Prosjektoppgave

NTNU
Norges teknisk-naturvitenskapelige universitet
Nasjonalt senter for matematikk i opplæringen

Mahmoud Hassaan

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Contents

Logical models are a promising method to predict the drug combinations without testing the massive number of possible combinations experimentally. In this project we analyzed two logical models the AGS cancer model CASCADE 1.0 and the generic cancer model CASCADE 2.0 to confirm the previously discovered targets in addition to discovering a new targets that can be used as future cancer treatment target. Using different methods like the synergy scores, determinative power data and COLOMOTO notebook to find the high frequency nodes among the synergy combinations and identify the druggable and non-druggable nodes. The effective model is the model that predict all the possible combinations without missing any true positive combination. By comparing the resulted nodes from each method, we can find the most common nodes that have high score in most of them this will give us a few numbers of possible combination that can be tested experimentally rather than testing all the possible combinations.

1. Introduction

Cancer, malignant tumors and neoplasms are synonyms of a group of diseases that affect any organ of the body with distinguishing characteristic; the quick development of aberrant cells that quickly outgrow their normal bounds and can infiltrate nearby body parts before metastasizing to other organs. The widespread occurrence of metastases in the body is considered as the primary cancer cause of death. In 2020 cancer led to nearly 10 million deaths or about one in six deaths worldwide. Breast, lung, colon, rectum and prostate cancers are the most common and dangerous types of cancers that can lead to death (WHO., 2020).

Most of the biomedical research now focuses on targeted therapy and since the beginning of using omics data the researchers become able to analyze enormous genomic, transcriptomic, proteomic and metabolomic data in short periods of time. All of this knowledge helped in facilitating the ability to identify new therapeutic targets and identification of synergistic targeted drug combinations for treatment of different tumor types (Gilad et al., 2021).

However, with this massive amount of information about drug targets and all of these biomedical researches there are a lot of limitations in the treatment options for many of deadly cancers. This is partially due to the fact that the present attempts to discover new drugs are primarily concentrated on protein families that have already been proven to be druggable like kinases (Jeon et al., 2014).

There are three different methods to generate target inhibitors which can lead to the reducing the drug development pipelines productivity failure. First, screening large chemical libraries to discover new small molecules that have effect on specific cancerous cell targets. Secondly, discovering a new therapeutic effect for the existing non-cancerous drugs. Finally, system biology recently used to discover a new therapeutic effect for existing medications by analyzing a lot of data that is related to cancer cell lines like drug disease networks, gene expression profiles, in addition to using target's structure similarities which plays pivotal roles in identification of novel medications (Jeon et al., 2014). Although using target therapies by single agent for treating cancer patients totally transformed cancer treatment strategies in the past, using single therapy has some drawbacks that decrease efficacy of this method like drug resistance and increased risk of producing side effects. So, many strategies have been developed to create models that have the ability to predict drug combinations using targeted anti-cancer inhibitors depending on biological, molecular and chemical information from cancer cell lines. Multiple pathways that have an important role in apoptosis, proliferation and cell survival will be targeted using drug combinations and this will overcome the resistance problems, increase therapeutic effects in addition the drug combinations will give the ability to reduce dose of each single drug which help in preventing side effects (Flobak et al., 2015, 2019; Gilad et al., 2021; Gregory et al., 2020; Jaaks et al., 2022; Menden et al., 2019).

Drug combination means the use of two or more agents that may increase or decrease efficacy of each other and produce a novel effect. By utilizing the synergistic pharmacological effects which means that the two drugs administered together have greater effect than the effect of each drug alone, the efficacy of anti-cancer therapy combination can be further increased without increasing the dose of single medication to level that led to producing side effect (Flobak et al., 2015).

Combinatorial anti-cancer treatments are considered as a promising strategy for treatment of most serious and complex cancer types, but due to the massive number of possible combinations it is a challenge to identify the most effective combination. It becomes impossible to test all of these combinations experimentally. Therefore, it's necessary to develop an in-silico methods for systematically identification of the most effective combination before testing it in the laboratory (Flobak et al., 2015; Xu et al., 2012)

The development of a logical model with no false positive which means that all the combinations that will be predicted to be synergistic will be confirmed using cell growth experiments and also

with no false negative synergies which means the combinations that will be predicted to be non-synergistic will not give a synergistic growth inhibition in the cell growth experiments. This can lead to the discovery of a drug combination that has a promising effect on preventing treatment resistance and reducing drug side effects. (Flobak et al., 2015; Niederdorfer et al., 2020)

Previous studies in the field of drug target combinations.

Many studies were performed to test the effect of drug target combinations in increasing cancer treatment efficacy and decreasing drug resistance. A study performed by *O'Neil et al* for 12 drugs used in combinations and targeted specific genes and proteins in 39 cancer cell lines (O'Neil et al., 2016). 104 FDA approved drugs were tested against the NCI-60 cell line panel by The National Cancer Institute (NCI). "DREAM challenge" AstraZeneca's drug combination data set which involves 118 drugs, including 59 targeted therapies, which form 910 pairwise drug combinations against 85 cancer cell lines (Menden et al., 2019).

In Flobak et al. study they used a Boolean and multilevel logical model (AGS model- CASCADE 1.0) to predict the synergies between 21 pairwise combinations of 7 chemical inhibitors (Flobak et al., 2015), while in (Flobak et al., 2019) a logical model was used to test the synergy between 19 combining small-molecule inhibitors.

High influence nodes are a group of nodes that control the state of the majority of the other nodes in the model so, a study that focused on the high influence nodes in the generic model CASCADE 2.0 to predict the synergistic drug combination of 19 inhibitors working on four cancer cell lines from gastric, colorectal and prostate cancer (Niederdorfer et al., 2020).

All of these studies give us logical models that can be used for predicting the effective drug combinations that can be used effectively and efficiently in cancer treatment in addition to preventing drug resistance and decreasing drug side effects.

Project aims: (1) Confirm the previously discovered combinations in different studies like Flobak et al., 2015, Neiderdorfer et al., 2020. (2) Analyzing the AGS cancer model CASCADE 1.0 and generic cancer model CASCADE 2.0 for discovering new drug combinations that have high synergy scores in the predicted data created using the two models. (3) Identifying the overrepresented molecular function among the high synergy scores combinations. (4) Identifying the percentage of the druggable and non-druggable nodes among the high synergy combinations.

(5) Confirming the new predicted combinations using pint, bioLQM and MaBoSS softwares in COLOMOTO notebook.

Questions and hypotheses

- What percentage of the drug synergy space is currently druggable?
- To what extent can this be increased if new targets can be included?
- Which molecular function other than protein phosphorylation provides the most candidate targets?
- Can a druggome analysis rationalize future drug development?
- Do all the predicted synergies relate to Cancer cells?
- Is there graph metric behavior similarity between synergy nodes and the other nodes in the models?
- How to identify new biomarkers that help in understanding the mechanism of synergy?

2. Materials and Methods

AGS cancer model CASCADE 1.0 and the generic cancer model CASCADE 2.0 network construction

The data of the two models in our study (AGS cancer model - CASCADE 1.0 & Generic cancer model - CASCADE 2.0) and the synergy data are available on the CASCADE pans (GitHub - druglogics/cascade-pan: Pipeline simulations for higher-order drug combinations in the CASCADEs).

CASCADE 1.0 depends on the (Flobak et al., 2015) data of AGS cell line. The model was created using GINsim software depending on the prior biological knowledge of the AGS intracellular signaling pathways, the model depends on pathways of AGS including PI3K/AKT, NFkB, JAK/STAT, CTNNB1/TCF, and MAPK pathways. The final network consists of 75 components and 149 direct interactions. The model used to predict the synergy between seven inhibitors each inhibitor targets a specific node in the network which leads to 21 pairwise combinations.

CASCADE 2.0 is a model created by (Niederdorfer et al., 2020) study and this study focused on 4 cell lines; AGS (gastric adenocarcinoma), COLO 205 (colorectal cancer), DU-145 (prostate cancer), and SW-620 (colorectal cancer) determined by KEGG and Reactome. The final model consists of 144 nodes and 366 interactions, including Prosurvival and Antisurvival as output nodes, generated using the prior-knowledge of 11 pathways related to the 4 cell lines. The model used to test 18 drug combinations in a set of 153 pairwise.

How was the simulation data created?

Gitsbe (*Generic Interactions To Specific Boolean Equations)* is a pipeline package that produces a group of Boolean models that are all fitted to a given set of steady-state or perturbation data using a genetic parameterization approach. Then, the output models from *gitsbe* used by *drabme (Drug Response Analysis to Boolean Model Ensembles)* which does a drug response analysis of a specified drug panel and generates anticipated synergy scores for each individual drug combination. Those two pipeline packages can be tested in one go using the *druglogics-synergy* package. The ensemble wise output file produced using drabme's launcher indicates how synergistic the combination was,

the more the negative number the more synergistic the combination (Supplementary materials 3 & 4).

Databases used to identify drugs available for each node.

The Drug Gene Interaction Database (*DGIdb - Mining the Druggable Genome*, n.d.) contains all the available drugs for each node so we got a massive number of drugs for each node (Supplementary materials 2). Then, the National Cancer Institute (NCI) (*NCI Drug Dictionary - NCI*, 2011), Therapeutic Target Database (Chen et al., 2002), Drug Bank (Knox et al., 2011) and Kinase Profiling Inhibitor Database (*Kinase Profiling Inhibitor Database | International Centre for Kinase Profiling*, n.d.), were used to identify the FDA approved, investigational and experimental drugs for each target, each node used as a search word and the resulting drugs were written on Excel sheet (supplementary materials 2).

Identification of high-influence nodes.

High-influence nodes are a group of nodes that create a small subnetwork and control the states of the majority of other nodes in the model (Pentzien et al., 2018). Numerous research suggested that a small number of nodes may be responsible for the dynamic behavior of an entire network (Weidner et al., 2021). As mentioned on (Niederdorfer et al., 2020) There is more than one method to identify the high influence nodes in the model for example the node influence can be determined by observing if a node's activity fixation or inversion altered the synergy predictions relative to the wild type (WT) analysis, pathway cross-talk inhibition index (PCI), closeness centrality or betweenness Centrality. Overrepresented molecular function can be used and compare the nodes in the highly represented GO with the synergy score to see if there is a connection between the Mol function and the node influence. The general principles for the selection of any drug combination can be used like no overlapping toxicity, prevention of cross resistance, the two drugs should have different mechanisms of action and each drug targets a different cell cycle or different pathway, moreover the drug-drug interaction that may alter kinetics have to be considered (Zhang et al., 2016). Nodes that have high betweenness centrality, closeness centrality, and reduced network efficiency if removed from the network were expected to have a great impact on the model predictions (Niederdorfer et al., 2020). Finally, the determinative power (DP) is one of the methods used to create a subnetwork from these small numbers of nodes that affect the behavior of the

majority of the network. DP calculated by adding together all mutual information quantities across all nodes that share the given node as same input (Pentzien et al., 2018).

Identification of the major pathways among the total nodes.

The adenocarcinoma cancer cell line (AGS) network which represents CASCADE 1.0 model constructed depending on the prior knowledge of the following pathways MAPK pathways (JNK, p38 MAPK and ERK), the PI3K/AKT/mTOR pathways, the Wnt/β-catenin pathway, and the NF-κB pathway and the connections between them (Flobak et al., 2015). KEGG, SIGNOR and PubMed used to extend CASCADE 1.0 network and created CASCADE 2.0 model by adding the following pathways: signaling by Rho GTPases, Signaling by RTKs, Apoptosis, Cell Cycle, JAK-STAT signaling pathway and TGF-beta signaling pathway focusing on the tested drug targets (Niederdorfer et al., 2020). As a result, from this extension we noticed that most of the pathways among the predicted combinations in CASCADE 1.0 nodes are the same in CASCADE 2.0 model prediction.

Identification of overrepresented molecular function.

G: profiler database was used to identify the overrepresented molecular function. First, nodes which found to have drugs and predicted to have high synergy scores used as an input then run inquiry for the two models (CASCADE 1.0 and 2.0). Then, the overrepresented molecular functions for the non-draggable nodes were identified to make a comparison and find if there are common characteristics among the druggable and non-druggable nodes in the network (supplementary materials 1). According to the nodes that have high score synergy we expect that **kinase activity** will be the overrepresented GO (molecular function), as the models and most of the nodes that have high synergy combinations and involved in a high number of combinations depend on kinases.

COLOMOTO notebook

For identification of new drug combinations that may have synergy, COLOMOTO notebook was used and many tools like *bioLQM* (Naldi, 2018) and *MaBoSS* (Stoll et al., 2017) were used to simulate drug perturbations, while *pint* (Paulevé, 2017) was used to predict new possible combinations that have good synergy and wasn't predicted by the previous studies, in addition to confirming the synergy of the high synergy scores combinations predicted by the two models.

First, in AGS cancer model - CASCADE 1.0 *BioLQM* perturbation and *MaBoSS* stochastic simulation used to confirm the results from (Flobak et al., 2015) study. The simulation was done by fixing the activity to be 0 (inactive) for all the 4 nodes that was predicted to have synergy (MEK or TAK1 inhibitors were combined with PI3K or AKT inhibitors) and comparing the change in the steady state to the wild type (WT) and calculating the growth inhibition (viability). The effect on viability was calculated for both single and double perturbation by subtracting the simulated value of Antisurvival output node from the output value of prosurvival node. According to the **Highest Single Agent model (HSA)** the drug combination was classified as a synergistic or non-synergistic combination if the viability of the double perturbation (combination) reduced more than the viability of each single perturbation alone, calculation was done using the following equation,

Viability (Drug A + Drug B) < min [Viability (Drug A, Drug B)] (Flobak et al., 2015; Niederdorfer et al., 2020).

Second, the same method used for generic cancer model - CASCADE 2.0 model to confirm the resulted combinations in (Niederdorfer et al., 2020) study.

finally, for both CASCADE 1.0 and CASCADE 2.0 *Pint* software was used to predict new combinations that may have synergy and the resulted prediction compared with the combinations synergy scores to see is there any overlap between the two predictions. BioLQM perturbation and MaBoSS stochastic simulation were used to confirm these predictions (Supplementary materials 7).

3. Results

Histogram for AGS cancer model - CASCADE 1.0 nodes combinations and synergy scores

The model predicted a total of 2926 pairwise combinations between the 75 nodes (Supplementary materials 3), ~16% (477 combinations) were predicted to have synergy. The combination considered as a good synergistic if the Highest Single Agent model (HSA) value is ≤ - 0.11 (Niederdorfer et al., 2020) (Materials and methods), so in CASCADE 1.0 model ~8% (222 combinations) predicted to have good synergy score between (-3 and -0.11) (Figure 1)

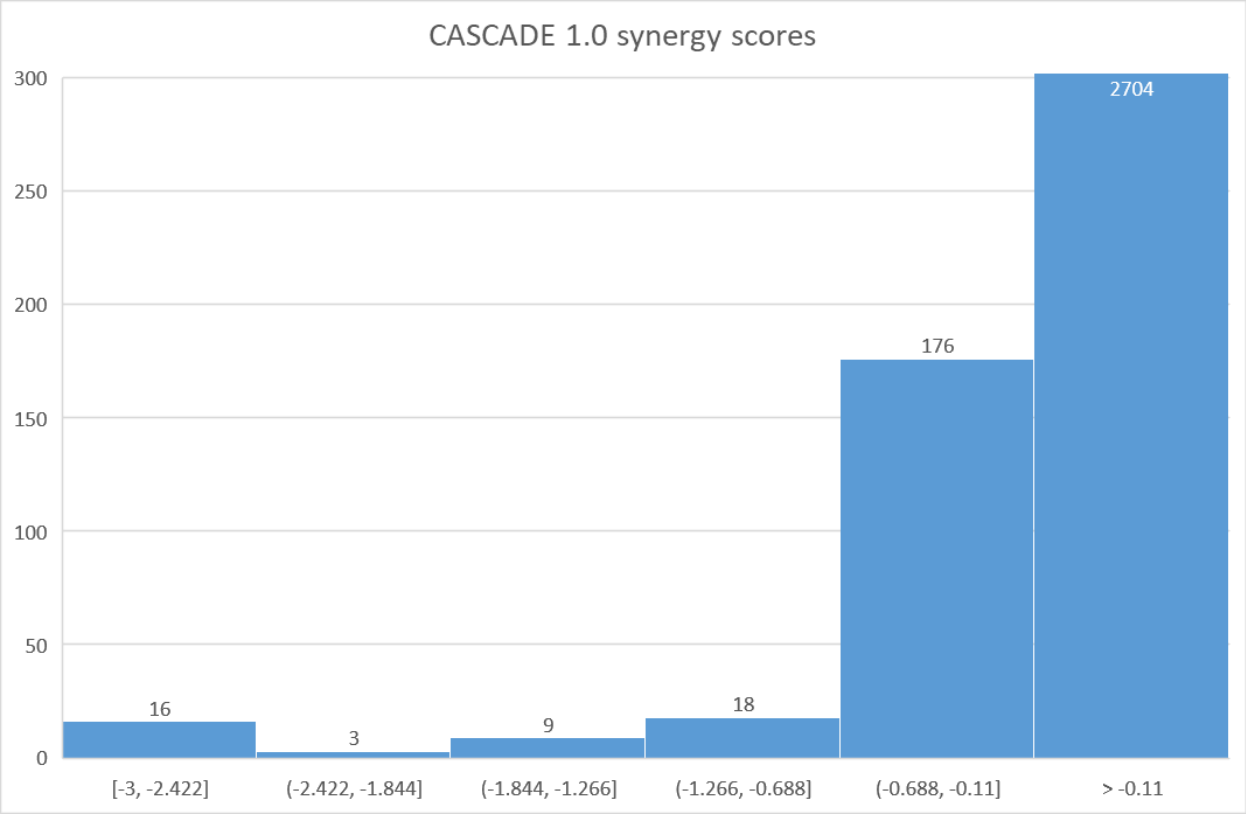

Figure 1. Bar chart represents the number of combinations in the CASCADE 1.0 model and the synergy score for each combination showing that there are ~8% of combinations predicted to have high synergy with scores ≤ - 0.11.

The AGS cancer model-CASCADE 1.0 high frequency nodes among the high score synergy combinations

Among the 222 combinations that were predicted to have high synergy scores according to Highest Single Agent model (HSA) we chose the high frequent nodes to do further analysis to prove if any of them can be used as a new cancer therapy target. A significant number of nodes were observed to have high synergy scores combinations, with high frequency and combined with nodes confirmed in a previous studies to be used as FDA approved targets. TCF7 for example significantly expressed in colorectal cancer according to the GEPIA website, in addition the histopathological grade from GEO data which confirmed that TCF7 was very related to colon cancer (Y. Guo et al., 2021). In the AGS cancer model (CASCADE 1.0) TCF7 was predicted to have combinations with high synergy scores (-2.8 to -0.12) (Supplementary materials 3) and most of the nodes combined with TCF7 like MEK, PI3K, PDPK1, RSK and MAP3K7 are previously discovered as drug targets with high synergy scores predicted by (Flobak et al., 2015; Niederdorfer et al., 2020), so by considering these studies in addition to the synergy scores we can predict TCF7 can be used as a new drug target.

LRP_f is a clinical trial target, it's a cell-surface coreceptor of Wnt/beta-catenin signaling and also located in the nucleus and cytosol. LRP protein plays several roles in the cell mediation like proliferation, differentiation and adhesion so it may induce tumor angiogenesis, adhesion or invasion through the effect on DVL1 polymers which inhibit AXIN1/GSK3-mediated phosphorylation and destruction of beta-catenin (Vania et al., 2019).

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase and has the ability to control cell growth and autophagy in addition to working as a mitogen, energy and nutrition level sensor, moreover its role in AKT1 phosphorylation. In vitro study about using AZD8055 as an inhibitor of mTORC kinase shows growth inhibition and antitumor activity which gives an indication about the role of mTORC in cancer progression and can be used as drug target (Chresta et al., 2010).

MDM2 (Ubiquitin-protein ligase E3 Mdm2) also called P53-binding protein due to the cellular inhibition of p53. The transcription factor p53 has an important role in DNA repair, metabolism, cell-cycle progression, apoptosis and senescence through controlling a large number of genes expression, so inhibiting the activity of p53 will suppress cancer growth. MDM2 is a clinical trial target used in treatment of acute myeloid leukemia, prostate cancer and solid tumor/cancer by

inhibiting p53 through three different mechanisms; promoting its degradation, prevent its binding to its DNA target or increasing its export out of the nucleus (Wang et al., 2017).

Many studies confirmed the participation of SHC1 in signaling by epidermal growth factor receptor-2 (HER-2), prolactin (PR), and estrogen receptor (ER) which are considered as biological markers in breast cancer initiation and progression. The polyomavirus middle T antigen (MT) model which used to study progression of mammary tumor gives evidence regards the involvement of shc1 gene in the initiation and progression of breast cancer through downstream signaling of RAS/MAPK and PI3K. In addition, the gene products accelerate tumorigenesis in the model that indicate the important role of SHC1 in cancer treatment (Wright et al., 2019, p. 52).

GRB2 (Growth factor receptor-bound protein 2) an adaptor protein involved in many cellular events like cell proliferation, metabolism and cell growth. However, GRB2 is still a clinical trial target, Grb2 participates in a number of tumor malignancies as a primary driver of oncogenesis and starts a variety of defective signaling cascades which gives an indication regards the importance of developing new cancer therapies targeting GRB2 (Ijaz et al., 2017., p. 2).

Yuan et al. study discussed the overexpression of miRNA-223-3p effect on the proliferation inhibition and enhancement of Mantle cell lymphoma (MCL) apoptosis through negative regulation of CHUK/NF-ĸB2 signaling pathway. Knocking down CHUK increases MCL cell proliferation in vitro, which gives an indication about the pivotal role of CHUK in Mantle cell lymphoma progression and can be considered as a promising target for tumor suppression (Yuan et al., 2021).

ERK is one of the MAPK family and a type of serine/threonine protein kinase, which has a role in cell division, development and growth regulation. ERK is usually located in the cytoplasm then transferred to the nucleus after activation through phosphorylation and regulates gene expressions and transcription factor activity regulations. The ERK1/2 phosphorylated (p-ERK1/2) normally increases in normal ovarian tissues and benign tumors, but Continuous activation of the ERK/MAPK signaling pathway leads to the formation of tumor cells. On the other side an invitro experiment confirmed that by inhibiting the ERK/MAPK signaling pathway helped on preventing the formation of the tumor cells and which can be used as cancer growth inhibitor in vivo (Y.-J. Guo et al., 2020).

Rac is one of the Rho family GTPases and plays a key role in cancer metastasis. Many studies reviewed the role of Rac in addition to Cdc42 pivotal role in different types of cancer. Rac and

Cdc42 become overactive when oncogenic growth factor receptors signals to the guanine nucleotide exchange factors that control their GDP/GTP. So, RAC can be used as a promising drug target in addition to decreasing resistance to cell surface receptor-targeted therapies (Maldonado et al., 2020).

DUSP1 (Dual-specificity phosphatase-1) overexpression leads to the inactivation of ERK, JNK, and p38 by dephosphorylation which are responsible for cell proliferation and apoptosis. Targeting JNK-induced apoptosis prompt carcinogenesis in various cancers like gastric, colon, bladder and prostate. Knocking down DUSP1 during treatment of ovarian cancer increased sensitivity to cisplatin. From this example DUSP1 targeting can be used to overcoming drug resistance and increase antitumor drugs efficacy by increasing drugs sensitivity (Shen et al., 2016, p. 1).

The dishevelled segment polarity protein (DVL) has a key role in mediating Wnt signals. Canonical and non-canonical Wnt/β-catenin signaling pathways are activated by dysfunction of DVL which lead to cancer formation in different types of cancer. DVL is highly expressed in Diffuse gastric carcinoma (DGC) comparing to the normal tissue, in addition DVL has role in regulation of CTNNB1 protein which make it a good choice to be a drug target in several cancer types (Sremac et al., 2021, p. 1).

Histogram for generic cancer model - CASCADE 2.0 node combinations and synergy scores

The model predicted 10296 pairwise combinations between the 144 nodes (Supplementary materials 4), ~16 % of the combinations (1704 combinations) were predicted to be synergized. Using the same concept of HSA by considering the good synergy scores to be ≤ - 0.11, so there are ~9% of the combinations (157 combinations) were predicted to have high synergy scores between (-1.36 and -0.11) (Figure 2).

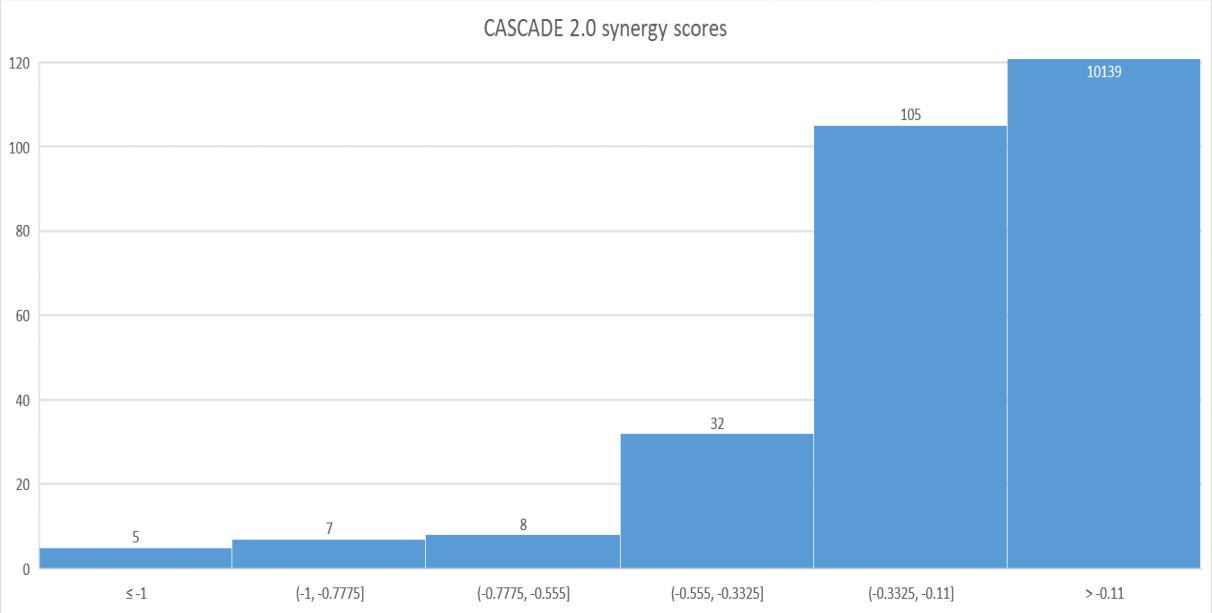

Figure 2. Bar chart represents the number of combinations in the CASCADE 2.0 model and the synergy score for each combination showing that there are 157 combinations predicted to have high synergy with scores ≤ - 0.11.

The generic cancer model-CASCADE 2.0 high frequency nodes among the high synergy scores combinations

From the 157 high synergy scores combinations we can expect that there will be some overlap between the predicted combinations by CASCADE 1.0 and CASCADE 2.0 models as the later one is an extension of the first model and includes all the nodes and all the pathways. However we can notice that there is many nodes have combinations in CASCADE 2.0 but not in CASCADE 1.0 which we will show in the next part.

MMP_f (Matrix metalloproteinase) is recognized to have important role in cancer progression as it was identified as a cell surface protease in the tumor cell which induce metastasis when its expression increased especially (MT1-MMP) (Knapinska & Fields, 2019). According to Therapeutic Target Database (TTP) MMP_f approved as a successful drug target in addition to having FDA approved drug (Prinomastat) for lung cancer treatment in addition to the clinical trial drug (Marimastat) for treatment of pancreatic cancer.

RTPK_f (Receptor Tyrosine-protein kinase) which is a successful target used for treatment of Colorectal cancer through the FDA approved drug (Regorafenib), Gastrointestinal stromal tumor

using (Ripretinib) and (Sorafenib) for treatment of unresectable hepatocellular carcinoma and advanced renal cell carcinoma (Wilhelm et al., 2004; Therapeutic Target Database).

CCND1 (G1/S-specific cyclin-D1) responsible for the phosphorylation and inhibition of members of the retinoblastoma (RB) protein family including RB1 and regulates the cell-cycle during G1/S transition, this phosphorylation leads to the release of the transcription factor E2F from the RB/E2F complex and target genes which are responsible for the progression through the G1 phase, so targeting CCND1 will prevent the tumor progression (Uniprot database).

The transcription factor LEF1 (Lymphoid enhancer-binding factor 1) is a part of the canonical Wnt/β-catenin signaling pathway and is involved in tumorigenesis and progression of multiple tumors. The expression of LEF1 significantly increased in colorectal cancer (CRC) and many other types of cancer, the negative correlation between LEF1 and Notch2 gives indication regards tumorigenesis, shorter overall survival time, and higher risk of death in CRC patients. Santiago et al. study demonstrated the crucial role of LEF1 in initiating and maintaining carcinogenesis (Santiago et al., 2017).

LRP_f, MDM2, CHUK, DUSP1 and ERK which are predicted in CASCADE 1.0 are also predicted in CASCADE 2.0 model with high frequency among the high synergy scores combinations.

The high influence nodes (Cytoscape Network analysis)

Cytoscape software used to analyze the 144 nodes network to identify the Betweenness Centrality, Average Shortest Path Length and Closeness Centrality (Supplementary Materials 6). The analysis confirmed the results from (Flobak et al., 2015; Niederdorfer et al., 2020; Jaaks et al., 2022) in these previous studies the nodes that used as drug targets have high Betweenness Centrality, Average Shortest Path Length and Closeness Centrality in addition to many other nodes that still clinical trials targets and some other targets have clinical trials drugs and have crucial role in cancer growth pathways like CCND1, CHUK, DUSP1, DVL_f, ERK_f, GRB2, LEF1, LRP_f, MDM2, MMP_f, RAC_f, RTPK_f, SHC1, TCF7_f and TP53.

Identification of the high influence nodes using determinative power analysis (DP)

Every large biological network includes a subnetwork which represents a small group of nodes that can draw conclusions about the characteristics of the original network and to comprehend the influence of network architecture on the network dynamics (Pentzien et al., 2018). By applying the determinative power analysis to the nodes in our models we noticed that most of the high DP score nodes comparing to the other nodes on the network are previously discovered in a number of studies and used as a cancer drug targets, AKT_f, MAP3K7, MEK_f and PIK3CA are previously discovered on (Flobak et al., 2015), while ROCK1, MAPK14, SYK, GSK3_f, JNK_f, MYC, CTNNB1, PDPK1, IKBKB and TGFBR2 are used as drug targets in (Niederdorfer et al., 2020) study. Moreover by comparing the synergy scores and the DP scores we discovered some nodes that have high score according to both of the two methods like ERK_f, RAC_f, SHC1, mTORC2_c, GRB2 and CCND1 in CASCADE 1.0, and ERK_f, RAC_f, LRP_f, RTPK_f, TCF7_f, MDM2 and CHUK in CASCADE 2.0. Although there are some nodes like TSC_f, TP53 and GAB_f that have low synergy scores, but they have high frequency among synergized combinations and are observed to have high DP in both CASCADE 1.0 and 2.0 (Supplementary Materials 5).

The major Pathways among the synergized combinations.

By connecting the pathways to the nodes participated in the high synergy combinations we noticed that MAPK signaling pathway patriciate with 37% among the combinations, PI3K-Akt signaling pathway 29%, Cell cycle pathway is one of the pathways among the high synergized combinations with 24%, 18% for Signaling by RTKs pathway, 17% for TGF-beta signaling, 11% for Wnt signaling pathway, while NF-kappa B signaling and mTOR signaling represent small percentage among the combinations this may be due to the low number of nodes in the models related to these pathways (Supplementary Materials 2).

Identification of novel pathways

Identification of new drugs for the new discovered targets or even in the previous studies lead to the appearance of some new pathways that can be used as target pathways. P53 signaling pathway is one of the new pathways that used by number of inhibitors as target, p53 is a tumor suppressor and any deletion or mutation in it leads to growth of different tumor types (Huang, 2021).

Interleukin (IL) signaling pathway is also noticed in the overrepresentation analysis of a large number of targets, interleukin (IL) is a proinflammatory cytokine that has a role in many pathological and physiological processes. The high expression of IL in the cell associated with cancer growth (Liu et al., 2017). Ras protein is widely spread in mammalian cells; each cell has at least 3 types of Ras proto-oncogenes. promoting Ras proteins mutation leads to increased cell proliferation and inhibits apoptosis. 30% of tumor types have oncogenic mutations in Ras gene so Ras signaling pathway is one of the common pathways that can be used as target for cancer treatment (Adjei, 2001).

The overrepresented Molecular functions

AGS cancer model-CASCADE 1.0 was constructed as a self-contained model and the regulatory network includes only the nodes that have regulation effect on each other (Flobak et al., 2015), so by doing the overrepresentation analysis we noticed that the molecular functions among all the targets are very similar and more related to the kinase and Protein serine/threonine kinase activity among the synergistic nodes in the two models and among all the nodes that have drugs. While for the targets that don't have drugs the overrepresented molecular function was related to enzyme, protein binding in addition to kinase binding and kinase activity which also have the same general characteristics of the total nodes in the network. For generic cancer model-CASCADE 2.0 as we mentioned that it is an extension of CASCADE 1.0 so we will notice that all the 75 nodes of CASCADE 1.0 are also included in CASCADE 2.0 (Supplementary materials 2) so by running the overrepresentation analysis we noticed that most of the overrepresented molecular functions are the same between the two models in addition to SMAD and I-SMAD binding are noticed in CASCADE 2.0 model (Table 1).

On the other hand, after connecting the synergistic nodes to the overrepresented MF we found that these overrepresented MFs were engaged in synergy combinations by ∼30% and most of the targets resulted in these MFs were confirmed as a druggable in previous studies (supplementary Materials 1), in addition to some new targets which have high synergy and high determinative power scores from our project for example CCND1, DUSP1, GRB2, SHC1, TP53 and CHUK.

Table (1) Represents comparison of the GO molecular function overrepresented between the targets (A) The overrepresented molecular function for the nodes having drugs (Approved or clinical trials drugs) among the total nodes. (B) Represent the molecular function for the nodes that don't have drugs among the total nodes. (C) The overrepresented molecular function for the synergized nodes in CASCADE 1.0 model. (D) The overrepresented molecular function for the synergized nodes in CASCADE 2.0 model.

Total Nodes		**Synergized Nodes**	
A) Druggable Nodes	**B) Non Druggable Nodes**	**C) CASCADE 1.0 model**	**D) CASCADE 2.0 model**
Enzyme binding	Enzyme binding	Protein serine/threonine kinase activity	Enzyme binding
Protein kinase binding	Protein kinase binding	Protein serine kinase activity	Protein serine/threonine kinase activity
Kinase binding	Kinase binding	Enzyme binding	Protein kinase binding
Protein binding	Catalytic activity, acting on a protein	Protein kinase activity	I-SMAD binding
Protein kinase activity	SMAD binding	Protein serine/threonine/ tyrosine kinase activity	Kinase binding
Phosphotransferase activity, alcohol group as acceptor	I-SMAD binding	Phosphotransferase activity, alcohol group as acceptor	Kinase activity
Protein -containing complex binding	Protein serine/threonine kinase activity	Catalytic activity, acting on a protein	Phosphotransferase activity, alcohol group as acceptor
Binding	Protein kinase activity	Kinase activity	Catalytic activity, acting on a protein
Catalytic activity, acting on a protein	Protein serine kinase activity	MAP kinase activity	SMAD binding
Kinase activity	Protein serine/threonine/tyrosine kinase activity	Protein kinase binding	Transferase activity, transferring phosphorus-containing groups

Predicted combination using COLOMOTO notebook

Pint predicted combinations

CASCADE 1.0

Using *bioLQM* and *MaBoSS* software the previously discovered combinations (Flobak et al., 2015) between MEK or TAK1 inhibitors and PI3K or AKT inhibitors were confirmed, in addition by running a *bioLQM* perturbation for all the nodes against each other we noticed that the stable state of the perturbation of TCF node changed comparing to the wild-type (WT), this gives an indication that it can be used as a new drug target in the model. *Pint* software used to confirm this observation in addition to predict a new synergistic combination in the model. The output results showed that TCF, ERK, RSK and CCND1 are predicted as targets that can affect model goal reachability after setting PI3K and MEK as a new initial state to be 0 (inactive) (figure 3). Pint predicted combinations are matched with the model synergy predictions since the predicted mutations using pint are also have high synergy scores among CASCADE 1.0 predicted combinations (Table 2).

```
Out[37]: [{'ERK': 0, 'TCF': 0},
          {'PTEN': 1, 'TCF': 0},
          {'PI3K': 0, 'TCF': 0},
          {'PDK1': 0, 'TCF': 0},
          {'RSK': 0, 'TCF': 0},
          {'cMYC': 0, 'CCND1': 0},
          {'TCF': 0, 'CCND1': 0}]
```

Figure 3. The list of mutations predicted by pint to have synergy.

Table 2. The synergy scores from CASCADE 1.0 model for the combinations predicted using pint software which shows that the predicted combinations have high synergy scores.

TCF7_fi	ERK_fi	-2.820915033
TCF7_fi	RSK_fi	-1.329411765
TCF7_fi	PDPK1i	-0.132880845
TCF7_fi	PIK3CAi	-0.132880845

Double perturbation of TCF and PI3K using bioLQM and MaBoSS showed that there is no growth inhibition more than the single perturbation of TCF so we tried to perform triple perturbation

between TCF, PI3k and MEK which showed that ~70% probability that the model will reach growth of -1 (Antisurvival_b1) this means that there is growth inhibition more than single and double perturbation (Supplementary materials 7).

(Note: The results until the prediction of triple perturbation of TCF was done during the BI8040 course, while prediction and confirmation of ERK, RSK and CCND1in addition to the work on CASCADE 2.0 in the coming section is my work in this project)

CASCADE 2.0

The COLOMOTO notebook Pint software was used to predict new combinations in the CASCADE 2.0 model, then the predicted combinations compared with the synergy scores from the model. We noticed that pint software didn't predict any double mutation, but the predicted triple mutation showed that there are many new targets discovered by this project participate in the combinations with one or more of the previously discovered targets and also by comparing the predicted combinations with the synergy scores we found that these new combinations have high synergy scores (Figure 5). PPP1CA (serine/threonine phospho-protein phosphatase) was noticed to be available in most of the combinations in its active state. Many signaling pathways are regulated by PPPCs family and any disturbance in this genes regulation leads to the growth of different types of cancer through uncontrolled proliferation, differentiation and metastasis (Xie et al., 2022). The role of PPP1CA is not totally clear in the predicted combinations but it may be due to the logical roles in the model which have to have this node active to give the synergy results.

{'CHUK': 0, 'MYC': 0, 'PPP1CA': 1},
{'CHUK': 0, 'PPP1CA': 1, 'PLK1': 0},
{'TCF7_f': 0, 'MYC': 0, 'PPP1CA': 1},
{'TCF7_f': 0, 'PPP1CA': 1, 'PLK1': 0},
{'MYC': 0, 'CCND1': 0, 'RTPK_f': 0},
{'MYC': 0, 'CCND1': 0, 'PDPK1': 0},
{'MYC': 0, 'CCND1': 0, 'MEK_f': 0},
{'MYC': 0, 'CCND1': 0, 'ERK_f': 0},
{'MYC': 0, 'CCND1': 0, 'RSK_f': 0},

Figure 5. List of targets that were predicted using Pint to have synergy.

In generic cancer model -CASCADE 2.0 the node CCND1 was predicted in many combinations but by running bioLQM single perturbation CCND1 had the antisurvival output value at its maximum value (-3) which gives an indication that this node can't be used in any combination as it will give no growth inhibition in the double or the triple combination more than CCND1 single perturbation and this was confirmed using bioLQM perturbation and MaBoSS stochastic simulation (Supplementary materials 7).

Table 3. The synergy scores predicted by CASCADE 2.0 model for Pint predicted combinations

RSK_fi	MYCi	-1
CHUKi	MYCi	-0.141643933
ERK_fi	MYCi	-0.056198437
TCF7_fi	MYCi	-0.05472272
CHUKi	PLK1i	-0.039062567
TCF7_fi	PLK1i	-0.001761186

The predicted results are overlapped very well with the synergy results which gives a good impression regards the new nodes that we predict to be promising drug targets

BioLQM perturbation and MaBoSS stochastic simulation confirmation of the predicted combinations.

In this section MaBoSS stochastic simulation used to confirm pint predicted combinations. By considering the high influence nodes and the synergy scores in addition to the drug availability, the nodes have been chosen to run MaBoSS stochastic simulation to confirm or reject the predicted combination (Supplementary materials 7).

CASCADE 1.0:

MaboSS stochastic simulation and bioLQM perturbation used to confirm the predicted combinations but by comparing the single perturbation with the double perturbation it showed that there is difference in growth inhibition so it gives an indication that there is no synergy in the predicted double perturbation except the combination between TCF7 and ERK_f. The single perturbation for the nodes has Antisurvival b1 value = -1 while in the double perturbation the Antisurvival value = -1,5 which means that there is more growth inhibition due to synergy effect.

CASCADE 2.0:

The single mutation done for all the nodes in the predicted combinations and the resulted growth was Antisurvival = -2. We then tried to run double simulation for the nodes that predicted to have high synergy scores and predicted by pint, but we noticed that there is no more growth inhibition than the single mutation. Finally, triple simulation was used and the results were Antisurvival at its maximum value = -3 which means that there is more growth inhibition than the single and double simulations and confirm all the resulted combinations.

4. Discussion

Although the great efforts made by the biomedical researchers to discover a new cancer therapy, it is very challengeable to be able to find a single drug that have less side effects in addition to avoiding the resistance problem (Flobak et al., 2015; Xu et al., 2012). The scientists started to focus on the drug combinations which give the opportunity to decrease the drugs doses to help in decreasing side effect and targeting more than one pathway which increase efficacy and decrease resistance (Flobak et al., 2015, 2019; Gilad et al., 2021; Gregory et al., 2020; Jaaks et al., 2022; Menden et al., 2019).

In the tech savvy era, creating a logical models that have the ability to predict the drug combinations without the need to test these combinations experimentally help in saving a lot of time and effort in addition to the ability to find the suitable combination for the patient within the timeframe limited by disease progression. Also, the massive number of predicted combinations make it not possible to test all of these combinations experimentally. Combinations that have synergistic pharmacological effect are currently the main focus of the biomedical researchers as the combination has greater effect than the effect of its component if used alone. In addition, creating a logical model where its predictions to be synergized can be confirmed experimentally to have synergy (True positive), and its prediction for non-synergy combinations also confirmed experimentally to have no synergy (True negative) help in predicting the right combination and not to lose any possible effective one (Flobak et al., 2015; Niederdorfer et al., 2020). AGS cancer model-CASCADE 1.0 and generic cancer model-CASCADE 2.0 predictions were tested against in vitro experiments and synergy prediction are confirmed by the experimental observations, this gives an indication regards the efficacy of the models (Niederdorfer et al., 2020).

The Drug Gene Interaction Database, National Cancer Institute (NCI) Therapeutic Target Database, Drug Bank (Knox et al., 2011) and Kinase Profiling Inhibitor Database are used in this project to identify all the possible FDA approved drugs for the nodes in the models. The total percentage of the currently druggable nodes which have FDA approved drugs among the synergized combinations is ~18% and by including the new targets that were discovered to have high synergy combinations in addition to having clinical trials or FDA approved drugs for treatment of different types of cancer the percentage increase to become ~24% among the synergized combinations.

MAPK signaling pathway plays a crucial role in the cell growth regulation, apoptosis and proliferation and any alteration in this pathway lead to tumorigenesis, so this pathway considered as potential target for cancer treatment. Many nodes in the models are related to MAPK pathway and also involved by a large percentage in the synergized combinations (Santarpia et al., 2012).

PI3K-Akt signaling pathway also involved with a high percentage among the combinations due to its role in cancer cells progression. After analyzing the network to identify the overrepresented molecular function the results showed that Protein serine/threonine kinase activity, Kinase activity And Kinase binding are the most common molecular function to the nodes in the models we can conclude the reason that most of the nodes in the network related to kinases. By comparing the synergy nodes with the other nodes in the models we can notice that all the nodes have the same graph metric behavior and share the same characteristics the confirm the synchronization between all the nodes in the models.

Table 4. The new nodes that were predicted to have high frequency among the high synergized combinations. A) The nodes that have high synergy scores predicted by the two models. B) The high influence nodes predicted using Cytoscape network analysis and Determinative power (DP). C) nodes predicted using pint and confirmed by bioLQM and MaBoSS simulation.

A) High synergy scores nodes		**B) High influence nodes**		**C) COLOMOTO predictions**	
CASCADE 1.0	**CASCADE 2.0**	**Cytoscape Network analysis**	**Determinative power**	**CASCADE 1.0**	**CASCADE 2.0**
CHUK	CCND1	CCND1	CCND1	ERK	CHUK
DUSP1	CHUK	CHUK	CHUK	RSK_f	ERK_f
DVL_f	DUSP1	DUSP1	ERK_f	TCF7_f	RSK_f
ERK	ERK	DVL_f	GRB2		RTPK_f
GRB2	LEF1	ERK_f	LRP_f		TCF7_f
LRP_f	LRP_f	GRB2	MDM2		
MDM2	MDM2	LEF1	mTORC2_c		
mTORC2	MMP_f	LRP_f	RAC_f		
RAC	RTPK_f	MDM2	RTPK_f		
SHC1		MMP_f	SHC1		
TCF7		RAC_f	TCF7_f		
		RTPK_f			
		SHC1			
		TCF7_f			

In this project we worked on analyzing the AGS cancer model-CASCADE 1.0 and the generic cancer model-CASCADE 2.0 to find the common features of the nodes in each model, identifying the druggable and non-druggable nodes and trying to discover a new drug targets. This was done through the use of different methods and software like the nodes that have high synergy scores or considered as high influence node within the network or predicted and confirmed using COLOMOTO notebook. Table 4 represents the results that we observed by using each method. We can summaries that there are some nodes noticed to be included in more than one method while others are only noticed in one method. CCND1, CHUK, DUSP1, ERK, GRB2, LRP_f, MDM2, SHC1, TCF7 and RTPK_f are group of nodes involved frequently in most of the analysis methods, while DVL_f, RAC, LEF1 and RSK_f appeared only in two of our analysis but they have very high synergy scores in addition to having high Betweenness Centrality, Average Shortest Path

Length and Closeness Centrality and have a crucial role in cancer progression which make them a promising candidate to be used as drug targets in the future.

5. Conclusion

Even with the ability to create an effective model without any false positive or false negative prediction, the predicted combinations are still very low and not easy to find the effective combination that can be used for treatment. We expect that druggome analysis is a promising field to be used for discovery and testing new drug combinations that can be used for treatment of the most dangerous tumors types. For future work we recommend to use COLOMOTO notebook for more analysis as there are many methods and software that can be used to predict the more efficient combination.

Supplementary materials

1. GO Mol function of total(Druggable and non-druggable) and synergized nodes of CASCADE 1.0 & CASCADE 2.0 https://docs.google.com/spreadsheets/d/1M6C7sxn4BSuPoitFpBQt6Fhx-_u_DbMrQ8MYKgzMP0c/edit#gid=50177526
2. Total nodes for CASCADE 1.0 and 2.0 and the Approved drugs https://docs.google.com/spreadsheets/d/1NwQvXHmC3jvMMeobiufLrFDawbkQsbBS/edit#gid=1705763928
3. CASCADE 1.0 synergy scores https://docs.google.com/spreadsheets/d/1Sh3KD83ecGiFZyLw4FPa_Ufhco68GfW0/edit#gid=1379667106
4. CASCADE 2.0 synergy scores https://docs.google.com/spreadsheets/d/1Sh3KD83ecGiFZyLw4FPa_Ufhco68GfW0/edit#gid=1340235167
5. Determinative power scores for identification of high influence nodes https://docs.google.com/spreadsheets/d/1opXIWCDnDDi6nXlp7b7oLhyqF1Q3PvVA/edit?usp=sharing&ouid=113691580111986064094&rtpof=true&sd=true
6. Cytoscape Network analysis to identify Betweenness Centrality, Average Shortest Path Length and Closeness Centrality https://drive.google.com/file/d/1QQP8HRD6AXCzjWMIws8Ohxpd0SdV3AhF/view?usp=sharing
7. COLOMOTO notebook: usage of pint, bioLQm and MaBoSS stochastic simulation to predict and confirm new combinations. https://drive.google.com/file/d/1aeNOB9PEWzFa51qggqLkXD7YExn8Ep4-/view?usp=sharing
8. AGS cancer model-CASCADE 1.0 : http://ginsim.org/node/194
9. Generic cancer model-CASCADE 2.0 : https://github.com/druglogics/cascade

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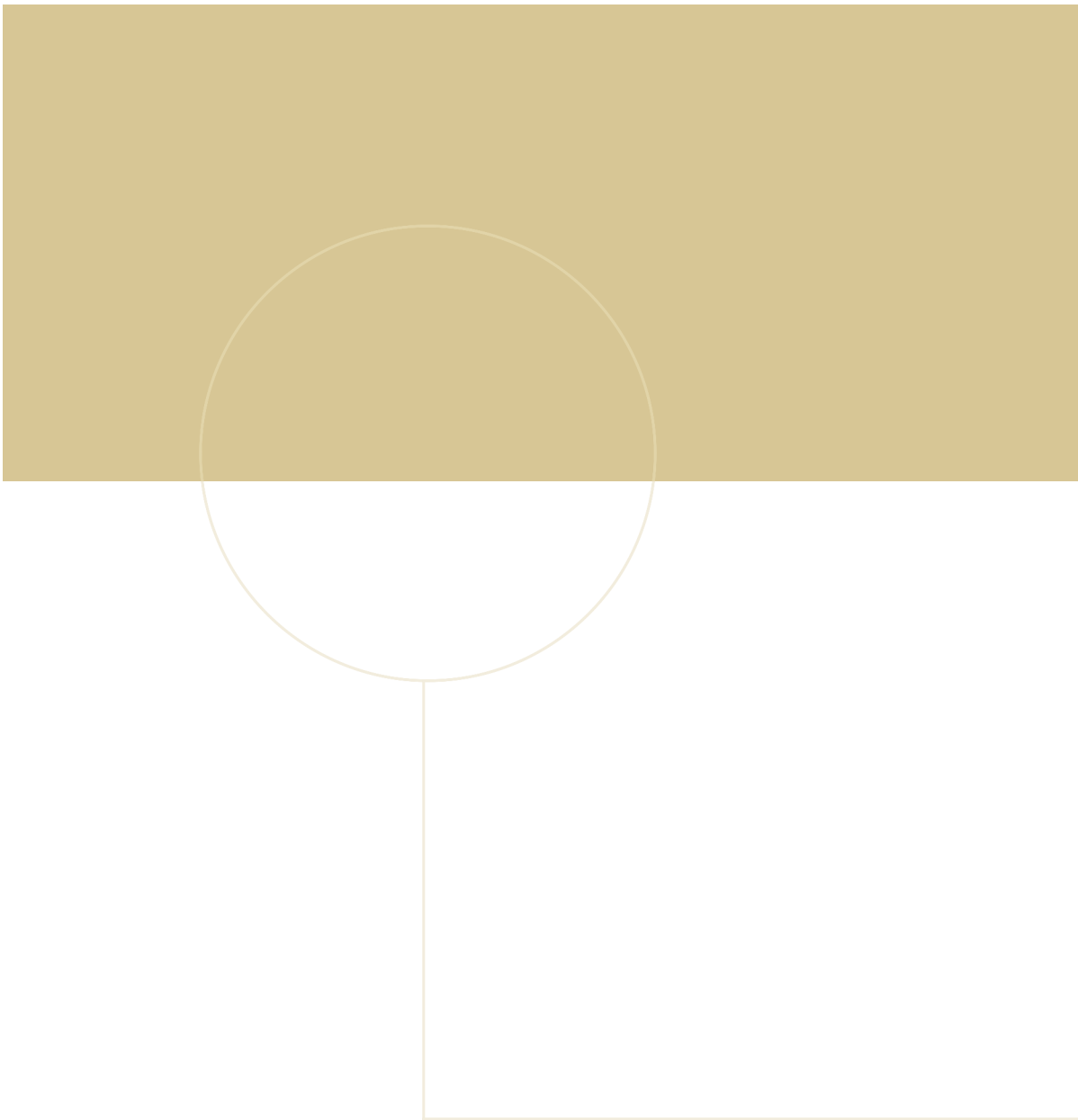

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