorubicin is deprotonated and hence has low water solubility, while a decrease in the pH led to the release of the drug from the SWCNT carrier due to its increased hydrophilicity. The cytotoxicity of the phospholipid-PEG wrapped SWCNT–doxorubicin construct was tested on human glioblastoma cancer cells. This derivative induced cell death, similarly to free doxorubicin at a concentration of 10 μM, although the observed IC_{50} value was higher (~8 μM for the nanotube conjugate compared to ~2 μM for the free doxorubicin). Furthermore, a targeted doxorubicin delivery was tested using a cyclic arginine–glycine–aspartic acid (RGD) peptide, which acts as a recognition motif for integrin $\alpha_V\beta_3$ receptors, overexpressed in a wide range of solid tumours. In the non-covalent SWCNT derivative, the targeting agent was bound on the PEG chain and doxorubicin was then loaded. This conjugate, tested on integrin $\alpha_V\beta_3$-positive cells, showed enhanced drug delivery compared to the derivative without RGD and a smaller IC_{50} value (~3 μM), compared to the constructs without RGD ligand. The behaviour of this phospholipid-PEG wrapped SWCNT–doxorubicin was further studied *in vivo* into SCID mice bearing Raji lymphoma xenografts.[25] The authors observed higher tumour uptake, probably due to the prolonged circulation half-life of the construct, and a greater inhibition of tumour growth compared to the free drug. However, the therapeutic efficacy of the SWCNT–Dox construct was not better than DOXIL® (a liposomal formulation containing doxorubicin). On the other hand, the SWCNT–Dox construct caused neither significant

Table 1 Schematic representation and characteristics of the CNT conjugates described in this article



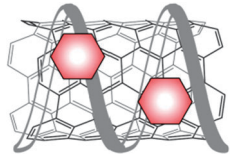
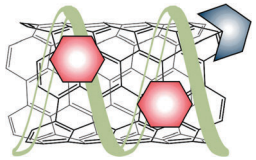
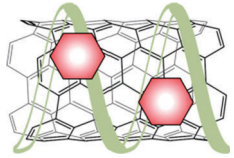
Compound	CNT type	Drug	Drug–CNT bond: Covalent	Drug–CNT bond: Non-covalent	Specific release mechanism (if any)	Specific targeting (if any)	Biological studies: *In vitro*	Biological studies: *In vivo*	Ref.
	MWCNTs	Doxorubicin		✓	—	—	Human breast cancer cells	—	23
	SWCNTs	Doxorubicin		✓	Acidic tumour or lysosomal pH	RGD peptide	Human glioblastoma cancer cells	—	24
	SWCNTs	Doxorubicin		✓	Acidic tumour or lysosomal pH	—	—	Lymphoma-bearing mice	25

Table 1 (*continued*)

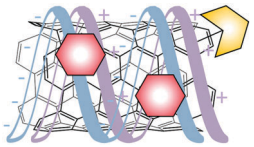
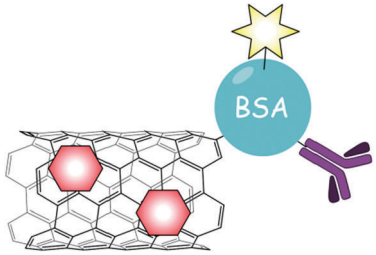
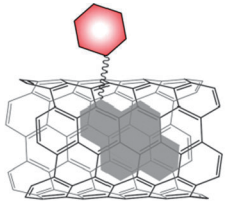
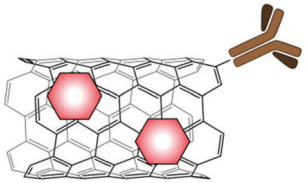
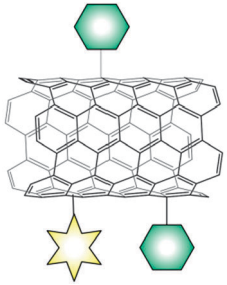
Compound	CNT type	Drug	Drug–CNT bond		Specific release mechanism (if any)	Specific targeting (if any)	Biological studies		Ref.
			Covalent	Non-covalent			*In vitro*	*In vivo*	
	SWCNTs	Doxorubicin		✓	Acidic tumour or lysosomal pH	Folic acid	Human cervical cancer cells	—	27
	SWCNTs	Doxorubicin		✓	Acidic tumour or lysosomal pH	Anti-carcino-embryonic antigen antibody	Human colon cancer cells	—	28
	SWCNTs	Doxorubicin		✓	Enzymatic cleavage of carbamate	—	Murine melanoma cells	Melanoma-bearing mice	29
	SWCNTs	Doxorubicin		✓	NIR radiation	Anti-P-glycoprotein antibody	Human leukemia cells	—	30
	MWCNTs	Methotrexate	✓		—	—	Human T lymphocytes	—	36

Table 1 (*continued*)

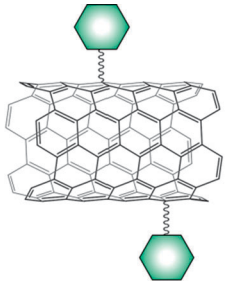
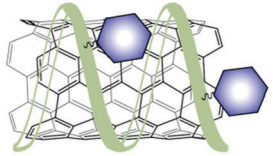
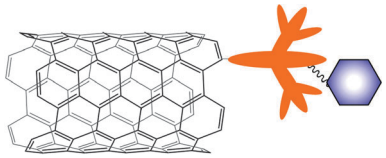
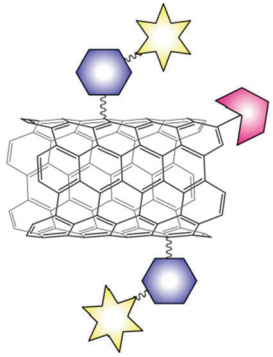
Compound	CNT type	Drug	Drug–CNT bond: Covalent	Drug–CNT bond: Non-covalent	Specific release mechanism (if any)	Specific targeting (if any)	Biological studies: *In vitro*	Biological studies: *In vivo*	Ref.
	MWCNTs	Methotrexate	✓		Enzymatic cleavage of peptidic linker	—	Human breast cancer cells	—	37
	SWCNTs	Paclitaxel		✓	Enzymatic cleavage of ester by carboxylesterases	—	Murine breast cancer cells	Murine breast cancer-bearing mice	38
	MWCNTs	Paclitaxel	✓		Acidic tumour or lysosomal pH and enzymatic hydrolysis of ester	—	Human ovary and lung cancer cells	—	39
	SWCNTs	Taxoid	✓		Reduction of the disulfide bond by endogenous thiols	Biotin	Murine leukemia cells	—	40

Table 1 (*continued*)

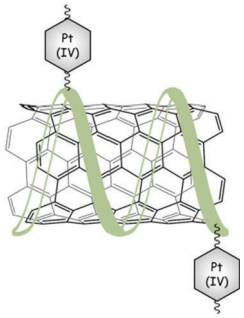
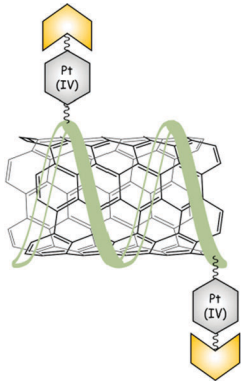
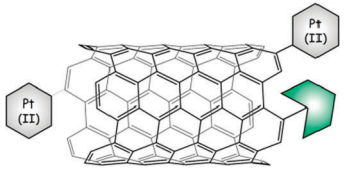
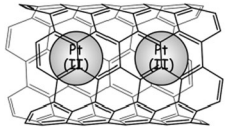
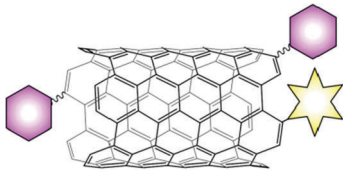
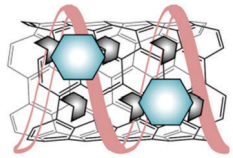
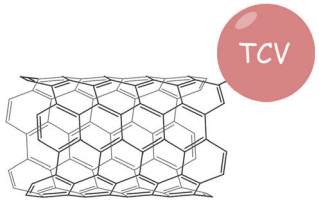
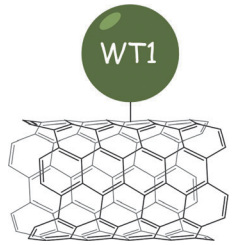
Compound	CNT type	Drug	Drug–CNT bond: Covalent	Drug–CNT bond: Non-covalent	Specific release mechanism (if any)	Specific targeting (if any)	Biological studies: *In vitro*	Biological studies: *In vivo*	Ref.
	SWCNTs	Cisplatin		✓	Reduction from Pt(IV) to Pt(II) due to endosomal acidic pH	—	Human testicular cancer cells	—	41
	SWCNTs	Cisplatin		✓	Reduction from Pt(IV) to Pt(II) due to endosomal acidic pH	Folic acid	Human choriocarcinoma, nasopharyngeal and testicular cancer cells	—	42
	SWCNTs	Cisplatin	✓		Ligand exchange (hypothesised)	Epidermal growth factor	Human head and neck squamous cancer cells	Human head and neck squamous carcinoma-bearing mice	43
	MWCNTs	Carboplatin		✓	—	—	Human bladder cancer cells	—	45
	SWCNTs	Cisplatin		✓	—	—	Human prostate cancer cells	—	46

Table 1 (*continued*)

Compound	CNT type	Drug	Drug–CNT bond: Covalent	Drug–CNT bond: Non-covalent	Specific release mechanism (if any)	Specific targeting (if any)	Biological studies: *In vitro*	Biological studies: *In vivo*	Ref.
	MWCNTs	Camptothecin	✓		Enzymatic cleavage of ester by esterases	—	Human gastric carcinoma cells	Hepatoma-bearing mice	47
	SWCNTs	Gemcitabine		✓	—	Magnetite nano-particles guided with an external magnetic field	—	Sprague–Dawley rats	48
	MWCNTs	Tumor lysate proteins (for TCV)	✓		—	—	—	Hepatoma-bearing mice	49
	SWCNTs	Wilm's tumour protein (WT1Pep427)	✓		—	—	Antigen presenting cells (dendritic cells and macrophages)	Healthy BALB/c mice	50

toxicity nor mortality, allowing the use of higher doses which is not the case with DOXIL®. Treatments with 10 mg kg^{-1} of SWCNT–Dox (dose normalised on doxorubicin) instead of 5 mg kg^{-1} led to improved efficacy, without causing any severe toxicity. In comparison, 5 mg kg^{-1} of free doxorubicin or DOXIL® resulted in a definite decrease in the weight of animals and increased mortality rates of 20% and 40% respectively. Possible reasons are that the larger size of the SWCNT–Dox constructs, compared to free doxorubicin, probably slowed down the excretion rates and the PEG coating of SWCNTs could have hidden doxorubicin from macrophages. Both these effects could determine prolonged blood circulation, thus enhancing tumour accumulation because of the EPR effect (Fig. 1). Moreover, the slightly acidic microenvironment of tumours is expected to facilitate doxorubicin detachment from the SWCNT as previously speculated.[24] The same authors, however, observed a slow *in vivo* dissociation of doxorubicin from the SWCNT after administration, which suggests that non-covalent constructs cannot guarantee proper *in vivo* stability. Another *in vivo* targeting study was performed with PEGylated SWCNT functionalised with RGD peptide. After tail vein injection into U87MG tumour-bearing mice, the nanotubes accumulated in tumours (13% ID g^{-1} in 24 hours), with no obvious toxicity or negative health effects for the animals.[26]

Zhang *et al.* prepared a different kind of SWCNT-based vector for doxorubicin.[27] CNTs were firstly oxidised and then derivatised by wrapping them with an anionic and a cationic polysaccharide, namely sodium alginate and chitosan, respectively. Doxorubicin was then loaded onto the modified SWCNT *via* electrostatic interactions and π–π stacking. The release of doxorubicin under acidic conditions (pH 5.5) was assessed in order to mimic the typical environment of lysosomes or cancer tissues. A release was found of almost 40% in 72 hours, while the construct was stable at pH 7.4. Finally, an identical conjugate bearing folic acid (linked to chitosan) was prepared to target tumour cells over-expressing the folate-receptor. *In vitro* cytotoxicity tests and cellular uptake studies were performed on human cervical carcinoma (HeLa) cells. The construct caused a significant reduction in cell viability compared to the drug itself, which proves its efficacy as a targeted delivery system.

A triply functionalised SWCNT derivative was prepared, bearing doxorubicin, fluorescein and a targeting antibody that recognises the carcinoembryonic antigen (CEA), a tumour marker for a variety of adenocarcinomas.[28] Doxorubicin was non-covalently loaded onto the oxidised SWCNT by π–π stacking. A fluorescein-labelled BSA was then covalently attached through its amines to the carboxylic groups present on the oxidised SWCNT, *via* an amidation reaction. Finally, the antibody was tethered through its amines to the BSA carboxylic groups again with an amidation step. In this way the authors designed a construct that targets CEA-over-expressing cancer cells and is then internalised by endocytosis releasing doxorubicin at lysosomal acidic pH. The fluorescein unit was introduced to localise the SWCNT construct by the fluorescent signal after doxorubicin release. The internalisation by WiDr human colon cancer cells was studied by confocal microscopy. After a 4 hour treatment, the fluorescently labelled SWCNTs were found in the cytoplasm, while doxorubicin was localised in the nucleus, where the drug exerts its activity. As a control, the fluorescein-labelled BSA did not show internalisation to the same high extent, indicating the good carrier ability of CNTs. Nevertheless, no information on the cytotoxicity of the complex was reported, nor any stability study to evaluate the release of doxorubicin from the CNT.

In a different approach, doxorubicin was linked to pyrene and this system was non-covalently attached to the SWCNT *via* π–π bonding driven by the polyaromatic unit of pyrene.[29] The linker between doxorubicin and pyrene was a short chain with a carbamate group, designed to be enzymatically cleaved inside lysosomes. *In vitro* studies on a mouse melanoma cell line were performed to assess the internalisation of the construct and its cellular toxicity. The construct was found to accumulate in the lysosomes and caused a time- and dose-dependent cytotoxicity. Furthermore, the therapeutic efficacy was evaluated *in vivo* on B16F10 melanoma-bearing mice. Free doxorubicin and SWCNT–doxorubicin induced similar reduction in tumour volume, with a significant decrease in the systemic toxicity for the CNT–Dox construct. In this case also the release of the drug from the CNT was studied, comparing the effect of incubation with cell lysate to the use of a normal buffer. Though the former system was significantly more efficient, proving that intracellular enzymes play a fundamental role, a release was also observed in the latter case, demonstrating that a non-covalent construct is not totally stable in a biological environment.

With the double aim of obtaining a targeted drug delivery system while overcoming the MDR effect, SWCNTs were functionalised *via* amidation with an antibody (Ab) directed towards P-glycoprotein (P-gp), one of the main players in MDR.[30] Subsequently the Ab–SWCNT construct was non-covalently loaded with doxorubicin, *via* π–π stacking. This construct was tested *in vitro* on human leukemia cells, over-expressing P-gp on their surface and therefore being resistant to doxorubicin. Cells were exposed to near-infrared (NIR) radiation to trigger doxorubicin release. As a consequence of NIR absorption by SWCNT the amount of doxorubicin released in 24 hours was tripled (nevertheless a partial release was observed without NIR radiation as well). The Ab–SWCNT construct appeared bound to the cell membrane, while doxorubicin was localised inside cells in much higher quantities with respect to treatments with the free drug. Doxorubicin alone or together with the anti P-gp antibodies (at a concentration of 5 μg mL^{-1}) could partially inhibit cell growth. Increasing the incubation time after treatment from 24 hours to 48 hours and 72 hours, cells gradually recovered and began to proliferate. The SWCNT construct, on the other hand, used with an equivalent concentration of Dox, inhibited cell growth more efficiently than the free drug and in a time-dependent manner, probably due to a slow drug release from the vector surface. Since the first publications by Ali-Boucetta *et al.*[23] and Liu *et al.*,[24] a lot of proof-of-principle studies on the use of CNT–Dox constructs for cancer therapy have been performed, while this research is still ongoing.[31–35]

2.2 CNT–methotrexate constructs

Another clinically used anticancer drug that was investigated with CNT for cancer therapy is methotrexate. Methotrexate (MTX)

suffers alike others from low bioavailability and toxic side effects. Preliminary studies of CNT-based drug delivery with this drug have been performed in our laboratories.[36] MWCNTs were covalently functionalised simultaneously with methotrexate and fluorescein (Table 1). Although a rapid internalisation of the constructs in human Jurkat T lymphocytes was observed, the efficacy of the CNT–MTX construct was lower than the free drug. In a more recent study, the same drug (MTX) was bound to the MWCNT through cleavable linkers.[37] The use of a peptide linker recognised by intracellular proteases led to a significant decrease in MCF-7 breast cancer cell viability compared to free MTX. It is therefore possible to conclude that the lower efficacy in the first approach was probably due to the lack of drug release once the construct was internalised.

2.3 CNT–taxane constructs

Taxanes represent another class of antineoplastic drugs that have already been conjugated to CNT as a new platform for cancer therapy. Paclitaxel (PTX), a representative molecule of this class, was covalently bound through an ester bond to the PEG of the non-covalently PEGylated SWCNT and intravenously (i.v.) injected into xenograft tumour-bearing mice (a PTX resistant 4T1 murine breast cancer mice model) to test the *in vivo* efficacy of the CNT–PTX construct (Table 1).[38] Results were promising, showing a tumour growth inhibition value of almost 60% for the SWCNT–PTX derivative, which is two or three times higher than that reported for Taxol® and PEG-PTX (28% and 21%, respectively). Higher apoptosis levels and lower cellular proliferation were observed with the SWCNT–PTX construct compared to the drug alone. It is important to underline the lack of toxicity in mice for these PEGylated SWCNTs themselves which has been demonstrated by the same authors in another study.[13] Evaluation of the pharmacokinetics showed that SWCNT–PTX has a longer blood circulation half-life coherently with the enhanced hydrophilicity of the conjugate with respect to the drug itself. However, a much higher PTX presence in reticuloendothelial system (RES) organs (liver/spleen) and intestine was also observed 2 hours after injection. This is a predictable behaviour for nanomaterials in general and could raise concerns about the toxicity towards these organs. Nevertheless, the authors reported differences between the biodistribution of SWCNT and PTX, indicating a rapid release of the drug from the conjugate probably due to ester cleavage by carboxylesterases. As a consequence, the drug seemed to be rapidly excreted, lowering its toxicity. At the same time, PTX levels in tumours were 10 times higher for the SWCNT–PTX derivative than for the free Taxol®, 2 hours post-injection, and the tumour-to-normal organ/tissue PTX uptake ratio was bigger, thus indicating a better selectivity of the CNT-delivered drug.

In a recently published paper another kind of CNT–PTX construct was prepared.[39] MWCNTs were first oxidised and covalently functionalised with hyperbranched poly citric acid (PCA). Then PTX was bound to PCA carboxyl groups with a cleavable ester bond, able to release the drug at acidic pH and through enzymatic hydrolysis. Finally, the *in vitro* activity of the construct towards SKOV3 ovary and A549 lung cancer cell lines was assessed. Earlier cytotoxic effects, even though comparable in intensity, were reported for the construct with respect to PTX alone, the fact being ascribed by the authors to an improved cell penetration for the CNT-based construct.

Another study with the same class of therapeutic compounds was performed. This was a case of a covalent SWCNT derivative bearing a taxoid molecule, derivatised with biotin as targeting unit towards cancer cells.[40] This construct was intended to be a prodrug, driven by the biotin to cancer cells which overexpress the specific receptor on their surface and thus internalised by receptor-mediated endocytosis. Once inside the cell, the prodrug liberates its cargo upon reduction of the disulfide bond by endogenous thiols such as glutathione (GSH), whose concentrations are typically more than one order of magnitude higher in tumour tissues than in blood plasma. The system should act specifically on cancer cells leading the taxoid to carry out its mitosis inhibition action only in the desired sites. By means of confocal fluorescence microscopy, thanks to the presence of a fluorescent tag covalently bound to the taxoid molecule, the authors demonstrated the actual internalisation of the whole conjugate in leukemia L1210FR cells. Moreover, in the case of a control SWCNT derivative without biotin, they observed a temperature-dependent but an energy-independent internalisation. This is coherent with a non-endocytotic mechanism, and is in accordance with the hypothesis of a needle-like diffusion of carbon nanotubes through cell membranes.[8] The biotin-conjugate, as foreseen, was instead internalised by receptor-mediated endocytosis, the uptake being far higher than in cells not expressing the biotin receptor. The release of the drug by GSH and its binding to the microtubule network, where it exerts its cytotoxic action, were efficient, with an IC_{50} value smaller than the one for the drug alone, probably due to an increase in intracellular delivery.

2.4 CNT–platinum constructs

Platinum (Pt) analogues have also been investigated for drug delivery using CNTs for cancer treatment. Feazell *et al.* reported that the preparation of a Pt(IV)-based SWCNT prodrug led to intracellular concentrations six times higher than those reached when treating the cells with the free drug (Table 1).[41] More recently, the same authors built another Pt-based targeted prodrug system using SWCNT as a long-boat for the delivery of Pt.[42] The derivative was prepared by binding folic acid, as targeting agent, to a Pt(IV) compound, and tethering this conjugate through an amide coupling with terminal amines of phospholipid-PEG chains wrapped around the CNT. The Pt(IV) can be reduced inside the endosome, then losing the two axial ligands and leading to the active Pt(II) compound. The authors demonstrated that the internalisation of this construct takes place by folate receptor-mediated endocytosis. The system was active in killing cancer cells (human choriocarcinoma and human nasopharyngeal carcinoma) with IC_{50} values more than eight times lower than cisplatin alone. The authors thus proved the actual ability of the system to act as a prodrug, generating the cytotoxic derivative (cisplatin) once internalised, and thus killing in a selective way folate receptor over-expressing cells.

In a later interesting paper, Bhirde *et al.* described the targeted killing of cancer cells using a cisplatin-delivery system based on drug–SWCNT covalent conjugates.[43] The interaction of an epidermal growth factor (EGF) ligand with its receptor (EGFR), which is over-expressed in cancer cells, was exploited to target CNTs both *in vitro* and *in vivo*. The efficient *in vitro* internalisation in head and neck squamous carcinoma cells of the SWCNT–EGF conjugate was proven and was found to be mediated by the EGF–EGFR interaction. Furthermore, quantum dot (QD)-functionalised SWCNTs were administrated to tumour-bearing athymic mice to study the short-term biodistribution profile of this construct. The EGF–CNT conjugate showed a much higher accumulation within the tumour compared to the control conjugate without EGF. Small amounts of CNT were found within spleen, lungs, liver, kidneys and heart, regardless of the presence of EGF. Moreover, the animals treated with SWCNT–cisplatin–EGF showed a decrease in tumour growth compared with the untargeted SWCNT–cisplatin conjugate. This demonstrated that the SWCNT can effectively and selectively deliver cisplatin towards EGFR over-expressing tumours.

Another interesting approach for the functionalisation of CNTs, also used in oncology, is the filling of the internal empty cavity of the nanotubes. This method was first investigated by the Green group in 1994 and involves the opening of MWCNT caps by nitric acid treatment and filling of the inner cavity with different materials through a wet chemistry approach.[44] By applying the same method, Hampel *et al.* filled the nanotubes with carboplatin, a widely used chemotherapeutic agent for cancer treatment.[45] This derivative was then tested on human bladder cancer cells and a reduced cell viability compared to the drug alone was observed. More recently, cisplatin was also encapsulated into a SWCNT.[46] The release of the drug in physiological solution was studied, and it was found to start after 24 hours and to continue up to 72 hours. However, the inhibition of cell growth with this construct on two prostate cell lines (DU145 and PC3) was comparable to the free drug.

2.5 CNT–camptothecine construct

Another antineoplastic drug, 10-hydroxycamptothecine (HCPT), was covalently attached to the MWCNT to study the influence of CNT on drug efficacy (Table 1).[47] MWCNTs were first oxidised, and the generated carboxylic groups underwent an amidation step to introduce a spacer bearing an amino group to which a tethered derivative of HCPT was bound through another amidic bond. The tether presented an ester linkage, hydrolytically unstable, and was introduced to subsequently trigger the release of the drug. The authors observed a reduced drug release ($<15\%$ after 5 days) under both acidic and basic conditions, but a much improved release (80% after 5 days) when incubating the construct with fetal bovine serum, which contains esterase that could catalyse the hydrolysis. Furthermore, a fluorescein moiety was bound to the unreacted amine after HCPT conjugation, for *in vitro* imaging on human gastric carcinoma cells. Fluorescence confocal microscopy showed that the constructs localise intracellularly with a uniform distribution in the cytoplasm. Cell viability (WST-1) assay revealed a significantly improved cytotoxicity with respect to equal concentrations of HCPT, whereas CNT alone did not cause any decrease in cell viability. Finally, a different construct bearing DTPA was prepared, in order to chelate a radioactive nuclide (Tc) for *in vivo* biodistribution studies in tumour-bearing mice. High uptake was found in the liver, spleen, lungs, kidneys, stomach, femur and tumour. In the latter, the maximum uptake level (3.6% ID g^{-1}) was reached within 4 hours post-injection. The observed blood circulation half-life was 3.6 hours, *versus* 30 minutes reported for HCPT. Since the *in vitro* drug release tests showed that 4 hours after incubation with serum only 12% of the HCPT was released, the authors considered that the majority of the conjugate could reach the different organs (and the tumour) without releasing a large amount of drug into the blood stream. The *in vivo* antitumour performance of the HCPT-bound MWCNT was also studied, finding tumour growth inhibition much more efficient than HCPT alone, without causing any severe toxicity to the animals.

2.6 CNT–gemcitabine construct

A MWCNT–gemcitabine construct was delivered to lymphatic vessels, exploiting the EPR effect together with the use of an external magnetic field (Table 1).[48] MWCNTs were first functionalised with poly(acrylic) acid and then decorated with magnetite nanoparticles ($FeO{\cdot}Fe_2O_3$) by a co-precipitation step with Fe^{2+} and Fe^{3+}. Three hours after subcutaneous injection in rats, the construct was able to reach popliteal lymph nodes without accumulation in the major organs such as the liver, spleen, kidneys, heart and lungs, simply by the EPR effect. The same CNTs were loaded with the antineoplastic drug gemcitabine by physical adsorption. The system was guided *in vivo* by applying a permanent magnet on the projection surface of one popliteal lymph node. Very high accumulation of gemcitabine was detected in the lymph nodes after 24 hours. At the same time blood concentration was lower, when compared to gemcitabine alone, to the treatment without the magnetic field, and also to a control represented by nanosized activated carbon decorated with magnetic nanoparticles.

3. Delivery of immunotherapeutics

CNT-based antitumour immunotherapy has also been explored. This approach employs tumour cell vaccines (TCV) made of inactivated cancer cells or dendritic cells presenting tumour antigens to trigger the immune response of the patient against the tumour itself.[49] With the aim of improving the efficacy of TCV, tumour lysate proteins were covalently coupled to oxidised MWCNTs *via* an amide bond (Table 1). The conjugate was injected subcutaneously into H22 hepatoma-bearing mice treated with TCV. Controls were performed with TCV + CNT only and TCV + tumour lysate proteins only. The cure rate was significantly increased with respect to animals treated only with TCV or with TCV + tumour lysate proteins, even if a partial effect was exerted by CNT alone. To assess whether the immunity was tumour-specific, the animals that survived after treatment with tumour lysate proteins-bearing MWCNTs were challenged again with a subcutaneous

injection of tumour cells. In the case of H22 cells the animals rejected the tumour, while they did not reject another mouse breast cancer, proving how the therapeutic system made them develop specific immune responses.

In the same therapeutic context, Villa *et al.* studied the ability of SWCNTs to act as antigen presenting carriers, in order to improve the response to weakly immunogenic peptides.[50] The Wilm's tumour protein (WT1), which attracted attention as a vaccine target for many human leukemias and cancers (currently in human clinical trials), was covalently conjugated with SWCNT. It was found that SWCNT–WT1 was rapidly taken up *in vitro* by antigen presenting cells, in a dose-dependent manner. Interestingly, *in vivo* immunisation of BALB/c mice using SWCNT–WT1 and an adjuvant caused a specific humoral immune response which was not seen against the peptide alone neither against the peptide mixed with the adjuvant, proving the potential of the SWCNT to deliver poorly immunogenic peptides to the immune system, thus improving vaccine therapy.

4. Delivery of nucleic acids

The way the CNTs interact with nucleic acids has been extensively studied for their potential applications. Both antisense oligonucleotides and small interfering RNA (siRNA) are very promising fragments for gene silencing, applicable for the treatment of many diseases. They can in fact inhibit protein expression, potentially blocking many cellular pathways. Cancer therapy is one of the possible applications, when the targets are oncogenes or genes involved for instance in angiogenesis or chemotherapy resistance. One of the first studies in this field was performed a few years ago.[51] Cationic SWCNTs were used to complex siRNA, able to silence the expression of telomerase reverse transcriptase and thus inhibit cell growth. This activity was proved *in vitro*, on different cell lines, both murine and human, and *in vivo*, after intra-tumour injection in xenografted mice.

The *in vivo* anti-tumour activity of a CNT-based siRNA delivery system was also assessed by our group, using a different kind of construct.[52] MWCNTs, covalently functionalised *via* 1,3-dipolar cycloaddition and bearing terminal amino groups, were used as cargos for a pro-apoptotic siRNA sequence (the proprietary siTOX). The system was injected within the tumour mass on human lung carcinoma (Calu 6) xenografted mice and compared with MWCNT with a non-coding sequence (siNEG), functionalised MWCNT alone, and both siTOX and siNEG delivered with cationic liposomes. MWCNT–siTOX conjugates significantly inhibited tumour growth and prolonged animal survival compared with the controls, while cationic liposome-based systems did not affect tumour growth or preserve the animals alive.

In the context of targeted delivery, a folate targeted DNA transporter based on CNT was prepared, as a system in principle exploitable to deliver nucleic acids to cancer cells.[53] In this case, the covalently functionalised SWCNT presented positive charges to form electrostatic interactions with nucleic acids. The authors then bound fluorescently labelled-double stranded DNA (dsDNA) to this derivative, proving by UV a strong enhancement in the loading of dsDNA for the positively charged SWCNT compared with the non-charged SWCNT. The derivative was further functionalised by wrapping a folic acid modified phospholipid around it. The complex was tested in mouse ovarian epithelial cells, showing an increased uptake for the derivative with the folic acid, compared to the one without the targeting unit. In addition, the fluorescently labelled-dsDNA alone was internalised only at a very poor level, thus demonstrating the carrier role of CNT. In a final experiment, HeLa cells were induced to overexpress folate receptors, culturing them with a folic acid-free medium. These cells showed a much higher internalisation of the derivative than normal HeLa cells, confirming the efficacy of the delivery system.

In another study, oxidised MWCNTs were complexed to polyethylenimine, and then coated with an oligonucleotide antisense sequence, by means of electrostatic interactions.[54] The oligonucleotide was coupled to fluorescent cadmium telluride QDs to follow the cellular trafficking of the complex. An efficient uptake was demonstrated while the complex exerted the expected apoptotic activity.

Dendrimer–CNT constructs have also been exploited for gene delivery. The anti-survivin oligonucleotide was complexed to the polyamidoamine (PAMAM) dendrimer covalently attached to the CNT and used to transfect MCF-7 cells.[55] The conjugate distributed mainly in the cytoplasm, endosomes and lysosomes of the cells and was able to release the antisense oligonucleotide, inducing apoptosis. The PAMAM dendron was also directly grown on the MWCNT surface and used as an anchoring point for siRNA, after introducing a trimethylammonium unit on each branch termination. Different dendron generations were prepared and an improved efficacy in delivering a fluorescent oligonucleotide was observed, as the degree of dendritic branching increased. Furthermore, the gene silencing capability of the system was confirmed.[56,57]

An interesting approach was developed combining the use of CNT as a nucleic acid delivery system with photodynamic therapy.[58] This therapy represents an option to cancer treatment and it is based on the delivery of a photosensitiser, which, upon activation by an appropriate light source, transfers light energy to tissue oxygen. Mainly singlet oxygen is generated, which can react rapidly with cellular components, triggering cellular damage. In this study an aptamer, a synthetic DNA/RNA probe able to recognise and bind a specific target, was covalently bound to chlorine e6 (Ce6), a well-known photosensitiser. The aptamer was subsequently wrapped around the SWCNT, the conjugate being able to quench 98% of the singlet oxygen generation (SOG) normally occurring upon excitation of Ce6. When involved in the binding to its target human α-thrombin, the aptamer was released from the CNT, and SOG was no longer quenched. Phototoxicity on human lymphoma cells treated with the construct was also studied, showing a reduction in cell viability comparable with treatment with Ce6 alone, when thrombin was added. This indicated the feasibility of a targeted SWCNT-based system for photodynamic therapy.

All the examples reported above demonstrate that both single- and multi-walled CNTs are good vectors for nucleic acid delivery and that the transported sequences retain their activity. These results are even more attractive considering

that CNTs also exert an important protective action against enzymatic digestion.[59] Indeed, oligonucleotides wrapped on SWCNTs are preserved from enzymatic cleavage and interference from nucleic acid binding proteins, increasing their stability in cells. Moreover, it has been reported that oxidised SWCNTs induce a stabilisation of the human telomeric i-motif of DNA.[60] Even though the mechanism still needs to be studied, it could be exploited in anti-neoplastic systems, since stabilisation of the i-motif inhibits telomerase, an essential enzyme for the proliferation of cancer cells.

5. Alternative anticancer strategies: thermal ablation and radiotherapy

Cancer treatments may deal not only with the administration of drugs but can cover other possibilities. Among these, a very interesting strategy, named thermal ablation, arises from the intrinsic optical properties of CNT that allows for the development of hyperthermia. The optical absorbance of CNT is in fact very high in the NIR region, 700–1100 nm, where biological systems are transparent.

It is worthy of note that gold nanoshells and gold nanoparticles, to the best of our knowledge, represent the only other materials which have provided good results in photothermal cancer treatment with NIR radiations.[61–63] The laser intensity and radiation time used, however, are often higher than those needed to kill the cells with CNT. This observation makes CNT an even more promising material in the field, opening the way for further exploration, to reach a maximum laser energy level corresponding to a peak fluence of 35–45 mJ cm^{-2}, established as the safety standard for medical lasers.[64]

Among the studies carried out so far, it is important to distinguish the non-targeted approaches, both *in vitro*[65,66] and *in vivo*, when CNTs were directly injected into the tumour[67,68] from those where CNTs were specifically targeted to tumour cells. The first paper reporting this use of CNT was published by the Dai group.[69] SWCNTs were non-covalently functionalised with phospholipid-PEG chains bearing a fluorescent tag or a folic acid molecule. The complex was then administered to HeLa cells overexpressing the folate receptor (FR^+ cells) and to normal HeLa cells as a control. The FR^+ cells showed a high internalisation of the folic acid–SWCNT derivative, imaged by fluorescence microscopy, while the normal cells showed poor uptake. Cells were then irradiated by an 808 nm laser (1.4 W cm^{-2}) for 2 minutes (Table 2). This treatment resulted in extensive FR^+ cell death, while normal proliferation behaviour was found for the cells which did not internalise carbon nanotubes.

Table 2 Summary of the laser radiation conditions used in the cited references

Ref.	Laser wavelength/nm	Peak intensity/W cm^{-2}	Pulse duration/min	Peak fluence/ J cm^{-2}[a]
69	808	1.4	2	168
70	808	0.8	3	144
71	808	5	7	2100
72	808	9.5	4	2280
73	808	2	5	600

[a] The peak fluence value was estimated according to the equations: peak fluence (J cm^{-2}) = laser pulse energy (J)/focal spot area (cm^2) and laser pulse energy (J) = laser peak power (W) × pulse duration (s).

A multi-component targeting system was also created by binding to the SWCNT two different monoclonal antibodies specific for breast cancer cell antigens IGF1R and HER2.[70] The double targeting ensures high efficacy and selectivity. The derivatives were prepared by functionalising CNT with 1-pyrenebutanoyl succinimide which is able to interact strongly with the aromatic surface of the nanotubes by π–π stacking, as already mentioned, and bears an anchoring point to link the antibodies. The cells incubated with this conjugate were excited by 808 nm photons at 800 mW cm^{-2} for 3 minutes (Table 2). After this treatment all cells incubated with the IGF1R and HER2 antibody–SWCNT hybrids were destroyed. At the same time, 80% of the cells incubated with non-specific antibody–SWCNT hybrids survived.

Non-covalent SWCNT–Ab derivatives were also prepared exploiting the strong binding between neutravidin attached to the Ab and biotin present on the polymer coated SWCNT.[71] The constructs were targeted to human cells presenting the specific antigen for the Ab and subsequently treated with a NIR laser (808 nm, 5 W cm^{-2}) for 7 minutes (Table 2). This resulted in a significant decrease in cell viability. Since the disadvantage of non-covalent constructs is, as mentioned, the possible dissociation in biological fluids, the same authors prepared a covalent SWCNT–Ab construct. They formed amide bonds between the carboxylic groups of oxidised SWCNT and the amines of two different monoclonal Abs (anti-CD22 or anti-CD25).[72] To assess the specific binding to human Burkitt's lymphoma cells ($CD22^+CD25^-$) and phytohemagglutinin-activated normal human peripheral blood mononuclear cells ($CD22^-CD25^+$), after incubation with the constructs, the cells were treated with fluorescein-conjugated goat anti-mouse immunoglobulin (FITC-GAMIg) and analysed through flow cytometry. The results clearly showed specific recognition of the correspondent cell receptor by the two Ab–SWCNT conjugates. Finally thermal ablation of the specifically targeted cells was achieved after exposure to NIR radiation (808 nm laser, 9.5 W cm^{-2}) for 4 minutes (Table 2).

Among cancer thermal ablation studies involving CNTs, another interesting option was proposed.[73] Targeting cancer could be translated more specifically into targeting cancer stem cells. These cells are responsible for the formation of metastases, resistance to therapies and restoration of tumours. They therefore represent a very important target to study and more efforts should be made in this direction. The chosen target was the $CD133^+$ glioblastoma cell line presenting high tumourigenicity and cancer stem-like properties. SWCNTs were wrapped with chitosan which was covalently coupled to a CD133 monoclonal antibody. These CNTs were tested *in vitro* on both $CD133^+$ and $CD133^-$ glioblastoma cells. Internalisation was observed only by the $CD133^+$ cells which were specifically killed after exposure to NIR laser radiation (808 nm, 2 W cm^{-2}) for 5 minutes (Table 2). Furthermore, the treatment with CNT followed by NIR radiation inhibited spheroid body formation, which represents an index of cell self-renewal. To partially translate the study *in vivo*, CNT-treated or untreated cells were used to induce tumour formation in nude mice. Two days after

a subcutaneous injection the mice were subjected to NIR laser radiation, resulting in significant inhibition of tumour growth when CNTs were used.

Another promising anticancer strategy is radiotherapy. As mentioned above, the main purpose of attaching radioisotopes to the CNT is for imaging studies, to determine their biodistribution following radioisotope traces. A therapeutic application however can also be envisaged. The preparation of a SWCNT derivative bearing an ^{111}In chelate was reported and its biodistribution was analysed, finding accumulation mainly in the kidney, spleen and liver.[74] The same derivative was then further functionalised with a tumour specific monoclonal antibody, and it was delivered *in vivo* to a murine model of disseminated human lymphoma, showing selective tumour targeting. Therefore, the replacement of indium with a proper radionuclide would render these constructs ideal for their application in radiotherapy. Indeed, the preparation of covalent CNT–Ab constructs intended to target tumour neovasculature for radiotherapy was subsequently reported by the same authors.[75] Angiogenic endothelial cells express on their surface a monomeric cadherin that, after forming dimers on nearby cells, constitutes the tight junctions of normal vascular endothelium. In tumours, these cells are poorly connected (this being one of the reasons for the EPR effect). Thus the cadherin is in monomeric form, which is the only one recognised by the antibody E4G10. Doubly functionalised SWCNTs were prepared, by binding to the CNT both the specific antibody and the alpha particle-emitting ^{225}Ac, able to kill the targeted cell and the tissue in its proximity. The conjugate was tested *in vivo, via* intravenous injection in xenografted tumour mice, demonstrating its specific ability to reduce tumour growth and improve mice survival compared with controls bearing a different antibody. The success of this approach seems to be very promising as in principle targeting of tumour vasculature renders these kinds of constructs suitable for any species of solid tumour.

6. Tumour targeted CNT

As seen from the studies already mentioned above, various ligands have been attached to CNTs to improve their tumour specificity and achieve active targeting onto specific cell receptors. Some other works focused mainly on the targeting issue, as a proof-of-principle, without proposing any specific therapeutic approach.

Specific recognition of membrane receptors has been achieved with Ab-functionalised SWCNTs.[76] Nanotubes were first functionalised with a phospholipid-PEG to increase solubility and to prevent non-specific binding in the biological environment. Then terminal amines of the PEG units were covalently functionalised with two different antibodies (rituximab and trastuzumab). After incubation, binding to the corresponding membrane receptors (CD20 on B-cells, and HER2/neu on breast cancer cells, respectively) was proved, exploiting the intrinsic near infrared fluorescence of CNT. This study demonstrated the feasibility of using antibodies as targeting agents in applications such as imaging or drug delivery.

Raman spectroscopy was also used as an imaging technique for CNT.[77] In this case a phospholipid-PEG–CNT construct bound to different antibodies or to an RGD peptide was prepared using SWCNTs with a different isotope composition, that display well-shifted Raman G-band peaks. As a consequence, it was possible to distinguish the different CNT constructs in mixed cellular populations, proving that they were able to selectively recognise their specific target. Raman imaging was used also for both *in vitro* and direct *in vivo* studies exploiting PEGylated SWCNT functionalised with RGD peptide.[26,78] *In vitro* data showed that $\alpha_V\beta_3$ integrin-positive tumour cells (U87MG) internalised the construct much better than the SWCNT without RGD and than $\alpha_V\beta_3$ integrin-negative cells (HT29) do. *In vivo* data showed selective prolonged accumulation of the RGD targeted SWCNT in the tumour, while for the non-targeted SWCNT an initial tumour accumulation was observed, followed by a rapid decrease. Using a different approach, SWCNTs with phospholipid-PEG-COOH were bound to protein A by amidation and then, an anti-integrin antibody, previously marked with fluorescein, was linked to the system exploiting the high affinity interaction of the Fc (fragment crystallisable) region of the antibody with protein A.[79] The chosen antibody recognises a specific integrin overexpressed on various cancer cells, rendering the construct suitable for cancer targeting. After demonstrating that the construct was not cytotoxic, its internalisation and targeting were studied using fluorescence confocal microscopy both in integrin-positive (U87MG human glioblastoma cancer cells) and integrin-negative (MCF-7 human breast cancer cells) cell lines. Only U87MG cells internalised the SWCNT construct, while no intracellular fluorescence was detected for MCF-7 cells nor for U87MG cells after a pre-treatment with the Ab alone. An integrin-mediated endocytosis uptake mechanism for CNTs was therefore hypothesised.

Bottini *et al.* studied the internalisation of SWCNT–QD–streptavidin complexes by CD3 positive-leukemia cells based on monitoring the intracellular QD fluorescence.[80] In this case the uptake mechanism was rather complex, being mediated by a multi-component recognition. Initially, a biotinylated anti CD3-antibody was allowed to bind to the CD3 receptor on the cell membrane. Then the streptavidin-loaded SWCNT connected to the biotinylated antibody, showing high internalisation. In contrast, poor uptake was observed when: (i) the antibody was absent; (ii) a non-biotinylated antibody was used; (iii) the experiments were carried out at 4 °C; and (iv) CD3 negative cell lines were used. All these data suggest a receptor-mediated endocytosis for this construct.

7. Conclusions

The utilisation of CNTs in cancer therapy has expanded dramatically in the recent years. Encouraging proof-of-principle studies have been conducted so far, showing great promise for CNT-based therapeutic systems in various cancer treatment modalities. The main advantage is related to the capability of CNTs to deliver cargos intracellularly and most efforts have concentrated on the development of specific tumour cell targeting following different therapeutic approaches. It has to be emphasised that the development of carbon nanotubes is not an end in itself, but part of a broader concerted effort from the wider research community to utilise the different capabilities

that various types of nanoscale systems have revealed and can offer to cancer therapy.[81] In that respect, CNTs have been shown to provide with: (a) capability to deliver biologically active molecules cytoplasmatically, by-passing a lot of biological barriers and acting as a cellular needle; (b) large surface area and internal cavity that can be decorated with targeting ligands and filled with therapeutic or diagnostic agents; (c) unprecedented electrical and thermal conductivity properties. However, it is not always possible to precisely compare the many therapeutic approaches, nor the different nanomaterials due to the great diversity among the constructs used. Further work is certainly necessary, both from the chemical point of view, in the preparation of well-characterised constructs with the desired properties, and in terms of biological activity, for a full comprehension of the potential CNT can offer to clinically-relevant cancer therapy.[21] We hope that future research will confirm the promise towards the achievement of this new technology platform, able to help fight cancer in a more selective and effective way.

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