International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR)
ISSN: 2582-7618 (Online), Volume-4 Issue-5, August 2024

Advancements in Peptide Vectors for Cancer Therapy and Tumor Imaging: A Comprehensive Review

Nasser Thallaj

Abstract: ***The objective of this work is to synthesize novel peptide vectors for cancer therapy and tumor imaging. Cancer is a complex disease characterized by uncontrolled cell proliferation and the ability of cancer cells to evade programmed cell death. It poses a significant health problem globally, with increasing incidence rates observed in both industrialized and developing countries. Early detection of cancer is crucial for effective treatment, and various techniques such as observation, palpation, biopsies, and medical imaging have been employed for this purpose. However, these methods have limitations in terms of early detection, precision, and availability. Therefore, the development of new treatments and diagnostic methods is a pressing need in cancer research. This study focuses on the synthesis of peptide vectors that can serve as effective tools for cancer therapy and tumor imaging. Peptide vectors offer several advantages, including their ability to specifically target cancer cells, their potential for multivalency, and their capacity for non-viral vectorization. The research aims to design and synthesize peptide vectors that target αVβ3 integrin, a protein overexpressed in tumor cells and associated with tumor neo-angiogenesis. The vectors will incorporate the cyclodecapeptide scaffold RAFT as a structural framework and will be functionalized with the -RGD- ligand, known for its affinity to αVβ3 integrin. The incorporation of specific features such as oxime ligation and disulfide bridges will enhance the stability and functionality of the vectors. Additionally, the use of FRET (Förster resonance energy transfer) will enable imaging capabilities for tumor detection. Overall, this work seeks to contribute to the advancement of cancer therapy and tumor imaging by synthesizing novel peptide vectors with targeted specificity and imaging capabilities. The successful development of these peptide vectors could potentially lead to improved early detection, targeted drug delivery, and more effective treatment strategies for cancer patients.***

Keywords: Anti-cancer therapy, Tumour imaging, Tumour neo-angiogenesis, αVβ3 integrin, Non-viral vectorization, Cyclodecapeptide scaffold RAFT, -RGD- ligand, Multivalency, Oxime ligation, Disulfide bridge, FRET

I. INTRODUCTION

The field of oncology, dedicated to the study and treatment of cancer, continues to be an area of intense research and innovation.

Manuscript received on 30 June 2024 | Revised Manuscript received on 13 July 2024 | Manuscript Accepted on 15 August 2024 | Manuscript published on 30 August 2024.

*Correspondence Author(s)
Prof. Dr. Nasser Thallaj*, Department of Pharmaceutical Chemistry and Drug Quality Control, Faculty of Pharmacy, Al-Rachid Privet University, Damascus, Syria. E-mail: profthallaj@gmail.com, ORCID ID: 0000-0002-6279-768X

Cancer is a complex disease characterized by the uncontrolled proliferation of cells that can evade programmed cell death, leading to the formation of abnormal cell populations that can spread throughout the body. The development and progression of cancer involve intricate biological processes such as angiogenesis, tumor escape, and metastasis [1-2]. Despite significant advancements in cancer detection and treatment, it remains a global public health concern, affecting millions of people worldwide.

The primary objective of this work is to synthesize novel peptide vectors for cancer therapy and tumor imaging. Peptide vectors hold immense potential in the field of oncology due to their ability to specifically target cancer cells while minimizing harm to healthy tissues. These vectors can be utilized for both therapeutic and diagnostic purposes, offering a promising avenue for improving cancer treatment outcomes and early detection [2-3]. One of the key areas of focus in this research is anti-cancer therapy. Traditional methods of cancer treatment, such as surgery, radiotherapy, hormone therapy, and chemotherapy, have limitations in terms of efficacy and potential side effects. The development of new therapeutic approaches is crucial to overcome these challenges and improve patient outcomes. By designing and synthesizing novel peptide vectors, it is possible to enhance the delivery of therapeutic agents specifically to cancer cells, thereby increasing treatment effectiveness while reducing systemic toxicity [4-5]. In addition to therapeutic applications, the synthesis of peptide vectors also aims to advance tumor imaging techniques. Early detection of cancer plays a vital role in successful treatment outcomes. Current imaging techniques, such as radiography, ultrasound, MRI, and PET scans, have significantly improved cancer detection, but they still have limitations in terms of sensitivity, specificity, and accessibility [6-7]. By developing peptide vectors specifically designed for tumor imaging, it is possible to enhance the accuracy and precision of cancer detection, enabling early intervention and improving patient prognosis. To achieve these objectives, several key factors will be considered during the synthesis of novel peptide vectors. The targeting of specific molecular markers expressed on cancer cells is crucial for effective vectorization. One such marker of interest is the αVβ3 integrin, which is overexpressed in various types of cancer and plays a significant role in tumor angiogenesis. Incorporating ligands that can selectively bind to αVβ3 integrin, such as the RGD motif, into the peptide vectors can enable targeted delivery to cancer cells.

Multivalency, a concept that involves the presentation of multiple ligands on a single vector, will be explored to enhance the binding affinity and specificity of the peptide vectors [8-9]. By incorporating multiple copies of the targeting ligands or utilizing scaffolds with inherent multivalent properties, the peptide vectors can achieve stronger interactions with cancer cells, leading to improved therapeutic efficacy and imaging sensitivity. Furthermore, the design of the peptide vectors will incorporate strategies to enhance stability and minimize premature degradation. The use of disulfide bridges and oxime ligation can provide structural stability, allowing the vectors to withstand enzymatic degradation and maintain their integrity during circulation. Additionally, the incorporation of fluorescence resonance energy transfer (FRET) systems within the vectors can enable real-time monitoring of their behavior and interaction with cancer cells, providing valuable insights into their biodistribution and targeting efficiency [10-11]. The synthesis of novel peptide vectors for cancer therapy and tumor imaging represents a promising avenue for advancing the field of oncology. By specifically targeting cancer cells and improving the accuracy of cancer detection, these peptide vectors have the potential to revolutionize cancer treatment and management. Through careful consideration of factors such as ligand selection, multivalency, stability enhancement, and real-time monitoring, this research aims to contribute to the development of more effective and precise strategies for combating cancer [9-13].

II. VECTORIZATION IN ONCOLOGY

A. Cancer: Current Status

Cancer is a group of diseases characterized by an unlimited proliferation of cells capable of escaping programmed cell death (called apoptosis), which leads to the formation of a population of abnormal cells that can disperse throughout the body. These diseases appear in the different parts of the human body: tissues, organs or cells associated with these tissues. There are therefore different types of cancers with distinct and diverse courses [13-14].

i. Complex Pathology with an Increasing Frequency

When cancer develops, there are three independent essential stages: initiation, promotion, and tumor progression. Carcinogenesis is accompanied by the establishment of other biological processes such as angiogenesis, tumor escape and the spread of malignant cells, explaining that the genesis of cancer is a complex and often long process [14-15].

Initiation is the stage in which a given cell acquires a permanent genetic abnormality. Initiators include viruses, chemicals and radiation. An "initiated" cell has not acquired growth autonomy but transmits to its offspring the irreversible genetic mutation resulting from the initiation. The damage created within an initiated cell can go unnoticed if other gene events do not take place to stimulate tumor progression. At this stage, the proliferating cells retain the specific characteristics of the tissues from which they originate [15-16].

The next step is tumor promotion. It corresponds to the clonal proliferation of "initiated" cells leading to cell clusters or benign tumors, following stimulation by tumor promoters that generally have no direct action on DNA (hormones, chronic inflammation, growth factors, etc.). These cells actively proliferate in the original tissue, but they have lost their differentiated cell characteristics [17].

The final stage is tumor progression resulting from one or more additional mutations that convert benign tumors to malignancies. The tumor cells then become able to invade the surrounding tissues and metastasize in organs far from the primary tumor. This last stage of evolution is called the "invasive cancer" stage [18].

The cancer cell implements mechanisms that are organized in an apparently sequential and precise way to be able to successfully implant, develop and progress. For cancer to develop, it is now accepted that changes must occur in the DNA of a single cell, whether through mutations or inappropriate chromosomal rearrangements [19]. Those

Genetic changes can be either spontaneous or induced by triggers of external origin. The gene alterations responsible for the development of cancer mainly affect the genes involved in cell cycle progression, cell adhesion phenomena, as well as DNA damage repair processes. The cancer cell then acquires certain properties that give it its malignancy (Figure 1) [20-22].

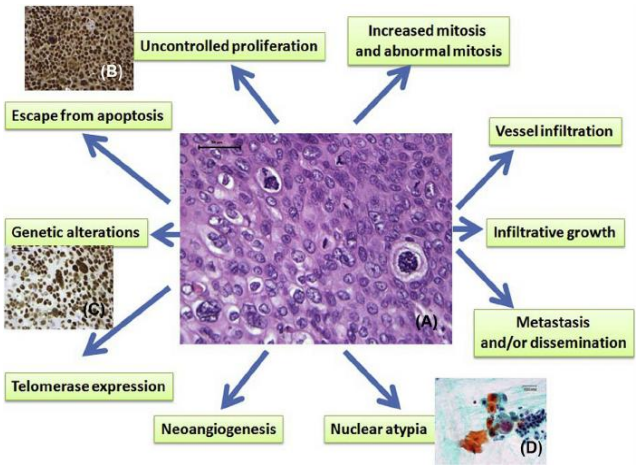


Figure 1: Properties Responsible for the Malignancy of Cancer Cells

Neither detected nor repaired by the body's monitoring and repair systems, genetic alterations are directly responsible for the uncontrolled proliferation of cancer cells, whether in the case of solid tumors or in that of "monoclonal" tumors of circulating cells. This disruption of some of the billions of cells that make up multicellular beings is a common pathology regardless of continent, ethnic origin or culture [23].

Today, cancer has become a public health problem in industrialized countries but also in developing countries. In 2000, more than 4.4 million cases were diagnosed in Asia, 2.8 million in Europe, 1.4 million in North America and 627,000 in Africa. Cancer accounted for 12% of deaths worldwide [24]. With an increasing number of new cases diagnosed in Europe estimated at 3.2 million in 2006, the incidence of cancers unfortunately seems to be on the rise.

This can be explained by the increase in life expectancy and the increase in risky behaviours (intensive exposure to UV rays, diet, tobacco, alcohol). However, this increase does not concern all types of cancer. Indeed, in industrialized countries, some cancers have declined significantly, thanks in particular to improved hygiene conditions (in the case of stomach cancer) and early detection (in the case of cervical and colon cancers) (Figure 2) [25].

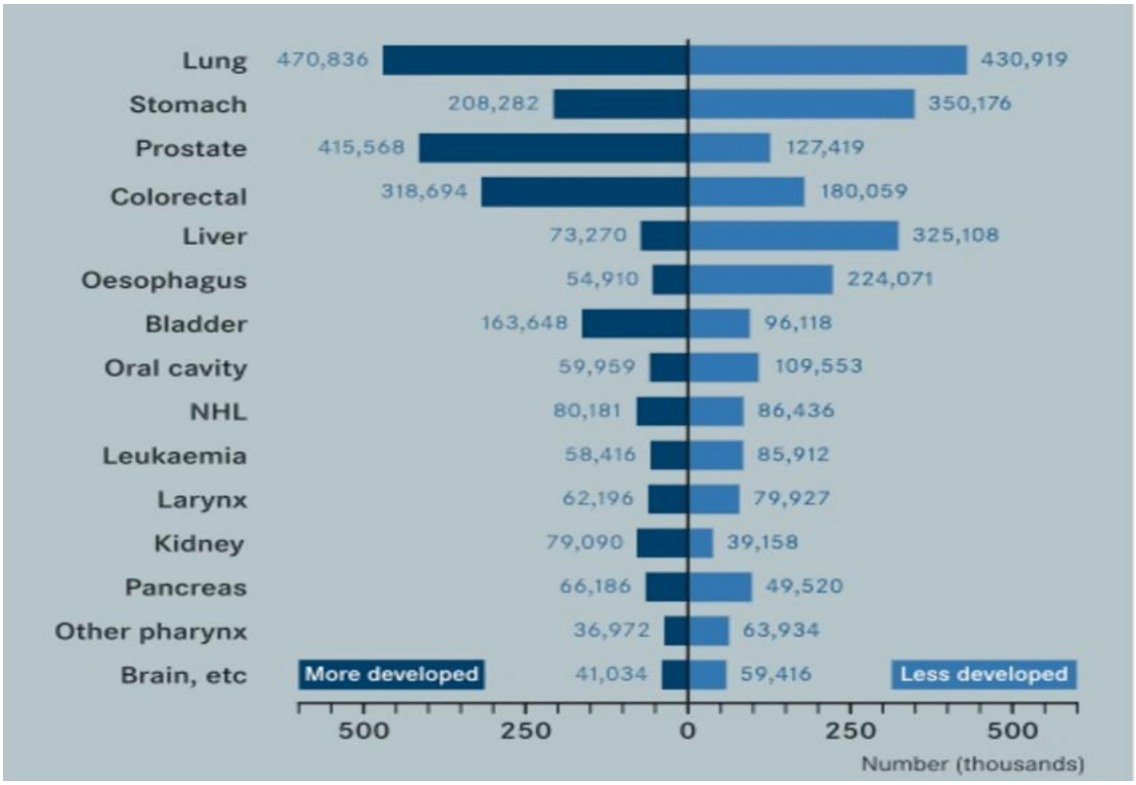


Figure 2: Comparison of the Most Common Cancers in the Most Developed and Least Developed Countries in 2000. Nhl = non-Hodgkin Lymphoma

Nevertheless, with more than 26 million people currently suffering from cancer worldwide, the development of new treatments and new diagnostic methods is at the center of research by the pharmaceutical industries and public research [26].

ii. Traditional Methods of Cancer Detection and Treatment

Cancer Detection

Early detection of cancers is essential to enable effective therapy. The number of tumor cells to be destroyed is lower and the cells do not yet possess all the genetic abnormalities that allow them to resist or adapt to therapeutic treatments. Conversely, the healing successes for well-established tumors are very limited. A number of techniques are now available for cancer detection [27].

Traditionally, the detection of tumors is done by observation and palpation. This technique does not allow for early detection. Indeed, the tumors detected are of the order of a centimeter and correspond to an advanced stage of cancer. Cell samples or biopsies are used to confirm the malignancy of a tumour, to determine its tumour stage and to carry out early detection of certain cancers (detection of precancerous lesions in cervical cancer). The conventional imaging methods used, radiography (X-rays), ultrasound (ultrasound), MRI (magnetic resonance), nuclear techniques (radioactivity) and endoscopy are effective techniques for detecting tumors of a few millimeters. They are particularly useful for the prevention of breast, lung and colon cancer. Other detection techniques have been developed, such as the search for genetic markers that analyze defective or missing genes ("hereditary" cancers). The search for serum markers, substances produced by tumors and released into the blood (hormones, enzymes, antigens) makes it possible to detect the presence of cancer cells and monitor their evolution, but only concerns a small number of cancers [28-29].

Among these different detection methods, medical imaging techniques have made enormous progress over the last thirty years. CT-Scan (or computed tomography) using X-rays, makes it possible to make cross-sections of the human body and to obtain precise images of an organ. PET Scan (Positron Emission Tomography) based on the detection of positron-emitting radioelements makes it possible to locate tumors with very high precision (exact shape and contours). The most commonly used radioactive tracer is 1 F-fluoro-deoxy-glucose (18F-FDG), a radioactive sugar analogue of glucose. This accumulates specifically in areas of high metabolic activity: tumors, metastases, brain. This functional imaging makes it possible to detect micrometastases invisible on X-rays or to monitor the evolution and response to anti-tumor treatment. This type of imaging is used, for example, to monitor the response of gastrointestinal tumors to treatment with Glivec® (Figure 3). Prior to treatment, the PET Scan shows intense 18F-FDG staining in the liver (single arrow), stomach (double arrow) and normal urinary excretion of the compound.

The CT scan confirms the presence of these lesions. One year after the start of therapy, the images show that the tumor masses have regressed and are no longer labeled with 18F-FDG indicating an absence of tumor activity and a response to therapy [30-31-32].

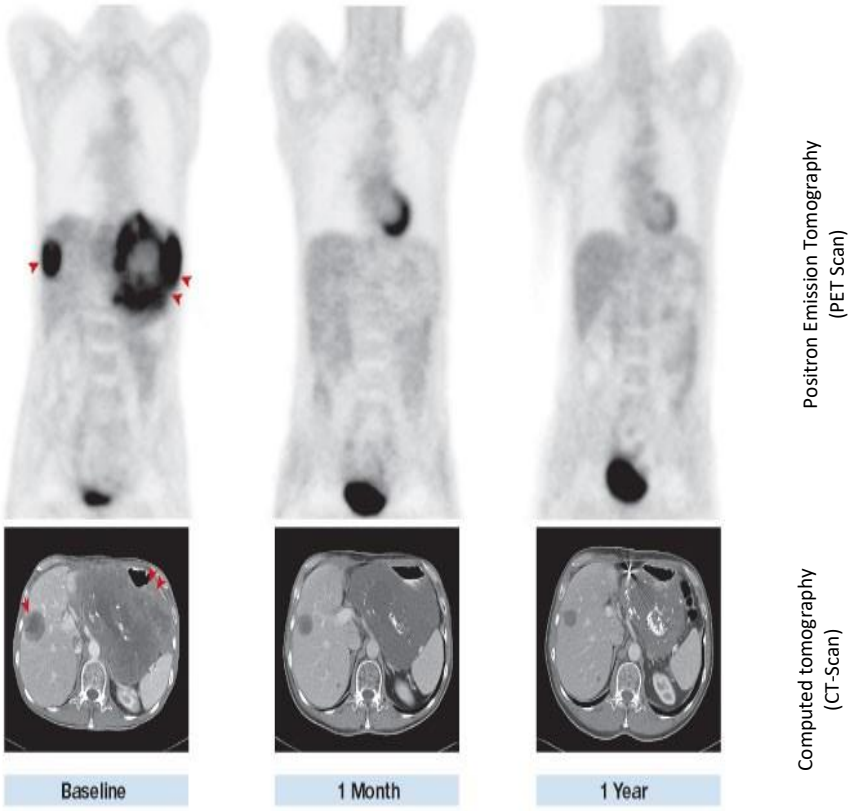


Figure 3: 18FDG PET SCAN and CT-Scan of the Response of Gastrointestinal Tumors to Glivec Treatment

Unfortunately, this type of imaging has certain constraints. The half-life of 18F is short (110 min) and requires extremely fast use of 18F-FDG. Routing between remote production and use sites remains a major problem with this type of marker. On the other hand, the PET Scan cannot be used to visualize tumors in organs with a high metabolism such as the brain or in organs involved in marker removal. However, these medical imaging techniques represent a considerable advance for oncology in the early detection of tumors, but remain very expensive technologies and only present in specialized centers [33].

B. Cancer Treatments

Once cancer is diagnosed, different methods are available to practitioners to treat the disease. Surgery is conventionally used to remove the primary tumor and allows the cure of a large number of early cancers. It is currently the most effective method for small tumor foci without metastasis, but remains problematic. Removing all cancer cells and preventing their spread during surgery can be difficult. Also for local treatments, radiotherapy, based on the action of "ionizing" radiation (X, alpha, beta or gamma) is used to treat tumors but poses the problem of the toxicity of "ionizing" radiation on the surrounding healthy tissues. Hormone therapy is prescribed to reduce hormone levels in the blood in hormone-sensitive cancers. Finally, chemotherapy corresponds to the use of natural or synthetic compounds that block cell proliferation (cytostatic treatments) or kill cells (cytotoxic treatments) [34].

Conventional chemotherapy uses various products grouped into several families, the most commonly used of which are described below. Alkylating agents represent the oldest class of anti-cancer drugs along with mustard gas derivatives (Cyclophosphamide). They act directly at the DNA level by establishing intra- or inter-strand bridging, thus blocking the cell cycle. Platinum salts (Cisplatin®) are also part of this class. Anti-metabolites, such as 5-fluorouracil, represent a second family of anti-cancer drugs that inhibit enzymes involved in DNA synthesis or are incorporated into DNA. We can then mention the family of topoisomerase inhibitors (I and II) that prevent DNA replication and cell cycle progression. Doxorubicin (anthracycline), which is still widely used in chemotherapy, is part of this class of anticancer drugs. A final major class of anticancer drugs is that of spindle "poisons", which bring together molecules that can act on the polymerization or depolymerization of the proteins that make up the microtubules of the mitotic spindle, thus preventing the continuity of the cell cycle. This family includes the derivatives of the alkaloids of periwinkle (Vinorelbine) and taxanes (Docetaxel). In addition, in the treatment of certain cancers, drug synergies have been shown by combining molecules belonging to different families (multidrug therapy) [35].

However, these chemotherapies are treatments that act specifically on all the cells that proliferate. They can therefore destroy healthy cells, especially cells with a high turnover rate such as blood cells. Thus, chemotherapy agents can lead to haematological toxicity (overall loss of blood cells, loss of iron, or loss of blood platelets), alopecia (hair loss), gonadal toxicity and cardiac toxicity (characteristic of antracyclines) to which can be added the development of secondary cancers. In view of the many toxicities associated with chemotherapy, palliative treatments are administered in parallel in order to allow the patient to survive (treatments stimulating the production of hematopoietic cells) or to limit side effects (pain, fatigue, vomiting) [36].

Surgery, radiotherapy and chemotherapy can be combined and used simultaneously. These combined methods often give good results in cancer remission without relapse. Nevertheless, whatever the treatment (surgery, chemotherapy, radiotherapy) it is often heavy and traumatic for the cancer patient [37].

C. Guidance in Cancer Detection and Treatment

Even today, the majority of tumors are diagnosed at advanced stages of the disease and are incurable, hence the need to develop increasingly refined and precise techniques to detect a large number of cancers as early as possible. The search for more efficient diagnostic means is turning to the development of probes capable of detecting the early stages of tumor progression and visualizing metastases of less than a millimeter in size. We are therefore moving towards molecular imaging, which consists of using probes that recognize specific biological phenomena of cancers and make it possible to image them using increasingly efficient medical imaging techniques [38]. Cancer treatments make it possible to reduce the volume of the tumor and improve the patient's survival (cure of cancers detected early). Chemotherapy, despite its toxicity, appears to be the only therapy to treat metastatic tumors. Unfortunately, in advanced stage cancers, it rarely cures. Here too, we are moving towards the development of new molecules targeting the specific abnormalities of cancer cells to limit their growth or to destroy them and reduce the toxicity of treatments. There is therefore a strong demand in oncology for earlier detection methods and less toxic and more effective therapeutic treatments. In order to meet this demand, research is increasingly moving towards targeted strategies for both diagnosis and therapy [38].

D. New targeted Strategies in Oncology

Numerous advances made over the last twenty years in the fields of molecular and cellular biology and genomics have contributed to a better understanding of the molecular mechanisms involved in the tumor phenomenon. This has enabled the development of new molecules targeting specific abnormalities in cancer cells [39].

i.Major targeted strategies

At present, several major targeted approaches have been developed to try to block the mechanisms involved in tumor development and progression. Targets can be intracellular (kinases, transferases, genes) and extracellular (growth factor receptors, cellular antigens localized on the surface of cancer cells or growth factors of the tumor extracellular environment) [39].

Genentech, EU

Table 1: Monoclonal Antibodies Currently Used in Oncology

L-Histidine (his/h) Commercial	L-Isoleucine (Ile / I)	Origine	L-Leucine (Leu / L)	L-Lysine (Lily/K)	L-Methionine (Met/M)
L-Phenylalanine (Phe/F)	L-Proline (Pro / P)	Humain	L-Serine (Ser/S)	L-Threonine (Thr/T) Cancer du colon	L-Tryptophan (Trp/W)
L-Tyrosine (Tyr/Y)	L-Valine (Val/V)	Humanisé	VEGF	2004 Cancer du colon	Genentech, EU
Erbitux™	Cetuximab	Chimérique	EGFR	2004 Cancer du colon	ImClone Systems, EU
Bexxar™	Tositumomab	131 I Souris	CD20	2003 Lymphome	GlaxoSmithKline, EU
Zevalin™	Ibritumomab	90 Y Souris	CD20	2002 Lymphome	Biogen Idec, EU
CamPath™	Alemtuzumab	Humanisé	CD52	2001 Leucémie	Ilex Genzyme, EU
Mylotarg™	Gemtuzumab-Ozogamicin	Humanisé	CD33	2000 Leucémie	Wyeth, EU
Herceptin™	Trastuzumab	Humanisé	Her2/neu	1998 Cancer du sein	Genentech, EU
Rituxan™	Rituximab	Chimérique	CD20	1997 Lymphome	Genentech, EU

Other compounds capable of inhibiting tumor neo-angiogenesis are in clinical studies, such as synthetic small molecules (Sorafenib, Sunitinib, Vatalanib and Thalinomide), a peptide (Cilengitide), recombinant proteins (Endostatin, Angiostatin) and a ribosym (Angiozyme). In addition, several small molecules that inhibit tyrosine kinase activity overexpressed in certain cancers have been validated (Imatinib, Gefitinib, Erlotinib). Other molecules that inhibit kinase or transferase activity specifically involved in the cell cycle and signal transduction of cancer cells are also under clinical investigation. As for the strategy targeting gene abnormalities in cancer cells, it is the subject of much research, but raises the problem of the lack of vectors allowing efficient transfection. Despite research efforts, the molecules currently validated in oncology are mainly compounds acting on extracellular targets or small organic molecules diffusing through the cell membrane to act on intracellular targets [40]. These molecules have a direct action on abnormalities in cancer cells by inducing their cell death or limiting their growth. Another possible avenue in the development of targeted strategies is the design of evolved macromolecules whose properties come from the assembly of different elements such as targeting agents, effector elements, molecular supports, and vectorization elements.

ii.Major Families of Cancer Targeting Agents

Recently, new avenues of research are moving towards the development of multifunctional systems combining tumor targeting properties and effector properties (therapy/diagnosis). Targeting can be ensured by molecules recognizing specific abnormalities of the extracellular membrane of cancer cells (cellular antigens, membrane receptors) or specific abnormalities of tumor tissue (tumor neo-vascularizations). The effector properties result from the coupling of therapeutic or detection elements to the targeting agent. These ligands are used to improve the pharmacoguiding properties of vectorization systems ensuring the targeted transport of effector elements [41].

E. Monoclonal Antibodies and Antibody Fragments

In general, monoclonal antibodies and antibody fragments have a very high specific affinity for certain elements presented on the surface of cells. In the case of recognition of elements presented by cancer cells, they become targeting agents of choice to reach tumor areas. Classically, once bound to cancer cells, these antibodies can induce an immune response (stimulation of the immune system) or another cellular response (endocytosis, proliferations). In order to provide or increase the therapeutic efficacy of antibodies, protein engineering techniques or chemical modifications have made it possible to arm these antibodies with toxins, radioactive isotopes, molecules used in traditional chemotherapy or cytokines. These targeting agents make it possible to specifically concentrate therapeutic agents at the tumor level, thus reducing their toxicity. The combination of antibodies and conventional chemotherapy molecules has been widely described in the literature. Among the ten monoclonal antibodies used in cancer therapy (Table 1), three were conjugated: Tositumomab, Ibritumomab and Gemtuzumab-Ozogamicin. In the case of Gemtuzumab-Ozogamicin (cytotoxic compound coupled to a humanized antibody), antibody/receptor recognition results in endocytosis of the antibody-drug complex and release of the active drug into cancer cells (Figure 4). This system is very effective in certain leukemias with acceptable toxicity [42].

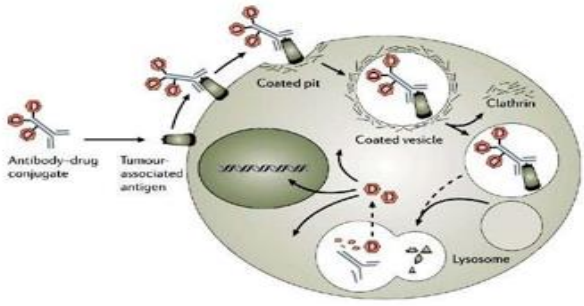


Figure 4: Principle of Action of Antibody Conjugates for Therapy

The coupling of a radioactive element to an antibody allows targeted irradiation of several layers of cells at the tumor level. The isotopes used are generally β emitters with a range of 1 to 7 mm. The radiolabeled antibody can thus irradiate cells that the antibody/radioelement complex has not been able to reach or cells that do not express the antigen. The efficacy of radioimmunotherapy (BexxarTM and ZevalinTM) has been demonstrated in relapsed and chemotherapy-resistant lymphomas. The therapeutic results obtained with these antibodies coupled with radioelements have been shown to be better than the naked antibody, but these conjugates are haematological toxic.

At the same time, for diagnostic experiments, antibodies were combined with radiotracers. They were also coupled with chromophores and provided very clear images of small tumor areas (less than 1 mm in diameter) that can be used in intraoperative situations. Although antibodies are highly effective as targeting agents, they have some disadvantages. Their large size leads to low intracellular penetration and intra-tumoral diffusion. They also have a strong immunogenic character if they are not human. In order to circumvent these drawbacks, antibody engineering has enabled the development of small antibody fragments on the one hand and humanized modified antibodies on the other. Their combination with detection or cytotoxic elements makes it possible to design effective macromolecules for the diagnosis and treatment of certain cancers [42].

F. Ligands of Tumor Membrane Receptors

A large number of membrane receptors are overexpressed on the surface of cancer cells. The natural ligands of these receptors bind to them with very good affinity and selectivity. They therefore emerged as potential targeters. The nature of these ligands is varied: protein (transferrin), saccharide (hyaluronic acid) or organic (folic acid). In addition, phage display33 and "One Bead One Compound" peptide library screening methods have made it possible to select new peptide ligands. Structural and chemical modifications of various ligands have optimized their properties (affinity, specificity) in order to obtain very good tumor targeting agents. Modeling studies based on ligand-receptor interactions are performed to develop and design synthetic ligands of high affinity. Today, there is therefore a panel of natural and synthetic ligands that can be used to direct therapeutic and detection elements at the tumor level. Thus, the coupling of the folate ligand to radiomarkers such as 111In, 99mTc and 65/68Ga allowed a significant in vivo accumulation of conjugates in tumors and kidneys and relatively low in other tissues [43].

G. Multivalence of targeting agents

Biological systems often involve multi-contact interactions: this is the case in particular during the management of a pathogen by macrophages during the immune reaction or during platelet adhesion to sites of inflammation in the coagulation phenomenon. These multiple interactions are responsible for specific biological responses, different from responses induced by a single contact. In the case of receptor targeting, multivalent ligand presentation systems can increase targeting efficiency addition, a modulation of the physiological response induced at the level of a receptor has also been observed as a function of the multivalence of the ligand (intensity and nature of the response). For example, a multivalent ligand presentation may induce a ligand/receptor complex endocytosis phenomenon not observed in the case of the monovalent ligand. There are several possible mechanisms that can account for the contribution of multivalence in a ligand-receptor recognition phenomenon (Figure 5) [44].

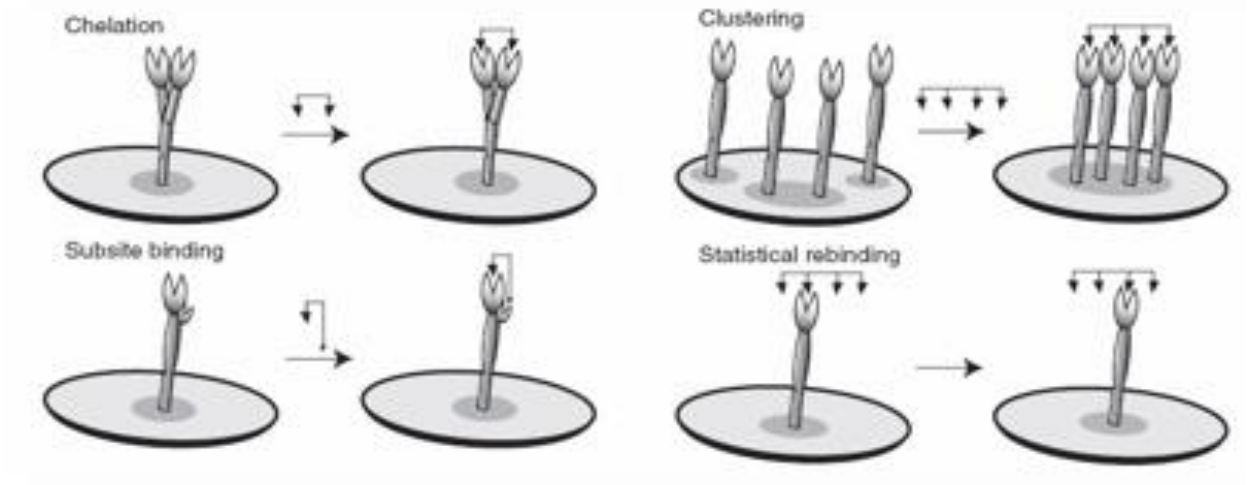


Figure 5: Mechanisms Involved in the Recognition of a Multivalent Ligand by its Target

The chelation effect and the subsite binding effect result from multiple contacts between the multivalent ligand and a receptor with multiple binding sites. The statistical rebinding effect corresponds to the ease of a multivalent ligand to bind again to the same receptor thanks to the high local concentration of ligand in the vicinity of the receptor. These three effects allow a decrease in the dissociation of the ligand/receptor system and increase the apparent affinity of the multivalent ligand for its receptor. Indirectly, the increase in the affinity of the ligand for its receptor can be the cause of endocytosis of the receptor/ligand complex. The clustering effect corresponds to a change in the proximity and orientation of several receptors caused by their binding to the multivalent ligand. This phenomenon can affect the signaling functions of receptors and induce cellular phenomena such as, again, endocytosis of the receptor/ligand system (Figure 5) [45]. Some multivalent systems can show a significant gain in terms of affinity and specificity for targeting but also a gain in terms of modulation of the biological response, with in particular induction of endocytosis. This last property of cellular internalization of multivalent systems is a special case. Traditionally, targeting agents concentrate effector elements near cancer cells, but few of them have vectorization properties. In the case where the target of the effector element is intracellular, it must be associated with a vectoring agent [45].

i.*Major Families of Cell Vectors*

There are mainly three main families of cell vectors: viral vectors, lipid systems and peptides or translocation proteins. Originally, these systems were particularly developed for gene therapy applications. Today, their use is generalized to all strategies requiring cellular entry.

H. Viral Vectors

Viruses are natural, highly evolved vectors that are highly efficient in penetrating host cells, transferring their genetic material and exploiting the cellular machinery for replication. To use them in therapy, non-pathogenic systems have been developed. In order to circumvent the lack of selectivity of these vectors for tumors, several strategies have been developed. Targeting agents were coupled to their surface, including bi-functional antibodies that recognize a protein in the virus envelope and a target cell receptor (Figure 6). This technique made it possible to introduce a "drug gene" into a cancer cell via an inactivated virus.

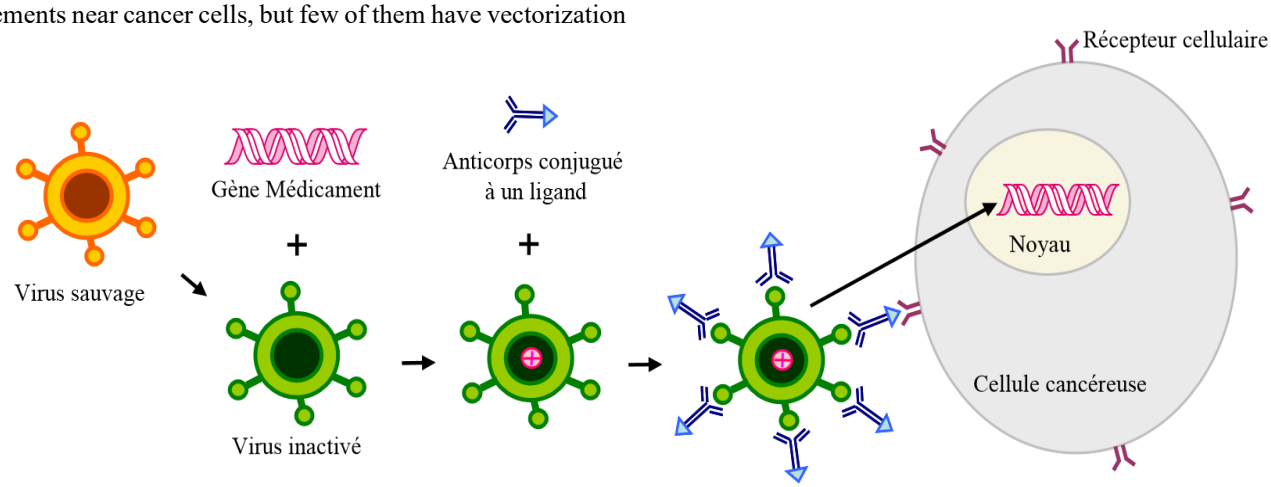


Figure 6: Principle of use of Targeted Viral Vectors for Gene Therapy

Targeting elements have also been introduced by genetic engineering by modifying viral fibers. The latter strategy was used to make a virus present peptides targeting receptors on the surface of cancer cells. Despite non-pathogenic viral material, the insertion of a gene into the genome of a host cell remains delicate and should not have an influence on the expression of other genes (genotoxic insertions likely to induce severe side effects) [45].

I. Lipid Systems, Nanoparticles

Thanks to the development of nanotechnologies, galenic research is at the origin of new delivery systems allowing the entry of active molecules into the cell. These systems also protect active molecules from degradation and control their release. These nanoparticles are mainly lipid systems. First-generation lipid vectors are vesicles formed by one or more layers of phospholipids. In order to limit their degradation by the immune system (opsonization), these were coated with hydrophilic polymers (second-generation vectors). In order to direct these vectors, they have been decorated with ligands (antibodies, peptides, sugars, folic acid). They are then able to selectively recognize antigens or cell receptors. These vectors have proven to be versatile tools because they are capable of vectorizing a wide range of effector elements with different physicochemical properties (hydrophilic, hydrophobic) (Figure 7). Thus, they have been used to deliver various elements inside cancer cells such as SiRNAs or cytotoxic agents. Many targeted lipid vectors show very satisfactory preclinical results. In 2007, a lipid complex containing fragments of antibodies directed against the transferrin receptor and vectoring a "drug gene" entered clinical phase I [46].

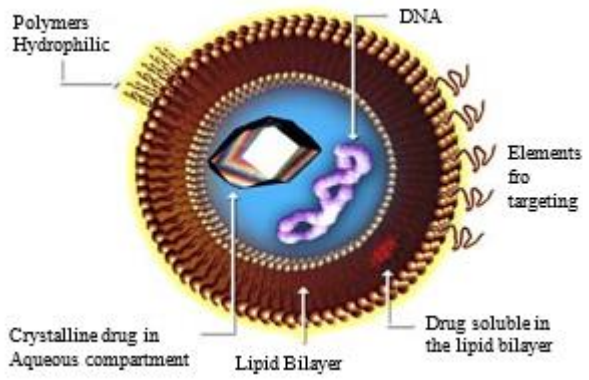


Figure 7: Diagram of Liposomes Targeted for Vectorization to Cancer Cells

Recently, polymeric nanoparticles have also been used for gene therapy but have shown only low transfection efficiency.

J. Translocation Peptides and Proteins

Synthesis *Cell Penetrating Peptide*) or small proteins (PTD, *Protein Transduction Domain*), with the property of crossing the cytoplasmic membrane of eukaryotic cells, have been used to vectorize macromolecules. Some of these sequences derive from natural proteins such as surface proteins of viruses and bacteria (Table 2).

Table 2: Examples of Translocation Agents

Agents de Translocation	Séquence d'acides aminés	Origines
Pénétratine	Rqikiwfqnrrmkwkk	Antennapedia
Tat (48-60)	Grkkrrqrrrppqc	HIV-1
Transportan	Gwtlnsagyllkinlkalaalakkil	Galanine et Mastoparan
Peptide amphiphile	Kalklalkalkaalkla	Synthèse
Arg_9	RRRRRRRRR	Synthèse

These translocation agents have been conjugated with a wide variety of compounds. Thus, they have enabled the cellular vectorization of small molecules, peptides, proteins, nucleic acids and nanoparticles. These systems show a high efficiency of *in vitro* and *in vivo* vectorization in small animals but their internalization mechanisms are not always clearly established [47]. Cell vectors as well as some multivalent targeting molecules are efficient systems for introducing various elements inside cells. The bifunctionality of the "multivalent targeting agents" vectors makes them particularly interesting, which has led many research teams to design molecules of this type.

K. Tools for Multivalent Vector Design

Over the past twenty years, the design of polyfunctional macromolecules intended to present ligands in a multivalent manner and to vectorize molecules of interest has led to the development of multivalent carriers and appropriate conjugation methods.

i.Multivalent Media for Targeting and Vectorization

The media used for targeting and vectorization, natural or synthetic, are called templates, molecular frames or "templates". These are mainly proteins, polymeric systems, nanoparticles, viruses or small molecules. Developed or modified by chemists, these objects are used to multivalently present ligands (recognition of cancer cells) and vectorize therapeutic and/or diagnostic elements [47].

L. Protein

Some globular proteins (Immunoglobulin, Albumin, Avidin) have been used as carriers for the presentation of several copies of a ligand. Ligands are typically introduced to the nucleophilic functions of the protein (lysine, histidine, cysteine or N-terminal residues) via functional spacer arms. This approach leads to an undefined chemical composition structure with an imprecise valence index, with no control of the distance between ligands or their orientation. In addition, ligands can also be introduced using the intrinsic properties of certain proteins. Avidin can be used for the multimeric presentation of ligands thanks to the combination of four biotinylated ligands. This strategy allows access to a chemical structure whose valence parameters are better controlled [48].

M. Lipid systems, Nanoparticles, Viruses

Due to their size and the possibility of associating different elements at the periphery, viruses, lipid systems and nanoparticles are, once decorated with ligands, multimeric presentation systems.

Nanoparticles commonly include objects between 1 and 100 nm in size. These systems can have different compositions (gold, quartz crystals, silica) with specific intrinsic properties (fluorescence, magnetism, X-ray opacity) and can be functionalized with multiple copies of a ligand. For example, inorganic " *quantum dot* " fluorescent nanocrystals multimerically presenting a tumor-addressing peptide (directed against a surface protein, nucleolin) have been used to vectorize therapeutic elements, such as siRNAs (Figure 8). These systems constitute a " *See and Treat* " approach that is particularly interesting in the study of new multivalent conjugates. They allow the evaluation of their cell vectorization efficiency, their mode of action, their pharmacokinetics and their bioavailability. The disadvantage of these systems is an inaccurate control of the number of ligands presented [49].

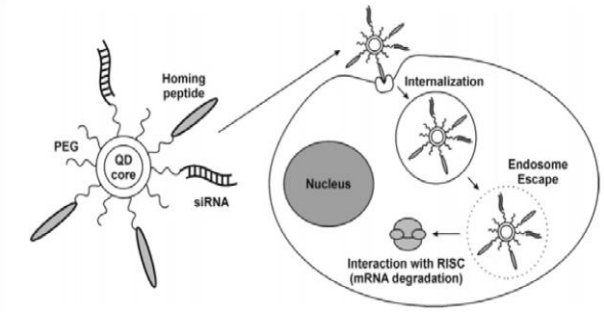
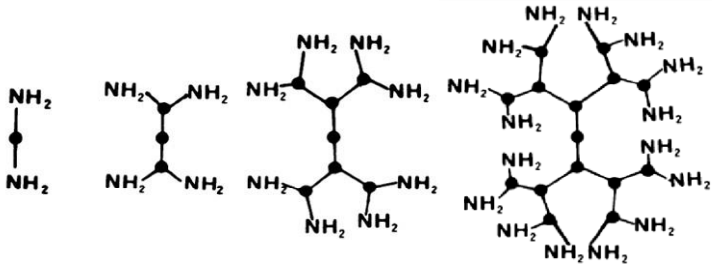


Figure 8: Diagram of Targeted Quantum Dot Nanoparticles for siRNA Vectorization

N. Polymeric Systems

Conventional polymerization techniques lead to multivalent ligands of large sizes, in which the ligands are distributed along a polymer. As polymerization reactions are difficult to control, the size of the macromolecule obtained is imprecise and its valence index is indeterminate [50]. Other controlled polymerization systems allow the synthesis of structures of defined size. These polymeric systems (dendrimers calixarenes, cyclodextrins) are used as molecular carriers for the multimeric presentation of ligands. example, MAP (Multiple Antigen Peptides) systems, originally developed to present multiple copies of an antigenic motif, are easily adaptable to the presentation of various ligands. These structures are based on a branched polylysine backbone whose amine functions of lysine residues (αNH2 and εNH2) constitute attachment points allowing access to multivalent systems with different valence indices (2, 4, 8, 16...) (Figure 9).



- *Peptide Backbone of Lysine Residues*

Figure 9: Diagram of Multiple Antigen Peptides

The possibility of using orthogonal protective groups for amine functions makes these systems particularly versatile. Indeed, the combination of different types of molecules allows the introduction of several functionalities. These multivalent architectures allow control of ligand density but not of macromolecule conformation (ligand orientation and distance between variable ligands).

O. Low Molecular Weight Molecules

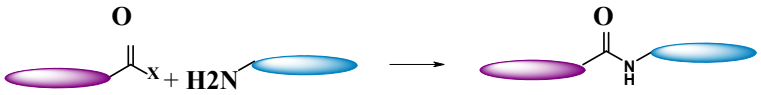
Some molecules of low molecular weights have also been used as molecular frames. The nature of these small compounds is very varied (monosaccharides, steroids or other small organic molecules) and they all have several chemical functions allowing the coupling of ligands. These molecules include objects that can have 2 to 10 copies of the ligand and whose size does not exceed 1000 Da. They therefore allow a control of the valence parameters. In addition, the structural properties of some frames also allow for ligand orientation control. Chemists therefore have a wide range of multivalent supports at their disposal to build new vectors for oncology. The carrier is chosen according to the desired biological effects: cell recognition, induction of a signaling pathway, induction of endocytosis [51].

i.Conjugation Methods for Macromolecule Assembly

There are a large number of possible chemical methods for assembling different elements to the multivalent support: traditional and chemoselective chemical ligations.

P. Traditional Chemical Bonds

Traditional chemical bonds make it possible to assemble two protected fragments: only the chemical functions involved in conjugation must be reactive. This therefore implies the protection of all the other functional groups, a constraint that is often limiting in biomolecule engineering. Among these bonds, the amide bond is conventionally used for the assembly of protected peptide fragments (Figure 10), but it is unsuitable for the conjugation of protected fragments of different origins (peptides, oligonucleotides, sugars) [52].



Protected Fragment Protected Fragment with a function With Conjugated Activated Acid an amine function

Figure 10: Schematic Diagram of the Coupling of two Elements by Peptide Bond

Indeed, the standard chemistry of the different biomolecules is not entirely compatible and does not allow the use of this type of bond. For example, in the case of a peptide-oligonucleotide coupling, the peptide fragments are deprotected by treatment in an acidic medium, a condition in which the oligonucleotides are unstable [53].

Q. Chemoselective Bonds

On the other hand, chemoselective methods offer a particularly interesting alternative to traditional syntheses for the conjugation of biomolecules from different families and the construction of complex conjugates of high molecular weight. They are based on the converging assembly of unprotected polyfunctional fragments of different types (peptides, proteins, saccharides, nucleic acids), both of which carry chemical functions with a reciprocal and specific reactivity ("complementary" electrophilic and nucleophilic functions). The formation of the chemoselective bond takes place mainly in an aqueous medium without coupling reagent. The ligation of two unprotected synthons makes it possible to circumvent the problems encountered during classical syntheses (low solubility and difficult purifications of protected fragments) and to considerably simplify synthesis strategies. There are different chemoselective methods involving various chemical functions: cycloadditions (1,3-dipole cycloadditions and Diels-Alder reactions), reactions involving electrophilicity of the carbonyl function (formation of oxime, hydrazones and thiosemicarbazones bonds), reactions involving thiol function (formation of thioether and disulfide bonds), reactions involving thiocarboxilic acid or thioester functions (formation of thiocarboxilic acid or thioester functions). thioester and amide bonds) and the reaction involving phosphine and azide functions (Staudinger ligation) (Figure 11).

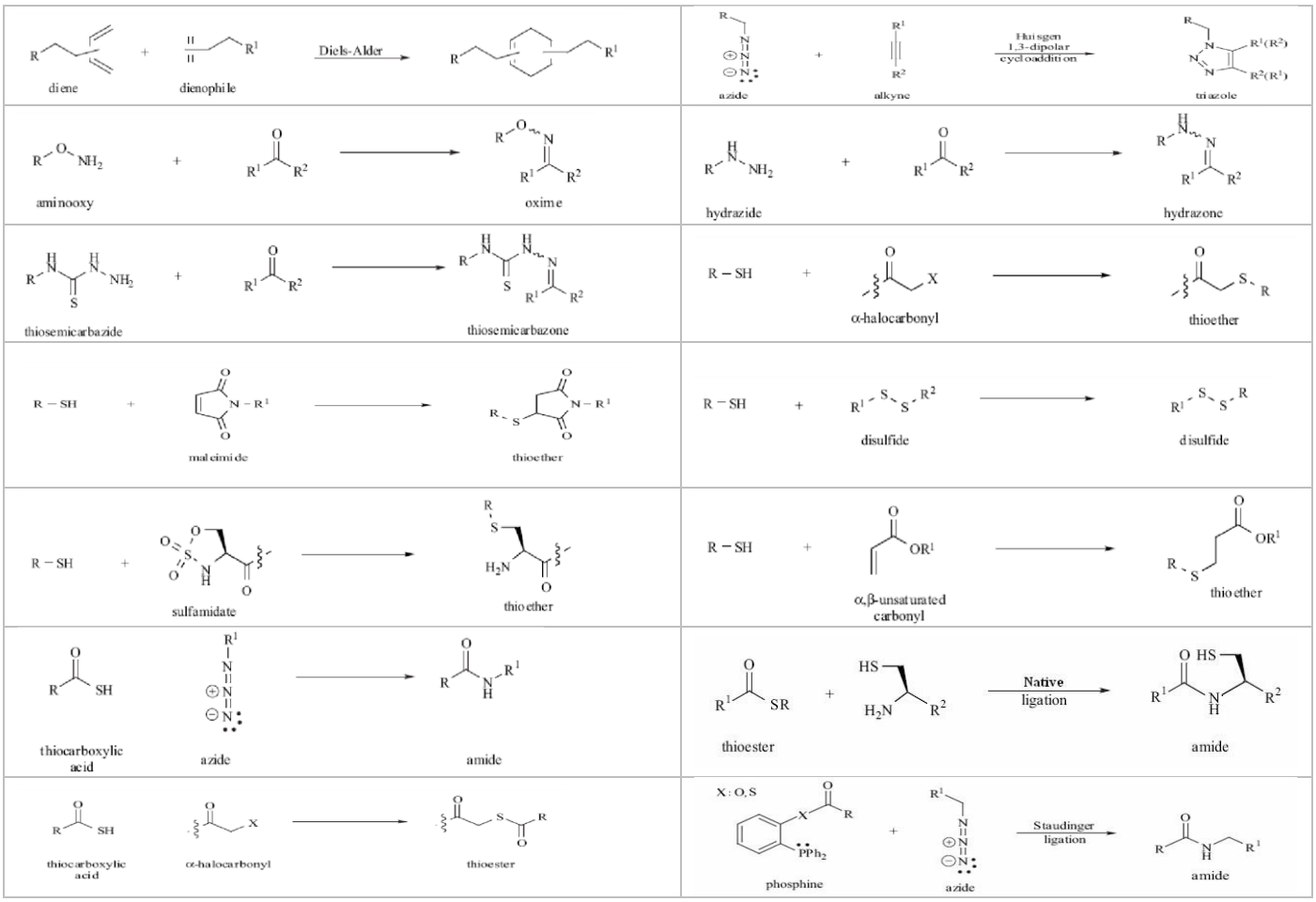


Figure 11: Diagram of the Different Chemoselective Reactions

Most of the bonds obtained are stable under physiological conditions and only some have stability properties dependent on the surrounding conditions (pH, enzymes). For example, under intracellular conditions, the disulfide bond can be reduced and cut. This rupture can be advantageously used in "pro-drug" strategies with intracellular release of the therapeutic elements. strategies are currently used for a number of anti-cancer drugs, such as the EC 145 conjugate (pro-drug folate/vinblastine using a disulfide bridge) which has successfully passed Phase I clinical studies [54].

III. DEFINITION OF THE RESEARCH PROJECT

The design of molecules capable of recognizing and focusing specifically in primary and metastatic tumor foci is essential for the development of more efficient diagnostic methods and more effective and better tolerated anti-tumor agents.

Although complementing the therapeutic arsenal against cancer, the new targeted strategies have certain limitations. The major problem with these strategies is that they are limited to a few tumor cell types. Indeed, by targeting a particular abnormality, these treatments are effective on cancers with this abnormality. Hence the need for an in-depth study of the characteristics of the tumor before prescribing treatment to the patient. The strategy corresponding to targeting tumor neo-angiogenesis seems much more generalizable since it is applicable to all vascularized tumors. Thus, it seemed very interesting to us to explore this path [55]. To this end, our work has been devoted to the design of synthetic vectors based on an original molecular chassis allowing a multimeric presentation of ligands of an overexpressed receptor at the tumor level for diagnostic and therapeutic applications against cancer.

A. Integrin αVβ3 Targeting Strategy for Tumor Neoangiogenesis

As detailed previously (Section I.1.1), during its development, a solid tumor acquires certain properties responsible for its malignancy, including the ability to induce neo-angiogenesis.

i.Tumor Neoangiogenesis

The formation of blood vessels is an important physiological process during embryogenesis and development. In adults, angiogenesis occurs only in rare normal physiological situations (menstrual cycle, embryonic development, wound healing) and in certain pathologies (rheumatoid arthritis, retinopathy, psoriasis, arteriosclerosis, cancer). Although observed since the first half of the 20th century tumor neoangiogenesis was not recognized as a critical step essential to the development of tumors until the early 1970s.

B. Mechanism of Tumor Blood Vessel Formation

Tumor neoangiogenesis is the formation of new vessels for tumor vascularization from pre-existing vessels (Figure 12). These new vessels will allow the necessary supply of nutrients and oxygen for the development of the tumor but also the passage of malignant cells into the bloodstream (metastases). Angiogenesis is a key process for tumor growth, survival and dissemination [56].

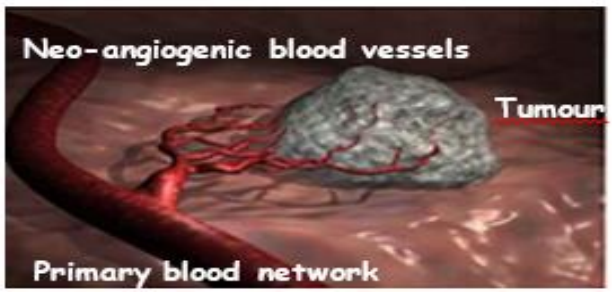


Figure 12: Diagram of the Establishment of Tumor Neovascularization from the Primary Vascular Network

The molecular mechanisms of tumor neovascularization are multiple and complex. They involve different actors and different regulatory voices. Not all of these mechanisms are clearly identified and some are controversial. The commonly accepted classical mechanism of tumor neoangiogenesis can be described in a simplified way as a succession of steps. The initiation of this process results from the dysregulation of the balance between pro- and anti-angiogenic factors (signals respectively inducing or inhibitory of angiogenesis). This deregulation, called "angiogenic switch", is mainly the result of metabolic stress (hypoxia, hypoglycemia), mechanical stress (pressure exerted by the proliferation of tumor cells), an inflammatory phenomenon or the deregulation of genes controlling the regulatory factors of angiogenesis. Under physiological conditions, inhibitory anti-angiogenic factors (thrombospondin-1, angiostatin, endostatin, platelet factor-4, tissue inhibitors of extracellular matrix metalloproteinases and interferon IFNα) are more important than activating factors. During the "angiogenic switch", the activating proangiogenic molecules become predominant and the inhibitors are repressed. Pro-angiogenic molecules are mainly growth factors: VEGF (Vascular Endothelial Growth Factor), EGF (Endothelial Growth Factor), FGF (Fibroblast Growth Factor), PDGF (Platelet-Derived Growth Factor) or tumor necrotizing factor: TNFα (Tumour Necrosis Factor α)). They are secreted by tumor cells, but also by the tumor stroma (fibroblasts, myofibroblasts), inflammatory cells or the extracellular matrix [56]. The "angiogenic switch" leads to the activation of endothelial cells near the tumor. This activation corresponds mainly to the overexpression of surface protein receptors (VEGFr, FGFr, EGFr, PDGFr) and molecules involved in cell adhesion (E-selectin, endoglin, αvβ3 and αvβ5 integrins) (Figure 13-A).

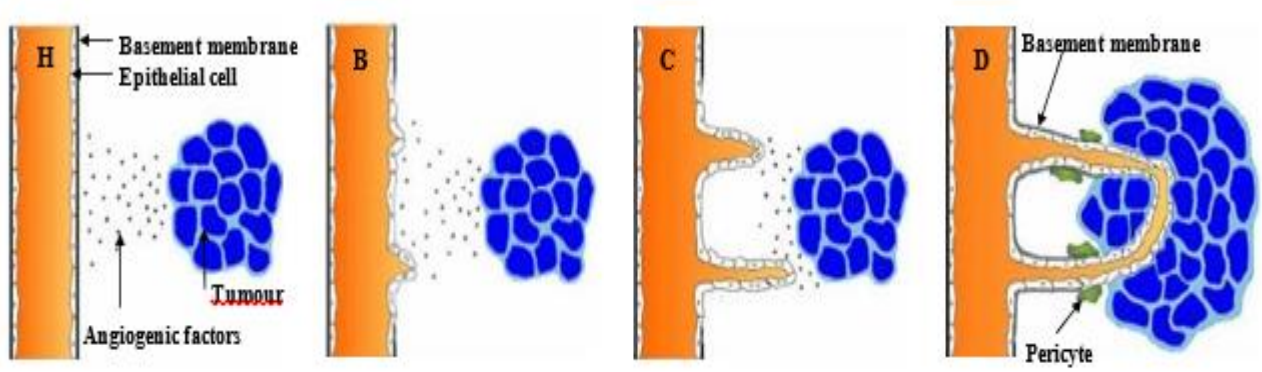


Figure 13: Diagram illustrating: A/ Emission of Pro-Angiogenic Factors and Activation of Endothelial Cells; B/ Degradation of the Basement Membrane; C/ Migration and Proliferation of Endothelial Cells; D/ Consolidation and Stabilization of Microvessels

At the same time, growth factors stimulate the secretion of proteolytic enzymes that degrade proteins in the extracellular matrix and basement membrane supporting the endothelium. This results in budding of the vessel in front of the tumor site (Figure 13-B). The endothelial cells then migrate to the source of the angiogenic stimulus leading to the creation of vascular branches (Figure 13-C). This step involves specialized adhesion molecules such as αvβ3 and αvβ5 integrins. The formation of microvessels continues with the rapid proliferation of endothelial cells, followed by the regeneration of the basal lamina and the recruitment of pericytes consolidating and stabilizing the vessel (Figure 13-D). Tumor neoangiogenesis leads to a disorganized and defective vascular architecture in the vicinity of the tumor [56]. The understanding of certain molecular and cellular mechanisms of tumor neoangiogenesis has made it possible to identify new therapeutic targets and to develop new strategies and molecules to reach tumors.

C. Anti-Tumor Strategies Anti-Angiogenic

Anti-angiogenic anti-tumor strategies can involve the different actors of neo-angiogenesis (neoplastic cells, tumor stroma cells, hematopoietic cells and neoformed endothelial cells) by acting on the process of tumor blood vessel formation. Inhibitory molecules have been developed:

- endothelial cell proliferation,
- Angiogenesis activators,
- the degradation of the extracellular matrix,
- interactions between endothelial cells and the extracellular matrix.

Angiogenesis inhibition strategies can be grouped into two categories in a simplified way: "anti-angiogenesis" and "tumor vascularization targeting". "Anti-angiogenesis" corresponds to the blocking of the functions of the endogenous regulators of angiogenesis. This results in an inhibition of the proliferation, migration and survival of vascular endothelial cells. Tumor vascularization targeting corresponds to the use of neovascularization targeting agents to deliver a therapeutic molecule near or inside endothelial cells. This leads to the death of vascular endothelial cells. The anti-angiogenic molecules tested in the clinical phase often show modest results as monotherapy, but show encouraging results in combination with conventional chemotherapy or radiotherapy. It seems that these molecules can "normalize" tumor vascularization, thus improving the action of chemotherapy or radiotherapy [57]. Among the different possible targeting strategies, the "targeting of tumor vascularization" *via* αVβ3 integrin overexpressed by neo-formed endothelial cells, but also by certain neoplastic cells with αVβ3 positive status, seemed particularly relevant to us.

i.Integrin αVβ3

The αVβ3 integrin is a player in the migration and adhesion of endothelial cells to the extracellular matrix for the formation of tumor neovascularization. Overexpressed by endothelial cells of neo-angiogenic vessels and by many human tumor lines, it is a very attractive target for the development of anti-cancer agents.

D. Characteristics and Roles of Integrin αVβ3

Integrins are an important family of cellular receptors responsible for cell-cell, cell-extracellular matrix and cell-pathogen interactions. They play an important role in the attachment of cells to their environment and in particular to the network of proteins of the extracellular matrix (collagen, laminin, fibronectin, vitronectin). Integrins are also involved in the transfer of signals across the plasma membrane and regulate many functions including cell differentiation, cell migration or wound healing [50-56]. Like the other members of this receptor family, αVβ3 integrin consists of two transmembrane glycoprotein subunits α and β, which are noncovalently associated. The α subunit is characterized by the presence, in the extracellular domain, of regions capable of associating with divalent cations (Ca2+, Mg2+). The extracellular domain of the β subunit has a cysteine-rich region and a folded N-terminal part forming a wide loop. These subunits bind to extracellular ligands via N-terminal domains (Figure 14-A/B).

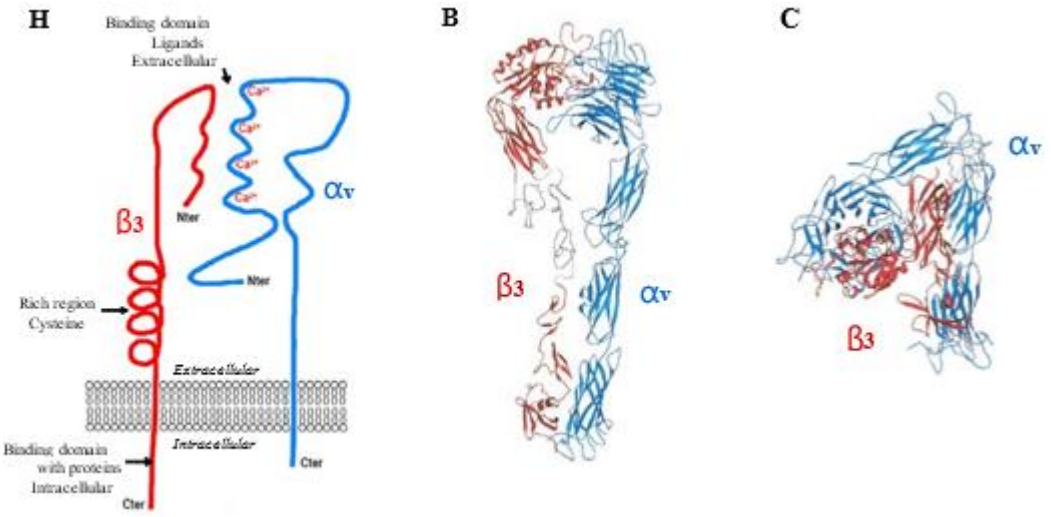


Figure 14: Representation of the αVβ3 Integrin.: a/ Simplified Diagram of Integrin; b/ Ribbon Representation of the Extracellular Domain of the Deployed Integrin; c/ Ribbon Representation of the Extracellular Domain of Integrin

The two chains α and β have small transmembrane parts consisting mainly of hydrophobic residues. The short intracellular regions contain the C-terminus ends of both chains and, unlike growth factor receptors, lack intrinsic enzyme activity (Figure 14-A). The cytoplasmic tail of the β subunit can associate with the actin filaments then connecting the receptor to the cytoskeleton. It can also form focal complexes with intracellular adaptor proteins (talin, vinculin, α-actinin) and establish a connection with cytoplasmic kinases or with growth factor receptors. The structure of the extracellular part of the αVβ3 integrin was resolved by X-ray (3.1 Å) (Figure 14-C). It reveals a compact V-shaped structure where each subunit is folded (Figure 15-Closed headpiece bent).99 Recently, studies have shown that this conformation corresponds to a physiological state of low affinity, whereas binding to a ligand induces a change in conformation in which the integrin deploys and presents a physiological state of high affinity.100,Integrin deployment increases its affinity state for ligands (Figure 15-Closed headpiece exptended). Binding of the ligand to integrin or binding of effector proteins (talin) to the cytoplasmic and transmembrane domains allows propagation of conformational signals resulting in concerted movement with the cytoplasmic domains of the integrin away (Figure 15-Open headpiece extended). The latter conformation activates the receptor [54-57].

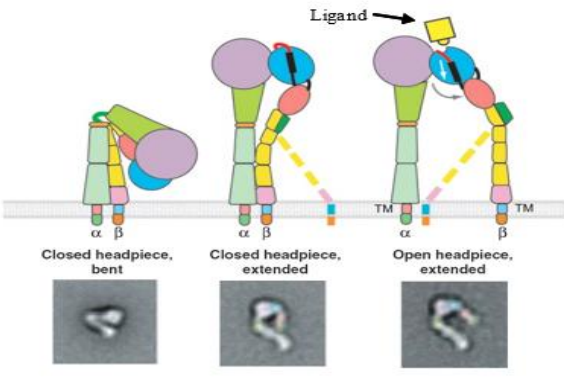


Figure 15: Electron Microscopy Diagrams and Images of the Conformational Changes of the Avβ3 Integrin Associated with the Regulation of its Affinity

Intracellular signals and ligand binding alter the conformation of integrin resulting in bidirectional signaling. In addition, the membrane distribution of integrin and the formation of receptor clusters seem to depend on the presence of substrates presenting ligands in a multivalent manner. Despite a better understanding of the conformational mechanisms regulating the affinity of αVβ3 integrin and its signaling, its role is not fully elucidated and seems complex. Integrin αVβ3 could regulate both pro-angiogenic and anti-angiogenic activities. High levels of expression of this receptor have been demonstrated specifically on the endothelium of the vessels forming in different models of angiogenesis. Immunohistochemical analyses show that αVβ3 integrin is highly overexpressed by neovascularization and some tumor lines compared to normal tissues (respectively more than 100000 versus less than 10000 integrins per cell). This difference in expression makes this receptor a particularly interesting target in anti-angiogenic research. Therapeutic levels of αVβ3 integrin antagonists have also been shown to disrupt newly formed vessels without affecting quiescent vascularization. These αVβ3 integrin antagonists have been shown to be able to inhibit tumor angiogenesis and disrupt metastasis. The affinity of these antagonists for the receptor has led to the use of these molecules as specific ligands of the αVβ3 integrin for its targeting [57].

E. Integrin-Specific Ligands αVβ3

The preferred endogenous ligand of αVβ3 integrin is vitronectin, but it also binds to many other adhesion proteins such as fibronectin, von Willebrand factor (vWF), fibrinogen or thrombospondin. The binding of αVβ3 integrin to these proteins is done through the amino acid consensus sequence "argininеglycine-aspartic acid" (-RGD-). This observation stimulated the development of peptides with this sequence -RGD- and the design of peptidomimetics mimicking this tripeptide.

The molecular ligands of αVβ3 integrin are mainly peptides, peptidomimetic small molecules and antibodies [57]. Among the antibodies developed in anti-angiogenic strategies, the most studied is Vitaxin® (MedImmune). This humanized monoclonal antibody is derived from the murine antibody LM 609 directed against αVβ3 integrin. In 2001, Vitaxin entered Phase I clinical studies in patients with resistant solid tumors. These studies showed good tolerability of Vitaxin and stabilization of tumor progression. Phase I and II clinical studies are currently under investigation on a second generation of Vitaxin antibodies, in patients with resistant solid tumors, lymphomas, advanced colorectal cancers, advanced melanomas, as well as advanced resistant prostate cancers. Recently, several classes of αVβ3 integrin peptidomimetics have been developed. These peptidomimetics consist of a rigid central chassis with a basic part and an acid part mimicking guanidine and carboxylic acid of the -RGD- sequence, respectively. Peptidomimetics that have a 2aminoimidazole group (mime of guanidine) and a 2,3-di-aminopropionate benzensulfonamide group (mime of carboxylate) have been shown to be very good ligands of αVβ3 integrin. These ligands quickly concentrate at the tumor level and exhibit mainly renal elimination [58].

As far as peptides are concerned, many systems presenting the sequence -RGD- have been developed, including linear peptides. Despite encouraging in vitro studies showing their efficacy in blocking cell adhesion at low concentrations (20 µM), clinical studies have shown a high accumulation in the liver without specific accumulation at the tumor level. These disappointing results result from the very nature of the compound: linear, these peptides can adopt many conformations that reduce the binding affinity with integrin and they are also particularly sensitive to proteolytic degradations. In order to limit these disadvantages, small cyclic peptides of conformation conformation capable of inducing activity have been developed.

F. Cyclic Pentapeptides -RGD-: Integrin-Specific Ligands αVβ3

i.Structural Optimization of Peptides -RGD- via Cyclization

In order to design ligands with high affinity and good specificity for αVβ3 integrin, the -RGD- motif was incorporated into constrained sequences of penta- and cyclic hexapeptides. These peptides have been evaluated by affinity assays for different purified integrins in which they compete with adhesion proteins. The affinity of a peptide for an integrin is quantified by an IC50 corresponding to the concentration of peptides necessary to have 50% inhibition of the binding of the integrin to an adhesion protein. This quantification is conventionally carried out by direct or indirect detection of the natural ligand. These affinity tests allowed the selection of a cyclopentapeptide, c[-RGDfV-]. The latter has a very good affinity for purified αVβ3 integrin (IC50 50 nM in competition with vitronectin), comparable to the affinity of vitronectin for integrin (IC50 25 nM in competition with vitronectin). c[-RGDfV-] also has very good specificity for αVβ3 integrin with much lower affinities for other integrins (IC50 29 µM for αIIbβ3 integrin in competition with fibrinogen; IC50 6.4 µM for α5β1 integrin in competition with fibronectin). In order to further increase the affinity of this ligand for αVβ3 integrin and to improve its pharmacological properties (stability), a systematic study of Nα-methylated derivatives of cyclopentapeptide was performed. These derivatives were evaluated and the compound methylated on the valine residue was found to be the most active (IC50 0.58 nM in competition with vitronectin) and the most specific of the αVβ3 integrin (IC50 860 nM for the αIIbβ3 integrin in competition with fibrinogen; IC50 37 nM for αVβ5 integrin in competition with vitronectin). The increase in the activity of the methylated compound results from the conformational stress provided by the methyl group, inducing a better interaction with the integrin. This cyclopentapeptide c[-RGDf(N-Me)V-] is now engaged, under the name Cilengitide (Merck KGaA), in phase I and II clinical studies for the treatment of glioblastoma multiforme, advanced melanomas and metastatic prostate cancers (Figure 16) [57-58].

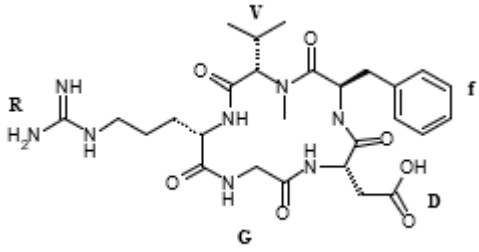


Figure 16: Structure of Cilengitide c[-RGDf(N-Me) V-].

Other ligands with the sequence -RGD- were cyclized by disulfide bridges or thioether bonds. Some disulfide bridge-cyclized ligands have been developed by the phage *display* method and show a high affinity and specificity for αVβ3 integrin. However, these are less stable than Cilengitide (*in vivo* instability of the disulfide bridge and multiple conformations), which makes these structures generally less interesting for the targeting of the αVβ3 integrin.

G. Structural Study of the Cyclopentapeptide/Integrin Interaction

In 2002, the crystal structure of the extracellular domain of αVβ3 integrin associated with the c[-RGDf(N-Me)V-] ligand in the presence of divalent manganese ions was resolved (Figure 17). It allowed to characterize the interaction of the -RGD- motif with integrin and to study the conformational changes caused by ligand binding.

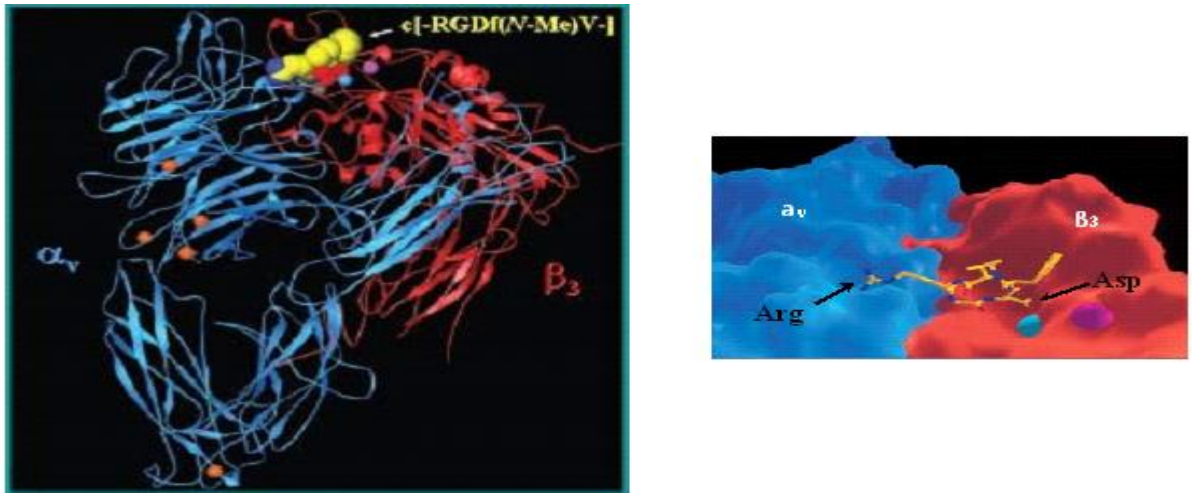


Figure 17: Representation of the Structure of the Extracellular Domain of the αVβ3 Integrin Associated with the c[-RGDf(N-Me) V-] Ligand in the Presence of Mn2+

The interaction between the ligand c[-RGDf(N-Me) V-] and the αVβ3 integrin requires the recruitment of 2 divalent cations. The -RGD- motif of the ligand associates with the receptor in a groove at the interface of the 2 αV and β3 chains and establishes contacts on either side of the 2 subunits (Figure 17). The arginine residue is oriented towards the αV chain and establishes salt bridges with two aspartic acid residues of integrin. The aspartic acid residue of the ligand is exposed to the β3 chain and is involved in polar interactions (coordination of a bivalent cation and hydrogen bonds with integrin). The intermediate glycine residue, at the interface between the two subunits, essentially establishes hydrophobic contacts with the β3 subunit. The two residues completing the ligand structure, -f(N-Me)V-, are exposed to the outside of the complex and do not make any contact with the receptor [58].

H. Properties of Cyclic Pentapeptides -RGD-

In different animal models, repeated administration of Cilengitide shows an anti-angiogenic and anti-tumor effect resulting in an inhibition of tumor growth without affecting quiescent vascularization. Cilengitide has also been evaluated in combination with radioimmunotherapy in preclinical models and has shown synergy between the two treatments, with no increase in toxicity. Cilengitide has been involved in clinical studies for various indications (solid tumors, gliomas or sarcomas). These studies have shown stabilization of the disease and a reduction in the spread of metastases in some patients. They also determined primarily renal elimination of the product, absence of haematological toxicity, tolerable side effects (nausea, fatigue, vomiting, rash), a plasma half-life of 3 to 5 h and the optimal dose to inhibit tumour growth (200 mg/m2). Clinical studies are continuing with numerous ongoing Phase II clinical studies to evaluate Cilengitide in various indications (prostate, pancreatic and lung cancers, advanced melanomas, acute myeloid leukemia, gliomas). To date, Cilengitide has not shown therapeutic efficacy against solid tumours whose stages may be too advanced for treatment to have a significant effect in terms of debulking or survival time.

The properties of cyclopentapeptides (RGDs) have been extensively studied using radiolabelled Cilengitide analogues. Of these, c[-RGDyV-] radiolabeled at 125I showed a rapid and significant accumulation of the compound at the tumor level (2-4% ID/g in 10 min) but shows hepatic elimination. To improve the pharmacokinetic properties of this type of compound, a saccharide part has been added to the peptide. The methylated valine of Cilengitide is replaced by a lysine carrying a 18F radio-labelled sugar. In vitro assays showed that changes in cyclopentapeptide -RGD- only slightly alter these affinity and selectivity properties for αVβ3 integrin (IC50 for αVβ3 of 5 nM in competition with vitronectin; IC50 6 µM for αIIbβ3 integrin in competition with fibrinogen; IC50 1 µM for αVβ5 integrin in competition with vitronectin). The biodistribution shows a predominantly renal elimination and a rapid and significant accumulation at the tumor level (3-4% ID/g in 10 min). In addition, other studies have shown that aspartic acid-supported cyclopeptide dimers supported by 18F and labeled with 18F increase accumulation as well as tumor retention compared to monovalent compounds. In addition, the dimeric version has the advantage of causing mainly renal elimination while the monomer is mostly eliminated by the hepatic route. It is also possible to decrease hepatic elimination by adding a hydrophilic polymer to cyclopentapeptide -RGD-. The addition of a co-ligand showed an improvement in the pharmacological properties of peptides -RGD- (increased tumor accumulation and metabolic stability) [59]. Finally, cyclopentapeptides (RGDs) have been widely used for the vectorization of therapeutic and detection elements. The observation of improved targeting properties of multiple ligands has also stimulated the development of multivalent compounds featuring these cyclopentapeptides for tumor targeting.

i. RAFT Template: New Multivalent Targeting and Vectorization Tool

Our laboratory uses an original molecular chassis, the RAFT, to design new vectors for cancer diagnosis and therapy.

ii. Origin of RAFT Templates

RAFT (Regioselectively Addressable Functionalized Template) templates were developed in the late 1980s under the name TASP (Template-Assembled Synthetic Protein) by Prof. Manfred Mutter. They are intended for the construction of small molecular systems capable of mimicking the functional properties of proteins (Figure 18). In this approach, the structural motifs of interest are adequately organized in space on the template in order to mimic a part of the protein.

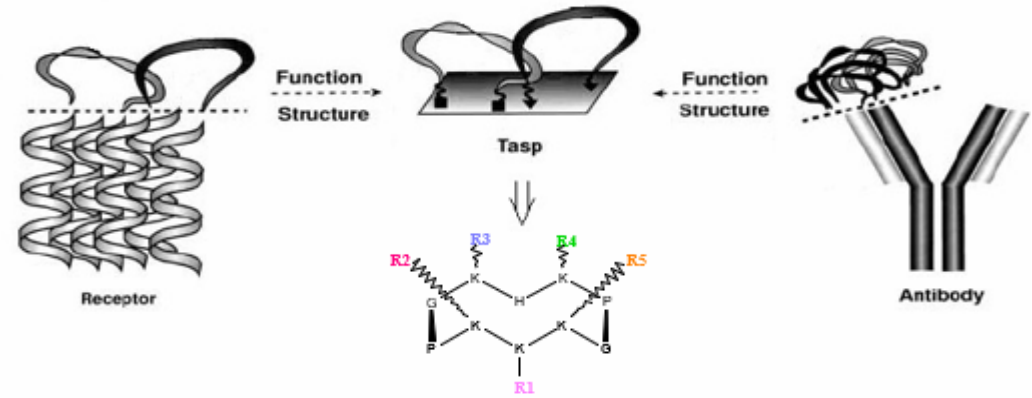


Figure 18: Schematic Diagram of the Construction of Protein Mimes and Structure of the Cyclic Peptide Chassis TASP

In the 1990s, these peptide frames that could be functionalized regioselectively thanks to chemically addressable lysine (K) residues were also called RAFTs. For the past thirty years, these molecular frames have been used to build multivalent architectures for various applications such as model structures mimicking proteins of interest such as ion channels, Class I MHC, amyloid fibers or synthetic anti-tumor vaccines [60].

iii. Structure of the FTRs

The RAFT backbone is a cyclic decapeptide with the sequence c[-Pro-Gly-Lys-LysLys-]2. This structure is inspired by that of Gramicidin S, an antibiotic with a symmetrical cyclodecapeptide structure: c[-DPhe-Pro-Val-Orn-Leu-]2. NMR studies supplemented by a crystal structure, have shown that the RAFT is composed of a sheet β antiparallels connected by two elbows -Pro-Gly- (type II β elbows) stabilizing the conformation of the cyclodecapeptide in solution (Figure 19). The cyclic structure of the chassis is also an advantage in the context of in vivo use. Indeed, cyclic peptides are described to confer greater resistance to proteolytic enzymes.

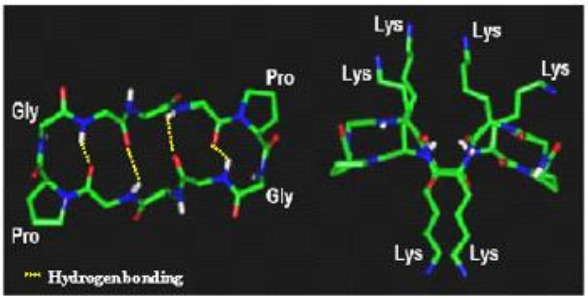


Figure 19: Molecular Modelling of the RAFT

In this structure with reduced conformational mobility, the side chains of the six lysine residues of the central part are oriented on either side of the mean plane of the cycle. The four lysine residues in the vicinity of the two bends -Pro-Gly- have their side chains that orient themselves on the same side of the mean plane of the ring (conventionally called the upper surface of the RAFT), while the two central lysine residues have their side chains that orient themselves towards the opposite side (lower side) (Figure 19) [55-60]. X-ray diffraction studies, performed on a RAFT chassis of the type c[-p-G-F(p-NO2)-A-F(p-NO2)-]2, allowed access to the size parameters of the template (L ~ 10 Å and l ~ 5-6 Å) and the distances that separate the atoms of the side chains from the central residues (Figure 20).Despite a reduced conformational flexibility of para-nitro phenylalalanines compared to lysines, these distances give an order of magnitude of the reduced degree of freedom of the lysine side chains on the RAFT template.

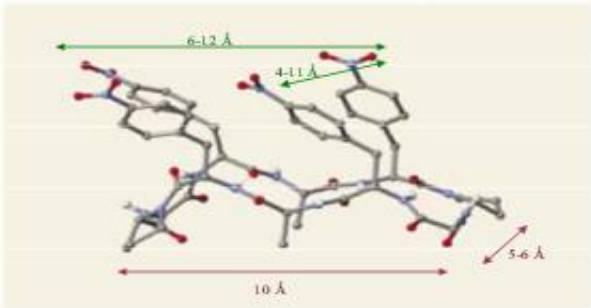


Figure 20: X-Ray Structure of the RAFT c[-p-G-F(p-NO2)-A-F(p-NO2)-]2

iv. RAFT Vector Design for Oncology

The originality of the RAFT lies in the presentation of two independently functionalizable faces with up to six anchoring sites of structural motifs (εNH2 of the side chains of the six lysine residues). These sites are chemically addressable by means of orthogonal protective groups, allowing the attachment of different patterns on both sides of the template. It is also possible to modulate the number of anchor sites on each of the two sides by replacing lysine residues with alanine residues. The properties of RAFT make it a particularly versatile tool in the construction of multivalent architectures [56-60].

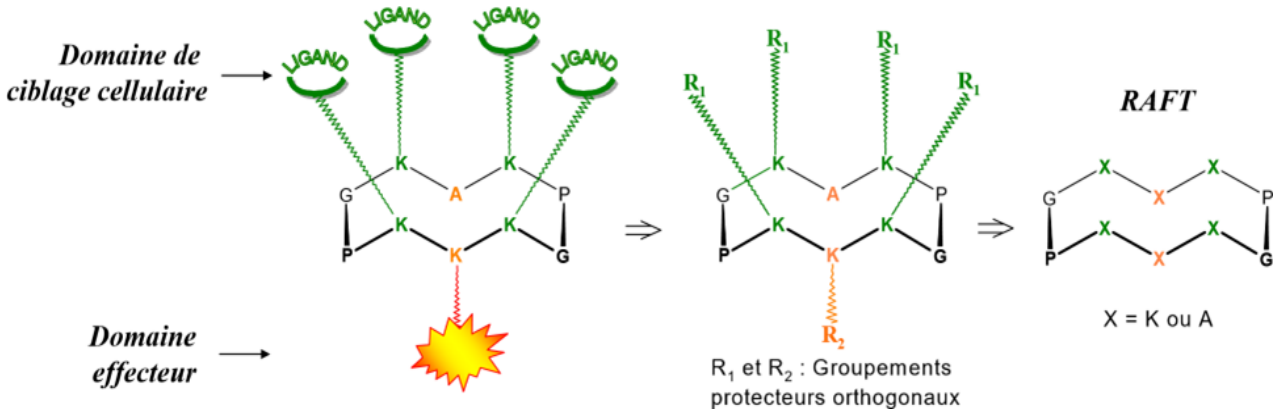


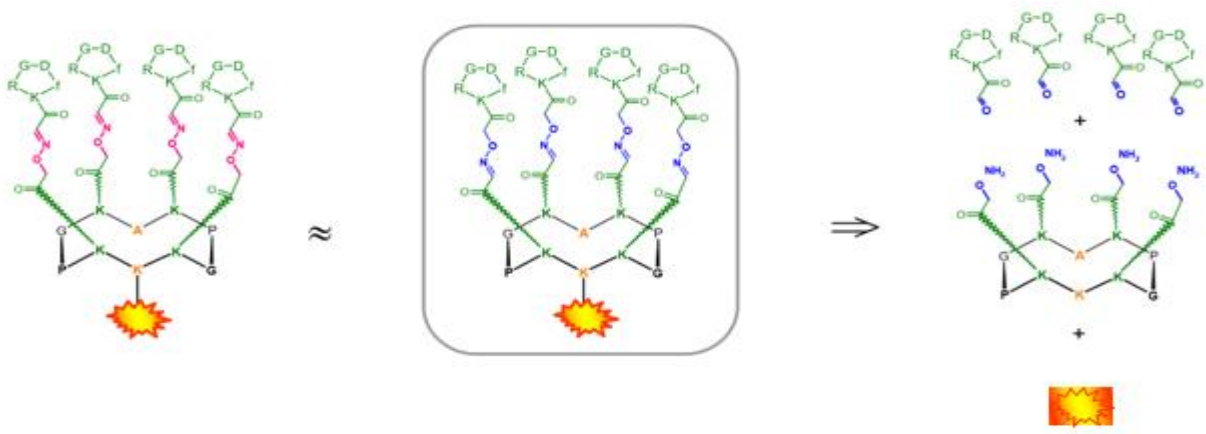
Figure 21: Diagram of the RAFT used for Vectorization in Oncology

In our work, we use the RAFT molecular chassis with four anchor sites on the upper side to construct a multivalent architecture of ligands targeting tumors and a single anchor site on the lower side for the attachment of an effector element (Figure 21). It is also possible to use this frame with two anchor sites on the underside for the vectorization of two effector elements. This RAFT system allows spatial separation of the targeting and effector modules, avoiding their interference. In addition, it is not immunogenic144 and its cellular metabolism does not generate toxic degradation products.

IV. EXPERIMENTAL APPROACH

A. Previous Results: RAFT(c[-RGDfK-]$_4$) New Tumor Targeting Tool

Our laboratory has recently developed a tetrameric structure RAFT(c[-RGDfK-]$_4$) whose biological properties have been evaluated by the Lung Cancer Research Group (INSERM U823, Institut Albert Bonniot, Pr. M.-C. Favrot).



Conjugated (Therapeutic Agent/Detection) RAFT(c[-RGDfK-]$_4$)

Figure 22: Diagram of the Construction of RAFT Conjugates (c[-RGDfK-]$_4$). Properties of RAFT(c[-RGDfK-]$_4$)

This multimeric compound is constructed from a RAFT molecular chassis to which four c[-RGDfK-] motifs are coupled by chemoselective oxime bonds (Figure 22). The syntheses of the RAFT template and the ligand -RGD- functionalized by oxyamine and aldehyde functions have been described and the coupling of these peptides by oxime ether bond has been extensively studied. Within our multivalent molecule, the oxime ether bond was found to be stable (no trans-oximation reactions corresponding to an exchange at the oxime bond between elements with aldehyde or oxyamine functions) and of E configuration (≤ 95%). This configuration was determined by NOESY-type NMR studies of RAFT(c[-RGDfK-]4).* The oxime bond allows conjugation with high efficiency between the two peptide partners. In addition, it has been shown that the orientation of the oxime bond in this macromolecule has no influence in terms of yield or in vitro targeting efficiency (Figure 22).

The study of the multivalence effect of different RAFT(c[-RGDfK-]n templates) has shown that the best structures in terms of binding and interaction with αVβ3 integrin are templates with three or four -RGD- motifs. The tetravalent compound is 10 times more affin for integrin than the monovalent compound (Figure 23-A). Given the structural properties of RAFT(c[-RGDfK-]4), this increase in affinity is most certainly related to an effect of local concentration of ligands -RGD- near the integrin when a multivalent ligand is associated with it, rather than to a clustering effect. In both in vitro and in vivo tests, RAFT(c[-RGDfK-]4) is an effective inhibitor of cell adhesion via αVβ3 integrins. In Nude mice, repeated intra-tumoral injections of low doses of RAFT(c[-RGDfK-]4) showed a reduction in tumor growth and confers anti-angiogenic and anti-tumor activity to this compound (Figure 23-B) [60].

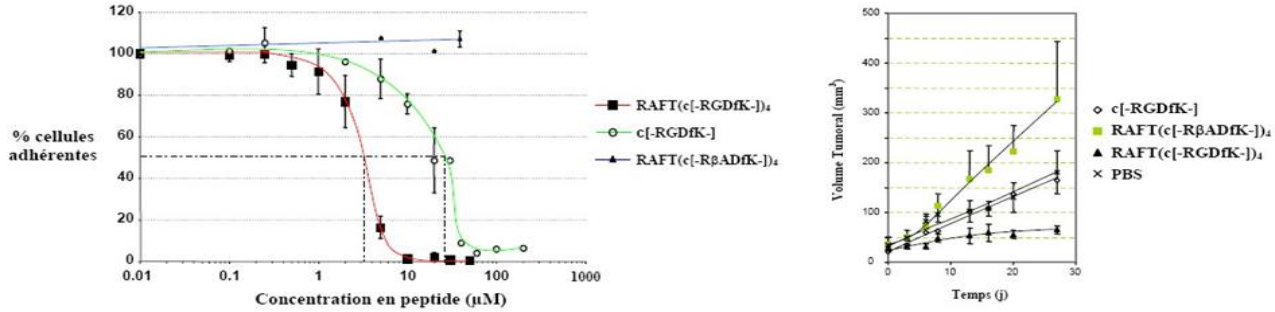
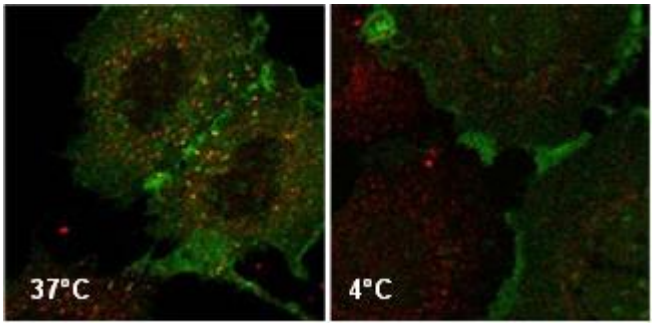


Figure 23: A/ Inhibition Curves of Monovalent (Ic50 = 30 Mm) and Tetravalent (Ci_{50} = 3 Mm) Ligands; B/ Curve of the in Vivo Anti-Proliferative Activity of Peptides -Rgd- Administered Intra-Tumorally to Mice Carrying Subcutaneous Tumors of the A549 Model

In addition, other *in vitro* studies have shown that RAFT(c[-RGDfK-]$_4$) exhibits internalizing properties in αVβ3-positive cells (Figure 24). Cellular entry of this multivalent compound occurs through receptor-dependent endocytosis, unlike cyclopentapeptide -RGD- which appears to be internalized by fluid-phase endocytosis. Furthermore, RAFT(c[-RGDfK-]$_4$) biotynilated on its underside has been shown to be capable of internalising a large protein, streptavidin (60 kDa). The very good affinity and specificity of RAFT(c[-RGDfK-]$_4$) for αVβ3 integrin and its receptor-dependent internalization properties have made it possible to envisage the use of this system for the vectorization of effector molecules (diagnostic and therapeutic).



: (FITC)RAFT(c[-RGDfK-]$_4$)
: Endosomes
: Endosome colocation and (PTIF) RAFT (c[-RGDfK-]4)
FITC: Fluorescein isothiocyanate

Figure 24: Fluorescence Imaging Images of the Study of the Mode of Internalization of the RAFT(c[-RGDfK-]$_4$) Ligand on the KEK293(β3) Cell Line

B. Tumor Targeting and Detection

For non-invasive imaging applications of tumors in small animals, Cyanine 5, a chromophore emitting in the near infrared and various radiotracers (125I-Tyr, 99m 157 18 150,153,154Tc, Gd, F-FDG) were coupled to the RAFT(c[-RGDfK-]4). Preliminary optical imaging results with Cyanine 5 conjugate in mouse models show that RAFT(c[-RGDfK-]4) effectively targets new tumor vasculature as well as αVβ3-positive status metastases (Figure 25) [53-60].

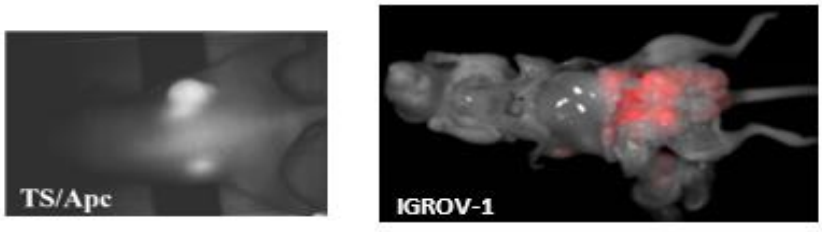


Figure 25: Non-Invasive Imaging Images of Tumors (Ts/Apc Status) And Metastases (Igrov-1 Status) in Mice (10 Nmol i.v. Injections of Conjugates/Mice)

In order to improve the detection of tumors and metastases by optical imaging, our laboratory has also developed new fluorescent probes that can be activated under intracellular conditions. These molecules are composed of our multivalent cyanile vector to which a quencher (QSY21) capable of absorbing the fluorescence emission of Cyanine 5 is conjugated by disulfide bridge. These compounds are not fluorescent, they become fluorescent after the disulfide bridge inside the cancer cells is reduced. This system increases the light contrast, thus improving tumor detection (Figure 26) [60].

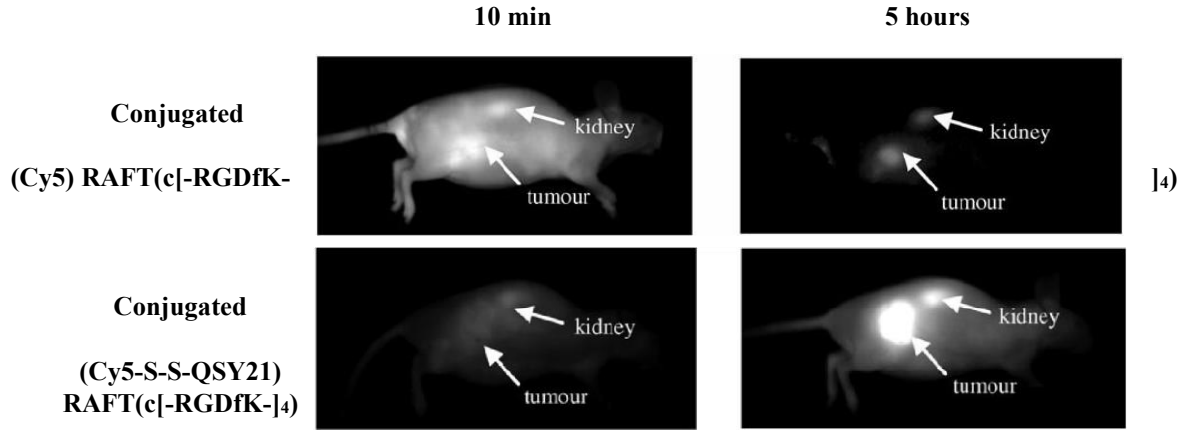


Figure 26: Fluorescence Imaging Images of Subcutaneous Tumors (Igrov-1) In Mice (10 Nmol Iv Injections of Conjugates/Mice)

i. Design of a New Generation of Molecules for Oncology

Preliminary results obtained with RAFT(c[-RGDfK-]4) have shown that this multivalent system represents a particularly effective tool for targeting tumor neoangiogenesis and tumors with αVβ3 positive status. This led us to design a new generation of molecules for oncology (Figure 27).

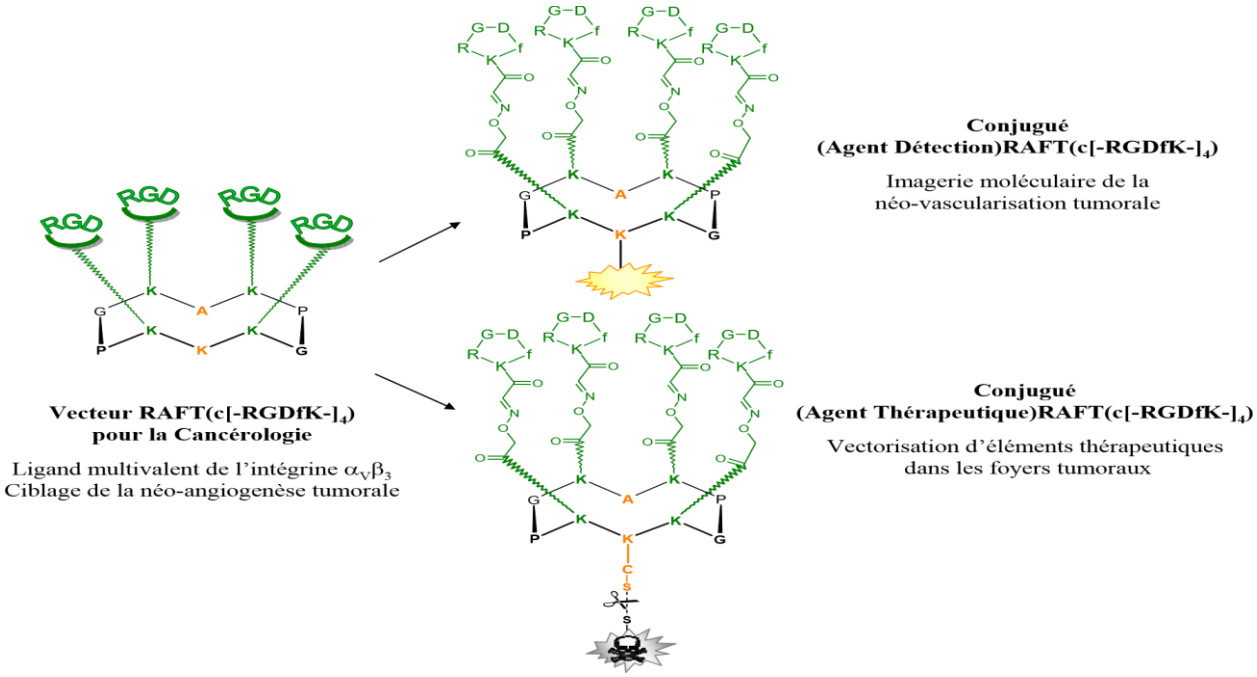


Figure 27: Schematics of New Conjugates for Oncology

V. CONCLUSION

In conclusion, the field of oncology has made significant progress in understanding and combating cancer. Cancer is a complex disease characterized by uncontrolled cell proliferation and the ability to evade programmed cell death. It is a global health concern, affecting millions of people worldwide and causing a significant number of deaths. The development of new treatments and diagnostic methods is crucial in the fight against cancer, and extensive research is being conducted in both the pharmaceutical industry and public research institutions.

Early detection of cancer is vital for effective therapy, as it allows for the treatment of tumors before they have spread and acquired resistance to treatments. Various techniques, such as observation, palpation, imaging methods (radiography, ultrasound, MRI, PET Scan), and the search for genetic and serum markers, are used for cancer detection. Medical imaging techniques, in particular, have advanced significantly, enabling the detection of tumors at earlier stages and providing precise information about their location and characteristics. Treatment options for cancer include surgery, radiotherapy, hormone therapy, and chemotherapy. Surgery is often used to remove the primary tumor, but it can be challenging to ensure the complete removal of all cancer cells.

Radiotherapy uses ionizing radiation to target and destroy cancer cells, but it poses challenges in terms of its effects on surrounding healthy tissues. Hormone therapy and chemotherapy aim to inhibit cell proliferation or induce cell death, targeting specific pathways or processes involved in cancer development and progression.

Chemotherapy, in particular, employs various classes of anti-cancer drugs, such as alkylating agents, anti-metabolites, topoisomerase inhibitors, and spindle "poisons." These drugs interfere with DNA replication, cell cycle progression, and protein polymerization or depolymerization, ultimately aiming to halt tumor growth and eliminate cancer cells. However, chemotherapy is associated with side effects and challenges, including drug resistance and toxicity.

To improve cancer therapy and tumor imaging, researchers are focusing on the development of novel peptide vectors. These vectors can serve as non-viral delivery systems for anti-cancer drugs or imaging agents, targeting specific molecular markers and enhancing their specificity and efficacy. The αVβ3 integrin, a protein involved in tumor neo-angiogenesis, has emerged as a promising target for peptide vectors. Strategies such as multivalency, cyclodecapeptide scaffold RAFT, -RGD- ligand, oxime ligation, disulfide bridge, and FRET are being explored to optimize the design and synthesis of these peptide vectors. The synthesis of novel peptide vectors for cancer therapy and tumor imaging holds great potential for improving treatment outcomes and patient prognosis. These vectors can enhance the specificity and selectivity of anti-cancer drugs, reduce off-target effects, and enable the visualization and monitoring of tumors. Furthermore, advancements in peptide synthesis techniques and drug delivery systems contribute to the development of personalized medicine approaches, tailoring treatments to individual patients and their specific cancer characteristics.

In summary, the fight against cancer requires a multidisciplinary approach, combining the efforts of scientists, clinicians, and pharmaceutical industries. The understanding of cancer biology, along with technological advancements in detection and treatment methods, continues to expand our capabilities in combating this devastating disease. The synthesis of novel peptide vectors represents a promising avenue for improving cancer therapy and tumor imaging, ultimately leading to better patient outcomes and a brighter future in the battle against cancer.

DECLARATION STATEMENT

Funding	No, I did not receive it.
Conflicts of Interest	No conflicts of interest to the best of our knowledge.
Ethical Approval and Consent to Participate	No, the article does not require ethical approval and consent to participate with evidence.
Availability of Data and Material	Not relevant.
Authors Contributions	I am only the sole author of the article.

REFERENCES

1. Machkour A, Thallaj NK, Benhamou L, Lachkar M, Mandon D.Chemistry. 2006 Aug 25;12(25):6660-8.P 6660-6661-6662-6663. https://doi.org/10.1002/chem.200600276
2. Thallaj, N., Machkour, A., Mandon, D., Welter, R., New. J. Chem., 2005, 29, 1555 – 1558. https://doi.org/10.1039/b512108f
3. Thallaj NK, Rotthaus O, Benhamou L, Humbert N, Elhabiri M, Lachkar M, Welter R, Albrecht-Gary AM, Mandon D. Chemistry. 2008;14(22):6742-53.P6745-6746-6747. https://doi.org/10.1002/chem.200701967
4. Wane A, Thallaj NK, Mandon D. Chemistry. 2009 Oct 12;15(40):10593-602. P10594-10595-10595. https://doi.org/10.1002/chem.200901350
5. Thallaj NK, Orain PY, Thibon A, Sandroni M, Welter R, Mandon D. Inorg Chem. 2014 Aug 4;53(15):7824-36. P7826-7827-7828. https://doi.org/10.1021/ic500096h
6. N. K. Thallaj, J. Przybilla, R. Welter and D. Mandon, J. Am. Chem. Soc. 2008, 130, 2414-2415. https://doi.org/10.1021/ja710560g
7. N. K. Thallaj, D. Mandon and K. A. White, Eur. J. of Inorg. Chem., 2007, 44–47. https://doi.org/10.1002/ejic.200600789
8. Thallaj, N.; International journal of applied chemistry and biological sciences 2021, 2 (4), 65-77.
9. Thallaj, N.; Indian Journal of Advanced Chemistry (IJAC)2021, 1 (2), . https://doi.org/10.54105/ijac.D2015.102222
10. Thallaj, N.; International Journal of Research Publication and Reviews (IJRPR)2021, 2, 10, 951-959
11. L. Labban, N. Thallaj, Z. Malek; International Journal of Medical Studies, 2020, 5, No 12, 23-36. https://doi.org/10.36648/1989-5216.12.3.309
12. L. Labban, M. Kudsi, Z. Malek, N. Thallaj; Advances in Medical, Dental and Health Sciences, 2020,3, 3,45-48. https://doi.org/10.11648/j.wjfst.20190304.11
13. L. Labban, N. Thallaj, M. Al Masri; Journal of Advanced Research in Food Science and Nutrition, 2020,3,1,34-41.
14. L. labban; N. thallaj; A. labban; archives of medicine, 2020, 12, 2:8, 1-5. https://doi.org/10.36648/1989-5216.12.3.309
15. L. labban; N. thallaj; international journal of herbal medicine,2020, 8, 2, 33-37.
16. L. Labban, N. Thallaj, Z. Malek; Journal of Medical Research and Health Sciences, 2019, 2, 11, 784-787.
17. L. labban N. Thallaj; acta scientific nutritional health, 2019, 3,10, 7-12.
18. Malek, Z.S.; Sage D.; Pevet, P.; Raison, S.; Endocrinology 2007, 148 (11), 5165-5173. https://doi.org/10.1210/en.2007-0526
19. Malek, Z.S.; Dardente, H.; Pevet, P.; Raison, S.; European Journal of Neuroscience 2005, 22 (4), 895-901. https://doi.org/10.1111/j.1460-9568.2005.04264.x
20. Malek, Z.S.; Pevet, P.; Raison, S.; Neuroscience 2004, 125 (3), 749-758. https://doi.org/10.1016/j.neuroscience.2004.01.031
21. Malek, Z.S.; Labban, L.; The International Journal of Neuroscience, 2020, 1-7.
22. Malek, Z.S.; Labban, L.; European Journal of Pharmaceutical and Medical Research 2019, 6 (11), 527-532.
23. Malek, Z.S.; Journal of AlBaath University 2018, 40 (4), 39-62.
24. Malek, Z.S.; Tishreen University Journal for Research and Scientific Studies, 2018, 40.
25. ZS Malek, LM Labban; International Journal of Neuroscience, 2021,131 (12), 1155-1161. https://doi.org/10.1080/00207454.2020.1782903
26. ZS Malek, LM Labban; Journal of current research in physiology and pharmacology, 2020, 4, (1),1-5.
27. L.M. Labban, M. M. Alshishkli, A. Alkhalaf, Z. Malek; J. Adv. Res. Dent. Oral Health, 2017, 2(3&4), 1-4.
28. L Labban, ZS Malek, Open Access Library Journal, 2018, 5 (07), 1-11. https://doi.org/10.4236/oalib.1104654
29. L Labban, ZS Malek, Ann Food Nutr Res J, 2019,1 ,1
30. Labban, L. and N. Thallaj, 2019. Acta Scient. Nutr. Health, 3: 7-12. https://doi.org/10.36648/1989-5216.12.3.309
31. Y. alhomush, Z. malek, A. Abboud, N.Thallaj, Research Journal of Pharmacy and Technology, 2022, 15, 10.
32. Thallaj, Nasser. "تخليق مشتقات 1، 3، 4-أوكسادیازول، ثیازولیدین بمساعدة المایکرویف." Tishreen University Journal-Medical Sciences Series44.1 (2022): 59-77.
33. N.Thallaj, Tishreen university journal,2022,44,2, 87-105.
34. N.Thallaj. Indian journal of advanced chemistry, 1, 3, 2022. 10-14. https://doi.org/10.54105/ijac.C2013.041322
35. Thallaj, Nasser. "Characterization of charge heterogeneity of antibody-drug conjugate by anion-exchange chromatofocusing." Tishreen University Journal-Medical Sciences Series 44.6 (2022): 21-29.
36. A.Abbood, Z.Malek, N.Thallaj. 2022 15, 11, 4935-4939. Research Journal of Pharmacy and Technology. https://doi.org/10.52711/0974-360X.2022.00829

37. L. Alallan, Z.malek, (2023). 45,(1) 73-81. Tishreen University Journal-Medical Sciences Series.
38. Thallaj, Nasser, and Mays Khazem. "Studying the efficacy of the plant extract of Tetracera scandens as an antidiabetic treatment." Tishreen University Journal-Medical Sciences Series 45.1 (2023): 33-44.
39. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2, 3,1-28https://doi.org/10.54105/ijapsr.C4018.042322
40. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2, 4,1-15. https://doi.org/10.54105/ijapsr.C4016.062422
41. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2, 6,1-12. https://doi.org/10.54105/ijapsr.C4015.102622
42. Thallaj, Nasser. "Review of a Few Selected Examples of Intermolecular Dioxygenases Involving Molecular Oxygen and Non-Heme Iron Proteins." Int. J. Adv. Parmacutical Sci. Res.(IJAPSR) 3 (2023): 1-18. https://doi.org/10.54105/ijapsr.C4011.023223
43. Thallaj, Nasser, and Ayat Abbood. " مقارنة بين مرتسمات الكروماتوغرافيا لمغايرات الشحنة لأضداد وحيدة النسيلة عارية ومرتبطة مع icIEFالبؤرية و دواء." Tishreen University Journal-Medical Sciences Series 45.2 (2023): 47-57. https://doi.org/10.54174/utjagr.v12i1.253
44. Dayoub, Leen, Yanal Alkuddsi, and Nasser Thallaj. "Investigating the interaction between some of Bipolaris sorokiniana's toxins and the Gα Subunit of the Wheat G-Protein using bioinformatics tools." University of Thi-Qar Journal of agricultural research 12.1 2023.
45. Thallaj, N; agha, M. I ,H;, nattouf; A.H; katib, CH; karaali, A; Moustapha, A; Labban L;open access library journal, 2020,7,5,1-21.. https://doi.org/10.4236/oalib.1106302
46. N.Thallaj. Indian journal of advanced chemistry, 1, 3, 2022. 10-14. https://doi.org/10.54105/ijac.C2013.041322
47. N.Thallaj.Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University, Natural Sciences Edition.2022.65, 06. 289-301.
48. N.Thallaj.Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University, Natural Sciences Edition.2022.65, 06. 313-328.
49. Z. MALEK, A. ABBOOD, N.THALLAJ. Xi'an ShiyouDaxueXuebao (ZiranKexue Ban)/ Journal of Xi'an Shiyou University, Natural Sciences Edition.2022.65, 06. 302-312.
50. N.Thallaj. 2022. 65, 7, 169-184. Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition.
51. N.Thallaj. 2022. 65, 7, 110-142. Xi'an Shiyou Daxue Xuebao (Ziran Kexue Ban)/Journal of Xi'an Shiyou University, Natural Sciences Edition.
52. N Thallaj, (2023). 44,(6),21-29. Tishreen University Journal-Medical Sciences Series.
53. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2022. 2, 6,1-12. https://doi.org/10.54105/ijapsr.C4015.102622
54. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2023. 3, 3,1-10. https://doi.org/10.54105/ijapsr.C4018.042322
55. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2024. 4, 1,32-52. https://doi.org/10.54105/ijapsr.D4042.04040624
56. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2024. 4, 3,6-19. https://doi.org/10.54105/ijapsr.D4042.04040624
57. N.Thallaj. International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR) 2024.4, 4,7-21. https://doi.org/10.54105/ijapsr.D4042.04040624
58. Besherb S, Alallan L, Hassan Agha MA, AIshamas I, Thallaj N. Influence of soil salinity on the chemical composition of essential oil of Rosmarinus Officinalis in Syria, Research J. Pharm. and Tech. 2024; 17(4). https://doi.org/10.52711/0974-360X.2024.00358
59. Isbera M, Abbood A, Ibrahim W. Weight and Content Uniformity of Warfarin Sodium Half Tablets. Research J. Pharm. and Tech. 2016; 9(3):215-218. doi: 10.5958/0974-360X.2016.00039.1https://doi.org/10.5958/0974-360X.2016.00039.1
60. Abbood A, Layka R. Weight and content uniformity Study of captopril half-tablets. Research J. Pharm. and Tech. 2017;10(6):1621-1626. doi: 10.5958/0974-360X.2017.00285.2. https://doi.org/10.5958/0974-360X.2017.00285.2

AUTHOR PROFILE

Dr. Nasser Thallaj, 2020- presente time: professor in the biomedical Science program.
2020- Present time: *Assistant professor* position. Faculty of pharma *alrashed university,* Damascus, syria
From 15 june 2019 – 31 July 2020: President Of *AlJazeera University*, Damascus, Syria.
From Abril 1st 2019 – 15jun: vice president for scientific affairs and Dean of Faculty of pharmacy AlJazeera University, Damascus, Syria.
From October 1st 2018-15 march 2020: Dean of Faculty of pharmacy *AlJazeera University,* Damascus, Syria.
2017-31 July2020: *Assistant professor* position. Faculty of pharmacy, *AlJazeera University,* Damascus, Syria
- 2015-2017: *Assistant professor* position. Faculty of pharmacy, *Syrian Private University,* Damascus, Syria
- 2015- 2016: Consultant in Ugarit Education Group: foundation of *AlManara University.*
- 2014-2015: vice president for scientific affairs (in charge), *University of Kalamoon,* Dier Attieh, Syria.
- 2014-2015: In charge of higher education affairs, *University of Kalamoon*, Dier Attieh, Syria.
- 2012-2014: Dean of Faculty of applied Sciences, *University of Kalamoon*. Dier Attieh, Syria.
- 2010-2013: Head of Department of Chemistry. Faculty of applied Sciences, *University of Kalamoon*. Dier Attieh, Syria
-2008: *Assistant professor* position. Faculty of applied Sciences, *University of Kalamoon.* Dier Attieh, Syria
2007-2008: Post-Doctoral position. *Laboratory of NanoOrganic Chemistry, and Supramolecular Materials,* thesis title: *drug delivery system Department* of Chemistry. *University Notre Dame de la Paix*. Namur, Belgium.