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Arava Center for Desert Plants Based Drug Discovery

a) Mission and Innovation:

**Mission:** To establish a center for drug discovery that will enable the identification of drug candidates, characterization of their mode of action and assessing their therapeutic efficacy on animal models for human diseases. Effective candidates will be considered for drug development and clinical trials. **Innovation:** To use a unique library of extracts/compounds derived mainly from plants native to the Negev-Arava Desert in Israel.

b) Specific aims:

1. To use cellular models of human diseases (cancer, neurodegenerative diseases and more) for screening libraries of natural entities (e.g. extracts/compounds of desert plants).

2. To screen the active candidates on vertebrates` animal models (zebrafish, c.elegans) for human diseases such as cancer, Alzheimer`s disease, Parkinsons` disease, Obesity and more.
c) Executive summary:

"Arava desert plant library"

Analysis of natural products as sources of new drugs indicates that many effective drugs (e.g. anti-cancer) may be traced to natural origin. Interestingly, the mainstream pharmaceutical research is moving from mono-molecular or single target approach to combinations and multiple target strategies. Perhaps multi-site mechanisms of action of crude desert plant extracts may offer greater chances for success where conventional single-site agents have been disappointing. The "Arava library" is composed from plants that belong to various families, including: SCLEPIADACEAL, MOLLUGINACEAL, POLYGONEAL, RHAMNACEAL, TAMARICACLAL, CUPRESSACEAL, CYPERACEAL, GERANIACEAL, GRAMINEAE, CUCURBITACEAL, LORANTHACEAL, ZYGOPHYLLACEAL, BORAGINACAL, AIGOACEAL, EUPHORBIACEAL, MALVACEAL AMARANTHACEAL, NEURADACEAL, CISTACEAL SCROPHULARIAL PLANTAGINACEAL, NYCTAGINACEAL, MORINGACEAL, COMPOSITAL, SOLANACEAL, SALVADORACEAL, LILIACEAL. Plant extracts prepared in polar solvent (water) and non-polar solvent (organic solvents) were used successfully against cell-based models of human diseases. Active compounds identified through such screening can provide 1) the starting point in the design of research tools that allow pharmacological probing of a molecular target in a disease process 2) leads for drug development.

Approaches and disease models

Current high-throughput screening methods for drug discovery face the problem that most of the hits generated during screenings turn out to be invalid when moving towards preclinical trials. To improve the process, screenings of chemical libraries are performed on whole animals. Murine model (Mus musculus) is one of the most commonly used animal models in biology; however, its cost and length of the experiments, limit its use in large-scale therapeutic screening. To date, the nematode Caenorhabditis elegans and the fish Danio rerio (“zebrafish”) are...
gaining momentum as screening tools. These organisms combine genetic amenability, low cost and culture conditions that are compatible with large-scale screens. Their main advantage is to allow high-throughput screening in a whole-animal context.

We plan to start with screening of libraries of compounds/extracts from desert plants on cellular models of human diseases (in tissue culture context) in order to identify hits to leads. The collection of drug candidates will be then subjected to screening on animal models.

d) The projects:

1. *Caenorhabditis elegans*: A versatile platform for screening a library of desert plants for drug discovery

Many brain diseases, including Huntington’s and Parkinson’s diseases, are characterized by the accumulation of damaged proteins with devastating consequences with no available cure. A novel source for drugs for these diseases could come from natural products. We are using the "Arava desert plant library" extracts, to screen for therapeutically active compounds affecting protein aggregation and we utilized *Caenorhabditis elegans* (*C. elegans*) Huntington’s disease model system. *C. elegans* have proven invaluable screening platform to study the mechanisms underlying neurodegenerative diseases. This proposal will focus specifically on the utilization of *C. elegans* models to identify desert plant extracts with potential therapeutics activity and to develop meaningful therapeutic strategies that either prevent or delay neurodegenerative disease onset or treat disease symptom. However, this system can be expanded in the future to...
examine other disease models using *C. elegans*. The main Goals: 1. to employ established screening protocols for the discovery of desert plant extracts with potential neuroprotective role against neurodegenerative disease models in *C. elegans*. 2. To domesticate the plants with functional activity in order to suggest a new crop of medicinal plant for the farmers of the Arava.

2. Scientific basis for folklore information on medicinal plants: development of platform for functional research and study of the benefits of edible desert medicinal plant *Asteriscus graveolens* as a model plant

We wish to develop a platform that will contribute to the process of "evidence-based complementary and alternative medicine" by adding scientific data for the mechanism of action of medicinal plants (whole plant vs. its constituents). The model plant in this project is the edible desert plant *Asteriscus graveolens* (from the "Arava desert plant library") as a potential anti-cancer medicinal plant. For that purpose we shall employ bioassay guided fractionation of the extract and identify the active compounds. We wish to learn whether the crude plant extract is as good as its active components (or even better). We shall evaluate several parameters that are indicative for potential anti-cancer activity of *Asteriscus graveolens*: programmed cell death (apoptosis), wound healing, antioxidant content. As people (in the Arava) drink tea from *Asteriscus graveolens* we hope that the results of this study will enable us to develop this plant into 1. An agricultural crop and 2. As a formula of medicinal plant with therapeutic entities.

3. Screening of Artemisia plant cultivates for anti-cancer pro-apoptotic activity

Various reports indicate that secondary metabolites from Artemisia species exert cytotoxicity against tumor cells. The following species from the Artemisia genus:
Artemisia arborescence, A. judaica, A. sieberi Besser, A. californica, A. vulgaris and A. dracunculus (adopted by Dr. Nativ Dudai, Neve Yaar, Vulcani Institute) will be studied for their effect on activating the genetic program of cell death in cancer cells. Every cell in our body contains a genetic program for its death. This genetic program termed: apoptosis is inactive in cancer cells. Treatment of cancer cells with chemotherapy sometimes face the problem that the cascade of events that should results in apoptosis, failed. Compounds that will activate apoptosis may act as anti-cancer drugs. In this project we look for such compounds by screening plant extracts and essential oils in vitro for those that inhibit selectively cancer cell growth and not normal cells. The active extracts/essential oils will be subjected for future studies with zebrafish model for cancer. The active compound will be identified by assay-guided fractionation.

4. Zebrafish as animal model for anti-cancer drug discovery:

I. Screening and elimination of toxic materials II. Pre-clinical studies with plant extracts active in in vitro models of cancer

In this project we shall use zebrafish as a discovery approach in which plant extracts/compounds from the "Arava desert plant library" are assayed for their effects on inhibiting human tumor growth in xenograft models. Zebrafish are vertebrate organisms that are of growing interest for preclinical drug discovery applications. Zebrafish embryos develop most of the major organ systems present in mammals, including the cardiovascular, nervous and digestive systems, in < 1 week and as such are suggested as a model system for pharmaceutical purposes. Many drugs developed by the pharmaceutical industry fail in clinical trials because of unanticipated toxic side effects. This project will be composed from two parts: 1. zebrafish as a platform for screening materials for toxic/non-toxic effects on early zebrafish development. 2. Zebrafish as a pre-clinical animal model for assessing...
selection of desert plant extracts/compounds that have shown by us to have anti-cancer activity in \textit{in vitro} models of cancer.

5. 'Personalized Target Cancer Therapy': Cultures of Putative Human Breast Cancer Stem Cells as platform for Screening "Arava desert plant library"

In the proposed study, we plan to form a platform to identify patient-specific breast-cancer drugs. We will culture patient putative cancer stem cells in “mammosphere” conditions (imitation of the growth of tumor in three dimensions, in vitro) and use these “mammosphere” for screening "Arava desert plant library" extracts/compounds. Active material maybe considered as patient-specific breast-cancer lead for drug.

Worldwide, breast cancer is the 5\textsuperscript{th} most common cause of death. Treatments for breast cancer many times fail to eradicate the tumor and reoccurrence of the tumors occur as metastasis. Recently it has been shown that cancer stem cells (CSC) are involved in many types of cancer and drugs that are useful in eradicating the bulk of the tumor are not effective against CSC. The aims of the present proposal: 1) to isolate and characterize CSCs from effluent and solid breast tumors, 2) to culture cancer stem cells in “mammosphere” conditions and 3) to screen the "Arava desert plant library" extracts/compounds for potential drugs against breast cancer, including CSCs and bulk tumor cells. The platform suggested here for screening effective, person-specific cancer drugs, is a major step toward customized treatment, the heart of the ‘Personalized Target Cancer Therapy’ concept.