**Personal Research Grants**

**Research Grant Application**

**General application information**

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| **Role** | **Name** | **Academic Rank** | **Department** | **Institute** |
| PI.1 | Dr. Aviad Zick MD | Lecturer | Oncology | Sharett Institute of Oncology, Hadassah-Hebrew University Medical Center |
| PI.2 | Prof. Yuval Tabach | Associate Professor | Developmental Biology and Cancer Research | Hebrew University - Faculty of Medicine |

**Research Title**

 CRISPR activation for applying synthetic lethality interactions inselected oncogenes

**Keywords**

CRISPR, sgRNA, Cas9, VPR, synthetic lethality, HER2+, breast cancer, drug discovery, gene overexpression, ERBB2, EGFR, amplicon, coamplification, PARP1, BRCA12/.

**Requested Budget in NIS**

 2

No. of Years

 150,000 NIS

Average Annual Budget

**Summery**

The aim of this experiment is to

find synthetic lethality in HER2+ cell lines BT474 and HCC1954 using CRISPR activation. We will start with 3 specific genes *ERBB2*, *MIEN1* and *ERN1* and will continue to all-genome functional genes. MCF7, an HER2 negative cancer cell line will be used as a negative control. We will try to unravel new way of killing HER2+ cancer cells by changing single gene copy number. The experiment will examine the survival of HER2+ cells after amplification of specific genes, using the CRISPR activation method, in order to cause cell death with synthetic lethality. Cells survival will be examined with XTT survival assay and clonogenic survival assay, DNA extraction will carrie using DNeasy blood and tissue kit, evaluation of genes copy number (CN) with digital droplet PCR, evaluation of protein expression levels by measuring the transcribed mRNA with northern blot.

**Background**

The HER2+ subtype of human breast cancer is associated with the malignant transformation of luminal ductal cells of the mammary epithelium. Human epidermal growth factor receptor 2 (HER2/*ErbB2*) is overexpressed in approximately 25% of human breast cancers. This HER2+ tumor subtype is associated with a gene expression signature that includes inflammation. Indeed, the increased expression of interleukin (IL) 1 and IL6 promotes HER2+ breast cancer development [1].

HER2 protein is encoded by *ERBB2* gene, a member of the epidermal growth factor receptors (EGFR) family. *ERBB2* amplification is defined as multiple copies of a DNA segment containing the *ERBB2* gene, is found in tumors and *ERBB2* amplified/ HER2 positive (HER2+) cancers. *ERBB2* amplification is a prognostic marker for aggressive breast tumors [2] and a predictive marker for prolonged survival of breast cancer patients treated with HER2 inhibitors [3]. Several 17q12-q21 genes are variably coamplified and coexpressed with *ERBB2*, this could influence the response to therapeutic. In some HER2+ tumors, *PPM1D*, which encodes wildtype p53-induced phosphatase, a protein phosphatase that regulates the DNA damage response pathway by inhibiting p53, is co-amplified. *MIEN1*, migration and invasion enhancer 1, is a Protein Coding gene which involved in negative regulation of apoptotic process and positive regulation of cell migration, is also co-amplified with *ERBB2* gene in some cases [4,5]. Cancer cells exploit the unfolded protein response (UPR) to mitigate endoplasmic reticulum (ER) stress caused by cellular oncogene activation. *ERN1*, which encodes IRE1, Inositol-requiring enzyme 1, is an important transducer of the unfolded protein response (UPR) that is activated by the accumulation of misfolded proteins in the endoplasmic reticulum (ER). human breast epithelial cells expressing an activated form of the *ERBB2* oncogene, leads to an overactivation of the UPR and markedly sensitizes these cells to ER stress-induced apoptosis have a sensitivity to ER stress [6].

The incentive to develop personalized therapy for cancer treatment is driven by the premise that it will increase therapeutic efficacy and reduce toxicity. Synthetic lethality is defined as a genetic interaction where the combination of two genetic events results in cell death or death of an organism. A synthetic lethal interaction occurs between two genes when the perturbation of either gene alone is viable but the perturbation of both genes simultaneously results in the loss of viability. Key to exploiting synthetic lethality in cancer treatment are the identification and the mechanistic characterization of robust synthetic lethal genetic interactions [7]. An example for synthetic lethality in cancer - Poly (ADP-ribose) polymerase (*PARP*) inhibitors, which Inhibiting its enzyme activity with small molecules thus preventing DNA repair in *BRCA1/2*-mutated tumors, have become the drug based on the synthetic lethal approach that is approved for clinical use to target [8].

CRISPR activation (CRISPRa) screen is a tool for gain-of-function studies in immortalized cell lines, however, deploying them at genome wide scale has been challenging. This is in part due to the difficulty of introducing three separate components (RNA-guided nucleases, transactivators and guide RNAs) simultaneously into cells to achieve CRISPRa [9]. A screening platform that combines pooled lentiviral Single guide RNA (sgRNA) delivery with Cas9 protein electroporation to enable loss and gain-of-function pooled screening at genome wide scale was applied to primary human T cells. Using this technology, gene modifications that promote T cell proliferation in response to stimulation are identified [10,11]. Recently, CRISPR interference (CRISPRi) study loss-of-function genome wide screen in HER2 positive breast cancer cell line found that inhibition of *FGFR4* gene dramatically increased cell and tumor susceptibility to anti-HER2 therapy, since *FGFR2* signaling serves as an escape pathway which is responsible for anti-HER2 therapy resistance in breast cancer [12].

The standard of care treatments for HER2+ breast cancers involve suppressing the overexpression of HER2 or targeting blockade of its activity such as the catalytic kinase activity or signaling activity. These therapeutic treatments cause the downregulation of cellular HER2 expression which induce cancer cell apoptosis by inhibiting cell proliferation and altering downstream signaling pathways [13]. Gene interactions in HER2+, which have been almost entirely focused on synthetic lethality in only few types of cancer or chemo-dependent synthetic lethality screen.

In our study we will conduct a CRISPRa system using a 3rd generation lenti vector constructed with single guide RNA (sgRNA), VPR (activating factors) fused with dCas9, to find gain-of-function synthetic lethality in HER2+ breast cancer cells, by finding overexpressed pair genes to better understand the HER2 pathway and perhaps gain further insight on treatment options.

**Research objectives**

**Hypothesis:** Synthetic lethal genetic interactions with tumor-specific overexpression genes can be exploited to develop anticancer therapeutics in HER2+ breast cancer.

**Aim:** In this study we will search for synthetic lethality in HER2+ breast cancer cells by finding overexpressed pair genes using CRISPRa. Our work extends previous investigations in HER2+ breast cancer cells, that has been focus on loss-of-function genes paired with HER2 treatment. The aim of this study is to find a new overexpressed synthetic lethality agents in HER2+ breast cancer cell lines. Genome-wide identification of synthetic lethality interactions in HER2+ cell lines by High-throughput screens will be performed. The specific aims are: **Aim1.** To study the effect of *ERBB2*, *MIEN1* and *ERN1* gene overexpression on growth of BTB474 cell line *in-vitro*. **Aim2.** To examine the effect of *ERBB2*, *MIEN1* and *ERN1* gene overexpression on growth of HCC1954 cell line *in-vitro*. **Aim3.** To investigate the effect of whole gene overexpression on growth of BTB474 and HCC1954 cell lines *in-vitro*.

**Study design:** We will establish a high-throughput CRISPRa screening platform in HER2+ breast cancer cells by using a 3rd generation lenti vector constructed with single guide RNA (sgRNA), VPR (activating factors) fused with dCas9 *in-vitro*. The CRISPRa system will introduce traceable sgRNA cassettes and an mCherry fluorescence protein reporter gene by lentivirus. Cells will be labeled with carboxyfluorescein succinimidyl ester (CFSE) to track cell divisions. After 4 days of stimulation, CFSE levels will reveal if cells had undergone multiple divisions. Cells will be sorted by FACS into two populations: (1) non-proliferating cells (CFSE high), and (2) highly proliferating cells (CFSE low). We will quantify sgRNA abundance from each population by deep sequencing of the amplified sgRNA cassettes. MAGeCK software will be used to systematically identify genes that were positively or negatively selected in the proliferating population of the cells. Top positive and negative regulators from the pilot screen will be confirmed in biological replicates with hits screens. Additionally, we will check cell survival in HER2 treated cells with MMT assay Clonogenic assay, a sensitive way to check the ability of a single cell to create a colony by cell division. Cells with a suspect to have a synthetic lethal agent, will undergo DNA extraction and qPCR will be performed in order to find the sgRNA candidate.

**Detailed Budget**

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| **Personnel NIS Amount Requested** |
| **Name** | **Role on Project** | **Title** | **Annual Base Salary** | **% Effort on Project** | **2 year period** | **Totals** |
| Aviad Zick | PI | M.D./Ph.D. | 36,000 | 40% | 72,000 | 72,000 |
| Yuval Tabach | CO-PI | Ph.D. | 24,000 | 15% | 48,000 | 48,000 |
| Dana Sherill-Rofe | Post-doctorate | Ph.D. | 0 | 10% | 0 | 0 |
| Lior Hagoel | Ph.D. student | M.Sc. | 48,000 | 100% | 96,000 | 96,000 |
| Myriam Maoz | Laboratory technician | M.Sc. | 0 | 10% | 0 | 0 |
| **Subtotals→→→→→** | 216,000 |
| **Materials, Supplies, and Consumables (Itemize by Category)** |
| **For *in-vitro* EXPERIMENTS** |
| Item | Price per sample | Per 870 samples  |
| Qiagen QIAamp nucleic acid purification | 5 | 4,350  |
| CRISPR Activation KIT | 15 | 13,050 |
| Cloning kit  | 10 | 8,700 |
| sgRNA Library and PRIMERS  | 35 | 30,450 |
| Survival and Proliferation KITS | 7 | 6,090 |
| Miscellaneous (tubes, chemicals, other consumables)  | 10 | 8,700 |
| **Total** | 82 | 71,340 |
| **Subtotals→→→→→** | 71,340 |
| Travel | 5000 |
| office equipment, publication fees | 2,000 |
| **Subtotals Direct Cost→→→→→** | 294,000 |
| Overhead 2% | 5,660 |
| **Total Direct Costs for Budget Period** | 300,000 |

**Budget justification:** The personnel costs are for time dedicated specifically for this research. Most of the budget is allocated for reagents used for testing the samples. The equipment and computer infrastructure are available on our campus and is routinely utilized by our groups. Experimental PhD student will perform *in-vitro* experiments, will be responsible for all aspects of data processing and developing novel integration techniques, he will be responsible for preparation of sequencing libraries, sequencing run and basic output processing. Dr. Zick Aviad will be the Chief advisor, as prof. Yuval Tabach will serve as co-advisor. Dana Sherill-Rofe is the most experienced person in the lab, she will guide and assist in all aspects in the lab and will support us at the study.

**Available Means for the Development and Conduction of the Research Project:** This grant will be the first in a hopefully massive collaboration between the laboratories of four young PIs with different and complementary expertise in cancer, computational biology, network biology, and extensive expertise in working in the oncology clinic as a medical oncologist.

**Statistical Analysis:**Dr. Zick, M.D/Ph.D, is a medical oncologist who specializes in molecular profiling of tumors. The co-PI Prof. Tabach has trained as a bioinformatician, and as such has extensive experience of data analysis and applications of statistical tests. Several statistical tests will be employed in this bioinformatics project. To find enrichment in a group of genes, we will use a hyper-geometric test. To find correlation between genes across evolution, we will use the Pearson correlation on normalized data. To identify genes that are significantly associated with cancer, we will use a Naïve Bayesian classifier. In addition, we will use a randomization process to calculate p-values at different stages of data analysis.

**Facilities Available:** The lab space (~80 m2) contains a room for 6-8 biologists and an additional room (of ~50 m2) is available for 6 bioinformatics team members, as well as a dark room. Incubators, microscopes, a PCR machine, centrifuges, refrigerators, gel electrophoresis apparatuses (DNA and proteins), freezers etc. are located within access to these facilities. The laboratory has a full computer system with access to our faculty’s computer cluster comprising 640 cores and a storage unit of 300 TB.

Software already installed includes Galaxy, R and Matlab. Databases that are going to be installed include the 1000 genome project and ENCODE. Other software and data can be installed upon request, and each user can upload his/her own data. We will also use the equipment located in the Unit of Interdepartmental Equipment, including both MiSeq and Nextseq desktop sequencers (Illumina). An ION PGM and ION Proton are available on campus in the pathology and genetic departments respectively. The obtained sequence data will be analyzed in‑house by our laboratory's bioinformatician and with the support of the bioinformatics unit.

**Literature References**

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