Machine Learning Discoveries of GINS1-X Synergy in ETC-1922159 Treated Colorectal Cancer Cells

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Posted Date: 13 January 2025

doi: 10.20944/preprints202501.0868.v1

Keywords: GISN1; porcupine inhibitor ETC-1922159; sensitivity analysis; machine learning; colorectal cancer

Article

Machine Learning Discoveries of GINS1-X Synergy in ETC-1922159 Treated Colorectal Cancer Cells

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† Aspects of unpublished work were presented in a poster session at the first Wnt Gordon Research Conference, from 6-11 August 2017, held in Stowe, VT 05672, USA.

Abstract: GINS1 (PSF1 homolog) forms a part of complex called GINS, named after numbers 5-1-2-3 (go-ichi-ni-san in Japanese) apropos the 4 protein subunits of the complex, namely, SLD5, PSF1, PSF2, and PSF3. It is a subgroup of the CDC45-MCM-(2 to 7)-GINS (CMG) complex, the enzyme that unwinds double-stranded DNA at replication forks. GINS1 has been found to be overexpressed in multiple cancers. In colorectal cancer (CRC) cells treated with ETC-1922159, GISN1 was found to be down regulated along with other genes. A recently developed search engine ranked combinations of GISN1-X (X, a particular gene/protein) at 2^{nd} order level after drug administration. Some of these combinations have been tested in wet lab, however many have been pointed out by the search engine that are yet to be explored/tested. These rankings reveal which GINS1-X combinations might be working synergistically in CRC. In this research work, I cover combinations of GINS1 with possible members of, origin recognition complex (ORC), minichromosome maintenance complex (MCM), DNA-directed DNA polymerases (POL), DNA-dependent RNA polymerase (POLR), topoisomerases (TOP), ubiquitin specific peptidase (USP), Fanconi anemia complementation group (FANC), forkhead box (FOX) and notch receptor (NOTCH) family.

Keywords: GISN1; porcupine inhibitor ETC-1922159; sensitivity analysis; machine learning; colorectal cancer

1. Introduction

1.1. Eukaryotic DNA Replication Machinery

Eukaryotic DNA replication restricts DNA replication once per cell cycle. The replication machinery coordinates many proteins at the site of replication, forming the replisome (Leman and Noguchi [1]). The replication process is a two step process in a given cell cycle - • during the G1-phase, there is loading of the replicative helicase, MCM-(2 to 7), onto chromatin by the ORC complex, where CDC6, CDT1, and MCM-(2 to 7) assemble at replication origins, and form pre-replicative complexes (pre-RCs). • In the subsequent S-phase, kinase activition allows fork establishment through CMG complex formation.

Considering the architecture, Li and O'Donnell [2] and Parker et al. [3] elucidate that CMG complex surrounds the DNA at origins in two steps - • in the G1 phase, two MCM-(2 to 7) rings are assembled around duplex DNA at the origin, by the concerted actions of the ORC, CDC6, and CDT1, thus forming a double hexamer. •, in S phase, CDC45 and GINS are assembled onto each MCM-(2 to 7) ring for production of two CMGs that ultimately form two replication forks that travel in opposite directions. Further, EM studies have revealed a 20-subunit core replisome with the leading POLϵ and lagging POLα, primase on opposite faces of CMG, thus forming a fundamentally asymmetric replisome architecture.

 doi:10.20944/preprints202501.0868.v1

1.2. GINS Complex and Cancer

Via biochemical analysis it has been shown that GINS works at the heart of the replication apparatus as a component of the CMG complex which facilitates the unwinding of duplex DNA ahead of the moving replication fork (MacNeill [4] and MacNeill [5]).

Xiang et al. [6] provide a comprehensive review regarding insight into the mechanisms and dynamics of how CMG is regulated during its assembly and activation in mammalian genomes, and how errors in CMG regulation due to oncogenic changes promote tumorigenesis. Seo and Kang [7] provide a review that emphasizes on molecular function of the CMG during replication and its relevance to cancers, based on published literature. They indicate that the CMG complex can be a potential target for a treatment of cancer and the feasibility of this replicative helicase as a therapeutic target has been tested recently.

In breast cancer cells, Nakahara et al. [8] found that GINS1 (PSF1) was over-expressed and knockdown of PSF1 expression slowed the growth of breast cancer cell lines by delaying DNA replication. Further, reduced PSF1 expression also inhibited anchorage-independent growth in breast cancer cell lines. Similar results regarding role of PSF1 in hepatocellular carcinoma tissues, was reported by Zhou et al. [9]. Chromosomal instability (CIN) is a trademark of cancer and comprises structural (S-CIN) and whole chromosomal (W-CIN). In human cancers, recent works have indicated that replication stress (RS), known to contribute to S-CIN, also affects mitotic chromosome segregation. Böhly et al. [10] showed that RS-induced increased origin firing was sufficient to trigger W-CIN and discovered that overexpression of these origin firing genes, like GINS1 and CDC45, correlated with W-CIN. In another study regarding hepatocellular carcinoma, Liang et al. [11] found that ZEB1 was a regulator of GINS1 induced epithelial mesenchymal transition (EMT), and GINS1 promoted EMT and tumor metastasis through β-catenin signaling. Finally, through immunohistochemistry studies in bladder cancer (BC) tissues, Fu et al. [12] demonstrate that increase in GINS1 expression. They show that GINS1 silencing arrested the cell cycle at the phase of G0/G1 phase, which inhibited BC cell growth both in vitro and in vivo, and GINS1 knockdown also hindered the AKT/mTOR pathway. In colorectal cancer (CRC) cells treated with ETC-1922159, GISN1 was found to be down regulated along with other genes.

GISN1 works in tandem with multiple components and some combinations of GISN1 have been confirmed in wet lab. However, many of the combinations have not been explored/tested or are known. To reveal these combinations, I use a modification of a recently published machine learning based search engine, details of which are given in the next section.

1.3. Combinatorial Search Problem and a Possible Solution

In a recently published work Sinha [13], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway. Readers are requested to go through the adaptation of the above mentioned work for gaining deeper insight into the working of the pipeline and its use of published data set generated after administration of ETC-1922159, Sinha [14]. The work uses SVM package by Joachims [15] in https://www.cs.cornell.edu/people/tj/svm_light/svm_rank.html. I use the adaptation to rank 2^{nd} order gene combinations.

2. Results & Discussion

2.1. GISN1 Related Synergies

2.1.1. GINS1 - ORC

Coulombe et al. [16] analysed the pre-RC proteomic interactome in human cells and found ORC-ubiquitin-ligase-1 (OBI1) to be associated with the ORC complex. OBI1 silencing resulted in defective origin firing, as shown by reduced CMG formation and OBI1 catalysed the multi-mono-ubiquitylation of a subset of chromatin-bound ORC3 and ORC5 during S-phase. Further, to determine which step of replication origin licensing or firing wass regulated by OBI1, they analysed the expression and chromatin association of key replication factors. Depletion of ORC1 lead to impaired recruitment of the

 doi:10.20944/preprints202501.0868.v1

MCM-(2 to 7) complex to chromatin, which confirmed defective origin licensing and as a consequence of this, chromatin binding of factors involved in the subsequent firing step, such as PSF1/GINS1 was reduced. Also, OBI1 knockdown impaired chromatin recruitment of replisome component PSF1/GINS1 by approximately 50%. These experiments confirm a possible synergy between ORC and GINS1. In colorectal cancer cells treated with ETC-1922159, ORC family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these ORC members along with GINS1.

Table 1 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 2 generated from analysis of the ranks in Table 1. The Table 1 shows rankings of ORC members w.r.t GINS1. ORC1 - GINS1 shows low ranking of 89 (laplace) and 224 (linear). ORC6 - GINS1 shows low ranking of 715 (laplace) and 419 (linear). ORC5 - GINS1 shows low ranking of 1451 (laplace) and 670 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, ORC2 and ORC3 showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Table 1. 2^{nd} order interaction ranking between GINS1 VS ORC members.

RANKING ORC FAMILY VS GINS1			
RANKING OF ORC FAMILY W.R.T GINS1			
	laplace	linear	rbf
ORC1 - GINS1	89	224	2514
ORC6 - GINS1	715	419	2320
ORC5 - GINS1	1451	2345	670
ORC2 - GINS1	2009	2147	178
ORC3 - GINS1	2338	1795	2154

One can also interpret the results of the Table 1 graphically, with the following influences - • ORC members w.r.t GINS1 with GINS1 $->$ ORC-1/6/5.

Table 2. 2^{nd} order combinatorial hypotheses between GINS1 and ORC members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ORC members w.r.t GINS1	
ORC-1/6/5	GINS1

2.1.2. GINS1 - MCM

As mentioned in one of the previous paragraphs, MCM family interacts with GINS in the CMG-complex formation. Brosh Jr and Trakselis [17] review the role of MCM10 in regulation of fork progression and regression and state that MCM10 crosslinks significantly with two members (PSF-1/2) i.e GINS-1/2, CDC45, and MCM-2/5/6 on the N-face. In colorectal cancer cells treated with ETC-1922159, MCM family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these MCM members along with GINS1.

Table 3 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 4 generated from analysis of the ranks in Table 3. The Table 3 shows rankings of MCM members w.r.t GINS1. MCM4 - GINS1 shows low ranking of 17 (laplace) and 63 (linear). MCM3 - GINS1 shows low ranking of 35 (laplace) and 90 (linear). MCM2 - GINS1 shows low ranking of 41 (laplace) and 143 (linear). MCM10 - GINS1 shows low ranking of 114 (laplace) and 139 (linear). MCM6 - GINS1 shows low ranking of 156 (laplace) and 231 (linear). MCM5 - GINS1 shows low ranking of 210 (laplace) and 509 (linear). MCM8 - GINS1 shows low ranking of 243 (laplace) and 849 (linear). MCM7 - GINS1 shows low ranking of 1383 (laplace), 184 (linear) and 34 (rbf). These rankings point to

 doi:10.20944/preprints202501.0868.v1

the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, MCMDC2 showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Table 3. 2^{nd} order interaction ranking between GINS1 VS MCM members.

RANKING MCM FAMILY VS GINS1			
RANKING OF MCM FAMILY W.R.T GINS1			
	laplace	linear	rbf
MCM4 - GINS1	17	63	2677
MCM3 - GINS1	35	90	2629
MCM2 - GINS1	41	143	2701
MCM10 - GINS1	114	139	2742
MCM6 - GINS1	156	231	2427
MCM5 - GINS1	210	509	2212
MCM8 - GINS1	243	849	2024
MCM7 - GINS1	1383	184	34
MCMDC2 - GINS1	1707	1454	2380

One can also interpret the results of the Table 3 graphically, with the following influences - • MCM members w.r.t GINS1 with GINS1 $->$ MCM-4/3/2/10/6/5/8/7.

Table 4. 2^{nd} order combinatorial hypotheses between GINS1 and MCM members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
MCM members w.r.t GINS1	
MCM-4/3/2/10/6/5/8/7	GINS1

2.1.3. GINS1 - POL/POLR

Bell and Labib [18] elucidate that DNA synthesis is carried out by three multisubunit DNA-directed DNA polymerases, POLα, POLδ), and POLϵ). Further, POLϵ and POLα are connected to the CMG helicase via GINS. Later, Denkiewicz-Kruk et al. [19] concluded that the impaired interaction between GINS and POLϵ required involvement of error-prone POLζ, and increased participation of recombination as a rescue mechanism for recovery of impaired replication forks. In colorectal cancer cells treated with ETC-1922159, POL family members and GINS1, were found to be down regulated and their regulation was recorded independently. Additionally, DNA-dependent RNA polymerase (POLR) family members were also observed as down regulated. I was able to rank 2^{nd} order combination of these POL/POLR members along with GINS1.

Table 5 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 6 generated from analysis of the ranks in Table 5. The Table 5 shows rankings of POL members w.r.t GINS1. POLϵ2 (or E2) - GINS1 shows low ranking of 87 (laplace) and 393 (linear). POLθ (or Q) - GINS1 shows low ranking of 203 (laplace) and 102 (linear). POLα1 (or A1) - GINS1 shows low ranking of 377 (laplace), 412 (linear) and 1100 (rbf). POLδ1 (or D1) - GINS1 shows low ranking of 407 (laplace), 691 (linear) and 1255 (rbf). POLδ2 (or D2) - GINS1 shows low ranking of 417 (laplace) and 323 (rbf). POLγ2 (or G2) - GINS1 shows low ranking of 918 (laplace) and 1129 (linear). POLϵ3 (or E3) - GINS1 shows low ranking of 1429 (laplace), 1265 (linear) and 810 (rbf). POLα2 (or A2) - GINS1 shows low ranking of 1295 (linear) and 811 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment. Further, POLβ (or B) showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

The Table 5 also shows rankings of POLR members w.r.t GINS1. POLR-3K - GINS1 shows low ranking of 459 (laplace), 993 (linear) and 222 (rbf). POLR-1C - GINS1 shows low ranking of 529 (laplace) and 806 (linear). POLR-1E - GINS1 shows low ranking of 670 (laplace), 588 (linear) and 888 (rbf).

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POLR-1B - GINS1 shows low ranking of 889 (laplace), 1019 (linear) and 280 (rbf). POLR-3E - GINS1 shows low ranking of 1209 (laplace) and 720 (rbf). POLR-2G - GINS1 shows low ranking of 1312 (laplace) and 1000 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment. Further, POLR-2K, POLR-3A, POLR-2F, POLR-1A, POLR-2D and POLR-1D showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Table 5. 2^{nd} order interaction ranking between GINS1 VS POL members.

RANKING POL FAMILY VS GINS1			
RANKING OF POL FAMILY W.R.T GINS1			
	laplace	linear	rbf
POLϵ2 (or E2) - GINS1	87	393	1829
POLθ (or Q) - GINS1	203	102	2743
POLα1 (or A1) - GINS1	377	412	1100
POLδ1 (or D1) - GINS1	407	691	1255
POLδ2 (or D2) - GINS1	417	1766	323
POLγ2 (or G2) - GINS1	918	1129	1955
POLϵ3 (or E3) - GINS1	1429	1265	810
POLα2 (or A2) - GINS1	2207	1295	811
POLβ (or B) - GINS1	2367	2046	1838
RANKING POLR FAMILY VS GINS1			
RANKING OF POLR FAMILY W.R.T GINS1			
	laplace	linear	rbf
POLR-3K - GINS1	459	993	222
POLR-1C - GINS1	529	806	2252
POLR-1E - GINS1	670	588	888
POLR-1B - GINS1	889	1019	280
POLR-3E - GINS1	1209	2203	720
POLR-2G - GINS1	1312	1699	1000
POLR-2K - GINS1	2028	2559	1521
POLR-3A - GINS1	2230	1772	1573
POLR-2F - GINS1	2309	2468	1396
POLR-1A - GINS1	2317	1157	1625
POLR-2D - GINS1	2425	1719	792
POLR-1D - GINS1	2612	2732	957

One can also interpret the results of the Table 5 graphically, with the following influences - • POL members w.r.t GINS1 with GINS1 $->$ POL-ϵ2 (or E2) / θ (or Q) / α1 (or A1) / δ1 (or D1) / δ2 (or D2) / γ2 (or G2) / ϵ3 (or E3) / α2 (or A2), and GINS1 $->$ POLR-3K/1C/1E/1B/3E/2G.

Table 6. 2^{nd} order combinatorial hypotheses between GINS1 and POL members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
POL members w.r.t GINS1	
POL-ϵ2 (or E2) / θ (or Q) / α1 (or A1) / δ1 (or D1) / δ2 (or D2) / γ2 (or G2) / ϵ3 (or E3) / α2 (or A2)	GINS1
POLR members w.r.t GINS1	
POLR-3K/1C/1E/1B/3E/2G	GINS1

2.1.4. GINS1 - TOP

Yang et al. [20] revealed through functional assays that GINS1 aggravated glioma malignant phenotypes in vitro and in vivo. Their study identified that GINS1 interacted with TOP2A and promoted glioma cell proliferation and migration through USP15-mediated deubiquitination of TOP2A protein. Day et al. [21] demostrated through biochemical and structural studies that GINS interacted with TOPBP1 through two separate binding surfaces, 1. involving a conserved amino acids in the

 doi:10.20944/preprints202501.0868.v1

TOPBP1-GINI region and 2. a surface on TOPBP1-BRCT4. These two surfaces bind to opposite ends of the A domain of the GINS subunit PSF1/GINS1. In colorectal cancer cells treated with ETC-1922159, TOP family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these TOP members along with GINS1.

Table 7 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 8 generated from analysis of the ranks in Table 7. The Table 7 shows rankings of TOP members w.r.t GINS1. TOP2A - GINS1 shows low ranking of 25 (laplace) and 896 (linear). TOP1MT - GINS1 shows low ranking of 366 (laplace) and 489 (linear). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, TOPBP1 and TOPB2 showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Also, as a synergy between TOP2A and GINS1, through USP15 was demonstrated in one of the forgoing literature, a ranking of the recorded members of USP family with GINS1 was also tabulated. The Table 7 shows rankings of USP members w.r.t GINS1, also. USP13 - GINS1 shows low ranking of 328 (laplace) and 75 (linear). USP28 - GINS1 shows low ranking of 1283 (laplace), 750 (linear) and 443 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, USP36, USP1, USP39 and USP10 showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Table 7. 2^{nd} order interaction ranking between GINS1 VS TOP members.

RANKING TOP FAMILY VS GINS1			
RANKING OF TOP FAMILY W.R.T GINS1			
	laplace	linear	rbf
TOP2A - GINS1	25	896	2393
TOP1MT - GINS1	366	489	1827
TOPBP1 - GINS1	1833	2405	1433
TOP2B - GINS1	1885	2177	1009
RANKING USP FAMILY VS GINS1			
RANKING OF USP FAMILY W.R.T GINS1			
	laplace	linear	rbf
USP13 - GINS1	328	75	2737
USP28 - GINS1	1283	750	443
USP36 - GINS1	1588	2080	1360
USP1 - GINS1	1682	1887	1929
USP39 - GINS1	2454	2122	175
USP10 - GINS1	2500	2573	1636

One can also interpret the results of the Table 7 graphically, with the following influences - • TOP members w.r.t GINS1 with GINS1 $->$ TOP-2A/1MT, and • USP members w.r.t GINS1 with GINS1 $->$ USP-13/28.

Table 8. 2^{nd} order combinatorial hypotheses between GINS1 and TOP members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TOP members w.r.t GINS1	
TOP-2A/1MT	GINS1
USP members w.r.t GINS1	
USP-13/28	GINS1

2.1.5. GINS1 - FANC

Although the CMG complex implies that interstrand crosslinks (ICLs) are absolute blocks to replisomes, recent studies indicate that cells can restart DNA synthesis on the side of the ICL distal to

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the initial encounter. Huang et al. [22] report that this restart requires ATR and is promoted by FANCD2 and phosphorylated FANCM. Following this, FANCM binds the replisome complex, with associated release of the GINS proteins. The results indicated that, there was a FANCM/FANCD2-dependent absence of GINS proteins from replisomes. Thus there might be a synergy between the FANC family and GINS. In colorectal cancer cells treated with ETC-1922159, FANC family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these FANC members along with GINS1.

Table 9 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 10 generated from analysis of the ranks in Table 9. The Table 9 shows rankings of FANC members w.r.t GINS1. FANCB - GINS1 shows low ranking of 37 (laplace) and 46 (linear). FANCM - GINS1 shows low ranking of 775 (laplace) and 420 (linear). FANCG - GINS1 shows low ranking of 1024 (laplace) and 927 (linear). FANCD2 - GINS1 shows low ranking of 1458 (laplace), 972 (linear) and 794 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, FANCI and FANCF showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

Table 9. 2^{nd} order interaction ranking between GINS1 VS FANC members.

RANKING FANC FAMILY VS GINS1			
RANKING OF FANC FAMILY W.R.T GINS1			
	laplace	linear	rbf
FANCB - GINS1	37	46	2678
FANCM - GINS1	775	420	229
FANCG - GINS1	1024	927	2558
FANCI - GINS1	1399	1566	1982
FANCD2 - GINS1	1458	972	794
FANCF - GINS1	2449	2484	656

One can also interpret the results of the Table 9 graphically, with the following influences - • FANC members w.r.t GINS1 with GINS1 $->$ FANC-B/M/G/D2.

Table 10. 2^{nd} order combinatorial hypotheses between GINS1 and FANC members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
FANC members w.r.t GINS1	
FANC-B/M/G/D2	GINS1

2.1.6. GINS1 - FOX

In diffuse large B-cell lymphoma (DLBCL), Chen et al. [23] found that GINS1 was upregulated and FOXP1 transcriptionally activated GINS1 expression. The FOXP1/GINS1 regulatory axis was validated in vivo xenograft lymphoma mouse model. Thus there might be a synergy between the FOX family and GINS. In colorectal cancer cells treated with ETC-1922159, FOX family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these FOX members along with GINS1.

Table 11 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 12 generated from analysis of the ranks in Table 11. The Table 11 shows rankings of FOX members w.r.t GINS1. FOXM1 - GINS1 shows low ranking of 21 (laplace) and 23 (linear) FOXJ1 - GINS1 shows low ranking of 454 (laplace) and 343 (linear). FOXD2-AS1 - GINS1 shows low ranking of 1437 (linear) and 313 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, FOXA2 showed high ranking with GINS1, thus indicating that they might not be working synergistically with GINS1, before the drug treatment.

 doi:10.20944/preprints202501.0868.v1

Table 11. 2^{nd} order interaction ranking between GINS1 VS FOX members.

RANKING FOX FAMILY VS GINS1			
RANKING OF FOX FAMILY W.R.T GINS1			
	laplace	linear	rbf
FOXM1 - GINS1	21	23	2661
FOXJ1 - GINS1	454	343	1611
FOXD2-AS1 - GINS1	1813	1437	313
FOXA2 - GINS1	2576	1942	123

One can also interpret the results of the Table 11 graphically, with the following influences - • FOX members w.r.t GINS1 with GINS1 $-$ > FOX-M1/J1/D2-AS1.

Table 12. 2^{nd} order combinatorial hypotheses between GINS1 and FOX members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
FOX members w.r.t GINS1	
FOX-M1/J1/D2-AS1	GINS1

2.1.7. GINS1 - NOTCH

In lung adenocarcinoma, Yishan et al. [24] found that the expression of GINS1 was up-regulated. They showed that knockdown of GINS1 inhibited proliferation, migration and invasion of the A549 cells, while overexpression of GINS1 enhanced the proliferation, migration and invasion of H1299 cells. Further, knockdown of GINS1 decreased the expression levels of NOTCH1 and NOTCH3, while overexpression of GINS1 increased the levels of NOTCH1 and NOTCH3. Thus there might be a synergy between the NOTCH family and GINS. In colorectal cancer cells treated with ETC-1922159, NOTCH family members and GINS1, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these NOTCH members along with GINS1.

Table 13 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in Table 14 generated from analysis of the ranks in Table 13. The Table 13 shows rankings of NOTCH members w.r.t GINS1. NOTCH4 - GINS1 shows low ranking of 418 (laplace), 230 (linear) and 572 (rbf). NOTCH1 - GINS1 shows low ranking of 661 (linear) and 976 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Table 13. 2^{nd} order interaction ranking between GINS1 VS NOTCH members.

RANKING NOTCH FAMILY VS GINS1			
RANKING OF NOTCH FAMILY W.R.T GINS1			
	laplace	linear	rbf
NOTCH4 - GINS1	418	230	572
NOTCH1 - GINS1	2219	661	976

One can also interpret the results of the Table 13 graphically, with the following influences - • NOTCH members w.r.t GINS1 with GINS1 $-$ > NOTCH-4/1.

Table 14. 2^{nd} order combinatorial hypotheses between GINS1 and NOTCH members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
NOTCH members w.r.t GINS1	
NOTCH-4/1	GINS1

 doi:10.20944/preprints202501.0868.v1

3. Conclusion

Presented here are a range of multiple synergistic GINS1 2^{nd} order combinations that were ranked via a machine learning based search engine. Via majority voting across the ranking methods, it was possible to find plausible unexplored synergistic combinations of GINS1-X that might be prevalent in CRC cells after treatment with ETC-1922159 drug.

Conflict of Interest

There are no conflicts to declare.

Author's Contributions

Concept, design, in silico implementation - SS. Analysis and interpretation of results - SS. Manuscript writing - SS. Manuscript revision - SS. Approval of manuscript - SS

Acknowledgements

Special thanks to Mrs. Rita Sinha and Mr. Prabhat Sinha for supporting the author financially, without which this work could not have been made possible.

Source of Data

Data used in this research work was released in a publication in Madan et al. [25].

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