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

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Abstract: Often, in biology, we are faced with the problem of exploring relevant unknown biological hypotheses in the form of myriads of combinations of factors/genes/proteins that might be affecting the pathway under certain conditions. In colorectal cancer (CRC) cells treated with ETC-1922159, many genes were found up and down regulated, individually. A recently developed search engine ranked combinations of Wnt-X (X, a particular gene/protein) at 2^{nd} order level after drug administration. These rankings reveal which Wnt-X combinations might be working synergistically in CRC. If found true, oncologists can further test the combination of interest in wet lab and determine the mechanism of functioning between the Wnt and X. In this research work, we cover combinations of Wnt with Achaete-scute complex homolog 2 (ASCL2), ATP-binding cassette (ABC) domain transporters, Interleukin (IL), ubiquitin conjugating enzyme E2 (UBE2) family, exosome (EXOSC), caspase (CASP), TP53 and B-cell lymphoma (BCL) family.

Keywords: WNT; porcupine inhibitor ETC-1922159; sensitivity analysis; colorectal cancer

1. Introduction

In the unpublished preprint [1], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway. Such combinations are of import due to the vast search space in which they exist and the difficulty to find them. The search engine facilitates in prioritizing the combinations as ranked biological hypotheses which the biologists might want to test in wet lab, to know if a synergistic combination is prevalent in a signaling pathway, in a direct or indirect manner. Interested readers are advised to go through unpublished preprints [1] and [2] for details regarding the search engine and the discoveries mentioned in there.

2. Materials and Methods

2.1. Combinatorial Search Problem and a Possible Solution

The issue of combinatorial search problem and a possible solution has been addressed in [3] and [2]. The details of the methodology of this manuscript have been explained in great detail in [3] & its application in [2]. Readers are requested to go through the same for gaining deeper insight into the working of the pipeline and its use of published data set generated after administration of ETC-1922159. In order to understand the significance of the solution proposed to the problem of combinatorial search that the biologists face in revealing unknown biological search problem, these works are of importance.

Briefly, from [2], the pipleline works by computing sensitivity indicies for each of these unique combinations and then vectorising these indices to connote and form discriminative feature vector for each combination. Since each combination is unique, the training and the test data are same. In the training data, the combinations are arranged and ranks from 1 to n are assigned. The ranking algorithm then learns the patterns from these combinations/sensitivity index vectors. Next the learned model is used to rank the test data by generating the ranking score for each of the unique

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combination. Sorting these shuffled scores of test data leads to prioritization of the combinations. [4] show an example of applying learned model to training data (same as the test data) in https://www.cs.cornell.edu/people/tj/svm_light/svm_rank.html. Note that these combinations are now ranked and give the biologists a chance to narrow down their focus on crucial biological hypotheses in the form of combinations which the biologists might want to test. Analogous to the webpage search engine, where the click of a button for a few key-words leads to a ranked list of web links, the pipeline uses sensitivity indices as an indicator of the strength of the influence of factors or their combinations, as a criteria to rank the combinations.

3. Results & Discussion

3.1. WNT Related Synergies

3.1.1. WNT10B-ASCL2

WNT10B has been found to be implicated in a range of cancers. In gastric cancer, the knockdown of WNT10B showed reduced expression of cell proliferation and migration as well as inhibition of epithelial-mesenchymal transition [5]. On the other hand, WNT10B is also involved in the formation of bone mass and progenitor maintenance of various kinds of tissue, while deletion of the same leads to loss of bone mass and mesenchymal progenitor cells [6]. Their contribution is also reported in axonal regeneration in injured CNS [7]. Furthermore, like WNT10B, WNT10A and WNT6 have shown to play a major role in inhibiting adipogenesis and stimulates osteoblastogenesis while regulating the mesenchymal stem cells [8] & [9]. Involvement in heptocellular carcinoma of WNT10B has been found wherein it is shown that stable silencing of WNT10B leads to significant reduction in proliferation, colony formation, migration and invasion in HepG2 HCC cell line [10]. Its implication in breast cancer [11] & [12] as well as endometrial cancer [13] has also been reported.

In colorectal cancer, WNT10B has shown to play a dual function of both oncogenesis promotion via β-catenin/TCF pathway and the inhibition of cell growth, possibly via FGF family of proteins [14]. Methylation of WNT10B has been found in the some of the cancer cell lines while its reversal has lead to over-expression of the WNT10B. However, the over-expression of WNT10B has lead to reduced cell growth in cancer, indicating a β-catenin independent component to be behind such a phenomena. Methylation of over-expressed WNT10B and synergistic work with FGF family of proteins later indicate the promotion of oncogenesis, as has been demonstrated in [14].

In a more recent work, ASCL2 has been found to play a major role in stemness in colon crypts and is implicated in colon cancer [15]. Switching off the ASCL2 leads to a literal blockage of the stemness process and vice versa. At the downstream level, ASCL2 is regulated by TCF4/β-catenin via non-coding RNA target named WiNTRLINC1 [16]. Activation of ASCL2 leads to feedforward transcription of the non-coding RNA and thus a loop is formed which helps in the stemness and is highly effective in colon cancer. At the upstream level, ASCL2 is known act as a WNT/RSPONDIN switch that controls the stemness [17]. It has been shown that removal of RSPO1 lead to decrease in the Wnt signaling due to removal of the FZD receptors that led to reduced expression of ASCL2. Also, low levels of LGR5 were observed due to this phenomena. The opposite happened by increasing the RSPO1 levels. After the drug treatment, it was found that ASCL2 was highly suppressed pointing to the inhibition of stemness in the colorectal cancer cells. Also, [17] show that by genetically disrupting PORCN or inducing a PORCN inhibitor (like IWP-2), there is loss of stem cell markers like LGR5 and RNF43, which lead to disappearance of stem cells and moribund state of mice. A similar affect can be found with ETC-1922159, where there is suppression of RNF43 and LGR5 that lead to inhibition of the Wnt pathway and thus the ASCL2 regulation. These wet lab evidences are confirmed in the relatively low ranking of the combination ASCL2-RNF43 via the inhibition of PORCN-WNT that leads to blocking of the stemness that is induced by ASCL2. Since ASCL2 is directly mediated by the WNT proteins, the recorded ASCL2-WNT10B combination showed low priority ranking of 488, 497 and 321

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for rbf, laplace and linear kernels, respectively, thus indicating a possible connection between WNT10B and ASCL2 activation. WNT10B might be playing a crucial role in stemness. This is further confirmed by wet lab experiments in [18], which show BVES deletion results in amplified stem cell activity and Wnt signaling after radiation. WNT10B has been implicated in colorectal cancer [14].

3.1.2. ABC Transporters - WNT Cross Family Analysis

[19] have shown the role of ABC transporters in progression and clinical outcome of colorectal cancer. Work by [20] show that Wnt-β catenin signaling regulates ABCC3 (MRP3) transporter expression in colorectal cancer. ABCA2 belongs to the category of ABC transporters that play an essential role in the development of resistance by the efflux of anticancer agents outside of cancer cells [19]. [19] observed that ABCA2 had no significant change/affect in colorectal cancer cases. [20] found ABCA2 to be downregulated in colorectal cancer case. In ETC-1922159 affected CRC cells, down regulation of ABCA2 was observed, after the inhibition of proliferation in respective cells. Multiple members of ABC transporters and WNTs were found to be UP regulated after ETC-159 in CRC cells and WNTs are known to regulate ABCs. Below, we show a range of up regulated, possible unknown and unexplored synergistic 2^{nd} order combinations that were ranked by the search engine. Note that the high numerical valued ranks (i.e nearing to 1800/2000 and above) indicate high potential of synergy that might be existing in CRC cells after the drug administration. Majority voting of rankings across the three different kernels point to the potential of the synergistic discovery. Wet labs investigations will assist in confirmation of these discoveries and if proven true, might lead to understanding of further mechanism between the components.

Tables 1 and 2 show the rankings of ABC family w.r.t to WNT family members and WNT family w.r.t to ABC family members, respectively. From these two tables, we derive the plausible influences that might be existing in a two way format that is depicted in Table 3. In Table 1, WNT2B - ABC-C3 combination shows a majority voting of 1853 (laplace) and 2498 (rbf). Similarly, WNT7B - ABC-C13 shows a majority voting of 2245 (linear) and 2298 (rbf). These two combinations are depicted in Table 3 as ABC members influenced by WNT members (see under ABC w.r.t WNT). Reversibily, in Table 2 ABC-A5 - WNT2B shows a majority voting of 2018 (linear) and 2132 (rbf), ABC-A5 - WNT4 shows a majority voting of 2436 (linear) and 2449 (rbf), ABC-A5 - WNT9A shows a majority voting of 1989 (laplace), 2209 (linear) and 2365 (rbf), WNT2B - ABC-C5 shows a majority voting of 1970 (laplace), 2309 (linear) and 2248 (rbf), ABC-C5 - WNT9A shows a majority voting of 2183 (linear) and 2480 (rbf), WNT2B - ABC-C13 shows a majority voting of 2150 (linear) and 2048 (rbf), WNT7B - ABC-C13 shows a majority voting of 2508 (laplace) and 1830 (linear), WNT7B - ABC-D1 shows a majority voting of 2238 (laplace) and 2021 (linear), WNT7B - ABC-G1 shows a majority voting of 1808 (linear) and 1866 (rbf), WNT7B - ABC-G2 shows a majority voting of 2334 (linear) and 2145 (rbf) and WNT9A - ABC-G2 shows a majority voting of 1919 (laplace) and 2003 (rbf). These point to WNT members influenced by ABC members (see under WNT w.r.t ABC). Hypothetically, what we find is that the synergies can be bi-directional also and might contain various intermitent factors through which the factors might be working synergistically. These hypothese form present themselves as important combinations that might be of interest to biologists/oncologists.

One can also interpret the results of the Table 3 graphically, with the following influences - • ABC w.r.t WNT with WNT-2B $->$ ABC-C3; WNT-7B $->$ ABC-C13; and • WNT w.r.t ABC with ABC-A5 $<-$ WNT-2B/4/9A; WNT-2B/9A $<-$ ABC-C5; WNT-2B/7B $<-$ ABC-C13; WNT-7B $<-$ ABC-D1; WNT-7B $<-$ ABC-G1; WNT-7B/9A $<-$ ABC-G2. Thus, in this way, we can utilize the search engine to derive the various probable combinations between the factors of interest and their interdependent influences through the two-way cross family analysis.

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Table 1. 2^{nd} order interaction ranking between ABC w.r.t WNT family members.

RANKING ABC FAMILY W.R.T WNT FAMILY

RANKING OF ABC FAMILY W.R.T WNT-2B				RANKING OF ABC FAMILY W.R.T WNT4			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - ABC-A5	2108	310	72	ABC-A5 - WNT4	359	1285	433
ABC-B11 - WNT2B	319	2132	18	ABC-B11 - WNT4	872	1284	867
WNT2B - ABC-C3	1853	262	2498	ABC-C3 - WNT4	10	617	296
WNT2B - ABC-C5	2213	1685	840	WNT4 - ABC-C5	1383	2119	215
WNT2B - ABC-C13	1149	1191	2175	WNT4 - ABC-C13	1649	1814	542
WNT2B - ABC-D1	1119	177	2163	ABC-D1 - WNT4	1041	1171	1740
WNT2B - ABC-G1	1068	1583	214	ABC-G1 - WNT4	1020	1146	2025
WNT2B - ABC-G2	1500	1533	172	ABC-G2 - WNT4	784	1431	435
RANKING OF ABC FAMILY W.R.T WNT-7B				RANKING OF ABC FAMILY W.R.T WNT-9A			
	laplace	linear	rbf		laplace	linear	rbf
ABC-A5 - WNT7B	1550	516	995	ABC-A5 - WNT9A	735	349	1479
ABC-B11 - WNT7B	968	599	324	ABC-B11 - WNT9A	843	1647	689
ABC-C3 - WNT7B	694	1668	695	ABC-C3 - WNT9A	1590	359	2136
WNT7B - ABC-C5	979	1715	2268	ABC-C5 - WNT9A	1295	368	2265
WNT7B - ABC-C13	950	2245	2298	ABC-C13 - WNT9A	1394	2294	1134
ABC-D1 - WNT7B	252	850	1215	ABC-D1 - WNT9A	910	2367	675
ABC-G1 - WNT7B	269	733	1160	ABC-G1 - WNT9A	426	2457	1074
ABC-G2 - WNT7B	1717	224	264	ABC-G2 - WNT9A	1108	2350	960

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Table 2. 2^{nd} order interaction ranking between WNT w.r.t ABC family members.

RANKING WNT FAMILY W.R.T ABC FAMILY							
RANKING OF WNT FAMILY W.R.T ABC-A5				RANKING OF WNT FAMILY W.R.T ABC-B11			
	laplace	linear	rbf		laplace	linear	rbf
ABC-A5 - WNT2B	1549	2018	2132	WNT2B - ABC-B11	1083	703	1887
ABC-A5 - WNT4	1375	2436	2449	WNT4 - ABC-B11	156	298	1517
ABC-A5 - WNT7B	2420	1527	460	WNT7B - ABC-B11	1134	204	2323
ABC-A5 - WNT9A	1989	2209	2365	WNT9A - ABC-B11	226	2134	1480
RANKING OF WNT FAMILY W.R.T ABC-C3				RANKING OF WNT FAMILY W.R.T ABC-C5			
	laplace	linear	rbf		laplace	linear	rbf
ABC-C3 - WNT2B	1127	1482	1905	WNT2B - ABC-C5	1970	2309	2248
ABC-C3 - WNT4	897	1454	489	WNT4 - ABC-C5	2129	229	230
ABC-C3 - WNT7B	656	2080	772	WNT7B - ABC-C5	1539	756	1258
ABC-C3 - WNT9A	2339	1616	814	ABC-C5 - WNT9A	213	2183	2480
RANKING OF WNT FAMILY W.R.T ABC-C13				RANKING OF WNT FAMILY W.R.T ABC-D1			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - ABC-C13	950	2150	2048	WNT2B - ABC-D1	1751	1370	1174
WNT4 - ABC-C13	538	326	2242	WNT4 - ABC-D1	45	1784	101
WNT7B - ABC-C13	2508	1830	1219	WNT7B - ABC-D1	2238	2021	1121
WNT9A - ABC-C13	738	2501	634	WNT9A - ABC-D1	732	1526	1759
RANKING OF WNT FAMILY W.R.T ABC-G1				RANKING OF WNT FAMILY W.R.T ABC-G2			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - ABC-G1	318	775	2040	WNT2B - ABC-G2	1342	1987	1230
WNT4 - ABC-G1	2169	157	39	WNT4 - ABC-G2	862	1352	1985
WNT7B - ABC-G1	587	1808	1866	WNT7B - ABC-G2	2334	2145	1526
WNT9A - ABC-G1	856	2350	920	WNT9A - ABC-G2	1919	1284	2003

Table 3. 2^{nd} order combinatorial hypotheses between ABC and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ABC w.r.t WNT	
WNT-2B	ABC-C3
WNT-7B	ABC-C13

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Table 3. *Cont.*

UNEXPLORED COMBINATORIAL HYPOTHESES	
WNT w.r.t ABC	
ABC-A5	WNT-2B/4/9A
WNT-2B/9A	ABC-C5
WNT-2B/7B	ABC-C13
WNT-7B	ABC-D1
WNT-7B	ABC-G1
WNT-7B/9A	ABC-G2

3.1.3. IL - WNT Cross Family Analysis

Interleukin (IL) has been found in cross talk with WNT pathway. [21] show that NFκB induced WNT signaling in colorectal cancer via interleukin-1β IL1B. Further, [22] have shown that nitric oxide mediates crosstalk between interleukin 1β and Wnt signaling in primary human chondrocytes by reducing DKK1 and FRZB expression. The role of IL-17 (Interleukin-17) family is known to be controversial in CRC, however there are cases were it has been reported to be a prognostic marker for colorectal cancer [23] & [24]. A homologue of the family, IL-17D a novel cytokine has been discovered [25] and found to play a role in many of the cancers. In cells treated with ETC-1922159, IL-17D was found to be down regulated and reversibly it must have been regulated in the colorectal cancer cases. Recently, crosstalk between WNT/β-Catenin and NF-κB signaling pathway during inflammation has been reported by [26]. [27] also show WNT/β-catenin negative feedback loop inhibits IL-1 induced matrix metalloproteinase expression in human articular chondrocytes. [28] conclude that WNT/β-catenin signaling promotes angiogenesis possibly via the induction of known angiogenic regulators such as Interleukin-8. In mouse colon, Interleukin-1 signaling is shown to mediate obesity-promoted elevations in inflammatory cytokines, WNT activation, and epithelial proliferation by [29]. In pulmonary fibrosis, [30] show that WNT/β-Catenin signaling induces IL-1β expression by alveolar epithelial cells. [31] show that IL-23 promotes the epithelial-mesenchymal transition of oesophageal carcinoma cells via the WNT/β-catenin pathway. Finally, [32] show that IL-6/WNT interactions in rheumatoid arthritis.

Family members belonging to each of the factors like WNT, IL etc, might be involved synergistically in pathological case or otherwise. IL and WNT members were found to be up regulated after the treatment of ETC-1922159 in colorectal cancer cells. We present here, multiple plausible and alternative synergistic combinatorial biological hypotheses for IL-WNT combination, which emerge after a cross family member analysis of the in silico revelations pertaining to the components under investigation.

Table 4 shows IL-WNT two way cross family analysis. The left side of the table contains rankings of IL family with respect to WNTs and the right side of the table contains rankings of WNT family with respect to ILs. Depicted in table are the plausible combinatorial hypotheses derived from majority voting of the rankings in table 4. On the left half, **w.r.t WNT2B**, IL-6ST/8/17REL show a synergy with WNT2B. These are reflected with rankings of 1797 (linear) and 2088 (rbf) for IL-6ST - WNT2B; rankings of 2107 (laplace), 1817 (linear) and 2088 (rbf) for IL-8 - WNT2B and rankings of 1824 (laplace) and 2241 (rbf) for IL-17REL - WNT2B, respectively. **W.r.t WNT4**, IL-1B/1RAP/15RA/17C show a synergy with WNT4. These are reflected with rankings of 1867 (laplace) and 1976 (linear) for IL-1B - WNT4; rankings of 2302 (laplace) and 1826 (linear) for IL-1RAP - WNT4; rankings of 1987 (laplace) and 2265 (linear) for IL-15RA - WNT4 and rankings of 2018 (laplace) and 1881 (linear) for IL-17C - WNT4, respectively. **W.r.t WNT7B**, IL-1RN/17REL show a synergy with WNT7B. These are reflected with rankings of 1882 (laplace) and 1796 (linear) for IL-1RN - WNT7B and rankings of 2053 (laplace), 2445 (linear) and 2489 (rbf) for IL-17REL - WNT4, respectively. **W.r.t WNT9A**, IL-1RAP/15RA show a synergy with WNT9A. These are reflected with rankings of 2273 (linear) and 2159 (rbf) for IL-1RAP - WNT9A and rankings of 1776 (laplace) and 2380 (linear) for IL-15RA - WNT9A, respectively.

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Table 4. 2nd order interaction ranking between ABC w.r.t IL family members.

RANKING IL FAMILY VS WNT FAMILY

RANKING OF IL FAMILY W.R.T WNT-2B				RANKING OF WNT-2B W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
IL1A - WNT2B	6	2363	924	IL1A - WNT2B	2290	1360	2427
IL1B - WNT2B	1015	1278	794	IL1B - WNT2B	847	2168	1369
IL1RAP - WNT2B	1481	1391	799	IL1RAP - WNT2B	2488	35	1892
IL1RN - WNT2B	1229	1967	1582	IL1RN - WNT2B	1307	43	2514
IL2RG - WNT2B	1434	1100	2335	IL2RG - WNT2B	1384	1255	1283
IL6ST - WNT2B	1157	1797	2088	IL6ST - WNT2B	776	242	1481
IL8 - WNT2B	2107	1817	2251	IL8 - WNT2B	2157	2025	593
IL10RB - WNT2B	961	2494	512	IL10RB - WNT2B	2419	856	1419
IL15 - WNT2B	1008	1214	1714	IL15 - WNT2B	1171	625	1215
IL15RA - WNT2B	728	1782	1382	IL15RA - WNT2B	2262	1021	657
IL17C - WNT2B	477	2357	1483	IL17C - WNT2B	1947	1304	1331
IL17REL - WNT2B	1824	12	2241	IL17REL - WNT2B	1980	919	1617
RANKING OF IL FAMILY W.R.T WNT-4				RANKING OF WNT-4 W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
IL1A - WNT4	2500	1346	955	IL1A - WNT4	507	221	91
IL1B - WNT4	1867	1976	1682	IL1B - WNT4	129	250	291
IL1RAP - WNT4	2302	1826	803	IL1RAP - WNT4	74	19	1553
IL1RN - WNT4	1314	856	104	IL1RN - WNT4	851	1218	2029
IL2RG - WNT4	1289	590	319	IL2RG - WNT4	520	920	424
IL6ST - WNT4	1315	273	2422	IL6ST - WNT4	991	1443	2454
IL8 - WNT4	1722	549	11	IL8 - WNT4	1980	2144	1267
IL10RB - WNT4	1700	153	1055	IL10RB - WNT4	1828	2259	1993
IL15 - WNT4	1012	871	1658	IL15 - WNT4	959	553	448
IL15RA - WNT4	1987	2265	819	IL15RA - WNT4	788	139	645
IL17C - WNT4	2018	1639	1881	IL17C - WNT4	406	276	232
IL17REL - WNT4	1019	425	893	IL17REL - WNT4	955	595	1689

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Table 4. *Cont.*

RANKING IL FAMILY VS WNT FAMILY

RANKING OF IL FAMILY W.R.T WNT-7B				RANKING OF WNT-7B W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
IL1A - WNT7B	662	950	149	IL1A - WNT7B	1058	2134	2312
IL1B - WNT7B	290	167	502	IL1B - WNT7B	1683	1871	1575
IL1RAP - WNT7B	872	1976	789	IL1RAP - WNT7B	381	1728	1517
IL1RN - WNT7B	1882	1796	503	IL1RN - WNT7B	1907	2162	1605
IL2RG - WNT7B	1381	446	482	IL2RG - WNT7B	1070	1695	2245
IL6ST - WNT7B	819	1284	1528	IL6ST - WNT7B	1268	1881	2020
IL8 - WNT7B	2232	220	701	IL8 - WNT7B	1551	58	2149
IL10RB - WNT7B	1318	1198	656	IL10RB - WNT7B	375	2145	803
IL15 - WNT7B	1000	290	245	IL15 - WNT7B	2307	1524	1687
IL15RA - WNT7B	1535	1054	2204	IL15RA - WNT7B	1575	191	1949
IL17C - WNT7B	515	263	113	IL17C - WNT7B	1956	2388	1982
IL17REL - WNT7B	2053	2445	2489	IL17REL - WNT7B	322	859	1631
RANKING OF IL FAMILY W.R.T WNT-9A				RANKING OF WNT-9A W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
IL1A - WNT9A	199	2228	1270	IL1A - WNT9A	597	1322	469
IL1B - WNT9A	305	2266	466	IL1B - WNT9A	776	652	1010
IL1RAP - WNT9A	1773	2273	2159	IL1RAP - WNT9A	2003	2179	964
IL1RN - WNT9A	2479	1506	1503	IL1RN - WNT9A	1363	1829	1632
IL2RG - WNT9A	1489	598	865	IL2RG - WNT9A	186	260	1276
IL6ST - WNT9A	2229	761	1103	IL6ST - WNT9A	2099	1416	1674
IL8 - WNT9A	346	1103	1910	IL8 - WNT9A	589	1751	1529
IL10RB - WNT9A	1836	1556	1006	IL10RB - WNT9A	1021	2127	1534
IL15 - WNT9A	168	1445	855	IL15 - WNT9A	1357	1025	1709
IL15RA - WNT9A	1776	206	2380	IL15RA - WNT9A	2149	2362	737
IL17C - WNT9A	72	2442	569	IL17C - WNT9A	1532	2465	1607
IL17REL - WNT9A	2512	24	580	IL17REL - WNT9A	2101	1940	313

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Table 5. 2^{nd} order combinatorial hypotheses between IL and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
IL w.r.t WNT	
IL-6ST/8/17REL	WNT-2B
IL-1B/1RAP/15RA/17C	WNT-4
IL-1RN/17REL	WNT-7B
IL-1RAP/15RA	WNT-9A
WNT w.r.t IL	
IL-1A/1RAP/8	WNT-2B
IL-8/10RB	WNT-4
IL-1A/1RN/6ST/17C	WNT-7B
IL-1RAP/15RA/17REL	WNT-9A

On the right half, **WNT2B w.r.t IL family**, IL-1A/1RAP/8 show a synergy with WNT2B. These are reflected with rankings of 2290 (laplace) and 2427 (rbf) for IL-1A - WNT2B; rankings of 2488 (laplace) and 1892 (rbf) for IL-1RAP - WNT2B and rankings of 2157 1824 (laplace) and 2025 (linear) for IL-8 - WNT2B, respectively. **WNT4 w.r.t IL family**, IL-8/10RB show a synergy with WNT4. These are reflected with rankings of 1980 (laplace) and 2144 (linear) for IL-8 - WNT4 and rankings of 1828 (laplace), 2259 (linear) and 1993 (rbf) for IL-10RB - WNT4; respectively. **WNT7B w.r.t IL family**, IL-1A/1RN/6ST/17C show a synergy with WNT7B. These are reflected with rankings of 2134 (linear) and 2312 (rbf) for IL-1A - WNT7B; rankings of 1907 (laplace) and 2162 (linear) for IL-1RN - WNT7B; rankings of 1881 (linear) and 2020 (rbf) for IL-ST - WNT7B; and rankings of 1956 (laplace), 2388 (linear) and 1982 (rbf) for IL-17C - WNT7B, respectively. **WNT9A w.r.t IL family**, IL-1RAP/15RA/17REL show a synergy with WNT9A. These are reflected with rankings of 2003 (laplace) and 2179 (linear) for IL-1RAP - WNT9A; rankings of 2149 (laplace) and 2362 (linear) for IL-15RA - WNT9A; and rankings of 2101 (laplace) and 1940 (linear) for IL-17REL - WNT9A, respectively. One can also interpret the results of the Table 5 graphically, with the following influences - • IL w.r.t WNT with IL-6ST/8/17REL $<-$ WNT-2B; IL-1B/1RAP/15RA/17C $<-$ WNT-4; IL-1RN/17REL $<-$ WNT-7B; IL-1RAP/15RA $<-$ WNT-9A and • WNT w.r.t IL with IL-1A/1RAP/8 $->$ WNT-2B; IL-8/10RB $->$ WNT-4; IL-1A/1RN/6ST/17C $->$ WNT-7B and IL-1RAP/15RA/17REL $->$ WNT-9A.

3.1.4. UBE2 - WNT Cross Family Analysis

[33] observed balanced ubiquitylation and deubiquitylation of Frizzled regulate cellular responsiveness to Wg/Wnt. Family members belonging to each of the factors like UBE2, WNT etc, might be involved synergistically in pathological case or otherwise. UBE2 and WNT members were found to be up regulated after the treatment of ETC-159 in colorectal cancer cells. However, not much is known about interation between the UBE2 family members and WNTs. Here we present a range of synergies that were ranked highly for up regulation. Table 6 presents the rankings of UBE family VS WNT family. Following this, is the Table 7 which derives the necessary influences via majority voting of rankings in Table 6.

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Table 6. 2^{nd} order interaction ranking between WNT w.r.t UBE2 family members.

RANKING UBE2 FAMILY VS WNT FAMILY

RANKING OF UBE2-A W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-A			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2A	1608	203	181	WNT2B - UBE2A	1677	899	1671
WNT4 - UBE2A	1293	2314	2279	WNT4 - UBE2A	424	1062	545
WNT7B - UBE2A	1139	1217	1961	WNT7B - UBE2A	392	2345	2151
WNT9A - UBE2A	443	1705	287	WNT9A - UBE2A	806	1581	1098
RANKING OF UBE2-B W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-B			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2B	1473	2220	599	WNT2B - UBE2B	2020	553	73
WNT4 - UBE2B	2260	2008	2141	WNT4 - UBE2B	301	334	47
WNT7B - UBE2B	2116	2206	1454	WNT7B - UBE2B	1336	2052	1903
WNT9A - UBE2B	2291	79	1381	WNT9A - UBE2B	2300	2476	2326
RANKING OF UBE2-F W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-F			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2F	1246	833	2387	WNT2B - UBE2F	1006	1917	49
WNT4 - UBE2F	2135	2505	1762	WNT4 - UBE2F	63	1109	664
WNT7B - UBE2F	2423	1673	2077	WNT7B - UBE2F	2236	1660	1751
WNT9A - UBE2F	2032	1165	128	WNT9A - UBE2F	1014	2251	2179
RANKING OF UBE2-H W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-H			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2H	1841	351	2178	WNT2B - UBE2H	2015	1019	1331
WNT4 - UBE2H	1090	778	1224	WNT4 - UBE2H	218	2248	2155
WNT7B - UBE2H	1505	1215	527	WNT7B - UBE2H	2294	1209	1367
WNT9A - UBE2H	605	332	2479	WNT9A - UBE2H	437	1202	2379
RANKING OF UBE2-J1 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-J1			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2J1	1539	1251	1814	WNT2B - UBE2J1	1500	1562	1255
WNT4 - UBE2J1	1583	2478	1604	WNT4 - UBE2J1	292	62	65
WNT7B - UBE2J1	2349	1207	2183	WNT7B - UBE2J1	552	1877	1846
WNT9A - UBE2J1	1835	2053	1652	WNT9A - UBE2J1	2471	2137	2469

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Table 6. *Cont.*

RANKING UBE2 FAMILY VS WNT FAMILY

RANKING OF UBE2-Z W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T UBE2-Z			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - UBE2Z	58	1756	1878	WNT2B - UBE2Z	1576	1171	1543
WNT4 - UBE2Z	2195	2468	938	WNT4 - UBE2Z	896	132	186
WNT7B - UBE2Z	2343	1973	723	WNT7B - UBE2Z	1972	1800	1399
WNT9A - UBE2Z	136	1986	4	WNT9A - UBE2Z	1149	865	813

On the left half, **w.r.t WNT family**, UBE2A show a synergy with WNT4. These are reflected with rankings of 2314 (linear) and 2279 (rbf) for UBE2A - WNT4; UBE2B show a synergy with WNT4/7B. These are reflected with rankings of 2260 (laplace), 2008 (linear) and 2141 (rbf) for UBE2B - WNT4 and rankings of 2116 (laplace) and 2206 (rbf) for UBE2B - WNT7B, respectively; UBE2F show a synergy with WNT4/7B. These are reflected with rankings of 2135 (laplace) and 2505 (linear) for UBE2F - WNT4 and rankings of 2423 (laplace) and 2077 (rbf) for UBE2F - WNT7B, respectively; UBE2H show a synergy with WNT2B. These are reflected with rankings of 1841 (laplace) and 2178 (linear) for UBE2H - WNT2B; UBE2J1 show a synergy with WNT-7B/9A. These are reflected with rankings of 2349 (laplace) and 2183 (rbf) for UBE2J1 - WNT7B and rankings of 1835 (laplace) and 2053 (rbf) for UBE2J1 - WNT9A, respectively. UBE2Z show a synergy with WNT-2B/4/9A. These are reflected with rankings of 1756 (linear) and 1878 (rbf) for UBE2J1 - WNT2B, rankings of 2195 (laplace) and 2468 (rbf) for UBE2J1 - WNT4, and 2343 (laplace) and 1973 (rbf) for UBE2J1 - WNT9A, respectively.

On the right half, **w.r.t UBE2**, UBE2A shows a synergy with WNT4. These are reflected with rankings of 2345 (linear) and 2151 (rbf) for UBE2A - WNT7B; UBE2B shows a synergy with WNT-7B/9A. These are reflected with rankings of 2052 (linear) and 1903 (rbf) for UBE2B - WNT7B and rankings of 2300 (laplace), 2476 (linear) and 2326 (rbf) for UBE2B - WNT9A, respectively; UBE2F shows a synergy with WNT-7B/9A. These are reflected with rankings of 2236 (laplace) and 1751 (rbf) for UBE2F - WNT7B and rankings of 2251 (linear) and 2179 (rbf) for UBE2F - WNT9A, respectively; UBE2H shows a synergy with WNT4. These are reflected with rankings of 2248 (linear) and 2155 (rbf) for UBE2H - WNT4; UBE2J1 shows a synergy with WNT-7B/9A. These are reflected with rankings of 1877 (llinear) and 1846 (rbf) for UBE2J1 - WNT7B and rankings of 2471 (laplace), 2137 (linear) and 2469 (rbf) for UBE2J1 - WNT9A, respectively. UBE2Z shows a synergy with WNT-9A. These are reflected with rankings of 1972 (laplace) and 1800 (linear) for UBE2Z - WNT7B, respectively.

One can also interpret the results of the Table 7 graphically, with the following influences - • UBE2 w.r.t WNT with WNT-4 $->$ UBE2-A; WNT-4/7 $->$ UBE2-B; WNT-4/7B $->$ UBE2-F; WNT-2B $->$ UBE2-H; WNT-7B/9B $->$ UBE2-J1; WNT-2B/4/7B $->$ UBE2-Z and • WNT w.r.t UBE2 with WNT-7B $<-$ UBE2-A; WNT-7B/9A $<-$ UBE2-B; WNT-7B/9A $<-$ UBE2-F; WNT-4 $<-$ UBE2-H; WNT-7B/9A $<-$ UBE2-J1; WNT-7B $<-$ UBE2-Z;

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Table 7. 2^{nd} order combinatorial hypotheses between UBE2 and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
UBE2 w.r.t WNT	
WNT-4	UBE2-A
WNT-4/7	UBE2-B
WNT-4/7B	UBE2-F
WNT-2B	UBE2-H
WNT-7B/9B	UBE2-J1
WNT-2B/4/7B	UBE2-Z
WNT w.r.t UBE2	
WNT-7B	UBE2-A
WNT-7B/9A	UBE2-B
WNT-7B/9A	UBE2-F
WNT-4	UBE2-H
WNT-7B/9A	UBE2-J1
WNT-7B	UBE2-Z

3.1.5. EXOSC - WNT10B Cross Family Analysis

Recently, emerging role of exosome (EXOSC) has been studied in WNT secretion and transportation by [34]. It has been found that exosomes play a critical role in morphogen signaling during embryonic development and cancer progression. In injured CNS, exosomes mediate mobilization of WNT10B to promote axonal regeneration as shown by [35]. [36] show the importance of exsosomes in WNT transportation. Emerging on these lines, we conducted a small two-way analysis of EXOSC components and WNT10B which were found to be down regulated in CRC cells after administration of ETC-1922159. Note that here, the interpretation of the rankings changes as the low numerical valued ranks (nearing to 1) are considered of high importance as they point to the synergistic down regulation after the drug administration. In line with the experiments, as ETC-1922159 a PORCN-WNT inhibitor block the transportation of WNTs, it might be that the affects of EXOSC components are also down regulated. These were rightly allocated with the low numerical valued in-silico ranks by the engine, thus pointing to the experimental down regulation in cells also. This confirmatory results also helps us in exploring the unknown combinations that might be prevailing synergistically when the WNT-EXOSC were up regulated before the administration of ETC-1922159 in CRC cells.

Table 8 shows the rankings of EXOSC family w.r.t WNT10B and vice versa. Followed by this is the unexplored combinatorial hypotheses in Table 9 generated from two-way analysis of the ranks in Table 8. On the left half of the Table 8, except for EXOSC7 - WNT10B, all other combinations of EXOSC family show high synergy with WNT10B. This is depicted by the low numerical valued ranks allocated by the search engine for EXOSC-2/3/5/6/8/9 with WNT10B, via majority voting across the ranking methods using laplace, linear and rbf kernels. This shows that EXOSC-2/3/5/6/8/9 had a critical role in the transport of WNT10B. On the right half of the same table, EXOSC-2/5/6/7/9 show synergistic affiliation with respect to WNT10B, via low numerical valued ranks. These are translated to graphical influences in Table 9. One can also interpret the results of the Table 9 graphically, with the following influences - • EXOSC w.r.t WNT10B with EXOSC-2/5/6/7/9 $<-$ WNT10B and • WNT10B w.r.t EXOSC with EXOSC-2/3/5/6/8/9 $->$ WNT10B. Further analyses of these combinations in wet lab might help biologists explore the deeper mechanism of exosome components and WNT10B in CRC cells.

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Table 8. 2^{nd} order interaction ranking between WNT w.r.t EXOSC family members.

RANKING EXOSC FAMILY VS WNT10B							
RANKING OF WNT10B W.R.T EXOSC FAMILY				RANKING OF EXOSC FAMILY W.R.T WNT10B			
	laplace	linear	rbf		laplace	linear	rbf
EXOSC2 - WNT10B	221	433	699	EXOSC2 - WNT10B	1695	1077	992
EXOSC3 - WNT10B	906	1292	860	EXOSC3 - WNT10B	610	2496	2428
EXOSC5 - WNT10B	919	484	997	EXOSC5 - WNT10B	832	1445	1589
EXOSC6 - WNT10B	407	1195	1747	EXOSC6 - WNT10B	1319	1738	1689
EXOSC7 - WNT10B	2599	2571	2584	EXOSC7 - WNT10B	2710	13	4
EXOSC8 - WNT10B	336	1437	391	EXOSC8 - WNT10B	451	2284	2493
EXOSC9 - WNT10B	222	701	732	EXOSC9 - WNT10B	1378	1501	1651

Table 9. 2^{nd} order combinatorial hypotheses between EXOSC and WNT10B family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
EXOSC w.r.t WNT10B	
EXOSC-2/5/6/7/9	WNT10B
WNT10B w.r.t EXOSC	
EXOSC-2/3/5/6/8/9	WNT10B

3.1.6. CASP - WNT Cross Family Analysis

[37] show that a caspase-dependent pathway is involved in Wnt/β-catenin signaling promoted apoptosis in Bacillus Calmette-Guerin infected RAW264.7 macrophages. [38] have shown that WNT11 promotes cardiomyocyte development by caspase-mediated suppression of canonical WNT signals. Additionally, [39] show that Wnt5a and Wnt11 inhibit the canonical Wnt pathway and promote cardiac progenitor development via the Caspase-dependent degradation of AKT. These findings indicate probable interplay of Caspase and WNTs in various pathological cases. In mice, caspase-1 activation and IL-1β secretion together have shown to contribute to inflammatory condition of acute arthritis (see [40]). Recently, Caspase-3 inhibition has been found to be a therapeutic approach in colorectal cancer as shown by [41]. [42] also show synergistic role of Caspase-8 and Caspase-3 expressions as biomarkers in colorectal cancer. Family members belonging to each of the factors like CASP, WNT etc, might be involved synergistically in pathological case or otherwise. CASP and WNT members were found to be up regulated after the treatment of colorectal cancer cells with ETC-1922159.

Table 10 shows the rankings of CASP family w.r.t WNTs and vice versa. Followed by this is the unexplored combinatorial hypotheses in Table 11 generated from two-way analysis of the ranks in Table 10. On the first three tabular rows of the Table 10 show rankings of CASP family w.r.t WNT family. Here we present the possible interdependent WNT-CASP combinations that might be working synergistically in CRC cells. Considering CASP5 w.r.t WNTs, CASP5 - WNT2B show up regulated synergy through rankings of 2171 (laplace) and 2366 (linear). Considering CASP9 w.r.t WNTs, CASP9 - WNT-4/7B/9A show up regulated synergy through rankings of 2472 (laplace) and 2200 (linear) for CASP9 - WNT4; 2196 (laplace) and 1935 (linear) for CASP9 - WNT7B; and 1863 (laplace) and 2002 (linear) for CASP9 - WNT9A, respectively. Finally, considering CASP16 w.r.t WNTs, CASP16 - WNT4 showed up regulated synergy with rankings of 2070 (laplace) and 1783 (linear).

The next three tabular rows show rankings of WNT family w.r.t CASP family. **W.r.t CASP4**, WNT-7B/9A show promise of up regulation. These are reflected with rankings of 2479 (linear) and

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1739 (rbf) for WNT7B - CASP4 and rankings of 2278 (linear) and 1939 (rbf) for WNT9A - CASP4, respectively. **W.r.t CASP5**, WNT-7B shows promise of up regulation. This is reflected with rankings of 2112 (laplace), 1919 (linear) and 2440 (rbf) for WNT7B - CASP5. **W.r.t CASP7**, WNT-2B/4/9A show promise of up regulation. These are reflected with rankings of 2505 (laplace) and 1891 (linear) for WNT2B - CASP7; rankings of 2456 (linear) and 2455 (rbf) for WNT4 - CASP7; and rankings of 2183 (laplace) and 1941 (linear) for WNT9A - CASP7, respectively. **W.r.t CASP9**, WNT-9A shows promise of up regulation. This is reflected with rankings of 2378 (laplace), 2396 (linear) and 2058 (rbf) for WNT9A - CASP9. **W.r.t CASP10**, WNT-4/9A show promise of up regulation. These are reflected with rankings of 1830 (laplace), 2229 (linear) and 1847 (rbf) for WNT4 - CASP10; and rankings of 2185 (laplace) and 1977 (linear) for WNT9A - CASP10, respectively. Finally, **w.r.t CASP16**, WNT-2B/4/9A show promise of up regulation. These are reflected with rankings of 2197 (laplace), 2489 (linear) and 1775 (rbf) for WNT2B - CASP16; rankings of 2508 (laplace), 1820 (linear) and 1867 (rbf) for WNT7B - CASP16; and rankings of 1943 (laplace) and 1839 (linear) for WNT9A - CASP16, respectively.

Table 10. 2^{nd} order interaction ranking between WNT VS CASP family members.

Ranking CASP family VS WNT family

Ranking of CASP4 w.r.t WNTs family				Ranking of CASP5 w.r.t WNTs family			
	laplace	linear	rbf		laplace	linear	rbf
CASP4 - WNT2B	2265	320	1517	CASP5 - WNT2B	975	2171	2366
CASP4 - WNT4	1050	1081	558	CASP5 - WNT4	1788	1356	569
CASP4 - WNT7B	622	9	632	CASP5 - WNT7B	716	978	606
CASP4 - WNT9A	446	1413	583	CASP5 - WNT9A	383	808	147
Ranking of CASP7 w.r.t WNTs family				**Ranking of CASP9 w.r.t WNTs family**			
	laplace	linear	rbf		laplace	linear	rbf
CASP7 - WNT2B	1152	305	248	CASP9 - WNT2B	1345	1501	1328
CASP7 - WNT4	936	1260	1787	CASP9 - WNT4	1344	2472	2200
CASP7 - WNT7B	901	1403	1303	CASP9 - WNT7B	2196	1935	1713
CASP7 - WNT9A	1330	1527	2436	CASP9 - WNT9A	1863	428	2002
Ranking of CASP10 w.r.t WNTs family				**Ranking of CASP16 w.r.t WNTs family**			
	laplace	linear	rbf		laplace	linear	rbf
CASP10 - WNT2B	1607	1108	739	CASP16 - WNT2B	240	621	193
CASP10 - WNT4	432	689	132	CASP16 - WNT4	2070	1783	711
CASP10 - WNT7B	1906	1171	1165	CASP16 - WNT7B	411	713	103
CASP10 - WNT9A	1611	2152	1451	CASP16 - WNT9A	14	2512	181

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Table 10. *Cont.*

RANKING CASP FAMILY VS WNT FAMILY							
RANKING OF WNTS FAMILY W.R.T CASP4				RANKING OF WNTS FAMILY W.R.T CASP5			
	laplace	linear	rbf		laplace	linear	rbf
CASP4 - WNT2B	609	1317	2372	CASP5 - WNT2B	1849	1192	1590
CASP4 - WNT4	105	711	1062	CASP5 - WNT4	890	682	714
CASP4 - WNT7B	1093	2479	1739	CASP5 - WNT7B	2112	1919	2440
CASP4 - WNT9A	456	2278	1939	CASP5 - WNT9A	315	1880	1437
RANKING OF WNTS FAMILY W.R.T CASP7				RANKING OF WNTS FAMILY W.R.T CASP9			
	laplace	linear	rbf		laplace	linear	rbf
CASP7 - WNT2B	2505	1891	1120	CASP9 - WNT2B	282	639	1414
CASP7 - WNT4	108	2456	2455	CASP9 - WNT4	572	1788	378
CASP7 - WNT7B	1380	1559	1681	CASP9 - WNT7B	979	901	676
CASP7 - WNT9A	2183	1941	1632	CASP9 - WNT9A	2378	2396	2058
RANKING OF WNTS FAMILY W.R.T CASP10				RANKING OF WNTS FAMILY W.R.T CASP16			
	laplace	linear	rbf		laplace	linear	rbf
CASP10 - WNT2B	625	1471	81	CASP16 - WNT2B	2197	2489	1775
CASP10 - WNT4	1830	2229	1847	CASP16 - WNT4	1382	954	1017
CASP10 - WNT7B	1965	937	147	CASP16 - WNT7B	2508	1820	1867
CASP10 - WNT9A	2185	1977	1350	CASP16 - WNT9A	1943	1154	1839

One can also interpret the results of the Table 11 graphically, with the following influences - • CASP w.r.t WNT with CASP5 $<-$ WNT2B; CASP9 $<-$ WNT-4/7B/9A; CASP16 $<-$ WNT4 and • WNT w.r.t CASP with. WNT-7B/9A $<-$ CASP4; WNT7B $<-$ CASP5; WNT-2B/4/9A $<-$ CASP7; WNT9A $<-$ CASP9; WNT-4/9A $<-$ CASP10; WNT-2B/7B/9A $<-$ CASP16.

Table 11. 2^{nd} order combinatorial hypotheses between CASP and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
CASP w.r.t WNT	
CASP5	WNT2B
CASP9	WNT4/WNT7B/WNT9A
CASP16	WNT4
WNT w.r.t CASP	
WNT7B/WNT9A	CASP4
WNT7B	CASP5
WNT2B/WNT4/WNT9A	CASP7
WNT9A	CASP9
WNT4/WNT9A	CASP10
WNT2B/WNT7B/WNT9A	CASP16

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3.1.7. TP53 - WNT Cross Family Analysis

[43] have shown that down regulation of β-catenin is activated by TP53. Wnt/β-catenin signaling is known to regulate the proliferation and differentiation of mesenchymal progenitor cells through the TP53 Pathway, as shown by [44]. [45] show that WNT activation by lithium abrogates TP53 mutation associated radiation resistance in medulloblastoma. In mouse cochlea, [46] show that WNT signaling activates TP53-induced glycolysis and apoptosis regulator and protects against cisplatin-induced spiral ganglion neuron damage. These range of interactions of TP53 with WNT points towards definite synergy. [47] show that TP53 protein regulates Hsp90 ATPase activity and thereby Wnt signaling by modulating Aha1 expression. Family members belonging to each of the factors like TP53, WNT etc, might be involved synergistically in pathological case or otherwise. TP53 and WNT members were found to be up regulated after the treatment of ETC-159 in colorectal cancer cells.

Table 12 contains rankings of TP53 w.r.t WNTs and vice versa. Followed by this is the unexplored combinatorial hypotheses in Table 13 generated from two-way analysis of the ranks in Table 12. On the left half of Table 12 are rankings of TP53 w.r.t WNTs and on the right half are the rankings of WNTs w.r.t TP53 family. Beginning with the left half, TP53I3 - WNT2B shows synergistic up regulation with rankings of 2056 (laplace) and 1712 (linear); TP53INP1 - WNT2B shows synergistic up regulation with rankings of 1805 (linear) and 2056 (rbf) and TP53BP2 - WNT9A shows synergistic up regulation with rankings of 2232 (linear) and 2143 (rbf). On the right half the table, TP53INP1 - WNT2B shows synergistic up regulation with rankings of 1853 (laplace) and 2089 (linear); TP53INP2 - WNT2B shows synergistic up regulation with rankings of 1723 (linear) and 2335 (rbf); TP53INP1 - WNT4 shows synergistic up regulation with rankings of 2414 (linear) and 2493 (rbf); TP53I3 - WNT7B shows synergistic up regulation with rankings of 1988 (laplace) and 2393 (rbf) and finally, TP53INP1 - WNT9A shows synergistic up regulation with rankings of 2045 (linear) and 2437 (rbf).

Table 12. 2^{nd} order interaction ranking between WNT VS TP53 family members.

RANKING TP53 FAMILY VS WNT

RANKING OF TP53 FAMILY W.R.T WNT2B				RANKING OF WNT2B W.R.T TP53 FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TP53BP2 - WNT2B	2286	234	1550	TP53BP2 - WNT2B	313	908	2457
TP53I3 - WNT2B	2056	1712	1461	TP53I3 - WNT2B	713	1223	1720
TP53INP1 - WNT2B	945	1805	2056	TP53INP1 - WNT2B	1853	2089	762
TP53INP2 - WNT2B	369	1277	453	TP53INP2 - WNT2B	754	1723	2335
RANKING OF TP53 FAMILY W.R.T WNT4				RANKING OF WNT4 W.R.T TP53 FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TP53BP2 - WNT4	1034	315	1734	TP53BP2 - WNT4	678	1464	2500
TP53I3 - WNT4	1738	1631	232	TP53I3 - WNT4	297	319	493
TP53INP1 - WNT4	645	498	450	TP53INP1 - WNT4	131	2414	2493
TP53INP2 - WNT4	671	1440	405	TP53INP2 - WNT4	529	467	154

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Table 12. *Cont.*

RANKING TP53 FAMILY VS WNT							
RANKING OF TP53 FAMILY W.R.T WNT7B				RANKING OF WNT7B W.R.T TP53 FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TP53BP2 - WNT7B	2333	1282	1673	TP53BP2 - WNT7B	1442	2217	1068
TP53I3 - WNT7B	324	712	284	TP53I3 - WNT7B	1712	1988	2393
TP53INP1 - WNT7B	1227	1585	1019	TP53INP1 - WNT7B	1226	1685	1497
TP53INP2 - WNT7B	845	1004	470	TP53INP2 - WNT7B	1017	1746	1925
RANKING OF TP53 FAMILY W.R.T WNT9A				RANKING OF WNT9A W.R.T TP53 FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TP53BP2 - WNT9A	908	2232	2143	TP53BP2 - WNT9A	1035	371	1218
TP53I3 - WNT9A	1707	2297	1018	TP53I3 - WNT9A	1351	1281	1695
TP53INP1 - WNT9A	447	243	1245	TP53INP1 - WNT9A	295	2045	2437
TP53INP2 - WNT9A	22	2497	1138	TP53INP2 - WNT9A	421	1765	1121

Table 13. 2^{nd} order combinatorial hypotheses between TP53 and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TP53 family w.r.t WNT	
TP53I3	WNT2B
TP53INP1	WNT2B
TP53BP2	WNT9A
WNT family w.r.t TP53	
TP53INP1	WNT2B
TP53INP2	WNT2B
TP53INP1	WNT4
TP53I3	WNT7B
TP53INP1	WNT9A

One can also interpret the results of the Table 11 graphically, with the following influences - • TP53 family w.r.t WNTs with TP53I3 $<-$ WNT2B; TP53INP1 $<-$ WNT2B and TP53BP2 $<-$ WNT9A; and • WNT family VS TP53 with TP53INP1 $->$ WNT2B; TP53INP2 $->$ WNT2B; TP53INP1 $->$ WNT4; TP53I3 $->$ WNT7B and TP53INP1 $->$ WNT9A.

3.1.8. BCL - WNT Cross Family Analysis

[48] observed that silencing Wnt2B by siRNA interference inhibits metastasis and enhances chemotherapy sensitivity in ovarian cancer. More specifically, [48] show that in the presence of Wnt2B siRNA treatment, the caspase-9/B-cell lymphoma 2 (BCL2)/B-cell lymphoma-xL (BCL-xL) pathway and the epithelial-mesenchymal transition/phosphorylated protein kinase B pathway were inhibited. [49] show that targeted disruption of the BCL9/β-catenin complex inhibits oncogenic WNT signaling. CDK1-mediated BCL9 phosphorylation inhibits clathrin to promote mitotic Wnt signaling as shown by [50]. These findings point to the existing synergy of BCL family with WNTs. Family members belonging to each of the factors like BCL, WNT etc, might be involved synergistically in pathological case or otherwise. BCL and WNT members were found to be up regulated after the treatment of ETC-159 in colorectal cancer cells.

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Table 14 contains rankings of BCL w.r.t WNTs and vice versa. Followed by this is the unexplored combinatorial hypotheses in Table 15 generated from two-way analysis of the ranks in Table 14. On the left half of Table 14 are rankings of BCL w.r.t WNTs. WNT4 - BCL2L2 shows high ranking with 2364 (laplace) and 2042 (linear); WNT7B - BCL2L2 shows high ranking with 1877 (laplace) and 2456 (linear); WNT9A - BCL2L2 shows high ranking with 1877 (laplace) and 2447 (linear); WNT4 - BCL2L13 shows high ranking with 1938 (laplace), 2425 (linear) and 1900 (rbf); WNT7B - BCL2L13 shows high ranking with 1993 (linear) and 2284 (rbf) and WNT2B - BCL10 shows high ranking with 2321 (laplace) and 2023 (linear).

On the right side are rankings of WNTs w.r.t BCL. WNT7B - BCL2L1 shows high ranking with 2213 (laplace) and 2266 (linear); WNT7B - BCL2L2 shows high ranking with 2456 (laplace), 2512 (linear) and 2286 (rbf); WNT9A - BCL2L2 shows high ranking with 1868 (laplace) and 2333 (rbf); WNT9A - BCL2L13 shows high ranking with 1858 (laplace), 2422 (linear) and 1934 (rbf); WNT2B - BCL3 shows high ranking with 1846 (laplace), 2056 (linear) and 1896 (rbf); WNT4 - BCL6 shows high ranking with 2483 (laplace) and 2488 (linear); WNT7B - BCL6 shows high ranking with 1893 (laplace) and 2284 (linear); WNT9A - BCL6 shows high ranking with 2098 (linear) and 1905 (rbf); WNT2B - BCL9L shows high ranking with 1918 (laplace) and 1882 (rbf) and WNT4 - BCL9L shows high ranking with 2498 (linear) and 2509 (rbf);

Table 14. 2^{nd} order interaction ranking between WNT VS BCL family members.

RANKING BCL FAMILY VS WNT

RANKING OF BCL2L1 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL2L1			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL2L1	1884	101	966	WNT2B - BCL2L1	1854	1666	1699
WNT4 - BCL2L1	98	1162	719	WNT4 - BCL2L1	21	107	16
WNT7B - BCL2L1	1434	1891	620	WNT7B - BCL2L1	2213	2266	1511
WNT9A - BCL2L1	1088	1020	1318	WNT9A - BCL2L1	1019	1462	1345
RANKING OF BCL2L2 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL2L2			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL2L2	625	2204	1677	WNT2B - BCL2L2	1574	2206	955
WNT4 - BCL2L2	2364	2042	1610	WNT4 - BCL2L2	160	590	316
WNT7B - BCL2L2	843	1877	2456	WNT7B - BCL2L2	2456	2512	2286
WNT9A - BCL2L2	1877	538	2447	WNT9A - BCL2L2	1868	2333	990
RANKING OF BCL2L13 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL2L13			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL2L13	201	1862	1353	WNT2B - BCL2L13	1256	1254	1490
WNT4 - BCL2L13	1938	2425	1900	WNT4 - BCL2L13	922	270	187
WNT7B - BCL2L13	1105	1993	2284	WNT7B - BCL2L13	1610	1319	954
WNT9A - BCL2L13	1855	268	2387	WNT9A - BCL2L13	1858	2422	1934

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Table 14. *Cont.*

RANKING BCL FAMILY VS WNT

RANKING OF BCL3 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL3			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL3	950	1328	2482	WNT2B - BCL3	1846	2056	1896
WNT4 - BCL3	1228	1562	1353	WNT4 - BCL3	591	359	1932
WNT7B - BCL3	591	615	553	WNT7B - BCL3	1687	2160	1428
WNT9A - BCL3	1037	1410	1102	WNT9A - BCL3	1539	1424	398
RANKING OF BCL6 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL6			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL6	455	2426	1529	WNT2B - BCL6	52	107	170
WNT4 - BCL6	256	486	787	WNT4 - BCL6	2483	2488	1273
WNT7B - BCL6	2147	1466	1105	WNT7B - BCL6	975	1893	2284
WNT9A - BCL6	1547	734	2012	WNT9A - BCL6	1558	2098	1905
RANKING OF BCL9L W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL9L			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL9L	2348	804	1558	WNT2B - BCL9L	1918	700	1882
WNT4 - BCL9L	1446	657	309	WNT4 - BCL9L	303	2498	2509
WNT7B - BCL9L	1539	253	1279	WNT7B - BCL9L	1608	811	2168
WNT9A - BCL9L	1923	677	688	WNT9A - BCL9L	941	1843	1238
RANKING OF BCL10 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T BCL10			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B - BCL10	2321	69	2023	WNT2B - BCL10	1951	1101	1599
WNT4 - BCL10	285	1170	465	WNT4 - BCL10	2032	34	406
WNT7B - BCL10	1847	606	1252	WNT7B - BCL10	1297	74	2009
WNT9A - BCL10	217	798	1649	WNT9A - BCL10	1771	335	861

One can also interpret the results of the Table 15 graphically, with the following influences - • BCL family w.r.t WNTs with WNT4 $->$ BCL2L2; WNT7B $->$ BCL2L2; WNT9A $->$ BCL2L2; WNT4 $->$ BCL2L13; WNT7B $->$ BCL2L13; WNT2B $->$ BCL10 and • WNT family w.r.t BCL with WNT7B $<-$ BCL2L1; WNT7B $<-$ BCL2L2; WNT9A $<-$ BCL2L2; WNT9A $<-$ BCL2L13; WNT2B $<-$ BCL3; WNT4 $<-$ BCL6; WNT7B $<-$ BCL6; WNT9A $<-$ BCL6; WNT2B $<-$ BCL9L; WNT4 $<-$ BCL9L.

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Table 15. 2^{nd} order combinatorial hypotheses between TP53 and WNT family members.

UNEXPLORED COMBINATORIAL HYPOTHESES	
BCL w.r.t WNT family	
WNT-4/7B/9A	BCL2L2
WNT-4/7B	BCL2L13
WNT-2B	BCL10
WNT family w.r.t BCL	
WNT-7B	BCL2L1
WNT-7B/9A	BCL2L2
WNT-9A	BCL2L13
WNT-2B	BCL3
WNT-4/7B/9A	BCL6
WNT-2B/4	BCL9L

Conclusions

Presented here are a range of multiple synergistic WNT 2^{nd} order combinations that were ranked via a search engine. Later, two way cross family analysis between components of these combinations were conducted. Via majority voting across the ranking methods, it was possible to find plausible unexplored synergistic combinations that might be prevalent in CRC cells after treatment with ETC-1922159 drug. The two-way cross family analysis also assists in deriving influences between components which serve as hypotheses for further tests. If found true, it paves way for biologists/oncologists to further investigate and understand the mechanism behind the synergy through wet experiments.

Conflict of Interest

There are no conflicts to declare.

Author's Contributions

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

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Source of Data

Data used in this research work was released in a publication in [51]. The ETC-1922159 was released in Singapore in July 2015 under the flagship of the Agency for Science, Technology and Research (A*STAR) and Duke-National University of Singapore Graduate Medical School (Duke-NUS).

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