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Posted Date: 9 January 2025

doi: 10.20944/preprints202501.0687.v1

Keywords: TOP2A; Porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer

 doi:10.20944/preprints202501.0687.v1

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Machine Learning Discoveries of TOP2A-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† Aspects of unpublished work were presented in a poster session at the first Wnt Gordon Research Conference, from 6-11 August 2017, held in Stowe, VT 05672, USA.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: DNA topoisomerase II α (TOP2A) belongs to the family of topoisomerases, which regulates the (un)winding of the DNA due to its double helical structure. The main function of TOP2A is to relieve the topological stress during DNA transcription, assist in separation of chromatids and condensation of chromosomes. In colorectal cancer (CRC) cells treated with ETC-1922159, TOP2A was found to be down regulated along with other genes. A recently developed search engine ranked combinations of TOP2A-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 TOP2A-X combinations might be working synergistically in CRC. In this research work, I cover combinations of TOP2A with WNT, nucleolar and spindle associated protein (NUSAP), Wolf-Hirschhorn syndrome candidate (WHSC), rhophilin Rho GTPase binding protein antisense RNA (RHPN-AS1), AT-rich interaction domain (ARID), DNA topoisomerase II binding protein (TOPBP), ERCC excision repair (ERCC), enhancer of zeste polycomb repressive complex 2 subunit (EZH), cyclin dependent kinase (CDK), origin recognition complex subunit (ORC), interleukin (IL), ubiquitin specific peptidase (USP), RAD54, zinc finger protein (ZNF), high mobility group box (HMGB), E2F transcription factor (E2F), GINS complex subunit (GINS), minichromosome maintenance (MCM), budding uninhibited by benzimidazoles mitotic checkpoint (BUB), DEAD/H-box helicase (DDX), H2A histone family member (H2A) and structural maintenance of chromosomes (SMC) family.

Keywords: TOP2A; Porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer

1. Introduction

1.1. Topoisomerases

Becuase of the double-helical structure of the DNA, coils are often formed during relication, transcription and translation. This leads to building up of tension in the DNA structure. To maintain the relaxed topology of the DNA, topoisomerases act as essential proteins providing the required functionality. Wang [1] first discovered the existance of topoisomerases. In a review, McKie et al. [2] describe that topoisomerases are classified into different types depending on whether they catalyse the formation and re-ligation of single-stranded (ss) or double-stranded (ds)DNA breaks. A detailed description of the structure and function of DNA topoisomerases can be found in Champoux [3].

1.2. DNA Topoisomerase II

The ATP-dependent Type II which requires Mg^{2+}, was identified in yeast by Goto and Wang [4]. Later, Adachi et al. [5] isolated the first conditional mutation in the mouse TOP2A. Watt and Hickson [6] particularly discuss the structure and function of type II. Of the two subtypes of type II, TOP2A has been found to be highly expressed in various malignancies, as reported by Zhou et al. [7]. In colon cancer, Zhang et al. [8] showed that the proliferation and invasion was suppressed by knockdown of TOP2A. Further, analysis by Carvalho et al. [9] revealed that TOP2A inhibitors were candidate

 doi:10.20944/preprints202501.0687.v1

drugs for rectal cancer treatment, based on drug repositioning. In colorectal cancer (CRC) cells treated with ETC-1922159, TOP2A was found to be down regulated along with other genes. TOP2A works in tandem with multiple components and some combinations of TOP2A 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 [10], 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 [11]. The work uses SVM package by Joachims [12] 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. TOP2A Related Synergies

2.1.1. TOP2A - WNT10B / NUSAP1 / WHSC1 / RHPN1-AS1 / ARID5B / TOPBP1

In non-small cell lung cancer, Wu et al. [13]'s experimental findings showed tha WNT3A, c-MYC, and β-catenin, expression levels were elevated when TOP2A was overexpressed and vice versa during TOP2A knockdown. Hu et al. [14] revealed that NUSAP1 gene silencing induced apoptosis in human glioblastoma, through the downregulation of the downstream molecule TOP2A. In hepatocellular carcinoma, Bao et al. [15] showed that activating transcription factor 2 (ATF2), bound to and promoted the transcription of WHSC1, which further increased the expression of TOP2A by inducing the dimethylation of histone H3 lysine 36 (H3K36me2). Zhou et al. [16] demostrated that RHPN1-AS1 negatively regulated miR-485-5p which lead to promotion of the TOP2A expression in ovarian cancer cells. Tsai et al. [17] show that ARID1A loss causes DNA replication stress associated with R-loops and transcription-replication conflicts in human cells. They show a model in which deletion of ARID1A reduces BRG1/BRM-associated factor (BAF) binding, thus failing to recruit TOP2A, which leads to accumulation of R-loops. Broderick et al. [18] identify TOPBP1 as an interactor of TOP2A, and show that it is required for TOP2A recruitment to resolve ultra-fine anaphase bridges (UFBs) during mitosis. These combinations with TOP2A which have been experimentally tested show a combinatorial synergy in various cases. In colorectal cancer cells treated with ETC-1922159, these components taken individually and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these individual members along with TOP2A.

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 individual members w.r.t TOP2A. WNT10B - TOP2A shows low ranking of 181 (laplace), 153 (linear) and 213 (rbf). NUSAP1 - TOP2A shows low ranking of 87 (laplace), 174 (linear) and 126 (rbf). WHSC1 - TOP2A shows low ranking of 444 (laplace), 711 (linear) and 541 (rbf). RHPN1-AS1 - TOP2A shows low ranking of 339 (laplace), 1381 (linear) and 371 (rbf). ARID5B - TOP2A shows low ranking of 464 (laplace), 855 (linear) and 355 (rbf). TOPBP1 - TOP2A shows low ranking of 702 (laplace), 1430 (linear) and 1032 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 1. 2^{nd} order interaction ranking between TOP2A VS INDIVIDUAL members.

RANKING INDIVIDUAL MEMBERS VS TOP2A			
RANKING OF INDIVIDUAL MEMBERS W.R.T TOP2A			
	laplace	linear	rbf
WNT10B - TOP2A	181	153	213
NUSAP1 - TOP2A	87	174	126
WHSC1 - TOP2A	444	711	541
RHPN1-AS1 - TOP2A	339	1381	371
ARID5B - TOP2A	464	855	355
TOPBP1 - TOP2A	702	1430	1032

One can also interpret the results of the table 1 graphically, with the following influences - • individual members w.r.t TOP2A with TOP2A $->$ WNT10B / NUSAP1 / WHSC1 / RHPN1-AS1 / ARID5B / TOPBP1 .

Table 2. 2^{nd} order combinatorial hypotheses between TOP2A and individual members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
Individual members w.r.t TOP2A	
WNT10B / NUSAP1 / WHSC1 / RHPN1-AS1 / ARID5B / TOPBP1	TOP2A

2.1.2. TOP2A - ERCC

Polo-like kinase 1-interacting checkpoint helicase (PICH), also known as excision repair cross-complementation group 6 like (ERCC6L), is a substrate if PLK1-interacting checkpoint helicase. Over-expression of PICH/ERCC6L is related to the proliferation of tumors and Li et al. [19] hypothesis that PICH can maintain genomic stability by regulating appropriate chromosome structure, ensuring proper chromosome segregation, and facilitating replication fork reversal, via PICH-PLK1-TOP2A axis. In colorectal cancer cells treated with ETC-1922159, ERCC family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these ERCC member along with TOP2A.

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 individual members w.r.t TOP2A. ERCC6L - TOP2A shows low ranking of 40 (laplace), 24 (linear) and 24 (rbf). ERCC8 - TOP2A shows low ranking of 870 (laplace) and 903 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 3. 2^{nd} order interaction ranking between TOP2A VS ERCC family.

RANKING ERCC FAMILY VS TOP2A			
RANKING OF ERCC FAMILY W.R.T TOP2A			
	laplace	linear	rbf
ERCC6L - TOP2A	40	24	24
ERCC8 - TOP2A	870	1714	903

One can also interpret the results of the table 3 graphically, with the following influences - • ERCC family w.r.t TOP2A with TOP2A $->$ ERCC-6L/8.

Table 4. 2^{nd} order combinatorial hypotheses between TOP2A and ERCC family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ERCC family w.r.t TOP2A	
ERCC-6L/8	TOP2A

2.1.3. TOP2A - EZH/CDK

In glioblastoma, Freitag et al. [20] show that EZH2 is overexpressed and combined EZH2-CDK4/6 inhibition increases antitumor activity against glioblastoma, by boosting cell death and cell stress, reverses stemness characteristics, disrupts endoplasmatic reticulum-mitochondrial homeostasis and reduces the invasion capability in GBM spheroids. Mechanistically, this was due to transcriptional changes in several genes, of which TOP2A is one of them. Slightly unrelated, but during retinal development in zebrafish Jin et al. [21] suggest that to regulate S phase entry, CDK1 interacts with cyclin A2 through phosphorylation of TOP2A. In colorectal cancer cells treated with ETC-1922159, EZH/CDK family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these EZH/CDK member along with TOP2A.

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 individual members w.r.t TOP2A. EZH2 - TOP2A shows low ranking of 1328 (laplace), 1463 (linear) and 1106 (rbf). CDK1 - TOP2A shows low ranking of 57 (laplace), 51 (linear) and 70 (rbf). CDK5RAP1 - TOP2A shows low ranking of 789 (laplace), 865 (linear) and 996 (rbf). CDK4 - TOP2A shows low ranking of 1277 (laplace), 948 (linear) and 1476 (rbf). CDK20 - TOP2A shows low ranking of 1370 (laplace), 1021 (linear) and 1421 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, CDK5RAP2 and CDK6 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 5. 2^{nd} order interaction ranking between TOP2A VS EZH-CDK family.

RANKING EZH-CDK FAMILY VS TOP2A			
RANKING OF EZH-CDK FAMILY W.R.T TOP2A			
	laplace	linear	rbf
EZH2 - TOP2A	1328	1463	1106
CDK1 - TOP2A	57	51	70
CDK5RAP1 - TOP2A	789	865	996
CDK4 - TOP2A	1277	948	1476
CDK20 - TOP2A	1370	1021	1421
CDK5RAP2 - TOP2A	1935	1717	2130
CDK6 - TOP2A	2544	1511	2151

One can also interpret the results of the table 5 graphically, with the following influences - • EZH-CDK family w.r.t TOP2A with TOP2A $->$ EZH2 and TOP2A $->$ CDK-1/5RAP1/4/20.

Table 6. 2^{nd} order combinatorial hypotheses between TOP2A and EZH-CDK family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
EZH/CDK family w.r.t TOP2A	
EZH2	TOP2A
CDK-1/5RAP1/4/20	TOP2A

2.1.4. TOP2A - ORC

In primary and immortalized glioma cells, Yang et al. [22] showed that depleting/knockout of ORC6 decreased cell viability and proliferation, disrupted cell cycle progression and mobility, and triggered apoptosis. Further, via in vivo experiments, they demonstrated that ORC6 depletion decreased expression of Cyclin A2/B2/TOP2A. In colorectal cancer cells treated with ETC-1922159, ORC family members and TOP2A, 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 TOP2A.

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 individual members w.r.t TOP2A. ORC1 - TOP2A shows low ranking of 258 (laplace), 145 (linear) and 279 (rbf). ORC6 - TOP2A shows low ranking of 1296 (laplace), 490 (linear) and 1449 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, ORC5, ORC3 and ORC2 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 7. 2^{nd} order interaction ranking between TOP2A VS ORC family.

RANKING ORC FAMILY VS TOP2A			
RANKING OF ORC FAMILY W.R.T TOP2A			
	laplace	linear	rbf
ORC1 - TOP2A	258	145	279
ORC6 - TOP2A	1296	490	1449
ORC5 - TOP2A	1583	2340	1380
ORC3 - TOP2A	2341	2220	2215
ORC2 - TOP2A	2464	2530	2429

One can also interpret the results of the table 7 graphically, with the following influences - • ORC family w.r.t TOP2A with TOP2A $-$ > ORC-1/6.

Table 8. 2^{nd} order combinatorial hypotheses between TOP2A and ORC family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ORC family w.r.t TOP2A	
ORC-1/6	TOP2A

2.1.5. TOP2A - IL

Through in vivo and in vitro experiments Li et al. [23] showed that knockdown of TOP2A inhibited inflammation and IL-17 signaling pathway, and promoted proliferation of ulcerative colitis (an inflammatory disease of the colonic mucosa). In colorectal cancer cells treated with ETC-1922159, IL family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these IL members along with TOP2A.

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 individual members w.r.t TOP2A. IL33 - TOP2A shows low ranking of 591 (laplace), 152 (linear) and 818 (rbf). IL17D - TOP2A shows low ranking of 1054 (laplace), 1194 (linear) and 853 (rbf). IL17RD - TOP2A shows low ranking of 1193 (laplace) and 1260 (rbf). IL1RL2 - TOP2A shows low ranking of 1237 (laplace) and 1328 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 9. 2^{nd} order interaction ranking between TOP2A VS IL family.

RANKING IL FAMILY VS TOP2A			
RANKING OF IL FAMILY W.R.T TOP2A			
	laplace	linear	rbf
IL33 - TOP2A	591	152	818
IL17D - TOP2A	1054	1194	853
IL17RD - TOP2A	1193	2235	1260
IL1RL2 - TOP2A	1237	2123	1328

One can also interpret the results of the table 9 graphically, with the following influences - • IL family w.r.t TOP2A with TOP2A $->$ IL-33/17D/17RD/1RL2.

Table 10. 2^{nd} order combinatorial hypotheses between TOP2A and IL family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
IL family w.r.t TOP2A	
IL-33/17D/17RD/1RL2	TOP2A

2.1.6. TOP2A - USP

Fielding et al. [24] show that deubiquitylase USP15 is required for TOP2A accumulation during G2, and USP15 depletion causes formation of anaphase chromosome bridges. In colorectal cancer cells treated with ETC-1922159, USP family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these USP members along with TOP2A.

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 individual members w.r.t TOP2A. USP13 - TOP2A shows low ranking of 49 (laplace), 63 (linear) and 43 (rbf). USP36 - TOP2A shows low ranking of 964 (laplace) and 1077 (rbf). USP28 - TOP2A shows low ranking of 1147 (laplace), 1546 (linear) and 1004 (rbf). USP1 - TOP2A shows low ranking of 1375 (linear) and 1241 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, USP39 and USP10 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 11. 2^{nd} order interaction ranking between TOP2A VS USP family.

RANKING USP FAMILY VS TOP2A			
RANKING OF USP FAMILY W.R.T TOP2A			
	laplace	linear	rbf
USP13 - TOP2A	49	63	43
USP36 - TOP2A	964	1738	1077
USP28 - TOP2A	1147	1546	1004
USP1 - TOP2A	1586	1375	1241
USP39 - TOP2A	2238	938	2224
USP10 - TOP2A	2695	2717	2707

One can also interpret the results of the table 11 graphically, with the following influences - • USP family w.r.t TOP2A with TOP2A $->$ USP-13/36/28/1.

Table 12. 2^{nd} order combinatorial hypotheses between TOP2A and USP family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
USP family w.r.t TOP2A	
USP-13/36/28/1	TOP2A

2.1.7. TOP2A - RAD54-ZNF

TOP2 form cleavage complexes (TOP2ccs) during their catalytic cycle to relieve topological stress, however they can be trapped by TOP2 poisons. Trapped TOP2ccs by action of TOP2 poisons, block transactions on DNA and generate genotoxic stress. Zhang et al. [25] uncovered RAD54L2 which mediates a TOP2-specific DNA damage avoidance pathway, by interacting with TOP2A/TOP2B and ZATT/ZNF451. In colorectal cancer, Gao et al. [26] showed that ZNF148 modulates TOP2A. In colorectal cancer cells treated with ETC-1922159, RAD54-ZNF family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these RAD54-ZNF members along with TOP2A.

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 individual members w.r.t TOP2A. RAD54B - TOP2A shows low ranking of 145 (laplace), 188 (linear) and 80 (rbf). RAD54L - TOP2A shows low ranking of 1233 (laplace) and 933 (rbf). ZNF815P - TOP2A shows low ranking of 45 (laplace), 72 (linear) and 38 (rbf). ZNF204P - TOP2A shows low ranking of 74 (laplace), 165 (linear) and 139 (rbf). ZNF367 - TOP2A shows low ranking of 106 (laplace), 37 (linear) and 96 (rbf). ZNF775 - TOP2A shows low ranking of 194 (laplace), 110 (linear) and 204 (rbf). ZNF485 - TOP2A shows low ranking of 309 (laplace), 936 (linear) and 349 (rbf). ZNF239 - TOP2A shows low

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ranking of 556 (laplace), 956 (linear) and 411 (rbf). ZNF511 - TOP2A shows low ranking of 621 (laplace), 612 (linear) and 737 (rbf). ZNF519 - TOP2A shows low ranking of 629 (laplace), 264 (linear) and 536 (rbf). ZNF771 - TOP2A shows low ranking of 677 (laplace), 636 (linear) and 542 (rbf). ZNF74 - TOP2A shows low ranking of 714 (laplace), 1371 (linear) and 726 (rbf). ZNF202 - TOP2A shows low ranking of 775 (laplace), 1331 (linear) and 782 (rbf). ZNF620 - TOP2A shows low ranking of 784 (laplace), 600 (linear) and 620 (rbf). ZNF22 - TOP2A shows low ranking of 826 (laplace), 666 (linear) and 735 (rbf). ZNF273 - TOP2A shows low ranking of 866 (laplace), 1684 and 935 (rbf). ZNF584 - TOP2A shows low ranking of 926 (laplace), 469 (linear) and 1120 (rbf). ZNF248 - TOP2A shows low ranking of 957 (laplace) and 1085 (rbf). ZNF90 - TOP2A shows low ranking of 975 (laplace), 968 (linear) and 1123 (rbf). ZNF740 - TOP2A shows low ranking of 1061 (laplace), 1219 (linear) and 1485 (rbf). ZNF138 - TOP2A shows low ranking of 1092 (laplace) and 1277 (rbf). ZNF48 - TOP2A shows low ranking of 1207 (laplace), 653 (linear) and 1477 (rbf). ZNF512 - TOP2A shows low ranking of 1214 (laplace), 807 (linear) and 1375 (rbf). ZNF695 - TOP2A shows low ranking of 1228 (laplace), 858 (linear) and 1393 (rbf). ZNF793 - TOP2A shows low ranking of 1243 (laplace), 1073 (linear) and 1139 (rbf). ZNF572 - TOP2A shows low ranking of 1257 (laplace), 1503 (linear) and 1481 (rbf). ZNF670 - TOP2A shows low ranking of 1258 (laplace), 541 (linear) and 1115 (rbf). ZNF32 - TOP2A shows low ranking of 1332 (laplace), 724 (linear) and 1213 (rbf). ZNF232 - TOP2A shows low ranking of 1344 (laplace) and 1098 (rbf). ZNF691 - TOP2A shows low ranking of 1394 (laplace), 1271 (linear) and 1172 (rbf). ZNF124 - TOP2A shows low ranking of 1361 (linear) and 1351 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, ZNF37BP, ZNF486, ZNF594, ZNF146, ZNF589, ZNF280D, ZNF738, ZNF736, ZNF428, ZNF330, ZNF174, ZNF780B, ZNF93, ZNF629, ZNF614, ZNF346, ZNF639, ZNF263, ZNF215, ZNF275, ZNF343, ZNF480, ZNF789, ZNF502, ZNF445 and ZNF689 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

 doi:10.20944/preprints202501.0687.v1

Table 13. 2^{nd} order interaction ranking between TOP2A VS RAD54-ZNF family.

RANKING RAD54-ZNF FAMILY VS TOP2A

RANKING OF RAD54-ZNF FAMILY W.R.T TOP2A						
	laplace	linear	rbf		laplace	linear	rbf
RAD54B - TOP2A	145	188	80	RAD54L - TOP2A	1233	1601	933
ZNF815P - TOP2A	45	72	38	ZNF204P - TOP2A	74	165	139
ZNF367 - TOP2A	106	37	96	ZNF775 - TOP2A	194	110	204
ZNF485 - TOP2A	309	936	349	ZNF239 - TOP2A	556	956	411
ZNF511 - TOP2A	621	612	737	ZNF519 - TOP2A	629	264	536
ZNF771 - TOP2A	677	636	542	ZNF74 - TOP2A	714	1371	726
ZNF202 - TOP2A	775	1331	782	ZNF620 - TOP2A	784	600	620
ZNF22 - TOP2A	826	666	735	ZNF273 - TOP2A	866	1684	935
ZNF584 - TOP2A	926	469	1120	ZNF248 - TOP2A	957	2135	1085
ZNF90 - TOP2A	975	968	1123	ZNF740 - TOP2A	1061	1219	1485
ZNF138 - TOP2A	1092	2251	1277	ZNF48 - TOP2A	1207	653	1477
ZNF512 - TOP2A	1214	807	1375	ZNF695 - TOP2A	1228	858	1393
ZNF793 - TOP2A	1243	1073	1139	ZNF572 - TOP2A	1257	1503	1481
ZNF670 - TOP2A	1258	541	1115	ZNF37BP - TOP2A	1270	1850	1708
ZNF32 - TOP2A	1332	724	1213	ZNF232 - TOP2A	1344	1662	1098
ZNF486 - TOP2A	1361	1826	1853	ZNF691 - TOP2A	1394	1271	1172
ZNF594 - TOP2A	1557	2110	1297	ZNF146 - TOP2A	1612	2050	1724
ZNF589 - TOP2A	1629	1679	1419	ZNF280D - TOP2A	1638	2471	1572
ZNF738 - TOP2A	1651	1788	1466	ZNF124 - TOP2A	1696	1361	1351
ZNF736 - TOP2A	1748	1559	1788	ZNF428 - TOP2A	1863	1513	2016
ZNF330 - TOP2A	1883	1583	2091	ZNF174 - TOP2A	1892	669	1773
ZNF780B - TOP2A	1919	1742	2089	ZNF93 - TOP2A	1958	2538	1930
ZNF629 - TOP2A	1990	2732	2049	ZNF614 - TOP2A	2016	2631	2476
ZNF346 - TOP2A	2078	721	1637	ZNF639 - TOP2A	2099	2238	2126
ZNF263 - TOP2A	2221	2741	2549	ZNF215 - TOP2A	2279	2155	2257
ZNF275 - TOP2A	2307	1517	2452	ZNF343 - TOP2A	2417	2001	2497
ZNF480 - TOP2A	2529	1990	2553	ZNF789 - TOP2A	2534	2696	2557
ZNF502 - TOP2A	2572	2002	2641	ZNF445 - TOP2A	2581	2640	2348
ZNF689 - TOP2A	2663	1551	2637				

One can also interpret the results of the table 13 graphically, with the following influences - • RAD54-ZNF family w.r.t TOP2A with TOP2A $->$ RAD54-B/L and TOP2A $->$ ZNF-815P / 204P / 367 / 775 / 485 / 239 / 511 / 519 / 771 / 74 / 202 / 620 / 22 / 273 / 584 / 248 / 90 / 740 / 138 / 48 / 512 / 695 / 793 / 572 / 670 / 32 / 232 / 691 / 124.

Table 14. 2^{nd} order combinatorial hypotheses between TOP2A and RAD54-ZNF family.

UNEXPLORED COMBINATORIAL HYPOTHESES

RAD54-ZNF family w.r.t TOP2A	
RAD54-B/L	TOP2A
ZNF-815P/204P/367/775/485/239/511/519/771/...	
74/202/620/22/273/584/248/90/740/138/...	
48/512/695/793/572/670/32/232/691/124	TOP2A

 doi:10.20944/preprints202501.0687.v1

2.1.8. TOP2A - HMGB

In carbon tetrachloride (CCl_4) induced liver fibrosis mouse model, Huang et al. [27] showed that deletion/inhibition of HMGB2 slowed the progression of CCl_4- induced liver fibrosis. Their RNA-seq analysis revealed the induction of CCl_4-activated genes, one of which was TOP2A (the activation of which was abolished in HMGB2$^{-/-}$ mice or in ICM-treated mice). In colorectal cancer cells treated with ETC-1922159, HMGB family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these HMGB members along with TOP2A.

Table 15 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 16 generated from analysis of the ranks in table 15. The table 15 shows rankings of individual members w.r.t TOP2A. HMGB2 - TOP2A shows low ranking of 378 (laplace), 330 (linear) and 314 (rbf). HMGB3 - TOP2A shows low ranking of 987 (laplace), 1173 (linear) and 827 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, HMGB1 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

Table 15. 2^{nd} order interaction ranking between TOP2A VS HMGB family.

RANKING HMGB FAMILY VS TOP2A			
RANKING OF HMGB FAMILY W.R.T TOP2A			
	laplace	linear	rbf
HMGB2 - TOP2A	378	330	314
HMGB3 - TOP2A	987	1173	827
HMGB1 - TOP2A	1920	2239	1855

One can also interpret the results of the table 15 graphically, with the following influences - • HMGB family w.r.t TOP2A with TOP2A $->$ HMGB-2/3.

Table 16. 2^{nd} order combinatorial hypotheses between TOP2A and HMGB family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
HMGB family w.r.t TOP2A	
HMGB-2/3	TOP2A

2.1.9. TOP2A - E2F

In gastric cancer cells Chen et al. [28] showed that overexpressed E2F1 increased TOP2A levels and vice versa. In colorectal cancer cells treated with ETC-1922159, E2F family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these E2F members along with TOP2A.

Table 17 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 18 generated from analysis of the ranks in table 17. The table 17 shows rankings

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of individual members w.r.t TOP2A. E2F7 - TOP2A shows low ranking of 7 (laplace), 20 (linear) and 8 (rbf). E2F8 - TOP2A shows low ranking of 291 (laplace), 85 (linear) and 373 (rbf). E2F2 - TOP2A shows low ranking of 436 (laplace), 219 (linear) and 550 (rbf). E2F1 - TOP2A shows low ranking of 606 (laplace), 284 (linear) and 562 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, E2F5 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

Table 17. 2^{nd} order interaction ranking between TOP2A VS E2F family.

RANKING E2F FAMILY VS TOP2A			
RANKING OF E2F FAMILY W.R.T TOP2A			
	laplace	linear	rbf
E2F7 - TOP2A	7	20	8
E2F8 - TOP2A	291	85	373
E2F2 - TOP2A	436	219	550
E2F1 - TOP2A	606	284	562
E2F5 - TOP2A	2448	2694	2536

One can also interpret the results of the table 17 graphically, with the following influences - • E2F family w.r.t TOP2A with TOP2A $->$ E2F-7/8/2/1.

Table 18. 2^{nd} order combinatorial hypotheses between TOP2A and E2F family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
E2F family w.r.t TOP2A	
E2F-7/8/2/1	TOP2A

2.1.10. TOP2A - GINS-MCM

GINS1 is a GINS complex subunit that interacts with the MCM2-7 complex and CCD45 in eukaryotic DNA replication. Gambus et al. [29] show that the GINS complex allows MCM helicase to interact with replisome progression complexes (RPCs) that are assembled during initiation and disassembled at the end of S phase. RPCs also interact with MCM10 and TOP1. In glioma cells and tissues, Yang et al. [30] showed that GINS1 expression level was upregulated and mechanistically it promotes proliferation and migration through USP15-mediated deubiquitination of TOP2A protein. In colorectal cancer cells treated with ETC-1922159, GINS-MCM family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these GINS-MCM members along with TOP2A.

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Table 19 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 20 generated from analysis of the ranks in table 19. The table 19 shows rankings of individual members w.r.t TOP2A. For GINS - GINS1 - TOP2A shows low ranking of 102 (laplace), 78 (linear) and 173 (rbf). GINS2 - TOP2A shows low ranking of 131 (laplace), 49 (linear) and 140 (rbf). GINS3 - TOP2A shows low ranking of 411 (laplace), 312 (linear) and 417 (rbf). GINS4 - TOP2A shows low ranking of 601 (laplace), 406 (linear) and 375 (rbf).

For MCM - MCM4 - TOP2A shows low ranking of 52 (laplace), 66 (linear) and 49 (rbf). MCM10 - TOP2A shows low ranking of 113 (laplace), 301 (linear) and 264 (rbf). MCM2 - TOP2A shows low ranking of 236 (laplace), 384 (linear) and 237 (rbf). MCM5 - TOP2A shows low ranking of 256 (laplace), 590 (linear) and 224 (rbf). MCM6 - TOP2A shows low ranking of 467 (laplace), 359 (linear) and 386 (rbf). MCM8 - TOP2A shows low ranking of 474 (laplace), 458 (linear) and 592 (rbf). MCM3 - TOP2A shows low ranking of 487 (laplace), 794 (linear) and 268 (rbf). MCM7 - TOP2A shows low ranking of 533 (laplace), 801 (linear) and 438 (rbf). These rankings point to the synergy existing between the two/three components, which have been down regulated after the drug treatment.

Table 19. 2^{nd} order interaction ranking between TOP2A VS GINS-MCM family.

RANKING GINS-MCM FAMILY VS TOP2A			
RANKING OF GINS-MCM FAMILY W.R.T TOP2A			
	laplace	linear	rbf
GINS1 - TOP2A	102	78	173
GINS2 - TOP2A	131	49	140
GINS3 - TOP2A	411	312	417
GINS4 - TOP2A	601	406	375
MCM4 - TOP2A	52	66	49
MCM10 - TOP2A	113	301	264
MCM2 - TOP2A	236	384	237
MCM5 - TOP2A	256	590	224
MCM6 - TOP2A	467	359	386
MCM8 - TOP2A	474	458	592
MCM3 - TOP2A	487	794	268
MCM7 - TOP2A	533	801	438

One can also interpret the results of the table 19 graphically, with the following influences - • GINS-MCM family w.r.t TOP2A with TOP2A $-$ $>$ GINS-1/2/3/4 and TOP2A $-$ $>$ MCM-4/10/2/5/6/8/3/7.

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Table 20. 2^{nd} order combinatorial hypotheses between TOP2A and GINS-MCM family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
GINS-MCM family w.r.t TOP2A	
GINS-1/2/3/4	TOP2A
MCM-4/10/2/5/6/8/3/7	TOP2A

2.1.11. TOP2A - BUB

Carvalhal et al. [31] confirm that BUB1 kinase activity promotes the centromeric localization of TOP2A. In colorectal cancer cells treated with ETC-1922159, BUB family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these BUB members along with TOP2A.

Table 21 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 22 generated from analysis of the ranks in table 21. The table 21 shows rankings of individual members w.r.t TOP2A. BUB1 - TOP2A shows low ranking of 19 (laplace), 41 (linear) and 44 (rbf). BUB1B - TOP2A shows low ranking of 211 (laplace), 154 (linear) and 99 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, BUB3 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

Table 21. 2^{nd} order interaction ranking between TOP2A VS BUB family.

RANKING BUB FAMILY VS TOP2A			
RANKING OF BUB FAMILY W.R.T TOP2A			
	laplace	linear	rbf
BUB1 - TOP2A	19	41	44
BUB1B - TOP2A	211	154	99
BUB3 - TOP2A	2203	1521	2167

One can also interpret the results of the table 21 graphically, with the following influences - • BUB family w.r.t TOP2A with TOP2A $-$ > BUB-1/1B.

 doi:10.20944/preprints202501.0687.v1

Table 22. 2^{nd} order combinatorial hypotheses between TOP2A and BUB family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
BUB family w.r.t TOP2A	
BUB-1/1B	TOP2A

2.1.12. TOP2A - DDX

Zhang et al. [32] suggest that DDX11-AS1 knockdown resulted in reduced resistance of esophageal cancer cells to paclitaxel by inhibiting TOP2A transcription via TAF1. In colorectal cancer cells treated with ETC-1922159, DDX family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these DDX members along with TOP2A.

Table 23 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 24 generated from analysis of the ranks in table 23. The table 23 shows rankings of individual members w.r.t TOP2A. DDX11-AS1 - TOP2A shows low ranking of 310 (laplace), 365 (linear) and 406 (rbf). DDX12P - TOP2A shows low ranking of 435 (laplace), 76 (linear) and 450 (rbf). DDX55 - TOP2A shows low ranking of 806 (laplace), 1389 (linear) and 919 (rbf). DDX11 - TOP2A shows low ranking of 829 (laplace) and 813 (rbf). DDX28 - TOP2A shows low ranking of 965 (laplace), 1108 (linear) and 871 (rbf). DDX18 - TOP2A shows low ranking of 1084 (laplace) and 1273 (rbf). DDX20 - TOP2A shows low ranking of 1369 (laplace), 393 (linear) and 1401 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, DDX54, DDX10, DDX31, DDX21, DDX51, DDX19A, DDX56, DDX46 and DDX27 showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

Table 23. 2^{nd} order interaction ranking between TOP2A VS DDX family.

RANKING DDX FAMILY VS TOP2A							
	RANKING OF DDX FAMILY W.R.T TOP2A						
	laplace	linear	rbf		laplace	linear	rbf
DDX11-AS1 - TOP2A	310	365	406	DDX12P - TOP2A	435	76	450
DDX55 - TOP2A	806	1389	919	DDX11 - TOP2A	829	1730	813
DDX28 - TOP2A	965	1108	871	DDX18 - TOP2A	1084	2242	1273
DDX20 - TOP2A	1369	393	1401	DDX54 - TOP2A	1759	2524	1974
DDX10 - TOP2A	1933	857	1829	DDX31 - TOP2A	1951	2386	1515
DDX21 - TOP2A	1979	606	2054	DDX51 - TOP2A	1987	1289	1949
DDX19A - TOP2A	2144	1726	2063	DDX56 - TOP2A	2324	1537	2326
DDX46 - TOP2A	2338	2584	2579	DDX27 - TOP2A	2580	1170	2591

One can also interpret the results of the table 23 graphically, with the following influences - • DDX family w.r.t TOP2A with TOP2A $->$ DDX-11-AS1/12P/55/11/28/18/20.

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Table 24. 2^{nd} order combinatorial hypotheses between TOP2A and DDX family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
DDX family w.r.t TOP2A	
DDX-11-AS1/12P/55/11/28/18/20	TOP2A

2.1.13. TOP2A - H2A

Zhang et al. [33] show that histone H2A phosphorylation modification generated by the mitotic kinase BUB1, is necessary and sufficient for the centromeric localization of TOP2A. In colorectal cancer cells treated with ETC-1922159, H2A family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these H2A members along with TOP2A.

Table 25 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 26 generated from analysis of the ranks in table 25. The table 25 shows rankings of individual members w.r.t TOP2A. H2AFV - TOP2A shows low ranking of 409 (laplace) and 798 (rbf). H2AFZ - TOP2A shows low ranking of 1178 (laplace) and 1108 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, H2AFX showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

Table 25. 2^{nd} order interaction ranking between TOP2A VS H2A family.

RANKING H2A FAMILY VS TOP2A			
RANKING OF H2A FAMILY W.R.T TOP2A			
	laplace	linear	rbf
H2AFV - TOP2A	409	1916	798
H2AFZ - TOP2A	1178	1962	1108
H2AFX - TOP2A	2602	1080	2458

One can also interpret the results of the table 25 graphically, with the following influences - • H2A family w.r.t TOP2A with TOP2A $->$ H2A-FV/FZ.

Table 26. 2^{nd} order combinatorial hypotheses between TOP2A and H2A family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
H2A family w.r.t TOP2A	
H2A-FV/FZ	TOP2A

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2.1.14. TOP2A - SMC

The TOP2A-dependent arrest is responsible ofr segregation of sister chromatids and has been identified as dysfunctional in various tumour cell lines. Deiss et al. [34] show that the SMC5/6 complex regulates the TOP2A-dependent G2 arrest and sister chromatid disjunction via NSE2-mediated SUMOylation of TOP2A. In colorectal cancer cells treated with ETC-1922159, SMC family members and TOP2A, were found to be down regulated and their regulation was recorded independently. I was able to rank 2^{nd} order combination of these SMC members along with TOP2A.

Table 27 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 28 generated from analysis of the ranks in table 27. The table 27 shows rankings of individual members w.r.t TOP2A. SMC2 - TOP2A shows low ranking of 335(laplace), 970 (linear) and 340 (rbf). SMC4 - TOP2A shows low ranking of 915(laplace), 446 (linear) and 986 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, SMC1A showed high ranking with TOP2A, thus indicating that they might not be working synergistically with TOP2A, before the drug treatment.

RANKING SMC FAMILY VS TOP2A			
RANKING OF SMC FAMILY W.R.T TOP2A			
	laplace	linear	rbf
SMC2 - TOP2A	335	970	340
SMC4 - TOP2A	915	446	986
SMC1A - TOP2A	2461	898	2353

Table 27. 2^{nd} order interaction ranking between TOP2A VS SMC family.

One can also interpret the results of the table 27 graphically, with the following influences - • SMC family w.r.t TOP2A with TOP2A $-$ > SMC-2/4.

UNEXPLORED COMBINATORIAL HYPOTHESES	
SMC family w.r.t TOP2A	
SMC-2/4	TOP2A

Table 28. 2^{nd} order combinatorial hypotheses between TOP2A and SMC family.

3. Conclusion

Presented here are a range of multiple synergistic TOP2A 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 TOP2A-X that might be prevalent in CRC cells after treatment with ETC-1922159 drug.

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

Data Availability Statement: Data used in this research work was released in a publication in Madan et al. [35].

 doi:10.20944/preprints202501.0687.v1

Acknowledgments: 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.

Conflicts of Interest: There are no conflicts to declare.

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