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A MODULAR NANOSYSTEMS PLATFORM FOR ADVANCED CANCER MANAGEMENT

An estimated 3.2 million new cancer cases and 1.7 million deaths per year in Europe define cancer as a crucial public health problem. The SaveMe project funded under the EU's FP7 Program is addressing major urgent needs for cancer diagnosis and treatment by design and development of a novel modular nanosystems platform integrating advanced functionalized nano-core particles and active agents. The modular platform will enable the design of diverse active nanosystems for diagnostic or therapeutic applications as defined by their active agent compositions.

The diagnosis and treatment of cancer remains one of the major health problems of Europe. One of the recent advances in medical care that is expected to transform medicine and may have a major impact on cancer is personalized medicine - the use of new methods of molecular analysis to better manage a patient's disease or predisposition to disease. By tailoring medical treatment to the individual characteristics of each patient and by classifying individuals into subpopulations that differ in their susceptibility to a particular disease or their response to a specific treatment, inroads may be made in both the diagnosis and therapy of cancer. Preventative or therapeutic interventions can then be concentrated on those who will benefit, sparing expense and side effects for those who will not. One aspect of personalized medicine is the application of "targeted medicine", the selection of specific therapeutic modalities based on clearly identified subpopulations of patients who are most likely to benefit from a specific therapy. The first example of targeted diagnostics and therapeutics was the introduction of the monoclonal antibody trastuzumab in 1999 [1]. By utilizing the HercepTest (DAKO, Carpinteria, CA) for patients with HER-2/neu overexpressing breast cancers, it was possible to identify patients with breast cancer responsive to a specific molecule. Another example of targeted therapy in which a diagnostic test directs cancer therapy is the detection of a specific chromosomal alteration (bcr/abl translocation) in patients with chronic myelogenous leukemia and the use of the tyrosine kinase inhibitor imatinab as specific therapy [2]. In addition to targeted therapy, early diagnosis of cancer is one of the crucial elements in maximizing successful treatment. In this report, we describe SaveMe, a research project funded by the Seventh Framework Programme that combines the concepts of personalized medicine, including targeted diagnostics and therapeutics [3]. The SaveMe consortium (Fig. 1) consists of nineteen partners from seven EU Member States (Germany, UK, Sweden, Belgium, Spain, Austria and Italy), one Associated State (Israel) and one ICPC (Russia). The consortium includes 3 SMEs, 2 large industries, 3 hospitals and 11 research institutes and universities. The partners come from different cultural backgrounds but more important from different scientific and technological disciplines, including nanochemistry and nanotechnology, in-silico modeling, toxicology and pharmacology, imaging, degradomics and molecular and cell biology. The SaveMe project will address current urgent needs for pancreatic cancer diagnosis and treatment by exploiting partners' expertise and most recent research achievements for the design and development of novel modular nanosystems platform integrating new functionalized nano-core particles and active agents. An estimated 3.2 million new cancer cases and 1.7 million deaths per year in Europe define cancer as a crucial public health problem [4]. While the SaveMe platform can be applied to a variety of different cancers, pancreatic cancer has been selected as the model system. Pancreatic cancer has the highest one-year mortality rate of any cancer and is Europe's sixth deadliest cancer [5]. The overall five-year survival rate is 4%, and has not improved during the last 25 years. Most pancreatic tumors are detected late, at metastatic stage and 85% are unresectable at the time of detection. This is due, in part, to the limitation of current imaging systems in diagnostic accuracy, particularly in determining resectability and evaluation of small lesions and metastatic disease.

State of the art

As a brief introduction to pancreatic cancer, it should be noted that the pancreas is a large organ located behind the stomach. It has both endocrine and exocrine functions, that is, secreting hormones into the blood that control glucose homeostasis, and producing enzymes that are released into the intestines that help digest and absorb nutrients, particularly fats and proteins. The pancreas is located deep in the abdomen in front of the spine and in close proximity to many important structures such as the small intestine (the duodenum) and the bile ducts, as well as important blood vessels and nerves. Pancreatic cancers can be divided into two major categories: (1) cancers of the endocrine pancreas (the part that makes insulin, glucagon or gastrin) are “islet cell” or “pancreatic neuroendocrine” cancers and (2) cancers of the exocrine pancreas (the part that makes enzymes). Islet cell cancers are rarer and usually grow slowly compared to exocrine pancreatic cancers. Cancers of the exocrine pancreas develop from the cells produce digestive enzymes and are called pancreatic adenocarcinomas. Adenocarcinoma of the pancreas comprises 95% of all pancreatic ductal cancers. The cause of pancreatic cancer is still unknown. It is more common in individuals with diabetes, long-term inflammation of the pancreas (chronic pancreatitis), obese individuals and smokers [6]. The risk increases with age. A small number of cases are genetic, and can be found clustered in certain families [7]. Pancreatic cancer is difficult to diagnose at an early stage. A tumor in the pancreas may grow without any symptoms for a long period of time, and many pancreatic cancers are often advanced when first found.

Early symptoms of pancreatic cancer may include fatigue and weakness, jaundice, dark urine and clay-colored stools, loss of appetite and weight loss, nausea and vomiting, and back pain or discomfort in the upper part of the abdomen. If a tumor of the pancreas is suspected, test that are useful to make a diagnosis include CT scan of the abdomen, endoscopic retrograde cholangiopancreatography (ERCP), endoscopic ultrasound, MRI of the abdomen and pancreatic biopsy. Unfortunately, pancreatic cancer is one of the faster spreading cancers, and only about 5% of patients can expect to survive five years after their diagnosis. The treatment of pancreatic cancer depends on the stage of diagnosis. If the cancer is diagnosed at an early stage and is localized to the pancreas, then surgical therapy can be effective. For now, early surgery is the only curative treatment for pancreatic cancer. The surgical procedure performed is pancreaticoduodenectomy, also known as “Whipple” procedure [8]. The operation consists of removal of a portion of the stomach, the duodenum (the first part of the small intestine), pancreas, bile ducts, lymph nodes, and gallbladder. If the cancer is discovered after it has grown into local structures but not yet spread to distant sites, this is described as locally advanced unresectable pancreatic cancer (stage III). Current care of this stage is a combination of low-dose chemotherapy plus irradiation to the pancreas and surrounding tissues. Clearly, the key to curing patients with pancreatic cancer is early diagnosis before the cancer has spread, and curative surgery. The main aims of the SaveMe Project therefore are directed at making an early diagnosis, as well as the development of novel therapies to treat cancers that have spread beyond the pancreas to local tissues or have metastasized.

Project Number [1]	263307	Project Acronym [2]	SaveMe

List of Beneficiaries

No	Name	Short name	Country	Project entry month[10]	Project exit month
1	TEL AVIV UNIVERSITY	TAU	Israel	1	48
2	VEREIN ZUR FOERDERUNG DER WISSENSCHAFTLICHEN FORSCHUNG AM WILHELMINENSPITAL DER STADT WIEN	WSP	Austria	1	48
3	UNIVERSITAETSKLINIKUM HEIDELBERG	UKH	Germany	1	48
4	BAR ILAN UNIVERSITY	BIU	Israel	1	48
5	ALMA MATER STUDIORUM-UNIVERSITA DI BOLOGNA	UNIBO	Italy	1	48
6	JOHANN WOLFGANG GOETHE UNIVERSITAET FRANKFURT AM MAIN	GU	Germany	1	48
7	RESEARCH AND PRODUCTION COMPLEX NANOSYSTEM LLC	Nan	Russian Federation	1	48
8	FUNDACION CIDETEC	CID	Spain	1	48
9	ASOCIACION CENTRO DE INVESTIGACION COOPERATIVA EN BIOMATERIALES	CIC	Spain	1	48
10	COLOROBBIA ITALIA SPA	COL	Italy	1	48
11	MEDICAL RESEARCH INFRASTRUCTURE DEVELOPMENT AND HEALTH SERVICES FUND BY THE SHEBA MEDICAL CENTER	SHEBA	Israel	1	48
12	UNIVERSITY OF EAST ANGLIA	UEA	United Kingdom	1	48
13	KLINIKUM RECHTS DER ISAR DER TECHNISCHEN UNIVERSITAT MUNCHEN	TUM-MED	Germany	1	48
14	KATHOLIEKE UNIVERSITEIT LEUVEN	KUL	Belgium	1	48
15	WEIZMANN INSTITUTE OF SCIENCE	WEIZMANN	Israel	1	48
16	SHEMYAKIN AND OVCHINNIKOV INSTITUTE OF BIOORGANIC CHEMISTRY - RUSSIAN ACADEMY OF SCIENCE	IBCh	Russian Federation	1	48
17	nHance Technologies Limited	nHance	United Kingdom	1	48
18	FILARETE SERVIZI SRL	FS	Italy	1	48
19	GEMS PET SYSTEMS AB	GE	Sweden	1	48
20	OSM-DAN LTD.	OSM	Israel	1	48

Fig. 1 - The nineteen partners of the SaveMe project

Objectives

As depicted in Fig. 2, the SaveMe project is designing a modular nanosystem platform for early diagnosis and therapy of cancer. The first step is the construction of a panel of nanoparticles that can be recognised by MRI, PET or combined PET/MRI scanning. Polymeric core nanoparticles developed within SaveMe will be of a 25-100 nm size range, water-compatible, biocompatible, biodegradable, surface functionalized and resistant to detrimental aggregation. Main chosen polymers are those known to be non-toxic and biodegradable. The polymeric core nanoparticles will be engineered to include various coordinating chemical groups:

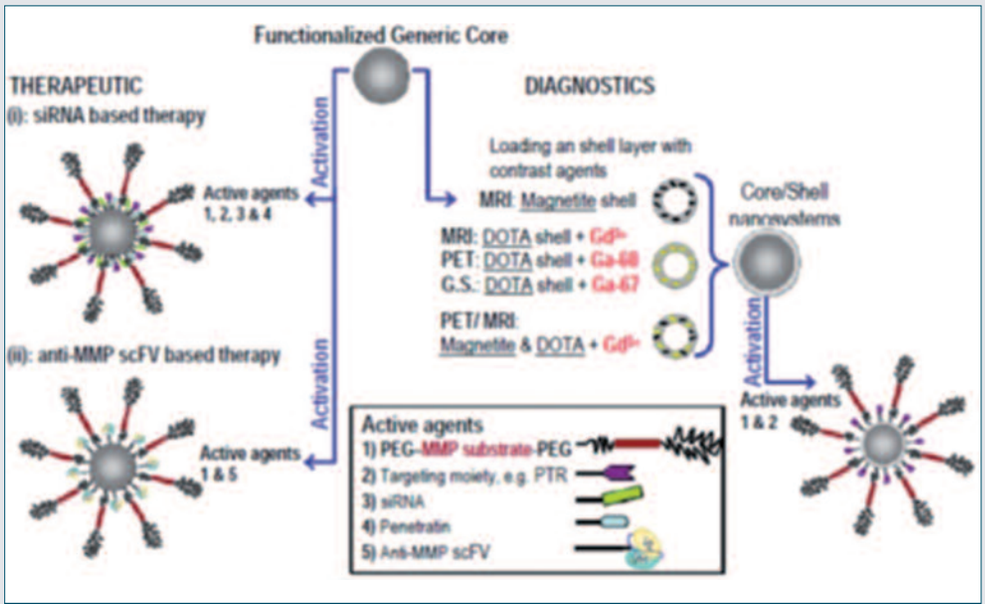

Fig. 2 - A schematic presentation of the novel modular nanosystems platform for efficient tumor diagnosis and therapy. The platform will be based on generic biocompatible polymeric core nanoparticles, adjusted per application via selective activation (loading specific active agents). Diagnostics nanosystems: A semi-confluent shell layer, containing advanced MRI contrast agents (iron oxide nanoparticles) and/or DOTA complexes for PET or Gamma Camera (GC) imaging, will be first loaded. The semi-confluency will leave uncovered core functionalized groups for linking active agents (PEG-MMP-substrate (see fig. 1.2) and PTR peptide) for tumor targeting and penetration. Therapeutics nanosystems: (1) siRNA based therapy: nanosystems will be loaded with PEG-MMP-substrate, PTR targeting moiety, siRNAs and Penetratin (see Fig. 2); (2) anti-MP based therapy-loading PEG-MMP-substrate & selective anti-MMP-scFVs

COOH, CHO, NH_2, SH, and/or OH, thus enabling covalent conjugation of peptides and nucleic acids (e.g. siRNA) per application. The nanosystems will be designed for intravenous (IV) administration. In order to allow the nanoparticles to localize only at the site of the tumor, the nanoparticles must be coated with active agents that will attach specifically to the tumor cells. Certain tumors contain on their surfaces proteins that are expressed only by the cancer cells and not by normal tissues. If the nanoparticles are "decorated" with corresponding molecules that recognize these specific cancer markers, then the nanoparticles will concentrate on the tumor and they then can be visualized with the scanning cameras, allowing recognition of the tumor. To date, SaveMe scientists have selected two tumor cell targeting peptides: (a) SSTR, a somatostatin analogue recently developed and patented (partner SHEBA); (b) targeting moieties based on newly emerging selective biomarkers, e.g. the receptor/ligand system Gal-1/tPA (partner UKH).

To be most effective, the nanoparticles used for imaging and therapeutics must penetrate tissues efficiently, reaching all the cancer cells that comprise the target population in a concentration sufficient to be visualized by the imaging modality and to exert a therapeutic effect. The size of the nanoparticle utilized for diagnosis and therapy is crucial. If the size is too large, the particle often may have difficulty accessing the deeper regions of a solid tumor, while if the particle is smaller, they can diffuse several-folds more rapidly, and thus have better entry into tumor mass. However, large particles have slower systemic clearance**:** clear slowly from the plasma (half time on the order of days) and exclusion from renal filtration due to large molecular size, while smaller particles such as Fabs, scFvs, and dAbs clear more rapidly with half times of minutes to hours -primarily through the kidneys. Conjugation of polyethylene glycol (PEG) chains to nanoparticles can increase their hydrodynamic radius above the kidney cutoff and greatly reduce systemic clearance rates. Thus, while large particle size will slow the systemic clearance on its way to the tumor site, there will be limited tumor perfusion. Smaller particles will potentially have a better tumor perfusion at the tumor site, yet will be lost significantly on their way as a result of more rapid clearance. To address this tradeoff between improved systemic distribution and optimal perfusion at tumor site, and as a complementary additional tumor specific targeting approach, SaveMe will develop the new nanosystems that are initially administered as relatively large PEGylated nanoparticles with low nonspecific binding, and then, at tumor site, transform to small highly specific particles for efficient tumor targeting and penetration. For these aims, the consortium will develop a specific active agent which will contain (1) a protective polymeric layer (PEG) for masking therapeutic and targeting active agents while providing an effective systemic transport and subsequent accumulation via the hyper-permeable vasculature at tumor site, and (2) a substrate-peptide which can be cleaved by cancer-secreted proteas-

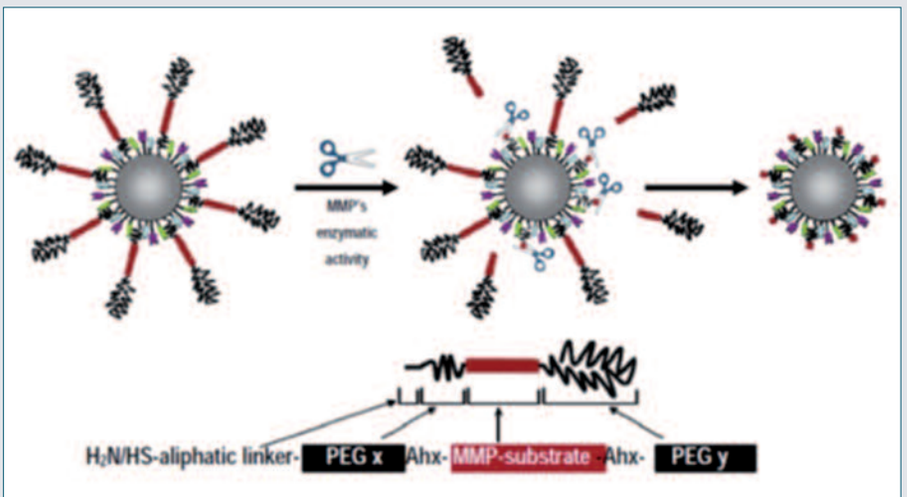

Fig. 3 - MMP specific dePEGylation at tumor site. Development of nanoparticles, which could be specifically dePEGylated in response to the presence of selective matrix metalloprotease (MMP) enzyme. The MMP substrate contained an MMPcleavable peptide sequence. The linker will allow stoichiometrically control of the PEGylation degree on the particles surface

es, thus removing the protective polymer layer and resulting in the unveiling of the hidden functional agents, together with a significant reduction in particle size reaching the required dimensions for optimal perfusion (Fig. 3). As mentioned above, currently cure of pancreatic cancer is based on recognition of the tumor at the earliest possible stage and total surgical resection of the cancer. Unfortunately, this is not usually possible. To treat more advanced pancreatic tumors, the SaveMe project is incorporating novel therapeutic agents into the nanoparticles. In studying the roles of proteases and related molecules, collectively called the "Degradome", primary focus is on secreted and cell surface proteases that shape the extracellular microenvironment. One family of molecules, the matrix metalloproteinases (MMPs) were originally identified as key effectors of tumor cell invasion and metastasis. This led to the development of synthetic MMP inhibitors (MPIs) as anti-cancer agents. However, broad-spectrum MPIs failed in the clinic for multiple reasons, primarily because MMPs carry out a much wider spectrum of roles than the "path-clearing" function they were originally assigned. Also, these inhibitors targeted numerous related metalloproteinases such as the metalloproteinase-disintegrins (ADAMs and ADAMTSs) whose existence was unknown at the time MPIs entered clinical use. It is known today that MMPs, ADAMs and ADAMTs regulate proteolytic and adhesive functions associated with the control of cell identity, division, location and fate. In particular, it has been shown that some metalloproteinases, such as MMP8 and ADAMTS15, act as suppressors of tumor invasion and metastasis, and thus their inhibition may have contributed to the failure of first-generation MPIs. The completion of the sequencing of mammalian genomes facilitated the full delineation of the complexity of the Degradome, which is one of the major achievements of the FP6 "CANCERDEGRADOME" Integrated Project, coordinated by partner UEA (Prof. Edwards). Today it is estimated that there are at least 572 proteases in the human genome, including 190 metalloproteinases. A major technical output from the CANCERDEGRADOME project was the development of tools and expertise for profiling the expression of Degradome genes in human tumors, which led to the identification of novel protease biomarkers and effectors of malignancy.

Conclusions and future perspectives

Thus SaveMe will develop a novel inhibition strategy of degradome related molecules for cancer therapeutic application. This strategy will consider and integrate the selection of individual degradome enzymes, the optimal site of inhibition, targeting a singular activity (e.g. catalytic) vs. multifunctional inhibition (inhibiting the activity of various functional domains), and substrate localization - extracellular, membrane-bound or intracellular. Recent studies from one of the SaveMe partners has identified human MMP-7 as a key protease involved in pancreatic carcinomas, disease progression and that its presence is associated with decreased survival [9]. Therefore, controlling the enzymatic activity of MMP-7 by a selective antagonist molecule is highly desirable as a potential therapeutic strategy. In addition to utilizing anti-MMPs, a further novel therapeutic option is to identify genes that can silence and pancreatic tumor cells. With this in mind, we have performed a large bioinformatic search and chose three model genes that are associated with pancreatic cancer, S100P, K-Ras and miR-21. By including silencing RNA for these genes on the surface of our nanoparticles, we anticipate that they will have a positive therapeutic action in pancreatic cancer.

Clearly, both the diagnostic and therapeutic effects of our active nanoparticles must be assed in vitro and in animal studies, and we anticipate initiation of these studies in the very near future.

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RIASSUNTO

Una piattaforma modulare nanosistemica per il trattamento dei tumori allo stadio avanzato

Il SaveMe è un progetto finanziato dalla Comunità Europea che ha avuto inizio nel marzo 2011. SaveMe è un acronimo per il titolo "A modular active nanoplatform for advanced cancer management". Il capofila dei 20 gruppi che lo compongono (tra università e industrie) è l'università israeliana di Tel Aviv. Il SaveMe durerà 4 anni ed ha come obiettivo generale la sintesi di innovative piattaforme modulari nanotecnologiche formate da sistemi nanoparticellari centrali (detti core nanosystems), decorate sulla superficie esterna da agenti attivi, in grado di permettere diagnosi e terapia di forme tumorali. Tra gli agenti attivi saranno studiate varie biomolecole (proteine, peptidi e anticorpi monoclonali) e agenti nanostrutturati metallici, tipo nanoparticelle magnetiche. L'obiettivo finale è ottenere nuovi sistemi nanotecnologici per diagnosi e terapia del tumore del pancreas.

L'unità di Bologna del Dipartimento di Scienze e Tecnologie della Chimica Industriale "Toso Montanari" è stata coinvolta in questo progetto, con l'obiettivo di sintetizzare e caratterizzare nuovi core nanosystems e di studiare le modalità di aggancio degli agenti attivi grazie alle competenze di chimica organica relative alla trasformazione di gruppi funzionali di sistemi nanostrutturati.