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Machine Learning Discoveries of FANCD2-X Synergy in ETC-1922159 Treated Colorectal Cancer Cells

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Abstract: Fanconi anemia complementation group D2 (FANCD2) is one of the members of FA complementation group, that is activated in response to DNA damage and is involved in DNA repair and regulation of genomic stability. FANCD2 works with other members of FANC group along with other proteins/genes, to carry out its required functionality. Further, disruption of FA/BRCA pathway has been implicated in cancer progression. In colorectal cancer (CRC) cells treated with ETC-1922159, FANCD2 was found to be down regulated along with other genes. A recently developed search engine ranked combinations of FANCD2-X (X, a particular gene/protein) at 2^{nd} order level after drug administration. Some of these combinations have been tested and established in wet lab, however many have been pointed out by the search engine that are yet to be explored/tested. These rankings reveal which FANCD2-X combinations might be working synergistically in CRC. In this research work, I cover combinations of FANCD2 with, REV3 like DNA directed polymerase zeta catalytic subunit (REV3L), Bloom syndrome RecQ like helicase (BLM), MRE11 homolog double strand break repair nuclease (MRE11A), Wnt family member 10B (WNT10B), ubiquitin like with PHD and ring finger domains 1 (UHRF1), hes family bHLH transcription factor 1 (HES1), BRCA DNA repair associated (BRCA), RAD51 recombinase (RAD51), ERCC excision repair endonuclease non-catalytic subunit (ERCC), KIAA, X-ray repair cross complementing (XRCC), structural maintenance of chromosomes (SMC), WD repeat domain containing (WDR), ubiquitin conjugating enzyme E2 (UBE2), cell division cycle (CDC), importin (IPO), aldehyde dehydrogenase family member (ALDH), H2A variant histone (H2A), heat shock protein (HSP), cyclin dependent kinase (CDK), dynein axonemal heavy chain (DNAH), forkhead box (FOX), ring finger protein (RNF), E2F transcription factor (E2F) and small nucleolar RNA host gene (SNHG) family.

Keywords: FANCD2; Porcupine inhibitor ETC-1922159; Sensitivity analysis; Colorectal cancer

1. Introduction

1.1. Fanconi Anemia

Fanconi anemia (FA) is an autosomal recessive genetic disease that causes impaired response to DNA damage in the FA/BRCA pathway. FA was first described in 1927 by Guido Fanconi. D'Andrea [1] give a perspective on the role of FA complementation group proteins that are encoded by FANC genes. Briefly, D'Andrea [1] sumarizes the basic working background of the FA/BRCA pathway as follows - The FA proteins (A, C, E, F, G) are assembled in a nuclear protein complex along with BLM as a subunit. This complex is then required for the monoubiquitination of the downstream FANCD2. Next, monoubiquitinated FANCD2 assembles with the breast cancer susceptibility proteins, BRCA1 and BRCA2. The nuclear foci also contain the RAD51 and the MRE11/RAD50/NBS1 (M/R/N) complex. Disruption of genes in this pathway results in cellular hypersensitivity to DNA cross-linking agents, chromosome instability, and a propensity to cancer progression.

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1.2. FANCD2

Houghtaling et al. [2] via FANCD2 knockout experiments in mice, showed that cellular sensitivity to DNA interstrand cross-links, perinatal lethality, germ cell loss, epithelial cancers and microphthalmia. Their work provides the first molecular link between the FA pathway and epithelial cancers. van Twest et al. [3] provide a detailed biochemical analysis and mechanistic basis of FANCI and FANCD2 monoubiquitination by the FA complex. They show that FANCB dimer coordinates the FANCD2 and FANCI monoubiquitination by two FANCL RING-ligases. FANCD2 was found to be down regulated in colorectal cancer cell lines after the treatment of ETC-1922159 drug, along with other genes, as observed in Madan et al. [4]. Many of the combinations of FANCD2 with other genes have been tested, established and reported in wet lab experiments, however there remain a range of combinations that are yet to be explored and tested. It would be nice to observe if there is any connection between the independently observed factors in the form of unknown biological hypotheses. To solve the issue, the next section discusses a solution to the problem.

1.3. Combinatorial search problem and a possible solution

In a recently published work Sinha [5], 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 [6]. The work uses SVM package by Joachims [7] 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. FANCD2 Related Synergies

2.1.1. FANCD2 - REV3L / BLM / MRE11A / WNT10B / UHRF1 / HES1

Bhat et al. [8] observe that experimental depletion of REV3 leads to increase in anaphase bridges, common fragile site (CFS) expression and chromosomal breaks/gaps. The genomic instability induced by REV3 depletion results in increased metaphase-specific FANCD2 foci formation and FANCD2 positive anaphase bridges, thus showing a connection between REV3 and FANCD2. Pichierri et al. [9] show that BLM and FANCD2 colocalise and co-immunoprecipitate in response to crosslinked DNA and stalled replication forks. Also, BLM and the FA core complex are both necessary for the assembly of the MRE11 complex. Further, the connection between MRE11 and FANCD2 is established as Roques et al. [10] show that inhibition of MRE11, NBS1 or RAD50 leads to a destabilization of FANCD2. Kaur et al. [11] found that FANCD2 was dependent on WNT/β-catenin signaling in WNT-high cancers, and treatment with a PORCN inhibitor creates a BRCA-like state. FA pathway is critical for the response to toxic DNA interstrand crosslinks (ICLs). Liang et al. [12] showed that reduction of cellular levels of UHRF1 by RNAi attenuates the FA pathway and knockdown cells cause a reduction in FANCD2 foci formation. They observed that UHRF1 is required for the recruitment of FANCD2 to ICLs, which allows the DNA repair process to initiate. Finally, Tremblay et al. [13] show that HES1 is an interactor of FA core complex and is required for FANCD2 monoubiquitination. Taken together, all these experiments show a direct connection/synergy of the involved members with FANCD2. In colorectal cancer cells treated with ETC-1922159, these individual members (or their variants) and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of individial members and FANCD2, that were down regulated.

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 FANCD2. BLM - FANCD2 shows low ranking of 184 (laplace) and 305 (rbf). WNT10B - FANCD2 shows low ranking of 365 (laplace) and 307 (rbf). UHRF1 - FANCD2 shows low ranking of 1356 (laplace), 880 (linear) and 1268 (rbf). HES6 - FANCD2 shows low ranking of 1495

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(laplace), 1182 (linear) and 1192 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, REV3L and MRE11A showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 1. 2^{nd} order interaction ranking between FANCD2 VS INDIVIDUAL family members.

RANKING INDIVIDUAL MEMBERS VS FANCD2			
RANKING OF INDIVIDUAL MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
REV3L - FANCD2	1872	1049	2677
BLM - FANCD2	184	2738	305
MRE11A - FANCD2	2042	1744	1344
WNT10B - FANCD2	365	1891	307
UHRF1 - FANCD2	1356	880	1268
HES6 - FANCD2	1495	1182	1192

One can also interpret the results of the table 1 graphically, with the following influences - • INDIVIDUAL family w.r.t FANCD2 with FANCD2 $->$ BLM, FANCD2 $->$ WNT10B, FANCD2 $->$ UHRF1, and FANCD2 $->$ HES6; .

Table 2. 2^{nd} order combinatorial hypotheses between FANCD2 and INDIVIDUAL family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
INDIVIDUAL members w.r.t FANCD2	
BLM / WNT10B / UHRF1 / HES6	FANCD2

2.1.2. FANCD2 - BRCA

DNA damage activates FA pathway, leading to monoubiquitination of FANCD2 protein and targeting nuclear foci containing BRCA1. Taniguchi et al. [14] show that this monoubiquitinated FANCD2 colocalizes with BRCA1 and RAD51 in S-phase–specific nuclear foci. Similar interaction was reported by Garcia-Higuera et al. [15]. Roy et al. [16] discuss that both BRCA1 and BRCA2 work in a common pathway, however they work at different stages in DNA damage response (DDR). BRCA1 functions in both checkpoint activation and DNA repair, while BRCA2 is a mediator of homologous recombination. In colorectal cancer cells treated with ETC-1922159, these BRCA family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of BRCA members and FANCD2, that were down regulated.

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 FANCD2. BRCA2 - FANCD2 shows low ranking of 354 (laplace) and 377 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Further, BRCA1 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 3. 2^{nd} order interaction ranking between FANCD2 VS BRCA family members.

RANKING BRCA MEMBERS VS FANCD2			
RANKING OF BRCA MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
BRCA2 - FANCD2	354	2668	377
BRCA1 - FANCD2	1772	244	2238

One can also interpret the results of the table 3 graphically, with the following influences - • BRCA family w.r.t FANCD2 with FANCD2 $-$ $>$ BRCA2.

Table 4. 2^{nd} order combinatorial hypotheses between FANCD2 and BRCA family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
BRCA members w.r.t FANCD2	
BRCA2	FANCD2

2.1.3. FANCD2 - RAD51

Based on the previous section where it was cited that Taniguchi et al. [14] show that the monoubiquitinated FANCD2 colocalizes with BRCA1 and RAD51 in S-phase–specific nuclear foci, it can be assumed that there is a synergy between RAD51 and FANCD2. In colorectal cancer cells treated with ETC-1922159, these RAD51 family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of RAD51 members and FANCD2, that were down regulated.

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 FANCD2. RAD51 - FANCD2 shows low ranking of 885 (laplace) and 1383 (rbf). RAD51AP1 - FANCD2 shows low ranking of 644 (linear) and 1291 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, RAD51C showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

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Table 5. 2^{nd} order interaction ranking between FANCD2 VS RAD51 family members.

RANKING RAD51 MEMBERS VS FANCD2			
RANKING OF RAD51 MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
RAD51 - FANCD2	885	1995	1383
RAD51AP1 - FANCD2	1734	644	1291
RAD51C - FANCD2	54	2399	2566

One can also interpret the results of the table 5 graphically, with the following influences - • RAD51 family w.r.t FANCD2 with FANCD2 $-$ > RAD-51/51AP1.

Table 6. 2^{nd} order combinatorial hypotheses between FANCD2 and RAD51 family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
RAD51 members w.r.t FANCD2	
RAD-51/51AP1	FANCD2

2.1.4. FANCD2 - ERCC

ERCC1 is identified as a participant in nucleotide excision repair and has a possible relationship with FA pathway. McCabe et al. [17] show that the lack of FANCD2 focus formation is greater with depletion of ERCC1 than the lack of monoubiquitinated FANCD2 formation. This suggests a relation between ERCC1 and FANCD2 and in particular FA pathway. In colorectal cancer cells treated with ETC-1922159, these ERCC family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of ERCC members and FANCD2, that were down regulated.

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 FANCD2. ERCC8 - FANCD2 shows low ranking of 371 (laplace) and 997 (rbf). ERCC6L - FANCD2 shows low ranking of 1209 (laplace) and 520 (linear). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Table 7. 2^{nd} order interaction ranking between FANCD2 VS ERCC family members.

RANKING ERCC MEMBERS VS FANCD2			
RANKING OF ERCC MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
ERCC8 - FANCD2	371	1951	997
ERCC6L - FANCD2	1209	520	1884

One can also interpret the results of the table 7 graphically, with the following influences - • ERCC family w.r.t FANCD2 with FANCD2 $-$ > ERCC-8/6L.

Table 8. 2^{nd} order combinatorial hypotheses between FANCD2 and ERCC family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ERCC members w.r.t FANCD2	
ERCC-8/6L	FANCD2

2.1.5. FANCD2 - KIAA

Kikuno et al. [18] document a range of over 2000 novel human genes that are designated KIAA plus a four-digit number. MacKay et al. [19] describe a highly conserved protein, KIAA1018 that interacts with, and is recruited to sites of DNA damage by, the monoubiquitinated form of FANCD2. In colorectal cancer cells treated with ETC-1922159, these KIAA family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of KIAA members and FANCD2, that were down regulated.

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 FANCD2. KIAA0586 - FANCD2 shows low ranking of 211 (laplace), 703 (linear) and 17 (rbf). KIAA1524 - FANCD2 shows low ranking of 225 (laplace) and 481 (rbf). KIAA1257 - FANCD2 shows low ranking of 749 (laplace), 1173 (linear) and 587 (rbf). KIAA0020 - FANCD2 shows low ranking of 979 (laplace) and 1332 (rbf). KIAA1324 - FANCD2 shows low ranking of 1466 (laplace), 413 (linear) and 1410 (rbf). KIAA1731 - FANCD2 shows low ranking of 1549 (laplace) and 1592 (linear). KIAA1586 - FANCD2 shows low ranking of 1233 (linear) and 277 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, KIAA0101, KIAA1143, KIAA1244 and KIAA1430 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

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Table 9. 2^{nd} order interaction ranking between FANCD2 VS KIAA family members.

RANKING KIAA MEMBERS VS FANCD2			
RANKING OF KIAA MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
KIAA0586 - FANCD2	211	703	17
KIAA1524 - FANCD2	225	2578	481
KIAA1257 - FANCD2	749	1173	587
KIAA0020 - FANCD2	979	2291	1332
KIAA1324 - FANCD2	1466	413	1410
KIAA1731 - FANCD2	1549	1592	2223
KIAA1586 - FANCD2	1667	1233	277
KIAA0101 - FANCD2	2118	570	2103
KIAA1143 - FANCD2	2279	1094	2043
KIAA1244 - FANCD2	2341	777	2453
KIAA1430 - FANCD2	2468	1138	2075

One can also interpret the results of the table 9 graphically, with the following influences - • KIAA family w.r.t FANCD2 with FANCD2 $-$ > KIAA-0586 / 1524 / 1257 / 0020 / 1324 / 1731 / 1586.

Table 10. 2^{nd} order combinatorial hypotheses between FANCD2 and KIAA family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
KIAA members w.r.t FANCD2	
KIAA-0586/1524/1257/0020/1324/1731/1586	FANCD2

2.1.6. FANCD2 - XRCC

XRCC2 is one of five somatic RAD51 paralogs and Andreassen and Hanenberg [20] state it appears to function downstream in the FA pathway, as it is not required for FANCD2 monoubiquitination. In colorectal cancer cells treated with ETC-1922159, these XRCC family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of XRCC members and FANCD2, that were down regulated.

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 FANCD2. XRCC6BP1 - FANCD2 shows low ranking of 1545 (linear) and 1200 (rbf). XRCC2 - FANCD2 shows low ranking of 111 (linear) and 1269 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Further, XRCC1 and XRCC6 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 11. 2^{nd} order interaction ranking between FANCD2 VS XRCC family members.

RANKING XRCC MEMBERS VS FANCD2			
RANKING OF XRCC MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
XRCC6BP1 - FANCD2	1720	1545	1200
XRCC2 - FANCD2	2002	111	1269
XRCC1 - FANCD2	2096	1096	2170
XRCC6 - FANCD2	2694	215	2539

One can also interpret the results of the table 11 graphically, with the following influences - • XRCC family w.r.t FANCD2 with FANCD2 $-$ > XRCC-6BP1/2.

Table 12. 2^{nd} order combinatorial hypotheses between FANCD2 and XRCC family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
XRCC members w.r.t FANCD2	
XRCC-6BP1/2	FANCD2

2.1.7. FANCD2 - SMC

Rossi et al. [21] show that SMC5 functions downstream of FANCD2 ubiquitylation with FANCC and SMC5/6 acts jointly with FANCD2 to mediate DNA repair and prevent genomic instability in human cells. Thus there is synergy between SMC and FANCD2. In colorectal cancer cells treated with ETC-1922159, these SMC family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of SMC members and FANCD2, that were down regulated.

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 FANCD2. SMC1A - FANCD2 shows low ranking of 1156 (laplace), 88 (linear) and 1502 (rbf). SMC4 - FANCD2 shows low ranking of 44 (laplace) and 83 (rbf). SMC2 - FANCD2 shows low ranking of 229 (laplace) and 236 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Table 13. 2^{nd} order interaction ranking between FANCD2 VS SMC family members.

RANKING SMC MEMBERS VS FANCD2			
RANKING OF SMC MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
SMC1A - FANCD2	1156	88	1502
SMC4 - FANCD2	44	2042	83
SMC2 - FANCD2	229	2541	236

One can also interpret the results of the table 13 graphically, with the following influences - • SMC family w.r.t FANCD2 with FANCD2 $->$ SMC-1A/4/2.

Table 14. 2^{nd} order combinatorial hypotheses between FANCD2 and SMC family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
SMC members w.r.t FANCD2	
SMC-1A/4/2	FANCD2

2.1.8. FANCD2 - WDR

Gurtan et al. [22] show that point mutations in the WD40 repeats of FANCL disrupt FA core complex and FANCD2 mono-ubiquitination. Further, WD40 region of FANCL interacts with the FA complex and this correlates with the level of FANCD2 mono-ubiquitination. In colorectal cancer cells treated with ETC-1922159, these WDR family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of WDR members and FANCD2, that were down regulated.

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 FANCD2. WDR76 - FANCD2 shows low ranking of 34 (laplace) and 575 (rbf). WDR62 - FANCD2 shows low ranking of 162 (laplace) and 765 (rbf). WDR74 - FANCD2 shows low ranking of 344 (laplace), 501 (linear) and 615 (rbf). WDR12 - FANCD2 shows low ranking of 403 (laplace) and 870 (rbf). WDR77 - FANCD2 shows low ranking of 544 (laplace), 1480 (linear) and 402 (rbf). WDR75 - FANCD2 shows low ranking of 563 (laplace) and 805 (linear). WDR36 - FANCD2 shows low ranking of 623 (laplace) and 1159 (rbf). WDR43 - FANCD2 shows low ranking of 738 (laplace) and 1464 (rbf). WDR91 - FANCD2 shows low ranking of 1069 (laplace), 1180 (linear) and 976 (rbf). WDR35 - FANCD2 shows low ranking of 1162 (laplace), 1042 (linear) and 109 (rbf). WDR92 - FANCD2 shows low ranking of 1228 (laplace) and 427 (rbf). WDR18 - FANCD2 shows low ranking of 1234 (laplace) and 924 (linear). WDR3 - FANCD2 shows low ranking of 1562 (laplace) and 344 (linear). WDR89 - FANCD2 shows low ranking of 1297 (linear) and 749 (rbf). WDR90 - FANCD2 shows low ranking of 1462 (linear) and 785 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Further, WDR41, WDR70, WDR27, WDR5, WDR46, WDR61 and WDR53 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 15. 2^{nd} order interaction ranking between FANCD2 VS WDR family members.

RANKING WDR MEMBERS VS FANCD2							
	RANKING OF WDR MEMBERS W.R.T FANCD2						
	laplace	linear	rbf		laplace	linear	rbf
WDR76 - FANCD2	34	2426	575	WDR62 - FANCD2	162	2485	765
WDR74 - FANCD2	344	501	615	WDR12 - FANCD2	403	1704	870
WDR77 - FANCD2	544	1480	402	WDR75 - FANCD2	563	805	1617
WDR36 - FANCD2	623	1641	1159	WDR43 - FANCD2	738	1775	1464
WDR91 - FANCD2	1069	1180	976	WDR35 - FANCD2	1162	1042	109
WDR92 - FANCD2	1228	2620	427	WDR18 - FANCD2	1234	924	2274
WDR3 - FANCD2	1562	344	1621	WDR89 - FANCD2	1662	1297	749
WDR41 - FANCD2	1709	2352	700	WDR70 - FANCD2	1825	1017	1849
WDR27 - FANCD2	1850	1609	602	WDR5 - FANCD2	2029	277	1561
WDR46 - FANCD2	2139	78	2500	WDR61 - FANCD2	2214	190	1741
WDR90 - FANCD2	2235	1462	785	WDR53 - FANCD2	2278	976	1627

One can also interpret the results of the table 15 graphically, with the following influences - • WDR family w.r.t FANCD2 with FANCD2 $-$ > WDR-76 / 62 / 74 / 12 / 77 / 75 / 36 / 43 / 91 / 35 / 92 / 18 / 3 / 89 / 90.

Table 16. 2^{nd} order combinatorial hypotheses between FANCD2 and WDR family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
WDR members w.r.t FANCD2	
WDR-76/62/74/12/77/75/36/43	FANCD2
WDR-91/35/92/18/3/89/90	FANCD2

2.1.9. FANCD2 - UBE2

Machida et al. [23] show that UBE2T is the ubiquitin-conjugating enzyme (E2) essential for FA pathway as it binds to FANCL , (the ubiquitin ligase subunit of the FA core complex) and is required for the monoubiquitination of FANCD2 in vivo. In colorectal cancer cells treated with ETC-1922159, these UBE2 family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of UBE2 members and FANCD2, that were down regulated.

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 of individual members w.r.t FANCD2. UBE2G2 - FANCD2 shows low ranking of 137 (laplace), 1276 (linear) and 183 (rbf). UBE2S - FANCD2 shows low ranking of 506 (laplace), 1347 (linear) and 635 (rbf). UBE2T - FANCD2 shows low ranking of 689 (laplace) and 266 (linear). UBE2C - FANCD2 shows low ranking of 829 (laplace), 108 (linear) and 1505 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Table 17. 2^{nd} order interaction ranking between FANCD2 VS UBE2 family members.

RANKING UBE2 MEMBERS VS FANCD2			
RANKING OF UBE2 MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
UBE2G2 - FANCD2	137	1276	183
UBE2S - FANCD2	506	1347	635
UBE2T - FANCD2	689	266	1718
UBE2C - FANCD2	829	108	1505

One can also interpret the results of the table 17 graphically, with the following influences - • UBE2 family w.r.t FANCD2 with FANCD2 $-$ > UBE2-G2/S/T/C.

Table 18. 2^{nd} order combinatorial hypotheses between FANCD2 and UBE2 family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
UBE2 members w.r.t FANCD2	
UBE2-G2/S/T/C	FANCD2

2.1.10. FANCD2 - CDC

Zhang et al. [24] show that CDC5L is required for the activation of downstream mediators of ATR checkpoint function such as FANCD2, CHK1 and RAD17. In colorectal cancer cells treated with ETC-1922159, these CDC family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of CDC members and FANCD2, that were down regulated.

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 FANCD2. CDCA2 - FANCD2 shows low ranking of 133 (laplace) and 329 (rbf). CDC25C - FANCD2 shows low ranking of 241 (laplace) and 76 (rbf). CDC6 - FANCD2 shows low ranking of 386 (laplace), 1327 (linear) and 14 (rbf). CDC25A - FANCD2 shows low ranking of 481 (laplace), 885 (linear) and 421 (rbf). CDCA7L - FANCD2 shows low ranking of 818 (laplace) and 1111 (rbf). CDCA8- FANCD2 shows low ranking of 909 (laplace), 1454 (linear) and 1220 (rbf). CDC7 - FANCD2 shows low ranking of 1269 (laplace), 695 (linear) and 1147 (rbf). CDCA7 - FANCD2 shows low ranking of 862 (linear) and 1373 (rbf). CDC20 - FANCD2 shows low ranking of 1088 (linear) and 1552 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, CDCA4, CDCA5, CDC45, CDC123, CDCA3 and CDC23 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

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Table 19. 2^{nd} order interaction ranking between FANCD2 VS CDC family members.

RANKING CDC MEMBERS VS FANCD2							
	RANKING OF CDC MEMBERS W.R.T FANCD2						
	laplace	linear	rbf		laplace	linear	rbf
CDCA2 - FANCD2	133	2743	329	CDC25C - FANCD2	241	2677	76
CDC6 - FANCD2	386	1327	14	CDC25A - FANCD2	481	885	421
CDCA7L - FANCD2	818	1991	1111	CDCA8- FANCD2	909	1454	1220
CDCA4 - FANCD2	1206	1615	1651	CDCA5 - FANCD2	1224	2014	2491
CDC7 - FANCD2	1269	695	1147	CDCA7 - FANCD2	1696	862	1373
CDC20 - FANCD2	1950	1088	1552	CDC45 - FANCD2	2401	248	1988
CDC123 - FANCD2	2515	469	2429	CDCA3 - FANCD2	2626	664	2470
CDC23 - FANCD2	2671	206	1997				

One can also interpret the results of the table 19 graphically, with the following influences - • CDC family w.r.t FANCD2 with FANCD2 $->$ CDC-A2 / 25C / 6 / 25A / A7L / A8 / 7 / A7.

Table 20. 2^{nd} order combinatorial hypotheses between FANCD2 and CDC family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
CDC members w.r.t FANCD2	
CDC-A2/25C/6/25A/A7L/A8/7/A7	FANCD2

2.1.11. FANCD2 - IPO

Wang et al. [25] demonstrate that C/EBPδ promotes monoubiquitination of FANCD2, by showing that the former interacts with FANCD2 and IPO4, thus mediating FANCD2–IPO4 association and augmenting nuclear import of FANCD2, which is a prerequisite for its monoubiquitination. In colorectal cancer cells treated with ETC-1922159, these IPO family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of IPO members and FANCD2, that were down regulated.

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 FANCD2. IPO11 - FANCD2 shows low ranking of 1040 (laplace) and 40 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, IPO5 and IPO9 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

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Table 21. 2^{nd} order interaction ranking between FANCD2 VS IPO family members.

RANKING IPO MEMBERS VS FANCD2			
RANKING OF IPO MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
IPO5 - FANCD2	418	1903	2055
IPO11 - FANCD2	1040	1863	40
IPO9 - FANCD2	2313	998	2085

One can also interpret the results of the table 21 graphically, with the following influences - • IPO family w.r.t FANCD2 with FANCD2 $-$ > IPO-11.

Table 22. 2^{nd} order combinatorial hypotheses between FANCD2 and IPO family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
IPO members w.r.t FANCD2	
IPO-11	FANCD2

2.1.12. FANCD2 - ALDH

Meng et al. [26] showed that ALDH1A1-knockdown resulted in decrease of KLF4 and p21 protein levels which caused S and G2 phase accumulation of cells. This increase in S and G2 cells shoed increased expression of FANCD2 and FANCJ. Thus, their results showed a correlation between ALDH1A1 and FANCD2. In colorectal cancer cells treated with ETC-1922159, these ALDH family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of ALDH members and FANCD2, that were down regulated.

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 FANCD2. ALDH3A1 - FANCD2 shows low ranking of 376 (laplace) and 543 (rbf). ALDH5A1 - FANCD2 shows low ranking of 419 (laplace), 1502 (linear) and 1249 (rbf). ALDH7A1 - FANCD2 shows low ranking of 581 (laplace), 1068 (linear) and 1352 (rbf). ALDH1B1 - FANCD2 shows low ranking of 262 (linear) and 1139 (rbf). ALDH9A1 - FANCD2 shows low ranking of 728 (linear) and 1157 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, ALDH3A2 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

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Table 23. 2^{nd} order interaction ranking between FANCD2 VS ALDH family members.

RANKING ALDH MEMBERS VS FANCD2			
RANKING OF ALDH MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
ALDH3A1 - FANCD2	376	1971	543
ALDH5A1 - FANCD2	419	1502	1249
ALDH7A1 - FANCD2	581	1068	1352
ALDH1B1 - FANCD2	1846	262	1139
ALDH9A1 - FANCD2	2107	728	1157
ALDH3A2 - FANCD2	2563	357	2398

One can also interpret the results of the table 23 graphically, with the following influences - • ALDH family w.r.t FANCD2 with FANCD2 $-$ > ALDH-3A1/5A1/7A1/1B1/9A1.

Table 24. 2^{nd} order combinatorial hypotheses between FANCD2 and ALDH family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ALDH members w.r.t FANCD2	
ALDH-3A1/5A1/7A1/1B1/9A1	FANCD2

2.1.13. FANCD2 - H2A

Bogliolo et al. [27] showed that phosphorylated H2AX was required for recruiting FANCD2 to chromatin at stalled replication forks. This binding of FANCD2 to phosphorylated H2AX is BRCA1-dependent. In colorectal cancer cells treated with ETC-1922159, these H2A family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of H2A members and FANCD2, that were down regulated.

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 FANCD2. H2AFX (or H2AX) - FANCD2 shows low ranking of 593 (laplace) and 324 (linear). H2AFV - FANCD2 shows low ranking of 791 (laplace), 1329 (linear). H2AFZ - FANCD2 shows low ranking of 1292 (laplace) and 634 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Table 25. 2^{nd} order interaction ranking between FANCD2 VS H2A family members.

RANKING H2A MEMBERS VS FANCD2			
RANKING OF H2A MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
H2AFX (or H2AX) - FANCD2	593	324	2008
H2AFV - FANCD2	791	1329	1659
H2AFZ - FANCD2	1292	1921	634

One can also interpret the results of the table 25 graphically, with the following influences - • H2A family w.r.t FANCD2 with FANCD2 $->$ H2A-FX/FV/FZ.

Table 26. 2^{nd} order combinatorial hypotheses between FANCD2 and H2A family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
H2A members w.r.t FANCD2	
H2A-FX/FV/FZ	FANCD2

2.1.14. FANCD2 - HSP

Oda et al. [28] showed that HSP90 associated with FANCA, and disruption of this association by treatment with 17-AAG induces proteasomal degradation and cytoplasmic relocalization of FANCA, which lead to impaired activation of FANCD2. Thus there is an established connection between HSP90 and FANCD2. In colorectal cancer cells treated with ETC-1922159, these HSP family members and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of HSP members and FANCD2, that were down regulated.

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 FANCD2. HSPE1 - FANCD2 shows low ranking of 336 (laplace), 1246 (linear) and 745 (rbf). HSPD1 - FANCD2 shows low ranking of 385 (laplace), 388 (linear) and 617 (rbf). HSPB6 - FANCD2 shows low ranking of 1000 (laplace) and 520 (rbf). HSPA9 - FANCD2 shows low ranking of 370 (linear) and 1514 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, HSPA4L and HSP4A showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 27. 2^{nd} order interaction ranking between FANCD2 VS HSP family members.

RANKING HSP MEMBERS VS FANCD2							
RANKING OF HSP MEMBERS W.R.T FANCD2							
	laplace	linear	rbf		laplace	linear	rbf
HSPE1 - FANCD2	336	1246	745	HSPD1 - FANCD2	385	388	617
HSPA4L - FANCD2	520	2211	2202	HSPB6 - FANCD2	1000	2421	520
HSPA9 - FANCD2	2078	370	1514	HSPA4 - FANCD2	2621	25	2650

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One can also interpret the results of the table 27 graphically, with the following influences - • HSP family w.r.t FANCD2 with FANCD2 $-$ > HSP-E1/D1/B6/A9.

Table 28. 2^{nd} order combinatorial hypotheses between FANCD2 and HSP family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
HSP members w.r.t FANCD2	
HSP-E1/D1/B6/A9	FANCD2

2.1.15. FANCD2 - CDK

Blazek et al. [29] show that a variant of cyclin-CDK complex namely CYCK/CDK12 regulated expression of a small subset of human genes. Depletion of CYCK/CDK12 resulted in decreased expression of BRCA1, FANCD2, ATR and FANCI. Via this regulation, it is thought that the CYCK/CDK12 protects the cells from genomic instability. In colorectal cancer cells treated with ETC-1922159, these CDK family member and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of CDK members and FANCD2, that were down regulated.

Table 29 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 30 generated from analysis of the ranks in table 29. The table 29 shows rankings of individual members w.r.t FANCD2. CDK5RAP1 - FANCD2 shows low ranking of 1305 (laplace), 566 (linear) and 1196 (rbf). CDK5RAP2 - FANCD2 shows low ranking of 34 (linear) and 921 (rbf). CDK6 - FANCD2 shows low ranking of 337 (linear) and 1409 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, CDK4 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 29. 2^{nd} order interaction ranking between FANCD2 VS CDK family members.

RANKING CDK MEMBERS VS FANCD2			
RANKING OF CDK MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
CDK4 - FANCD2	918	1632	1890
CDK5RAP1 - FANCD2	1305	566	1196
CDK5RAP2 - FANCD2	2064	34	921
CDK6 - FANCD2	2463	337	1409

One can also interpret the results of the table 29 graphically, with the following influences - • CDK family w.r.t FANCD2 with FANCD2 $-$ > CDK-6/5RAP1/5RAP2.

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Table 30. 2^{nd} order combinatorial hypotheses between FANCD2 and CDK family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
CDK members w.r.t FANCD2	
CDK-6/5RAP1/5RAP2	FANCD2

2.1.16. FANCD2 - DNAH

Chang et al. [30] show involvement of DNAH2 in the regulation of ubiquitination and nuclear localization of FANCD2 upon the DNA damage. In colorectal cancer cells treated with ETC-1922159, these DNAH family member and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of DNAH members and FANCD2, that were down regulated.

Table 31 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 32 generated from analysis of the ranks in table 31. The table 31 shows rankings of individual members w.r.t FANCD2. DNAH11 - FANCD2 shows low ranking of 762 (laplace), 1463 (linear) and 567 (rbf). DNAH14 - FANCD2 shows low ranking of 1252 (laplace) and 229 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Table 31. 2^{nd} order interaction ranking between FANCD2 VS DNAH family members.

RANKING DNAH MEMBERS VS FANCD2			
RANKING OF DNAH MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
DNAH11 - FANCD2	762	1463	567
DNAH14 - FANCD2	1252	1703	229

One can also interpret the results of the table 31 graphically, with the following influences - • DNAH family w.r.t FANCD2 with FANCD2 $->$ DNAH-11/14.

Table 32. 2^{nd} order combinatorial hypotheses between FANCD2 and DNAH family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
DNAH members w.r.t FANCD2	
DNAH-11/14	FANCD2

2.1.17. FANCD2 - FOX

Li et al. [31] report the functional interaction of FANCD2 and the forkhead transcription factor forkhead box (FOX) O3a i.e FOXO3A. FOXO3A formed a complex with FANCD2 foci in response to oxidative stress. In colorectal cancer cells treated with ETC-1922159, these FOX family member and

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FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of FOX members and FANCD2, that were down regulated.

Table 33 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 34 generated from analysis of the ranks in table 33. The table 33 shows rankings of individual members w.r.t FANCD2. FOXJ1 - FANCD2 shows low ranking of 830 (laplace) and 972 (rbf). FOXD2-AS1 - FANCD2 shows low ranking of 1154 (laplace) and 611 (linear). FOXM1 - FANCD2 shows low ranking of 44 (linear) and 1142 (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 FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 33. 2^{nd} order interaction ranking between FANCD2 VS FOX family members.

RANKING FOX MEMBERS VS FANCD2			
RANKING OF FOX MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
FOXJ1 - FANCD2	830	2003	972
FOXD2-AS1 - FANCD2	1154	611	2023
FOXM1 - FANCD2	1868	44	1142
FOXA2 - FANCD2	2101	1184	2240

One can also interpret the results of the table 33 graphically, with the following influences - • FOX family w.r.t FANCD2 with FANCD2 $-$ > FOX-J1/D2-AS1/M1.

Table 34. 2^{nd} order combinatorial hypotheses between FANCD2 and FOX family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
FOX members w.r.t FANCD2	
FOX-J1/D2-AS1/M1	FANCD2

2.1.18. FANCD2 - RNF

Yan et al. [32] show that FAAP20 binds the ubiquitin product of RNF8-UBC13. RNF8-FAAP20 cascade is needed for efficient FANCD2 monoubiquitination. In colorectal cancer cells treated with ETC-1922159, these RNF family member and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of RNF members and FANCD2, that were down regulated.

Table 35 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 36 generated from analysis of the ranks in table 35. The table 35 shows rankings of individual members w.r.t FANCD2. RNF157-AS1 - FANCD2 shows low ranking of 399 (laplace) and 533 (linear). RNF166 - FANCD2 shows low ranking of 659 (laplace) and 526 (rbf). RNF20 - FANCD2 shows low ranking of 1227 (laplace), 247 (linear) and 1331 (rbf). RNF26 - FANCD2 shows low ranking of 1476 (laplace), 873 (linear) and 1540 (rbf). RNF144B - FANCD2 shows low ranking of 1504 (laplace)

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and 1095 (rbf). RNF157 - FANCD2 shows low ranking of 1553 (laplace), 417 (linear) and 449 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

Further, RNF220 and RNF43 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 35. 2^{nd} order interaction ranking between FANCD2 VS RNF family members.

RANKING RNF MEMBERS VS FANCD2			
RANKING OF RNF MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
RNF157-AS1 - FANCD2	399	533	1742
RNF166 - FANCD2	659	1764	526
RNF20 - FANCD2	1227	247	1331
RNF43 - FANCD2	1366	1571	2048
RNF26 - FANCD2	1476	873	1540
RNF144B - FANCD2	1504	1733	1095
RNF157 - FANCD2	1553	417	449
RNF220 - FANCD2	2716	14	2668

One can also interpret the results of the table 35 graphically, with the following influences - • RNF family w.r.t FANCD2 with FANCD2 $-$ > RNF-157-AS1/166/20/26/144B/157.

Table 36. 2^{nd} order combinatorial hypotheses between FANCD2 and RNF family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
RNF members w.r.t FANCD2	
RNF-157-AS1/166/20/26/144B/157	FANCD2

2.1.19. FANCD2 - E2F

Mitxelena et al. [33] showed that E2F7 modulates the DNA repair program as knockdown experiment of E2F7 lead to a reduction in 53BP1 and FANCD2 foci. In colorectal cancer cells treated with ETC-1922159, these E2F family member and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of E2F members and FANCD2, that were down regulated.

Table 37 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 38 generated from analysis of the ranks in table 37. The table 37 shows rankings of individual members w.r.t FANCD2. E2F1 - FANCD2 shows low ranking of 200 (laplace) and 469 (rbf). E2F2 - FANCD2 shows low ranking of 472 (laplace) and 140 (rbf). E2F7 - FANCD2 shows low ranking

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of 482 (laplace) and 985 (rbf). E2F8 - FANCD2 shows low ranking of 192 (linear) and 582 (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 FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 37. 2^{nd} order interaction ranking between FANCD2 VS E2F family members.

RANKING E2F MEMBERS VS FANCD2			
RANKING OF E2F MEMBERS W.R.T FANCD2			
	laplace	linear	rbf
E2F1 - FANCD2	200	1944	469
E2F2 - FANCD2	472	2345	140
E2F7 - FANCD2	482	2500	985
E2F8 - FANCD2	1873	192	582
E2F5 - FANCD2	2537	435	2079

One can also interpret the results of the table 37 graphically, with the following influences - • E2F family w.r.t FANCD2 with FANCD2 $->$ E2F-1/2/7/8.

Table 38. 2^{nd} order combinatorial hypotheses between FANCD2 and E2F family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
E2F members w.r.t FANCD2	
E2F-1/2/7/8	FANCD2

2.1.20. FANCD2 - SNHG

In hepatocellular carcinoma, [34] showed that SNHG1 upregulated the expression of FANCD2 and G6PD, thus inhibiting ferroptosis. In colorectal cancer cells treated with ETC-1922159, these SNHG family member and FANCD2, were found to be down regulated and recorded independently. I was able to rank 2^{nd} order combinations of SNHG members and FANCD2, that were down regulated.

Table 39 shows rankings of these combinations. Followed by this is the unexplored combinatorial hypotheses in table 40 generated from analysis of the ranks in table 39. The table 39 shows rankings of individual members w.r.t FANCD2. SNHG3 - FANCD2 shows low ranking of 339 (laplace) and 1259 (linear) SNHG6 - FANCD2 shows low ranking of 551 (laplace) and 74 (rbf). SNHG10 - FANCD2 shows low ranking of 866 (laplace) and 1442 (rbf). SNHG5 - FANCD2 shows low ranking of 1140 (laplace) and 674 (rbf). SNHG7 - FANCD2 shows low ranking of 1220 (laplace) and 694 (linear) SNHG17 - FANCD2 shows low ranking of 1524 (laplace), 454 (linear) and 1198 (rbf). SNHG15 - FANCD2 shows low ranking of 1464 (linear) and 322 (rbf). These rankings point to the synergy existing between the two components, which have been down regulated after the drug treatment.

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Further, SNHG16, SNHG1, SNHG18 and SNHG8 showed high ranking with FANCD2, thus indicating that they might not be working synergistically with FANCD2, before the drug treatment.

Table 39. 2^{nd} order interaction ranking between FANCD2 VS SNHG family members.

RANKING SNHG MEMBERS VS FANCD2							
	RANKING OF SNHG MEMBERS W.R.T FANCD2						
	laplace	linear	rbf		laplace	linear	rbf
SNHG3 - FANCD2	339	1259	1759	SNHG6 - FANCD2	551	1943	74
SNHG10 - FANCD2	866	1960	1442	SNHG5 - FANCD2	1140	1961	674
SNHG7 - FANCD2	1220	694	1862	SNHG17 - FANCD2	1524	454	1198
SNHG16 - FANCD2	1600	2275	882	SNHG15 - FANCD2	1669	1464	322
SNHG1 - FANCD2	1705	2006	1185	SNHG18 - FANCD2	2276	614	1734
SNHG8 - FANCD2	2407	1232	2160				

One can also interpret the results of the table 39 graphically, with the following influences - • SNHG family w.r.t FANCD2 with FANCD2 $->$ SNHG-3/6/10/5/7/17/15.

Table 40. 2^{nd} order combinatorial hypotheses between FANCD2 and SNHG family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
SNHG members w.r.t FANCD2	
SNHG-3/6/10/5/7/17/15	FANCD2

3. Conclusion

Presented here are a range of multiple synergistic FANCD2 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 FANCD2-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. [4].

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References

1. D'Andrea, A.D. The Fanconi road to cancer. *Genes & development* **2003**, *17*, 1933–1936.
2. Houghtaling, S.; Timmers, C.; Noll, M.; Finegold, M.J.; Jones, S.N.; Meyn, M.S.; Grompe, M. Epithelial cancer in Fanconi anemia complementation group D2 (Fancd2) knockout mice. *Genes & development* **2003**, *17*, 2021–2035.
3. van Twest, S.; Murphy, V.J.; Hodson, C.; Tan, W.; Swuec, P.; O'Rourke, J.J.; Heierhorst, J.; Crismani, W.; Deans, A.J. Mechanism of ubiquitination and deubiquitination in the Fanconi anemia pathway. *Molecular cell* **2017**, *65*, 247–259.
4. Madan, B.; Ke, Z.; Harmston, N.; Ho, S.Y.; Frois, A.; Alam, J.; Jeyaraj, D.A.; Pendharkar, V.; Ghosh, K.; Virshup, I.H.; et al. Wnt addiction of genetically defined cancers reversed by PORCN inhibition. *Oncogene* **2016**, *35*, 2197.

 doi:10.20944/preprints202501.0902.v1

5. Sinha, S. Machine learning ranking of plausible (un) explored synergistic gene combinations using sensitivity indices of time series measurements of Wnt signaling pathway. *Integrative Biology* **2024**, *16*, zyae020.
6. Sinha, S. Sensitivity analysis based ranking reveals unknown biological hypotheses for down regulated genes in time buffer during administration of PORCN-WNT inhibitor ETC-1922159 in CRC. *bioRxiv* **2017**, p. 180927.
7. Joachims, T. Training linear SVMs in linear time. In Proceedings of the Proceedings of the 12th ACM SIGKDD international conference on Knowledge discovery and data mining. ACM, 2006, pp. 217–226.
8. Bhat, A.; Andersen, P.L.; Qin, Z.; Xiao, W. Rev3, the catalytic subunit of Polζ, is required for maintaining fragile site stability in human cells. *Nucleic acids research* **2013**, *41*, 2328–2339.
9. Pichierri, P.; Franchitto, A.; Rosselli, F. BLM and the FANC proteins collaborate in a common pathway in response to stalled replication forks. *The EMBO journal* **2004**, *23*, 3154–3163.
10. Roques, C.; Coulombe, Y.; Delannoy, M.; Vignard, J.; Grossi, S.; Brodeur, I.; Rodrigue, A.; Gautier, J.; Stasiak, A.Z.; Stasiak, A.; et al. MRE11–RAD50–NBS1 is a critical regulator of FANCD2 stability and function during DNA double-strand break repair. *The EMBO journal* **2009**, *28*, 2400–2413.
11. Kaur, A.; Lim, J.Y.S.; Sepramaniam, S.; Patnaik, S.; Harmston, N.; Lee, M.A.; Petretto, E.; Virshup, D.M.; Madan, B. WNT inhibition creates a BRCA-like state in Wnt-addicted cancer. *EMBO molecular medicine* **2021**, *13*, e13349.
12. Liang, C.C.; Zhan, B.; Yoshikawa, Y.; Haas, W.; Gygi, S.P.; Cohn, M.A. UHRF1 is a sensor for DNA interstrand crosslinks and recruits FANCD2 to initiate the Fanconi anemia pathway. *Cell reports* **2015**, *10*, 1947–1956.
13. Tremblay, C.S.; Huang, F.F.; Habi, O.; Huard, C.C.; Godin, C.; Lévesque, G.; Carreau, M. HES1 is a novel interactor of the Fanconi anemia core complex. *Blood, The Journal of the American Society of Hematology* **2008**, *112*, 2062–2070.
14. Taniguchi, T.; Garcia-Higuera, I.; Andreassen, P.R.; Gregory, R.C.; Grompe, M.; D'Andrea, A.D. S-phase–specific interaction of the Fanconi anemia protein, FANCD2, with BRCA1 and RAD51. *Blood, The Journal of the American Society of Hematology* **2002**, *100*, 2414–2420.
15. Garcia-Higuera, I.; Taniguchi, T.; Ganesan, S.; Meyn, M.S.; Timmers, C.; Hejna, J.; Grompe, M.; D D'Andrea, A. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. *Molecular cell* **2001**, *7*, 249–262.
16. Roy, R.; Chun, J.; Powell, S.N. BRCA1 and BRCA2: different roles in a common pathway of genome protection. *Nature Reviews Cancer* **2012**, *12*, 68–78.
17. McCabe, K.M.; Hemphill, A.; Akkari, Y.; Jakobs, P.M.; Pauw, D.; Olson, S.B.; Moses, R.E.; Grompe, M. ERCC1 is required for FANCD2 focus formation. *Molecular genetics and metabolism* **2008**, *95*, 66–73.
18. Kikuno, R.; Nagase, T.; Nakayama, M.; Koga, H.; Okazaki, N.; Nakajima, D.; Ohara, O. HUGE: a database for human KIAA proteins, a 2004 update integrating HUGEppi and ROUGE. *Nucleic acids research* **2004**, *32*, D502–D504.
19. MacKay, C.; Déclais, A.C.; Lundin, C.; Agostinho, A.; Deans, A.J.; MacArtney, T.J.; Hofmann, K.; Gartner, A.; West, S.C.; Helleday, T.; et al. Identification of KIAA1018/FAN1, a DNA repair nuclease recruited to DNA damage by monoubiquitinated FANCD2. *Cell* **2010**, *142*, 65–76.
20. Andreassen, P.R.; Hanenberg, H. XRCC2 (X-ray repair cross complementing 2). *Atlas of genetics and cytogenetics in oncology and haematology* **2019**, *23*, 1.
21. Rossi, F.; Helbling-Leclerc, A.; Kawasumi, R.; Jegadesan, N.K.; Xu, X.; Devulder, P.; Abe, T.; Takata, M.; Xu, D.; Rosselli, F.; et al. SMC 5/6 acts jointly with Fanconi anemia factors to support DNA repair and genome stability. *EMBO reports* **2020**, *21*, e48222.
22. Gurtan, A.M.; Stuckert, P.; D'Andrea, A.D. The WD40 repeats of FANCL are required for Fanconi anemia core complex assembly. *Journal of Biological Chemistry* **2006**, *281*, 10896–10905.
23. Machida, Y.J.; Machida, Y.; Chen, Y.; Gurtan, A.M.; Kupfer, G.M.; D'Andrea, A.D.; Dutta, A. UBE2T is the E2 in the Fanconi anemia pathway and undergoes negative autoregulation. *Molecular cell* **2006**, *23*, 589–596.
24. Zhang, N.; Kaur, R.; Akhter, S.; Legerski, R.J. Cdc5L interacts with ATR and is required for the S-phase cell-cycle checkpoint. *EMBO reports* **2009**, *10*, 1029–1035.
25. Wang, J.; Sarkar, T.R.; Zhou, M.; Sharan, S.; Ritt, D.A.; Veenstra, T.D.; Morrison, D.K.; Huang, A.M.; Sterneck, E. CCAAT/enhancer binding protein delta (C/EBPδ, CEBPD)-mediated nuclear import of FANCD2 by IPO4 augments cellular response to DNA damage. *Proceedings of the National Academy of Sciences* **2010**, *107*, 16131–16136.

 doi:10.20944/preprints202501.0902.v1

26. Meng, E.; Mitra, A.; Tripathi, K.; Finan, M.A.; Scalici, J.; McClellan, S.; da Silva, L.M.; Reed, E.; Shevde, L.A.; Palle, K.; et al. ALDH1A1 maintains ovarian cancer stem cell-like properties by altered regulation of cell cycle checkpoint and DNA repair network signaling. *PloS one* **2014**, *9*, e107142.
27. Bogliolo, M.; Lyakhovich, A.; Callén, E.; Castellà, M.; Cappelli, E.; Ramírez, M.J.; Creus, A.; Marcos, R.; Kalb, R.; Neveling, K.; et al. Histone H2AX and Fanconi anemia FANCD2 function in the same pathway to maintain chromosome stability. *The EMBO journal* **2007**, *26*, 1340–1351.
28. Oda, T.; Hayano, T.; Miyaso, H.; Takahashi, N.; Yamashita, T. Hsp90 regulates the Fanconi anemia DNA damage response pathway. *Blood, The Journal of the American Society of Hematology* **2007**, *109*, 5016–5026.
29. Blazek, D.; Kohoutek, J.; Bartholomeeusen, K.; Johansen, E.; Hulinkova, P.; Luo, Z.; Cimermancic, P.; Ule, J.; Peterlin, B.M. The Cyclin K/Cdk12 complex maintains genomic stability via regulation of expression of DNA damage response genes. *Genes & development* **2011**, *25*, 2158–2172.
30. Chang, L.; Gao, X.; Wang, Y.; Huang, C.; Gao, M.; Wang, X.; Liu, C.; Wu, W.; An, W.; Wan, Y.; et al. DNAH2 facilitates the homologous recombination repair of Fanconi anemia pathway through modulating FANCD2 ubiquitination. *Blood Science* **2021**, *3*, 71–77.
31. Li, J.; Du, W.; Maynard, S.; Andreassen, P.R.; Pang, Q. Oxidative stress–specific interaction between FANCD2 and FOXO3a. *Blood, The Journal of the American Society of Hematology* **2010**, *115*, 1545–1548.
32. Yan, Z.; Guo, R.; Paramasivam, M.; Shen, W.; Ling, C.; Fox, D.; Wang, Y.; Oostra, A.B.; Kuehl, J.; Lee, D.Y.; et al. A ubiquitin-binding protein, FAAP20, links RNF8-mediated ubiquitination to the Fanconi anemia DNA repair network. *Molecular cell* **2012**, *47*, 61–75.
33. Mitxelena, J.; Apraiz, A.; Vallejo-Rodríguez, J.; García-Santisteban, I.; Fullaondo, A.; Alvarez-Fernández, M.; Malumbres, M.; Zubiaga, A.M. An E2F7-dependent transcriptional program modulates DNA damage repair and genomic stability. *Nucleic acids research* **2018**, *46*, 4546–4559.
34. Zhou, L.; Zhang, Q.; Cheng, J.; Shen, X.; Li, J.; Chen, M.; Zhou, C.; Zhou, J. LncRNA SNHG1 upregulates FANCD2 and G6PD to suppress ferroptosis by sponging miR-199a-5p/3p in hepatocellular carcinoma. *Drug Discoveries & Therapeutics* **2023**, *17*, 248–256.