الأكاديمية العربية للعلوم والتكنولوجيا والنقل البحري
Arab Academy for Science, Technology & Maritime Transport

AASTMT RESEARCH

2020 Call for Collaboration Research and Innovation Project

Novel Nano-delivery system: Targeted Bacterial Ghost Associated Nano-particles Encapsulating Promising Chemotherapeutic Strategy for Breast Cancer Therapy.

Proposal's Theme:

Medical sciences/Pharmaceutics

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Proposal Details

Title: Novel Nano-delivery system: Targeted Bacterial Ghost Associated Nano-particles Encapsulating Promising Chemotherapeutic Strategy for Breast Cancer Therapy.

Short Title or Acronym: BGs Nano-System.

Keywords: Drug delivery, Betaine, Bacterial Ghosts, Breast Cancer, Nanoparticles.

Funding and Duration: 500,000 pound and period of 12 Months.

Total cost: 500,000 pounds.

Research Theme: Medical Sciences, Pharmaceutics, Treatment strategy, Molecular Biology.

Proposal Summary: English and Arabic.

English Proposal Summary:

Background: Recently, decreasing of breast cancer incidence and finding new cancer treatment options is a priority and big challenge for the scientific research community. Breast cancer remains the most common invasive cancer among women worldwide. In Egypt, breast cancer is the most prevalent cancer among Egyptian women with the second-highest incidence rate of cancers worldwide with remarkable incidence and mortality rates in Egypt. As a move for improvement of breast cancer treatment options, a proposed delivery system is suggested to be applied on different breast cancer cell lines.

Main Goal: a proposed study with a strong contribution can be focused on formulation of a new delivery system to deliver two different chemotherapeutic compounds exhibiting two different mechanistic roles on breast cancer.

Methodology: To reach our goal, we will focus on the following: First, using bacterial ghost to deliver one chemotherapeutic compound and another will be formulated as a nano-particle to deliver the second chemotherapeutic compound. Both delivery systems will be associated together through either chemical attachment or coating system. Second, we will achieve three separate phases as follows: a) Bacterial Ghost preparation and

characterization, acting as a drug delivery system. b) Nano-particles formulation and drug encapsulation followed by coating or attachment, c) *in-vitro* cytotoxicity assessment.

Outcome: The proposed study aims to match the AAST grant goals through: 1) pushing the young researchers and AASTMT demonstrators for a better future through supporting them with the current available chances, 2) building a wider strong network for the AASTMT with the research institutes of Alexandria for further collaboration and research work cooperation, 3) publishing novel results for a new idea based on previous achieved preliminary studies, 4) raising the awareness about the importance of the research and sustained development projects to solve many current gaps and more importantly find more clear solutions with minimal undesirable effects for the problem of cancer, which acts as a second leading cause of death worldwide.

Arabic summary.

الخلفية: في الآونة الأخيرة ، يعد تقليل حالات الإصابة بسرطان الثدي وإيجاد خيارات جديدة لعلاج السرطان أولوية وتحديًا كبيرًا لمجتمع البحث العلمي. يظل سرطان الثدي أكثر أنواع السرطانات شيوعًا بين النساء في جميع أنحاء العالم. في مصر ، يعتبر سرطان الثدي أكثر أنواع السرطانات انتشارًا بين النساء المصريات حيث يحتل المرتبة الثانية من حيث معدل الإصابة بالسرطان في جميع أنحاء العالم مع معدلات حدوث ووفيات ملحوظة في مصر, كخطوة لتحسين خيارات علاج سرطان الثدي ، يُقترح تطبيق نظام توصيل لعدة علاجات تستهدف انواع سرطان الثدي المختلفة.

الهدف الرئيسي: دراسة مقترحة ذات مساهمة قوية يمكن أن تركز على صياغة نظام توصيل جديد لتقديم مركبين مختلفين للعلاج يظهران دورين و ألية عمل مختلفة لعلاج سرطان الثدي.

المنهجية: للوصول إلى هدفنا ، سنركز على ما يلي: أولاً ، استخدام شبح بكتيري لإيصال مركب علاج كيميائي وآخر سيتم صياغته كجسيم نانوي لإيصال مركب العلاج الكيميائي الثاني. سيتم ربط كلا نظامي التوصيل معًا إما من خلال ربط كيميائي أو نظام أخر يدعى نظام "الطلاء" . ثانيًا ، سوف نحقق ثلاث مراحل منفصلة على النحو التالي: أ) إعداد وتوصيف الشبح البكتيري ، ليكون بمثابة نظام توصيل الدواء. ، ب) صياغة الجسيمات النانوية وتغليف الأدوية متبوعًا بالطلاء أو التعلق ، ج) تقييم السمية الخلوية في المعمل.

النتائج المتوقعة: تهدف الدراسة المقترحة لمطابقة أهداف منحة الأكاديمية من خلال: 1) دفع الباحثين الشباب والمتظاهرين بالأكاديمية نحو مستقبل أفضل من خلال دعمهم بالفرص المتاحة حاليًا ، 2) بناء شبكة قوية أوسع للأكاديمية مع معاهد البحث. الإسكندرية لمزيد من التعاون والتعاون في العمل البحثي ، 3) نشر نتائج جديدة لفكرة جديدة بناءً على الدراسات الأولية المحققة سابقًا ، 4) رفع مستوى الوعي حول أهمية البحث ومشاريع التنمية المستدامة لحل العديد من الثغرات الحالية والأهم من ذلك إيجاد حلول أكثر وضوحًا مع الحد الأدنى من الآثار غير المرغوب فيها لمشكلة السرطان ، والتي تعمل كسبب ثانٍ للوفاة في جميع أنحاء العالم.

Introduction/Background

Breast cancer is the second leading cause of cancer deaths among women, the development of breast cancer is a multi-step process involving many cell types, and its prevention remains challenging in the whole world [1]. There is a huge difference in breast cancer survival rates worldwide, with an estimated 5-year survival of 80% in developed countries to below 40% for developing countries [2]. It is one of the most common cancers in women worldwide, accounting for approximately 570,000 deaths only in 2015 [1]. In

Egypt, breast cancer (BC) accounts for 38.85% of total female cancer cases and is the most prevalent cancer among Egyptian women [3]. Furthermore, breast cancer is a metastatic cancer and can commonly transfer to distant organs such as the bone, liver, lung and brain, which is the main reason for its incurability [1]. However, in Egypt the case is getting worse because early and fast detection of breast cancer are generally not as accrued as it should be [3]. Decline in breast cancer mortality have been slowed recently, although the past few years have witnessed exciting advances in targeted and immunotherapeutic management of all breast cancer subtypes through many recent trends, drugs and natural products [4].

Recently, the main target behind the worldwide current research work is to discover, make use and combine the good properties of different new strategies together, in order to reduce the toxicity along with targeted delivery of different drugs separately and simultaneously to enhance the treatment options for the persistent types of cancer specially the breast cancers [5].

One of the developed promising strategies for better drug delivery is to recruit the evacuated bacterial cells which are known as Bacterial Ghosts (BGs) for drug delivery [6]. Bacterial Ghosts are empty cell-envelopes of bacteria which is used as vehicles for drugs in tumor therapy, and due to specific targeting of tumor cells allow a higher specificity of treatment and a reduction of the total amount of drug per application [7]. BGs are known for their safety which depends on nature since they are empty and evacuated of all the cytoplasmic content. Importantly, the efficacy arises from the good loading capacity available per each ghost, which is estimated to be around 250 femtoliters/ each evacuated cell [8]. Moreover, the existence of a very powerful aiding property within the BGs, which is the ability of the researcher to manipulate and associate valuable therapeutic particles to the ghost's surface [6]. Discovery of bacterial ghosts as drug delivery system to use bacteria as an oncolytic agent for cancer treatment, this was attributed to the inherent ability of some pathogenic bacteria to localize in cancerous cells [8].

Since toxicity of the anti-cancer drugs is a major concern [9]. One of the promising approaches in order to minimize the undesirable side effects and to ensure efficient targeted delivery of the valuable therapeutic agents to specific site of action is the development of unique nano-delivery systems, new trends have been developed conquering the scientific research field, such as: polymeric micro- and nanoparticles, liposomal systems and erythrocyte ghosts [10]. Nano-delivery systems offers: (1) improved bioavailability by enhancing aqueous solubility, (2) increasing resistance time in the body (increasing half-life for clearance/increasing specificity for its cognate receptors and (3) targeting drug to specific location in the body (its site of action), resulting in concomitant reduction in quantity of the drug required and dosage toxicity, enabling the safe delivery of toxic therapeutic drugs and protection of non-target tissues and cells from severe side effects [11].

Synthesized nano-carriers along with BGs that have been named as naturally inspired nano-carriers that are able to be loaded with different types of therapeutic agents can be used as a perfect delivery systems and delivery complexes to deliver different chemotherapeutic agents to its site of action [7]. New drug categories discovery and the ability of drug loading, natural products have been used for the treatment of various diseases and are becoming an important research area for drug discovery.

These products have been extensively studied and have exhibited anti-carcinogenic activities by interfering with the initiation, development and progression of cancer through the modulation of various mechanisms including cellular proliferation, differentiation, apoptosis, angiogenesis, and metastasis. This concept is gaining attention because it is a cost-effective alternative to cancer treatment [12]. Betaine is a constant and non-toxic natural substance with three additional methyl groups [13]. Previous studies has shown an anti-inflammatory and anti-angiogenic effect that accounts for Betaine, which is considered as a perfect agent to prevent further growth and metastasis through preventing the formation of a new blood vessels from the already existing ones around the tumor [13, 14]. Furthermore, betaine was coated before with a super paramagnetic nano-carrier, which has shown a great effect in the purpose it was used for [15]. Moreover, the hypothesis of Epigenetic vulnerability of cancer cells and epigenetic mechanisms' modification can bring us closer to carcinogenesis comprehension [16]. Based on the previous reported studies, a class of drugs named epigenetic modifiers known as histone deacetylase (HDAC) inhibitors have shown cytostatic effect for their chromatin remodeling property through inhibition of proliferation of tumor cells in culture and in vivo by inducing cell cycle arrest [17]. A further advantage, the HDAC inhibitors has shown the potential to be transferred and delivered via nano-carriers [18]. The HDAC enzyme family comprises four subclasses based on the amino-acids sequence similarity [19]. However, although HDACs have been shown to have a wide range of anticancer activities, cancers can have varying degrees of resistance to the inhibitors, resistance to current HDAC inhibitors forms in cancer cells may involve both "intrinsic" and "acquired" mechanisms [20]. The proposed idea aims at co-operating both mechanisms of HDAC inhibitors and Betaine along with the nano-particle and Bacterial Ghost approach to target the breast cancer.

Questions and Objectives

1) Why should we develop, discover and advance new and current cure options for the breast cancer disease?

2) How to solve the problem of drug resistance of breast cancers that develops against many different drugs?

3) What are the current available cooperative approaches to overcome the problem of drug toxicity, targeting alterations and expensive costs of preparation?

4) What is the innovation strategy within the current proposed research study using BGs, nano-particles formulation and their applications for breast cancer treatment?

To find a clear obvious answers and solutions for the mentioned questions, we are going to focus on three different and independent aims:

Aim (1): Nano-particle preparation and efficacy check: The present work aims at preparing one or more of targeted nano-carrier based delivery system for cancer therapy encapsulating betaine, mainly a super paramagnetic betaine nanoparticle and/or others.

- Nanoparticles are generally used to enhance the pharmacokinetic and pharmacodynamic properties of various types of drugs.
- Nanoparticles can be classified into different types according to the size, morphology, physical and chemical properties to carbon-based nanoparticles, ceramic nanoparticles, metal nanoparticles, semiconductor nanoparticles, polymeric nanoparticles and lipid-based nanoparticles.
- Nanocarriers are known to infiltrate tumor tissue by the enhanced permeation and retention effect (EPR) due to the highly permeable nature of the tumor vasculature.
- As nanocarriers circulate and encounter this area, characterized also by poor lymphatic drainage, leakage into and retention in the interstitium resulted in gradual accumulation.
- The nanoscale, surface properties, and shape govern drug loading and release pattern, in addition to biodistribution and cellular uptake from these carriers. Therefore, delivering anticancer drugs to specific site using nano-size drug carrier (or particle) have been extensively studied for the purpose of reducing side effects and increasing the efficacy of the drug.

Aim (2): Bacterial ghost preparation and testing: The present work aims at preparing a bacterial ghost to be loaded with one chemotherapeutic agent.

- The intention of the proposed study is to determine the cytotoxic impact of the entrapped compound and to compare its efficiency in different cancer cell lines.
- BG production is characterized by the introduction of a small hole in the envelope of the bacteria and release of the cytoplasmic content driven by the osmotic pressure difference between the cytoplasm and the outside growth medium of the bacteria.
- Bacterial ghosts representing novel advanced delivery and targeting vehicles suitable for the delivery of hydrophobic or hydrophilic drugs.
- Bacterial ghosts exhibit excellent natural or engineered adhesion properties with versatile carrier functions for drugs, proteins and DNA plasmids or DNA mini-circles.
- The simplicity of both bacterial ghost production and packaging of drugs and/or DNA makes them particularly suitable for the use as a delivery system.
- Further advantages of bacterial ghost delivery vehicles include high bioavailability and a long shelf life without the need of cold-chain storage due to the possibility to freeze-dry the material.
- BGs have inherently bio-adhesive properties, and are excellent cargo delivery vehicles because they can entrap biomolecular loads within themselves. These loads can gain entry through their tunnels but cannot easily escape.
- The drug can be encapsulated inside the hollow BGs where it can non-covalently bind to the inner surface through electrostatic charge interactions.

- The BG is also excellent targeting vehicles for primary immune cells, endothelial cells of blood vessels or intestine, or to recognize tumour tissue by specific ligand receptor interaction.
- BGs are efficiently internalized by phagocytic cells such as macrophages and dendritic cells (DCs)

Aim (3): Testing the efficacy of the combination complex, nanoparticle and bacterial ghost and application of breast cancer treatment: the present work aims at assessing the anti-cancer activity of two compounds (Betaine) as a natural compound and HDAC inhibitor, FDA approval for breast cancer treatment as an adjuvant, to be delivered, using a new formulated delivery system.

- Betaine is a constant and non-toxic natural substance with three additional methylgroups.
- Betaine is an essential biochemical molecule of the methionine/homeocystein cycle and is synthesized by conversion of choline.
- According to the previous studies, betaine suppresses inflammation through NF-κB activation during aging [13]. As a result, it was found that betaine suppressed tube formation, migration, and invasion of human umbilical vein endothelial cells (HUVECs).
- Histone deacetylase inhibitors (HDIs) are a class of prominent epigenetic drugs that are currently being evaluated in many research studies and clinical trials against a variety of diseases [21].
- Regarding the HDAC inhibitors' mechanism of action as an anti-cancer agents, they induce cancer cell cycle arrest, differentiation and cell death, reduce angiogenesis and modulate immune response [16].
- Mechanisms of anticancer effects of HDAC inhibitors are not uniform; they may be different and depend on the cancer type, HDAC inhibitors and doses.
- HDAC inhibitors seem to be promising anti-cancer drugs particularly in the combination with other anti-cancer agents, **which is "Betaine" in our proposed research design.**
- Since the proposed nano-system is a super paramagnetic one and/or others, it has been demonstrated that the size of iron oxide particle and the coating have a notable influence on its magnetic property, cellular uptake and cell viability [15].

Project Description

- The main goal of the proposed is to target Bacterial Ghost associated nano-particles encapsulating promising therapeutic agent for breast cancer therapy.

General approach overview (vision):

This project is multi-disciplinary merging Microbiology, Pharmaceutics and Drug Delivery, Biotechnology, Biochemistry and Molecular Cancer Therapeutics. The main goal of the proposed study is settled to co-operate different specialties with the advantages of new strategy for drug delivery and different treatment options to get the maximum benefits of it. The outcomes are expected to be promising on many levels starting with the scientific level of every participant passing through the associations participating in the project and finally the effect on Egypt as a country having a high incidence rate of breast cancer.

Several challenges are expected to be faced, including the novelty of the idea relevant to the current research work on the BGs. Furthermore, the idea of the project strongly challenges the current knowledge and research practice through applying theoretically possible steps that haven't been practically implemented before.

The expected impact and benefit for patient care, disease prevention, and health service organizations is that a new approach for breast cancer treatment is coming to the field proving its efficacy based on the previous preliminary carried out studies. Along with the good quality of research output as a short-term achievement and as a long term one, the opened doors for the pharmaceutical companies to consider the proposed succeeded idea for releasing a new platform of drugs that are composed of our small evacuated bacterial cells loaded and/or attached with the drug of choice within it.

Team composition and assembly has the right set of individuals with relevant expertise and experience to accomplish the project goals and tasks. Diversity is at the heart of being a team; the team has been composed of individuals with different roles, who work independently at some parts and in co-operation in another. Team is assembled for the proposed scientific research idea and is aimed at solving complex problems such as: minimizing the toxicity and undesirable side effects of chemotherapeutic agents, combination of different strategies for obtaining the maximum benefits of it and discovery of the combined effect of two different chemotherapeutic agents for breast cancer treatment. The partnership of different associations and individuals of different backgrounds is a crucial factor for the project's success; in return this will open the door for the member Sara Hassan Kamel to hold her master degree, moreover, well established relationships between the team members who belongs to different research working places, which hopefully can contribute in running further projects and research work with different scientific theme.

Project's mission:

The mission of the project is to; 1) establish a new drug delivery system taking all of its capabilities in consideration. 2) introduce new protocols of work to the Egyptian research field. 3) co-operate many associations together, each in its proper place to carry out each and every step precisely. 4) Filling specific current knowledge gaps (specially the gap of minimizing drug toxicity and altered delivery).

Project relevancies:

1) Relevance of the Associations:

The project is supposed to be completed within three phases as the following:

Phase I: will be concerned with the microbiological aspects of the project, will take place in The Institute of Graduate Studies and Research under the supervision of prof. Ahmed Abd El-Rehiem to perform the following: a) creation of Bacterial Ghosts, b) characterization for the BGs, c) lyophilization of BGs to be used later on the drug loading phase.

Phase II: will be concerned with the pharmaceutical and drug loading aspects, will take place in the AAST- Alexandria campus under the supervision of Dr. Passent Ehab and Dr. Mai Ali to perform the following: a) nano-carrier formulation, b) nano-carrier drug encapsulation, c) nano-carrier characterization, d) BGs drug loading, e) testing of BGs drug entrapment, f) combination of the BGs and nano-carrier by coating and/or attachement.

Phase III: will be concerned with the signaling pathways and the *in-vitro* cytotoxicity assessment of the loaded chemotherapeutic agents, will take place in the faculty of science under the supervision of Prof. Ahmed Sultan will take place in the faculty of Science (at the Research Unit of the Stem cells and Molecular Cancer Therapeutics) in collaboration with the AAST Lab, Abu-Qir campus. to examine the efficacy of the new formulated nano-particles and Ghost delivery system on different breast cancer cell lines ranging from dormant to highly metastatic breast cancer cell lines.

Each contributing association from the above mentioned ones is highly adaptable to each phase. Adaptability comes from the presence of well trained staff members, widely equipped labs and previously published research work that is fortunately related to the points of work in each phase.

2) Relevance of the labs:

Phase one: The microbiology labs within the institute of research and graduate studies are well equipped with the needed reagents, instruments and information to perform the first steps successfully till reaching a lyophilized form of the BGs. Specially that there were previously prepared BGs at the same labs for many research work.

Phase two: The pharmaceutics labs of the AAST are also safe, well-equipped and newly constructed.

Phase three: The *in-vitro* cytotoxicity testing will take place in professor/ Ahmed Sultan's lab, the lab is highly prepared with all the needed criteria starting from the preservation of the cell line tissues proceeding to the use with the minimal contamination possibilities.

3) Relevance of the team members:

- The team members are gathered from three different associations which are:

a) AASTMT, Abu Qir campus - Represented by: Dr. Passent Ehab (PI) and Dr. Mai Ali (Co-PI).
b) Institute of graduate studies and research, Department of Biotechnology - Represented by: Prof. Ahmed Abd El-Rehiem Hussien (Vice-Dean for the Institute of Graduate Studies and Research, Alexandria University.) and Ph. Sara Hassan. (Biotechnology department master student).
c) Faculty of Science, Alexandria University- Represented by Prof. Ahmed Sultan's research team (Research Unit for Molecular Cancer Therapeutics).

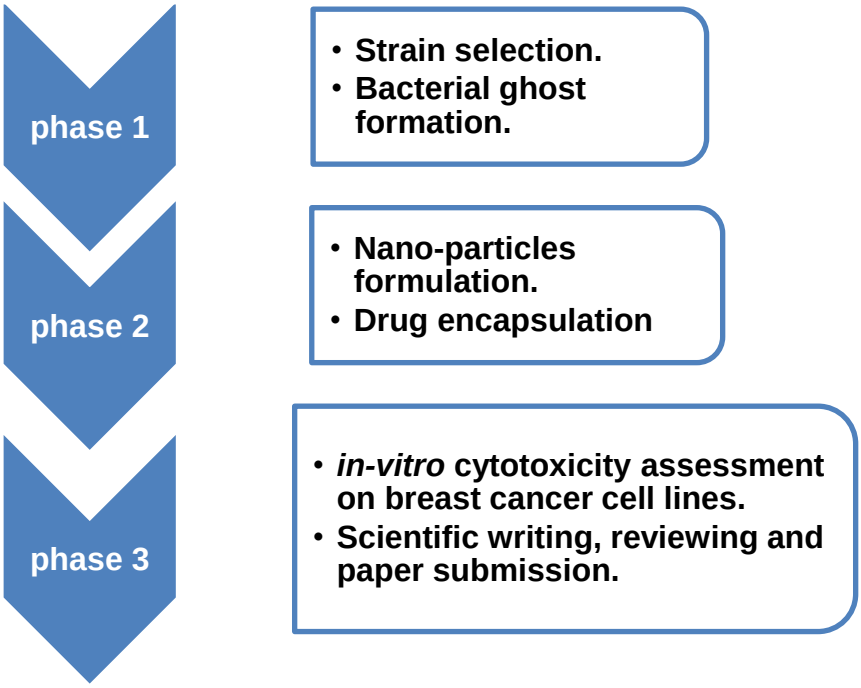
- The team members are the most suitable for the project due to the suitability for the tasks to be assigned to each member along the phases of the project as it will be discussed in the team management sector and the history of each team member.
- The team members have different scientific backgrounds and specialties, and this in return will provide a multiple diverse scientific contribution which is not only concerned with one type of science or specialty.
- The project will also help the member Ph. Sara Hassan to earn her master degree, and this is what makes her highly dedicated to the project which is highly relevant to her career, studies and future plans.

Methodology justification

The chosen methods are the best to be selected for the following reasons:

1) Availability of the methods' requirements with an easy access for its usage.
2) Novelty of the chosen protocols and methods.
3) Feasibility of the requirements and online purchase easiness.
4) Previous encouraging results of carried out research studies that used the same protocols.
5) Previous knowledge of the working staff about how to deal with any reagents, equipment and the protocol of method flow.

Research Design and Methods



Phase I: As previously mentioned, phase one will be concerned with the microbiological aspects of the project.

Target of phase one:

a) **Bacterial DNA completely degradation by several steps with quantification to the amount of DNA and proteins released to ensure its safety followed by sub-culturing to ensure the killing of the whole population.**
b) **BGs lyophilized vials ready use.**

Methodology of phase one:
Exposing the bacterial cells to one of a three widely used protocols in order to get a safe super-clean ghost.

Methods of phase one:

The process will go according to the following proposed steps:

Selection of the target Strain to be formulated as a ghost:

Choice can depend on many available strains, for Example: *E-coli K12, listeria Monocytogenes, Salmonella enterica serovar typhimurium* ATCC strain and/or others.

It is strongly advice the use of *Salmonella* for the following reason:

Salmonella was shown to be targeted to tumor regions selectively, various studies have shown successful antitumor activity when *Salmonella* was combined with chemotherapeutics [22]. *Salmonella* could selectively migrate to the tumor region; this targeting capability was accounted for the following reasons involving: serine, aspartate and galactose/ribose receptor on surface of the *Salmonella*, those receptors attracted *Salmonella* toward cancerous cells, therefore, *Salmonella* was shown to selectively accumulates at a ratio of >1000:1 in tumor tissues in comparison with normal tissues [23].

Protocol to be used: (no hazardous materials)

The available protocols are three, based on the methods and reagents:

a) Gene E mediated lysis protocol [24].

- BGs are envelopes from Gram-negative bacteria which can be produced by controlled expression of the cloned lysis gene E.
- Gene *E* codes for a 91-aa polypeptide which in contrast to lytic proteins from other phages.
- E represents a membrane protein with the ability to oligomerize into a trans-membrane tunnel structure for cytoplasmic content expulsion.

b) Sponge-method protocol [25].

- The protocol based on minimizing the effect of each of SDS, NaOH, $CaCO_3$, and H_2O_2, which are used as an alternative for the *E* lysis gene to prepare the BGs.
- The protocol is based on using active chemical compounds in concentrations less than the Minimum Inhibition Concentration (MIC).
- Those chemical compounds are SDS, NaOH, and H2O2.
- Plackett-Burman experimental design was used to map the best conditions for BGs production.

c) "A novel protocol for bacterial ghosts' preparation using tween 80" [26].

- A new protocol based on exposing the bacterial cells to tween 80 for an extended period of time followed by sudden reduction of the surrounding pH.
- The cells were incubated in 7% $^{v}/_{v}$ tween 80 solution in Muller-Hinton broth for 24 h at 37 °C then pH was decreased to 3.6 by adding lactic acid for one hour.
- The bacterial pellets are then to be separated by high speed centrifugation, and then washed three times by half normal saline solution.

Major equipment to be used:

NanoDrop (spectrophotometer), Scanning electron microscope, Lyophilizier, Centrifuge, Incubator, Gel Electrophoresis (maybe).

Phase II:

Target of phase two:

1) The proposed work aims at preparing one or more of nano-delivery systems such as super paramagnetic nano-particles based delivery system of the chemotherapeutic agent "Betain" for cancer therapy.
2) Complete physicochemical characterization of the developed formulation.
3) Loading the BGs with the drug of choice "HDAC inhibitor".
4) Either coating/attachment of the super paramagnetic nano-particle encapsulating "Betaine" with the previously prepared BGs, this is possible according to many techniques [27].

Methodology of phase two:

1) Preparation of super paramagnetic nano-particles and/or others, according to the following protocol, or adoption of other preparation techniques for the formulation of other nano-delivery system/s:

1) For the preparation of magnetite nanoparticles, 1 M FeCl3.6H2O (>99%) was mixed with 2 M FeCl2.4H2O (>99%) at the ratio 2:1 and then 28.6% (w/w) Betaine hydrochloride will be dissolved in the solution by stirring.
2) The pH of reaction solution will be adjusted with the 3M NaOH until reaching a pH value of 11.
3) After stirring vigorously for 15 min, the solution pH will be adjusted to 4 by adding 2M HCl dropwise.
4) The betaine coated Fe3O4 particles will be collected by magnetic separation by applying a permanent magnet for 30 min and washed with deionized water three times.
5) To remove excess ions and betaine further, the product will be dialyzed against with deionized water overnight.
6) Following this, the solution will be centrifuged at 3500 g at RT for 10 min. Finally the product will pass through a 0.22 μm filter and used for investigations.

2) Trials to be carried in order to reach the best choice for combination, coating and/or attaching of nano-particles of "Betaine" to bacterial ghost cells encapsulating the HDAC inhibitor.

1) Cell-membrane coating approach [28] :

- Membranes isolated from different source **(in the proposed case it will be a BG)** cells by various methods and coating this onto core NPs by
  a) co-incubation,
  b) sonication, or
  c) extrusion.
- Nanoparticles (NPs) coated with cell membranes offer an opportunity to unite natural cell membrane properties with those of the artificial inner core material.

2) **New approach: Delivery of Nanoparticles on Surfaces [27].**

1) **Mechanical-based delivery.**
2) **Electrical-based delivery.**

There is more than one method within each category, trials will be carried out to reach the best option and the favored linking method.

Materials:

1) Polymers such as polylactide (PLA), poly (D, L-lactide–co-glycolide) (PLGA), poly Ɛ caprolactone and / or others.

2) Different lipids such as glyceryl palmitostearate, glyceryl behenate, monostearin, tristearin and / or others.

3) Stabilizers and surfactants such as poloxamers, tweens and polyvinyl alcohol and / or others.

4) Ferrous chloride and ferric chloride.

5) Anticancer drugs to be encapsulated/coated (Betaine).

Phase III:

Cytotoxicity assessment and testing the effect of new formulated nano-particles and/or Bacterial Ghost on the proliferation of breast cancer cell lines in-vitro:

Target of the phase III:

- To investigate the efficacy of the new formulated of nano-particles and/or Bacterial Ghost as a new strategy targeting breast cancer proliferation and metastasis. For that sake, we will use different breast cancer cell lines ranging from a quit (dormant) to highly metastatic breast cancer cell line to investigate the efficacy of our new treatment strategy on breast cancer cell lines.

Methodology of phase three:

A) *In vitro* cell culture assays

Cell culture:

HCC cells will be cultured in DMEM medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (complete medium). All cells will be grown in a humidified 37°C incubator in 5% CO2.

Cell proliferation assay:

Cell proliferation will be analysed by Cell Titer 96 AQ_{ueous} One Solution Cell Proliferation Assay Kit (Promega, Madison, WI, USA) following the manufacturer's instructions, and as detailed elsewhere [29]. Data will be calculated as a percentage of untreated control cells.

Cytotoxicity assay:

MTT assay will be used for the measurement of the cytotoxicity of treated compounds as described previously [30]. In brief, approximately 1×10^5 cells per well will be seeded into 96-well culture plates in 200 μl of complete medium at 37°C with 5% CO_2. After overnight growth, the cells will be washed with the same medium, and then will be replaced with new medium containing varying concentrations of nano-particles and/or Bacterail Ghost for different time intervals (0–72 h). After 24 h treatment, 10 μl of 5 mg/ml MTT (Sigma) solution will be added to the culture and incubated for 2.5 h at 37°C. MTT reaction will be terminated by the addition of 40 mM HCI in isopropanol. The MTT formazan formed will be measured spectrophotometrically at 570 nm according to the method described by Mosmann (1983) [31]. IC_{50} value will be calculated by Bliss method [32].

Cell cycle analysis:

Two breast cancer cell line (ER-positive cell line (T-47D and ZR-175) and Triple negative cell lines cells (MDA-MD-231 and MDA-MD-861) (3×10^5 cells will be seeded per well in 24-well plates) will be treated with BRP (5 and 10 μM) for 48 h. The cells will be washed twice with PBS and will be fixed with 70% ethanol for 24 h at -20°C. Fixed cells will be washed twice with PBS and will be stained with 50 μg/ml of propidium iodide in PBS containing 20 μg/ml RNase A for 30 min in the dark. For determination of DNA content at various phases in the cells, flow cytometry will be used. At least 10,000 cells will be collected in each experiment.

Ultrastructural studies using Transmission electron microscopy (TEM):

As described previously [33], cells will be harvested and washed in PBS and cell pellets will be fixed with 2.5% glutaraldehyde for 2h at 4°C and then will be incubated with 1% osmium tetroxide for 1h at 4°C. After dehydration in series concentrations of ethanol and infiltration in propylene oxide, cells will be embedded in Epon. Ultrathin sections (60 nm) will be stained with uranyl acetate and lead citrate, then cell Ultrastructural features will be observed by TEM.

Immunohistological methods:

Cell proliferation and metastatic markers in tumors will be evaluated. Briefly slides will be deparaffinized, heated in a microwave oven for 15 min in citric buffer (0.01%), and will be covered with antibody for 1 h. The antibody is HPR-conjugated; therefore, after 2x PBS wash, the slides will be stained with DBA and counterstained with hematoxylin for characterization of tumor morphology.

Anticipated Results and Evaluation Criteria

Phase I – Anticipated results:

1) A successfully intact to be produced BGs that will be used as a drug delivery system.
2) Full in-vitro characterization.
3) Properly lyophilized ghost vials for testing on a cancer cell lines in later stages.

Phase I – evaluation criteria:

1) The released DNA and protein amount tested by the Nano-drop. **(Evaluation for safety) "The release of reasonable quantities of proteins and DNA is a strong indicator for effective cellular evacuation." (16)**
2) Confirmed killing of the bacterial strain by sub-culturing. **(Evaluation for safety).**
3) Punctures formation using scanning electron microscope. **(Evaluation for ghost's integrity)**

How the data will be analyzed? :

1) **Released DNA amounts:** Beside the Nano-drop, gel electrophoresis could be used for testing the increase in bands' intensity. As a way of analysis, as long as the band's intensity increases- the liberated amount increases too which indicates a safe ghost for use.
2) **Sub-culturing:** As a way of analysis, the result of least colonies observed indicates the least viable count. No colonies observed (empty agar plates) is the best anticipated result.
3) **Punctures formation.** Analysis in this step depends on the holes and punctures observed per ghost on the images obtained by scanning electron microscope.The good obvious puncture ensures that the cytoplasmic contents were successfully expelled out (Safe ghost).

Phase II- Anticipated results:

- Formulated Nano-carrier based delivery system encapsulating "Betaine" as an anti-cancer drug.
- Successful combination between the previously prepared bacterial ghosts encapsulating the HDAC inhibitor and the and developed nanocarrier such as super paramagnetic nano-particle and/or others.

Phase II - Evaluation criteria:

- Characterization of the prepared Nano-carriers in terms of the following criteria [34]:
  1) **Particle size and charge** – using "Malvern zetasizer".
- The PS, polydispersiy index (PDI) and ζ potential (ZP) of the prepared formulations will be measured using dynamic light scattering (DLS) technique. Samples will be diluted (1:100) and sonicated for 5 min prior examination using Malvern Zetasizer .
  2) **Particle shape and morphology** – Transmission electron microscope "TEM", Scanning electron microscope "SEM".

- TEM examination will be carried out for samples diluted with deionized water (1:20). A drop of the diluted sample was dried on a carbon-coated copper grid forming a thin film. The films will be then imaged and photographed.

3) **Drug interaction** – using infrared spectroscopy "FT-IR".
4) **Drug entrapment efficiency,**
5) **Drug release and/ or other characterization techniques such as;** Magnetism, in case super paramagnetic nanoparticles were developed using "VSM", vibrating sample magnetometer. The measurements will be carried out in an external field up to 20 kOe at room temperature.
6) **Stability studies of the developed nano-delivery system.**

Phase III – Anticipated results and evaluation criteria: An important evaluation criterion: Western blotting

- Immunoblotting analysis will be carried out to investigate the effect of nano-particles treatment on the expression of pro- and antiapoptotic markers, major cell proliferative pathways, and the activity of specific oncogenes in breast cancer cell line. Briefly, cells will be plated and cultured in a complete medium and will be treated with different conc. of nano-particles for various time intervals. The cells will be then washed and detached with trypsin-EDTA. Cells will be collected by centrifugation and disrupted in lysis buffer supplemented with protease cocktail inhibitor for 30 min [35].

- For immunoblotting, Protein concentrations of the cell lysates will be measured using the Bradford Bio-Rad assay. 40-60 mg of protein will be separated via SDS-PAGE and will be transferred to a PVDF membrane for immunoblotting. Membranes will be blocked in Tris-buffered saline containing 5% non-fat dry milk and will be incubated overnight at 4°C with primary antibody (at recommended dilutions in TBST, 5% non-fat drymilk) with gentle agitation.

- After three washes for 5 min each with TBST, the membranes will be probed with horseradish peroxidase-labeled goat anti-rabbit or rabbit anti-mouse antibody in TBST, 5% non-fat dry milk) for 1 h at room temperature. After additional washing steps, membranes will be incubated with a chemiluminescent substrate for 1 min at room temperature. To remove bound antibody between each antibody incubation, membranes will be incubated in Restore Western blot stripping buffer (Pierce) according to the manufacturer's protocol and reprobed. Primary antibodies, secondary antibodies, horseradish peroxidase-labeled goat anti-rabbit and rabbit anti-mouse will be purchased from Santa Cruz Biotechnology,Santa Cruz, CA, USA.

Anticipated results: by the end of this phase, we will be able to clear up the following,

1- To introduce a promising data about identification of the molecular role of new formulated nano-particles and loaded Bacterial Ghost as a new strategy to control breast cancer progression, and target and eliminate oncogenic molecular signaling pathways that involve in breast cancer proliferation *in vitro* model.

2- It is expected that the materials and data of this project will be included within the MSc thesis of our graduate student, who will be selected to help the PI and Co-PI for the final data publication and introducing the promising data internationally.

Expected Project Outcomes and Impact to AASTMT.

I- Technical output and Impact:

1) The project will facilitate the practical work of one MSc theses of Ph. Sara Hassan, part-time Demonstrator, College of Pharmacy, AASTMT (Abu Kir Campus) in collaboration with the Institute of Graduate Studies and Research- Biotechnology department, under the supervision of Prof. Ahmed Abd El-Rehiem Hussien with Prof. Ahmed Sultan as an external supervisor.
2) One or more originally reviewed novel research paper accepted for publication (received a DOI) in a Q1-Q2 journal or its equivalent, more than one paper is a highly predicted outcome.
3) Addressing the needs of the current research field through filling the gap of "the major drawbacks of cytotoxic chemotherapy as a cancer treatment".

II- Financial feasibility & Socio-economic Impact:

- Speaking of the Egyptian societal impact:

- Since Egypt has got high incidence rate of breast cancer, the proposed research idea is expected to increase awareness and recognition within and between scientific communities and the whole country.
- Moreover, not only in Egypt but worldwide, the proposed idea will have a main impact on innovative products and processes in industry since the pharmaceutical companies are heading nowadays to the production of new therapies with lesser side effects.
- Spreading awareness for patients perceived that breast cancer is a spiritual disease that is contagious and disgraceful. Mostly, patients stopped work to cater for themselves, and as a result, they encountered financial challenges. Their challenges were compounded with conscious efforts to keep diagnosis secret to avoid being stigmatized.
- The project offers the integration between nanotechnology and biomedical science, which can generate interesting experimental novel data. These experimental data could be valuable source for starting much international collaboration, grant money attraction; opening new channels between Alexandria University and pharmaceutical companies.

III – Publication:

1) At least one journal papers and one conference papers are expected from this project. The expected outcome is at least one peer-reviewed original research paper accepted for publication (received a DOI) in a Q1-Q2 journal or its equivalent.
2) At least one patent (national and/or international) is expected. Prof. A. Sultan has a good experience in submitting US patents. However, it will be expected to submit one patent in Egypt as a preparation for the future step of converting the proposed pilot study into a prototype for the Egyptian market.

Resources.

The available labs are four labs:

1) Pharmaceutics lab –College of pharmacy, AASTMT (Abu Qir) campus. (PI and Co PI-lab)
2) Microbiology lab – College of pharmacy, AASTMT (Abu Qir) campus.
3) Microbiology and biotechnology lab – Institute of graduate studies and research.
4) Cell line lab (Prof. Ahmed Sultan lab) – Faculty of science.

Office and Computer Facilities:

- Available computer labs are found at the AASTMT (Abu Qir) campus.
- **Important programs**: mainly the EndNote program and plagiarism checker.

  Both programs are installed and ready for use in the AASTMT (Abu Qir) campus.

Common features between the four labs (lab space description):

1) All labs are well aerated with fire extinguishers.
2) Presence of multiple benches with basic reagents for disinfection and sterilization.
3) Presence of technicians who are responsible for checking the availability of the reagents and carrying out the starting steps (e.g.: autoclaving the petri-dishes for Agar plates preparation, nano-delivery system formulation), especially in lab 1 and lab 2.
4) Labs are available for work six/five days per week.

Lab	Equipment's Available
Phase I labs: Microbiology labs.	1) Microbiology, immunology and biotechnology Department, microbiology lab, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus. 2) Microbiology and biotechnology lab, institute of graduate studies and research.
	- Shaking incubator. - Autoclave. - Laminar air flow cabinet. - Vortex. - UV/visible spectrophotometer. - Incubator. - Deep freezer (-20 c). - Light microscope. - Real-time PCR. - Conventional-PCR. - Lyophilizer. - Oven. - Glasswares (conical flasks, test tubes, pipettes …… etc.) - Submarine agarose gel electrophoresis unit. - UV-transilluminator. - Different types of fermenters.
Lab	**Equipment's Available**
Phase II lab: Pharmaceutical sciences lab.	Pharmaceutics Department, pharmaceutical Sciences lab, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus.
	- Rotary evaporator. - Bath sonicator. - Magnetic stirrer - Homogenizer. - UV spectrophotometer. - Cooling centrifuge. - Shaking water Bath.

Lab	Equipment's Available
Phase III lab: Research Unit – Alexandria University.	Three separated Unites: One: for Stem cells and cancer Molecular therapeutics. Second: for Molecular Biology and Gene expression. Last: for Animal research Unit.
	- Western blotting System with its scanner (Bio-rad device) - Real Time-PCR (Applied Biosystems, Step One Plus) - Micro-volume Spectrophotometer (Denovix, USA) - Conventional PCR - Cooling micro centrifuge - ELISA micro-plate reader - Shaking water-bath - Revolver Refrigerator - Micro-Pipettes - Vortex - CO2 incubator - 80C Ultra-Freezer - 20C deep freezer - Liquid Nitrogen Tissue bank - Falcon tubes - Spectrophotometer - Mini-Spinning Centrifuge. - Digital balance 3 and 4 digits. - CBC analyzer - IHC Unit with Complete digital imaging system - RNA-DNA Molecular Gene Expression and Manipulation Unit.

Team Information.

*** The team is composed of 5 members:**

1) **Dr. Passent Mohamed Ehab Gaafar (PI).**
   Assistant Professor – Pharmaceutics Department, Pharmaceutical Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus.

2) **Dr. Mai Mahmoud Ali (Co-PI).**
   Assistant Professor - Pharmaceutics Department, Pharmaceutical Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus.

3) **Prof. Ahmed S. Sultan.**
   Professor of Biochemistry and Molecular Therapeutics. Ex: Chairman of Biochemistry Department, Faculty of Science, Alexandria University, Egypt. PI & Director of Stem Cell and Molecular Cancer Therapeutics Unit Alexandria University. Adjunct Professor, Oncology Department, LCCC, Georgetown University Medical Center, Washington DC, USA.

4) **Prof. Ahmed Abd-ElRehim Hussien.**
   Professor of Biotechnology, Vice-Dean for the Institute of Graduate Studies and Research, Alexandria University.

5) **Ph. Sara Hassan Kamel.**
   Part-time demonstrator at the Microbiology, Immunology and Biotechnology Department, Clinical and Biological Sciences Division, College of Pharmacy, AASTMT Abu Qir campus.

1- Dr. Passent Mohamed Ehab Gaafar (PI).

1. Basic Information	
Full Name in Arabic باسنت محمد ايهاب مصطفى جعفر.	**Full name in English:** Passent Mohamed Ehab Moustafa Gaafar.
Title: Doctor of Philosophy.	**Field of specialization: Nanotechnology, cancer therapy and stimuli responsive nano-carriers.**
Affiliation:	Arab Academy for Science, Technology and Maritime Transport. Alexandria. Egypt. (AASTMT).
Current Position:	Assistant Professor – Pharmaceutics Department, Pharmaceutical Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus.
Contact Information: **Mobile Phone:** 01009077838	**Fax: -**

2. Scientific Achievements:		
h* index:** ***2	**Citations :** **46**	**Total number of Int. publications :** **3**

Last three recent relevant publications

Authors (underline your name), year, title, Journal, vol. and pages

1.	Farid, R. M., **Gaafar, P. M. E.,** Hazzah, H. A., Helmy, M. W., & Abdallah, O. Y. (2020). Chemotherapeutic potential of L-carnosine from stimuli-responsive magnetic nanoparticles against breast cancer model. *Nanomedicine*, *15*(9), 891-911.
2.	Atia, N. M., Hazzah, H. A., **Gaafar, P. M.,** & Abdallah, O. Y. (2019). Diosmin Nanocrystal–Loaded Wafers for Treatment of Diabetic Ulcer: In Vitro and In Vivo Evaluation. *Journal of pharmaceutical sciences*, *108*(5), 1857-1871.
3.	**Gaafar, P. M.,** Abdallah, O. Y., Farid, R. M., & Abdelkader, H. (2014). Preparation, characterization and evaluation of novel elastic nano-sized niosomes (ethoniosomes) for ocular delivery of prednisolone. *Journal of liposome research*, *24*(3), 204-215.

2- Dr. Mai Mahmoud Ali (Co-PI).

1. Basic Information	
Full Name in Arabic مي محمود علي أحمد.	**Full name in English:** Mai Mahmoud Ali Ahmed.
Title: Doctor of Philosophy.	**Field of specialization: Nanotechnology, targeted therapy and taste masking and formulation optimization.**
Affiliation:	Arab Academy for Science, Technology and Maritime Transport. Alexandria. Egypt. (AASTMT).
Current Position:	Assistant Professor – Pharmaceutics Department, Pharmaceutical Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) -Abu Qir Campus.
Contact Information: **Mobile Phone:** 01001994153	**Fax: -**

2. Scientific Achievements:		
h* index:** ***2	**Citations :** **90**	**Total number of Int. publications :** **3**

Last three recent relevant publications

Authors (underline your name), year, title, Journal, vol. and pages

1.	El-Salamouni, Noha S., **Mai M. Ali**, Sherien A. Abdelhady, Lamia S. Kandil, Gihan A. Elbatouti, and Ragwa M. Farid. "Evaluation of chamomile oil and nanoemulgels as a promising treatment option for atopic dermatitis induced in rats." *Expert opinion on drug delivery* 17, no. 1 (2020): 111-122.
2.	Wen, Ming Ming, Noha S. El-Salamouni, Wessam M. El-Refaie, Heba A. Hazzah, **Mai M. Ali**, Giovanni Tosi, Ragwa M. Farid, Maria J. Blanco-Prieto, Nashiru Billa, and Amira S. Hanafy. "Nanotechnology-based drug delivery systems for Alzheimer's disease management: Technical, industrial, and clinical challenges." *Journal of Controlled Release* 245 (2017): 95-107.
3.	**Ali, M**., M. A. El-Massik, O. Y. Abdallah, and H. Abdelkader. "Reduction of bitterness and enhancing palatability of cetirizine oral liquid dosage forms by cyclodextrins." *Journal of Pharmaceutics & Drug Development* 1, no. 3 (2014): 1-8.

3- Prof. Ahmed S. Sultan.

1. Basic Information	
Full Name in Arabic أحمد سمير سلطان	**Full name in English:** Ahmed Samir Ahmed Sultan.
Title: Professor & Director	**Field of specialization:** Biochemistry & Molecular Cancer Therapeutics & Cancer Stem Cells
Affiliation:	Faculty of Science, Alexandria University
Current Position:	Professor & Director of Stem Cells and Molecular Cancer Therapeutics Unit. Ex. Biochemistry Department Chairman Adjunct Professor, Lombardi Cancer Center, Georgetown Univ. Med. Center, USA
Contact Information: **Mobile Phone:** +201222267463 EG and +1 2027518264 US	**Fax: +**2035839256

2. Scientific Achievements:		
***h* index:** **22**	**Citations** **1478**	**Total number of Int. publications:** **96 and three books**

Last three recent relevant publications

Authors (underline your name), year, title, Journal, vol. and pages

1.	**Sultan AS**, M.A. Marie, S.A. Sheweita, Novel mechanism of cannabidiol induced apoptosis in breast cancer cell lines, The Breast (2018), doi: 10.1016/j.breast.2018.06.009 (Q1)
2.	Elsherbini, A.M. Sheweita, S.A., **Sultan, A.S** Pterostilbene as a Phytochemical Compound Induces Signaling Pathways Involved in the Apoptosis and Death of Mutant P53-Breast Cancer Cell Lines. Nutrition and Cancer, 2020 (Q2)
3.	Sheweita SA, Rania GA, Sally AS, **Sultan AS.** Letrozole and zoledronic acid changed signalling pathways involved in the apoptosis of breast cancer cells. JTUMS. 2021 (16):112-120. doi.org/10.1016/j.jtumed.2020.10.017
4.	Rana M. A. R., Hedayat A. G., Afrah F. A, Mahmoud I. K., Noha A., Attalla F. El-Kott and **Sultan AS**. Dipteran Carboxymethyl Chitosan as an Inexhaustible Derivative with a Potential Antiproliferative Activity in Hepatocellular Carcinoma Cells. Evidence-Based Complementary and Alternative Medicine. 2021, 28: 4396305. doi: 10.1155/2020/4396305 (Q1)
5.	Abdelmeguid NE, Khalil MI, Badr NS, Alkhuriji AF, El-Gerbed MSA, **Sultan AS.** Ameliorative effects of colostrum against DMBA hepatotoxicity in rats. JTUMS. 2021) in press) (doi.org/10.1016/j.jtumed.2020.10.01
6.	Mohamed HSH, Morsy A. Abu-Youssef, Yousry M. G, Hesham MAS, Johannes Haybaeck, and **Sultan AS.** Potential antimicrobial and anticancer activity of silver nanoparticles conjugated with pyridine and pyrimidine derivatives. Eur. J. Med. Chem. (in press 2021) (Q1)

4- Prof. Ahmed Abd El-Rehim Hussien.

1. Basic Information	
Full Name in Arabic: أحمد عبد الرحيم حسين	**Full name in English:** Ahmed Abd El-Rehim Hussien.
Title: Professor and Vice-Dean.	**Field of specialization**: Microbiology, Molecular Biology and Medical Biotechnology
Affiliation:	- University of Alexandria, Department of Biotechnology, Institute of Graduate Studies & Research.
Current Position:	Vice-Dean for the Institute of Graduate Studies and Research, Alexandria University.
Contact Information: **Mobile Phone:** 002- 01227922071 and 806-224-5567 **Fax:** 002-03-4285792	

2. Scientific Achievements:		
h index: **21**	**Citations:** **1,063**	**Total number of Int. publications:** **103**

Last three recent relevant publications

Authors (underline your name), year, title, Journal, vol. and pages

1.	Chong, L., Feghaly, J., Zoglman, J., & **Hussein, A.** (2020). A DILEMMA IN DEVICE THERAPY. *Journal of the American College of Cardiology*, *75*(11_Supplement_1), 2615-2615.
2.	Bashandy, T., **Hussein, A**., Solma, M., Kassab, A., & Hamdon, H. (2020). Molecular Evaluation of Three Populations of Farafra Sheep in Comparison to Ossimi and Rahmani Sheep Breeds. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *68*(6), 929-936.
3.	**Hussein, A**., Al-Sabagh, A., Noah, A. Z., El-arabi, A., & EL Safty, M. (2020). Enhancing Oil Based Drilling Fluids Parameters Using Secondary Synthetized Emulsifiers and Nanoparticles. *Egyptian Journal of Chemistry*, *63*(10), 2-6.
4.	Mar, P. L., Kumar, S., **Hussein, A**., Lakkireddy, D., & Gopinathannair, R. (2020). Tachycardia cycle length alternans in orthodromic reciprocating tachycardia due to mutually dependent dual AV node physiology and retrograde supernormal conduction. *Journal of Interventional Cardiac Electrophysiology*, 1-2.
5.	Horbal, P. J., Hassanieh, I., Daher, G., Duderija, E., Kichura, A., & **Hussein, A.** (2020). MANAGEMENT OF A LARGE LEFT VENTRICULAR MASS EXTENDING FROM THE MITRAL TO AORTIC VALVE. *Journal of the American College of Cardiology*, *75*(11_Supplement_1), 2772-2772.
6.	Amro, A. A., Neama, A. J., **Hussein, A**., Hashish, E. A., & Sheweita, S. A. (2014). Evaluation the surface antigen of the Salmonella typhimurium ATCC 14028 ghosts prepared by "SLRP". *The Scientific World Journal*, *2014*.

Research Team Information Table

Name of Res. Team Member in English	Name of Res. Team Member in Arabic	University / Institute In English	Position / Title	% of time spent on project	No. of months	Incentive per month (LE)	Number of other projects and their IDs	Total % of time spent on other projects	Contact No
Passent Mohammed Ehab Gafaar	د. باسنت محمد ايهاب جعفر جعفر.	AASTMT/ College of Pharmacy (PI)	Lecturer, College of Pharmacy, AASTMT (Abu Qir)	40	12	1500	-	-	+201009077838 Passent.ehab@aast.edu
Mai Mahmoud Ali	د. مي محمود علي.	AASTMT/ College of Pharmacy (Co- PI)	Lecturer, College of Pharmacy, AASTMT (Abu Qir)	40	12	1400	-	-	+201001994153 drmaiali@aast.edu
Prof. Ahmed Abdelrehim Hussien .	ا.د. أحمد حسين عبد الرحيم.	Institute of graduate studies and research, Alexandria university.	Prof. of Biotechnology.	20	12	1300	-	-	+201033999805 Ahmed.hussein@alexu.edu.eg
Prof. Ahmed Sultan.	ا.د. أحمد سلطان.	Faculty of science, Alexandria university.	Prof. of Biochemistry and Molecular therapeutics	20	12	1300	-	-	+201222267463 dr_asultan@alexu.edu.eg as4048@georgetown.edu
Sara Hassan	ص. سارة حسن.	AASTMT	Part-time demonstrator.	60	12	500	-	-	+201281044280 sara_hassan975@yahoo.com

Project Management

PLAN ON PROJECT'S ACTIVITIES (*GANTT CHART*):

Activity Name	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Main 1: Bacterial ghost production.	#	#	#									
Sub 1.1: Strain selection	#	#										
Sub 1.2: Ghost formation	#	#	#									
Sub 1.3: Ghost characterization.	#	#	#									
Main 2: Nanoparticle part.	#	#	#									
Sub 2.1: Nanoparticle formulation.	#	#	#	#								
Sub 2.2: Drug entrapment/loading.			#	#								
Sub 2.3: Coating/attaching.			#	#								
Main 3: *In-vitro* cytotoxicity assessment.				#	#	#	#	#	#			
Sub 3.1: Sub Task 3.1												
Sub 3.2: Sub Task 3.2												
Sub 3.3: Sub Task 3.3												
Main 4: Scientific writing.					#	#	#	#				
Sub 4.1: Results analysis.							#	#				
Sub 4.2: Manuscript preparation.		#	#	#	#	#	#	#	#	#		
Sub 4.3: Reviewing and submission						#	#	#	#	#	#	#

Main Task 1: responsibility of:

1) Prof. Ahmed Hussien.
2) Ph. Sara Hassan.

Main Task 2: responsibility of:

a) Dr. Passent Ehab.
b) Dr. Mai Ali
c) Ph. Sara Hassan.

Main Task 3: responsibility of:

a) Prof. Ahmed Sultan.
b) Ph. Sara Hassan.

Main Task 4: responsibility of:

- **Results analysis:** Each member is concerned with the analysis of his/her assigned task
- **Scientific writing:** Ph. Sara Hassan with the instructions of the four members.
- **Reviewing:** The whole team.

➢ **Information flow and communication:** Consortium periodical meetings either zoom meetings or placed meetings at one of the three associations.

Allowable Project Costs

i. **Personnel costs of the research staff and other personnel**: 72,000 L.E.

ii. **Mobility costs (internal travel):**

- **For example: For phase II:** Measuring magnetism by VSM will require travelling to Cairo.
  - **Total cost:** 2,000 L.E.

Iii- Acquisition of material and small-scale research equipment: all equipment and non-disposable materials will serve Al-Alamein campus.

- **For Phase I:**

Target strain (ATCC strain, maybe: *Salmonella enterica serovar typhimurium*).	15,500 L.E.
Staphylococcal nuclease enzyme.	16,000 L.E.
Reagents: SDS, H2O2, tween 80, Muller-Hinton agar, lactic acid, KOH, NaOH, CaCO3, gram-stain reagents (iodine, safranin, crystal violet, ethyl alcohol, immersion lens oil), agar jelly powder.	7,500 L.E.
Petri-dishes, metal and/or disposable loops, Cryovials, Pipettes and glass-wares. Small centrifuge beach-top 15,000 r.p.m.	1,000 L.E. 25,000 L.E.
Drug to be loaded into the ghost (HDAC inhibitor, maybe: SAHA)	10,000 L.E.

- **Total cost for phase I: 75,000 L.E.**

- **For Phase II:**

Reagents: Hydrated $FeCl_3$, hydrated $FeCl_2$, ammonia, HCL, Tween 80, Tween 60, Span 40, Span 60, Cholesterol, Polaxomer 407, different lipids (mainly: Phospholipids such as "Lipoid s100"), different polymers.	10,000 L.E.
Liposome extruder holding a sack of two polycarbonate membrane filters with a pore size of 200 nm.	7,600 L.E.
Dialysis tubes, Visking® 36/32, 28 mm, MWCO 12000-14000, Serva, USA.	3,000 L.E.
Centrisart ultracentrifuge tube, molecular weight cutoff [MWCO] 20,000, Vivaproducts, Inc, Littleton, MA, USA. (2 boxes).	4,000 L.E.
Small neodymium-iron magnet (10*10*3 mm). Probe Sonicator.	400 L.E. 25,000 L.E.
Drug: Betaine	7,000 L.E.
Full physicochemical characterization	10,000 L.E.
Supelcosil HPLC C18 column (5 m; 250 mm × 4.6 mm, Supelco Corporation, PA, USA)	10,000 L.E.

Total cost:	**77,000 L.E.**

- **For Phase III:**

ITEM	1 Year
I. Equipment's (40,000 LE), including Egyptian Taxes **(a).** Instruments= 25, 000 LE **(b).** Spare parts= 15,000 LE	1-UV lamp clean cabinet 2-Double distal water system filters. 3-Hepa filters (FFU cleanroom supplies) for biological cabinets, Co_2 incubators' filters, suction pump and Co_2 incubators' regulators (2). 4-Liquid nitrogen tissue tank reservoir 35L. 5-Micropipette (French version) 1, 200, 20, 1 micro-letter. 6-Steril plastic and T.C. glass wears. 7-TC-Horse and Fetal serum 8- TC plastic wears.
II. Expendable Supplies & Materials (119,000 LE). Including Egyptian Taxes a-Materials/supplies b-Kits c-Disposables d-Media and Buffers	1-Tissue culture chemicals, medias, protein and DNA markers, labeling, growth factors, histological embedding and stains, kits, media and sterilization supplies. 2-Molecular biology reagents (buffers, proteinase inhibitors, Agarose gels and Matrigel (3D TC). 3-Primer for PCR and gene isolation kits. 4-Histon release detection kit 5-Caspases color detection kit 6-TMP Western detection kit 7-Liquid Nitogen tank.

	Items	Number of Units	Unit Price (L.E)	Total Price (L.E)
ANTIBODIES	Mouse MAB-GLUT4	10,705	100 UL	10,705
	MAB-CEBPβ	10,460	100 UL	10,460
	E-cadherin antibody	6550	100 µg	6550
	β-catenin antibody	6550	1 mL	6550
	Cleave caspase-3 Antibody	6550	100 µg	7550
	p53 antibody	12976	200 UL	12976
	PPARγ antibody	8040	50 UG	8040
	Acrp30 antibody	9610	100 UG	9610
	p-21 antibody	9765	100 UG	9765
	Second antibody anti-mouse	1745	0.5 ml	1745
	Human recombinant TGFb1	5535	5 UG	5535
TOTAL EXPENSES FOR 1st YEAR				**89,955**

Table of Eligible Cost

Eligible costs	Break downs		AASTMT support (L.E.)
(A) Staff Cost	Dr. Passent Mohammed Ehab Gafaar. (PI)		18,000 L.E.
	Dr. Mai Mahmoud Ali. (Co-PI) Prof. Ahmed Abd El-Rehim Hussien. Prof. Ahmed S. Sultan. Ph. Sara Hassan Kamel.		16,800 L.E. 15,600 L.E. 15,600 L.E. 6,000 L.E.
	Technicians and/or Labor		-
	Consultation fees		-
	Total		72,000 L.E.
(B) Equipment	Equipment **(explained in details for phase II and III allowable project costs).**		82,600.
	Spare parts **(explained in details for phase III).**		15,000.
	Total Equipment		97,600 L.E.
(C) Expendable Supplies & Materials	Stationary		-
	Miscellaneous Laboratory, Field supplies, Materials. **(explained in the allowable project cost part).**		154,900.
	Total expendable Supplies & Materials		154,900 L.E.
(D) Travel	Internal Transportation		2,000.
	Accommodation		-
	Total travel		2,000 L.E.
(E) Other Direct Costs	Services	Manufacture of specimens and prototypes.	-
		Acquiring access to specialized reference sources databases or computer software	-
		Computer services	-
	Report preparation		-
	Publications & patent Costs		17,000 L.E.
	Workshops organization or Training		8,000 L.E.
	Others (explain): **antibodies purchase, anti-cancer drugs, target strain purchase, Physicochemical characterization and SNUC enzyme purchase**.		148,500 L.E.
	Total other direct costs		173,500 L.E.
(G) Total Costs			**500,000**

Plans for Disseminating Research Results / Sustainability of the action.

Dissemination plan.

- The process of sharing research findings is essential for uptake and use to ensure the success and sustainability of practice-based research networks as a long-term goal.

- **The purpose of dissemination:**
  1) Raising awareness for two important issues, the first issue is the current resistance that may develop against several chemotherapeutics which results in less achievable results, and with no doubts the second issue is the increasing incidence and mortality rates of breast cancer.
  2) Inform the scientific community and to whoever is interested in the research field with the new trends of drug delivery systems and how these trends can be joined together for creating a better treatment option.
  3) Getting the feedback from the community which in return will help in the future steps to be taken regarding the work around the same point of interest.
  4) Promotion, selling and showing the obtained output and results to the scientific community in order to maintain the sustainable development in the cancer research field.

- **Targeted audience and stakeholders:**
  1) Internal Audiences: mainly members of the project consortium and the AASTMT committee where a report should be handed.
  2) External Stakeholders: to reach out to people who will benefit from the outcomes of the project, such as teachers, researchers, and journalists, who can act as catalysts for the dissemination process.

- **Methods:**
  1) Regarding the team members: consortium placed/online meetings to be held periodically.
  2) Regarding the AASTMT: report submission with the latest achievements, to be handed each three months.
  3) Regarding the research community: the journal article to be published by the end of the project.

Declaration of original submission and Other Grant(s).

This proposal did not and will not be submitted in whole or part for funding; twice within the same cycle, or to other funding programs within AASTMT, or other funding agencies.

The corresponding PI did not receive any fund or project grants over the last three years.

Acknowledgment Form

By signing below, I acknowledge that I have read, understand and accept to comply with all the terms of the foregoing application, mentioned in AASTMT general conditions and guidelines for submitting a research proposal, including, but not limited to:

- The total number of the application pages should not exceed **30 pages** excluding a cover page, as well as all sections of the proposal (as mentioned in AASTMT General Conditions and Guidelines for Submitting Research Proposal).
- At any time, a contracted AASTMT project team member should only be participating in a maximum of 3 projects (or a maximum of 2 projects as a PI).
- Allowable budget maximum limit should be strictly adhered to in the project proposal. In all cases, requested budget has to be justified in detail.
- AASTMT guidelines, IPR rules, code of ethics, etc. (www.aast.edu), should be read carefully and adhered to. These are integral parts of the contract.
- All proposals – in addition to PI and other data - must be uploaded to the AASTMT website by the designated deadline. Uploaded PI data should conform to the corresponding data in the application form. The PI must be a PhD holder.

Applications will not be considered eligible and will be discarded in the following cases:

- Proposals submitted by e-mail or sent as hard copies or uploaded to the AASTMT website after the deadline.
- Proposals not conforming to the designated format.
- Proposals whose uploaded PI data does not conform to PI data in the proposal file.
- Proposals in which the allowable budget maximum limit has been exceeded.
- Proposals in which maximum allowable contracted AASTMT project participation limit has been exceeded.
- Proposal letter does not include a scanned copy of the signed and stamped PI institution endorsement letter in case of team member work outside AASTMT.
- Submitted applications will be evaluated and the applicant will be informed with the evaluation result of his/her proposal within 3-4 months.
- AASTMT technical decisions made by remote reviewers are final.
- Proposal does not include a scanned copy of the signed acknowledgment form.

Date & Signature: Passent Gaafar 14-3-2021

Key Publications and references

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March 10, 2021

ENDORSEMENT LETTER

On behalf of Alexandria University, Faculty of Science, I would like to express my pleasure to join the new established multidisciplinary, consortium, research team, to submit an innovated research proposal titled "Novel Nano-delivery system: Targeted Bacterial Ghost Associated Nano-particles Encapsulating Promising Chemotherapeutic Strategy for Breast Cancer Therapy" to AASTMT RESEARCH-2020 Call for Collaboration Research and Innovation Projects, PI; Dr. Passant Mohamed Ehab Gafaar, Ph.D., College of Pharmacy of the Arab Academy for Science, Technology and Maritime Transport (AASTMT), Alexandria, Egypt.

Herein, I would like to confirm my enthusiastic collaboration and full support of the above proposed study. As I have stated in the proposed study that I will be responsible for the Phase III of the study and will provide Dr. Passant with the possible technical and materials support that are important to achieve the main goal of the proposed study such as Tissue Culture facilities (2D and 3D culture), different breast cell lines, and the Molecular Biology techniques to investigate the Molecular mechanism of the objectives of the proposed study at the level of gene expression.

The proposed work of Phase III will be performed in my research Unit at the Faculty of Science and I has carefully reviewed the application and agreed to the objectives of the proposal and the Institutional's role at the level of participation, and I am willing to complete the assigned activities.

Finally, I will support Dr. Passant Gafaar's proposed research study and provide her with all possible facilities to guarantee the success of the proposed research.

Best regards,

Dr. Ahmed Sultan, M.Sc., Ph.D., MPH

Ahmed Sultan

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جامعة الإسكندرية
ALEXANDRIA UNIVERSITY

معهد الدراسات العليا والبحوث
Institute of Graduate Studies & Research

March 10, 2021

ENDORSEMENT LETTER

To whom it may concern,

This letter confirms the support of Institute of Graduate Studies & Research, Alexandria university, Biotechnology Department Represented by Prof. Dr. Ahmed Abd El-Rehiem Hussien, main supervisor for the master candidate: Sara Hassan Kamel, to collaborate with Dr. Passant Mohamed Ehab Gafaar from the college of Pharmacy of the Arab Academy for Science, Technology & Maritime Transport (AASTMT), Alexandria (Egypt), in research projects under the Call for Collaboration Research and Innovation Project, to submit an innovated research proposal titled "Novel Nano-delivery system: Targeted Bacterial Ghost Associated Nano-particles Encapsulating Promising Chemotherapeutic Strategy for Breast Cancer Therapy". The team member has carefully reviewed the application, agrees to the objectives of the project and Institute's role and level of participation in it, and is willing and able to complete the activities of phase I. It is envisaged that the principal terms of the proposed collaboration would be substantially as follows to achieve the objectives of the AASTMT Grant Call: - Promote multidisciplinary collaborative research and development projects in the area of innovative drug delivery systems. - Support research outputs exploitation and innovation with closer links to regional industries to develop new therapeutic options for treatment of breast cancer- Promote the key issues of competitiveness and collaboration - Support applied research projects that contribute to the achievement of sustainable development - Raise awareness on the importance of research and innovation in developing new technologies and their economic and social impacts.

The team member's approval:

A. Hussein

Prof. Dr. Ahmed Hussein
Department of Biotechnology
Institute of Graduate studies
& Research
Alexandria University