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Shriprakash Sinha *

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Article

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

shriprakash sinha

Independent Researcher; 104-Madhurisha Heights Phase 1, Risali, Bhilai-490006, India; sinha.shriprakash@yandex.com

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 Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB)-X (X, a particular gene/protein) at 2^{nd} order level after drug administration. These rankings reveal which NFκB-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 NFκB and X. In this research work, we cover combinations of caspase (CASP) with receptor interacting serine/threonine kinase (RIPK) family, mucin (MUC) family with RIPK, tumor necrosis factor (TNF) with NF-κB family and NF-κB-Inhibitor (NF-κB-I), NFκB-2/I with STAT family, Iκ B kinase ε (IKBKE) with STAT family, IKBKE with conjugal transfer protein (TRAF), ATP-binding cassette (ABC) domain transporters with NFκB, IKBKE with ubiquitination modifier enzyme and ubiquitination conjugating enzymes (UBA/UBE) and REL-A/B with NFκB .

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

1. Introduction

In the unpublished preprint Sinha [1], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway.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 Interested readers are advised to go through unpublished preprints Sinha [1] and Sinha [2] for details regarding the search engine and the discoveries mentioned in there.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 Sinha [3] and Sinha [2]. The details of the methodology of this manuscript have been explained in great detail in Sinha [3] & its application in Sinha [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 Sinha [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 combination. Sorting these shuffled scores of test data leads to prioritization of the combinations.

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Joachims [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. NF-κB Related Synergies

3.1.1. CASP - RIPK Cross Family Analysis

Tables 1 and 2 show the rankings of CASP family w.r.t RIPK and vice versa, respectively. Followed by this is the derived influences between CASP and RIPK via two way analysis of majority voting of rankings in the two foregoing tables. These influences are tabulated in table 3. In table 1, only CASP9 - RIPK3 combination showed up regulation with rankings of 2133 (laplace), 2030 (linear) and 2295 (rbf). In table 2, RIPK1 showed up regulation with CASP-4/10 with rankings of 2363 (laplace) and 1805 (rbf) for CASP4 - RIPK1; and 2438 (laplace) and 1915 (linear) for CASP10 - RIPK1, respectively. RIPK2 showed up regulation with CASP-5/9/16 with rankings of 1776 (linear) and 2247 (rbf) for CASP5 - RIPK2; 2000 (laplace), 2476 (linear) and 2138 (rbf) for CASP9 - RIPK2; and 2006 (linear) and 2046 (rbf) for CASP16 - RIPK2; Finally, RIPK4 showed up regulation with CASP-16 with rankings of 2273 (laplace) and 2023 (linear) for CASP16 - RIPK4.

Table 1. 2nd order interaction ranking between CASP w.r.t RIPK family members.

Ranking CASP family w.r.t RIPK family							
Ranking of CASP4 w.r.t RIPK family				Ranking of CASP5 family w.r.t RIPK			
	laplace	linear	rbf		laplace	linear	rbf
CASP4 - RIPK1	1154	1259	147	CASP5 - RIPK1	490	152	1818
CASP4 - RIPK2	559	2147	434	CASP5 - RIPK2	1274	2485	608
CASP4 - RIPK3	111	131	41	CASP5 - RIPK3	523	1047	317
CASP4 - RIPK4	187	1048	1039	CASP5 - RIPK4 7 1176	2361	1292	
Ranking of CASP7 w.r.t RIPK family				Ranking of CASP9 family w.r.t RIPK			
	laplace	linear	rbf		laplace	linear	rbf
CASP7 - RIPK1	2445	1289	1253	CASP9 - RIPK1	1726	1304	1480
CASP7 - RIPK2	1584	406	155	CASP9 - RIPK2	2079	291	1647
CASP7 - RIPK3	1406	1057	2091	CASP9 - RIPK3	2133	2030	2295
CASP7 - RIPK4	1739	231	2332	CASP9 - RIPK4	2037	1627	363
Ranking of CASP10 w.r.t RIPK family				Ranking of CASP16 family w.r.t RIPK			
	laplace	linear	rbf		laplace	linear	rbf
CASP10 - RIPK1	758	846	1405	CASP16 - RIPK1	73	1046	1887
CASP10 - RIPK2	1535	2312	884	CASP16 - RIPK2	20	932	1189
CASP10 - RIPK3	1530	250	2181	CASP16 - RIPK3	30	359	717
CASP10 - RIPK4	954	415	1547	CASP16 - RIPK4	493	2507	519

The caspase - receptor interacting protein kinases (RIPK) has an intricate mechanism which has not yet been discovered and many views exist about their synergistic interaction. Green *et al.* [5] presents a review of RIPK-dependent necrosis and its regulation by CASPs. Furthermore, Lin *et al.* [6] show that cleavage of the death domain RIPK by CASP-8 prompts TNF-induced apoptosis. RIPK1 is known to promote death receptor-independent CASP-8 mediated apoptosis under unresolved ER stress conditions, as shown by Estornes *et al.* [7]. Weng *et al.* [8] show that CASP-8 and RIPK

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regulate bacteria-induced innate immune responses and cell death. Also, Moriwaki *et al.* [9] show that RIPK3-CASP8 complex mediates atypical pro-IL-1β processing. Recent work by Declercq *et al.* [10] shows RIPK importance in cell death and survival along with CASP influence. These interactions point to a definite synergy between the CASP - RIPK. Chaudhary *et al.* [11] showed activation of NF-κB pathway via Caspase-8 (CASP-8) and its homologs. Additionally, Caspase-8 was found to interact with Receptor-interacting serine/threonine-protein kinase 1 (RIPK1). Family members belonging to each of the factors like CASP, RIPK etc, might be involved synergistically in pathological case or otherwise. CASP and RIPK members were found to be up regulated after the treatment of ETC-1922159 in colorectal cancer cells.

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

Ranking RIPK family w.r.t CASP family							
Ranking of RIPK family w.r.t CASP4				Ranking of RIPK family w.r.t CASP5			
	laplace	linear	rbf		laplace	linear	rbf
CASP4 - RIPK1	2363	1374	1805	CASP5 - RIPK1	7	82	131
CASP4 - RIPK2	1713	2349	1261	CASP5 - RIPK2	1577	1776	2247
CASP4 - RIPK3	1397	768	1008	CASP5 - RIPK3	574	14	30
CASP4 - RIPK4	2215	1334	1425	CASP5 - RIPK4	2448	1178	810
Ranking of RIPK family w.r.t CASP7				Ranking of RIPK family w.r.t CASP9			
	laplace	linear	rbf		laplace	linear	rbf
CASP7 - RIPK1	1341	2005	1131	CASP9 - RIPK1	820	140	611
CASP7 - RIPK2	1287	727	1143	CASP9 - RIPK2	2000	2476	2138
CASP7 - RIPK3	579	595	775	CASP9 - RIPK3	1550	430	97
CASP7 - RIPK4	852	1586	595	CASP9 - RIPK4	1565	862	209
Ranking of RIPK family w.r.t CASP10				Ranking of RIPK family w.r.t CASP16			
	laplace	linear	rbf		laplace	linear	rbf
CASP10 - RIPK1	2438	1915	1039	CASP16 - RIPK1	924	686	587
CASP10 - RIPK2	1526	1800	1228	CASP16 - RIPK2	1613	2006	2046
CASP10 - RIPK3	419	1481	2001	CASP16 - RIPK3	827	494	328
CASP10 - RIPK4	1303	947	785	CASP16 - RIPK4	2273	2023	1698

Table 3. 2^{nd} order combinatorial hypotheses between CASP and RIPK.

Unexplored combinatorial hypotheses	
CASP w.r.t RIKP family	
CASP9	RIPK3
RIPK w.r.t CASP family	
RIPK1	CASP4/CASP10
RIPK2	CASP5/CASP9/CASP16
RIPK4	CASP16

One can also interpret the results of the table 3 graphically, with the following influences - • CASP w.r.t RIKP family with CASP9 $<-$ RIPK3 and • RIPK w.r.t CASP family with RIPK1 $<-$ CASP-4/10; RIPK2 $<-$ CASP-5/9/16 and RIPK4 $<-$ CASP16.

3.1.2. MUC - RIPK Cross Family Analysis

In a recent work Sheng *et al.* [12] show that MUC13 promoted tumor necrosis factro (TNF)-induced NF-κB activation by interacting with TNFR1 and the E3 ligase, cIAP1, to increase ubiquitination of Receptor-interacting serine/threonine-protein kinase 1 (RIPK1). Family members belonging to each of the factors like MUC, RIPK etc, might be involved synergistically in pathological case or otherwise.

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MUC and RIPK members were found to be up regulated after the treatment of ETC-1922159 in colorectal cancer cells.

Tables 4 and 5 show the rankings of MUC family w.r.t RIPK family and vice versa, respectively. Followed by this is the derived influences between MUC and RIPK. In table 4, MUC1 was found to be highly upregulated with RIPK1. This is reflected in the rankings of 2027 (linear) and 2249 (rbf) for MUC1 - RIPK1. MUC3A was found to be highly upregulated with RIPK3. This is reflected in the rankings of 2208 (laplace) and 2017 (rbf) for MUC3A - RIPK3. MUC12 was found to be highly upregulated with RIPK4. This is reflected in the rankings of 2249 (linear) and 2130 (rbf), for MUC12 - RIPK4. MUC20 was found to be highly upregulated with RIPK3. This is reflected in the rankings of 2192 (laplace), 2288 (linear) and 1796 (rbf) for MUC20 - RIPK3.

Table 4. 2^{nd} order interaction ranking between MUC w.r.t RIPK family members.

Ranking MUC family w.r.t RIPK family							
Ranking of MUC1 w.r.t RIPK family				Ranking of MUCA3 w.r.t MUC3A			
	laplace	linear	rbf		laplace	linear	rbf
MUC1 - RIPK1	2027	2249	218	MUC3A - RIPK1	945	186	1508
MUC1 - RIPK2	248	1802	389	MUC3A - RIPK2	840	2390	1653
MUC1 - RIPK3	342	410	342	MUC3A - RIPK3	2208	2017	689
MUC1 - RIPK4	176	162	853	MUC3A - RIPK4	714	1494	797
Ranking of MUC4 w.r.t RIPK family				Ranking of MUC12 w.r.t RIPK family			
	laplace	linear	rbf		laplace	linear	rbf
MUC4 - RIPK1	358	2384	690	MUC12 - RIPK1	317	2437	167
MUC4 - RIPK2	371	500	408	MUC12 - RIPK2	286	2178	76
MUC4 - RIPK3	809	371	1096	MUC12 - RIPK3	747	366	136
MUC4 - RIPK4	652	1863	1248	MUC12 - RIPK4	176	2249	2130
Ranking of MUC13 w.r.t RIPK family				Ranking of MUC17 w.r.t RIPK family			
	laplace	linear	rbf		laplace	linear	rbf
MUC13 - RIPK1	379	2241	227	MUC17 - RIPK1	858	932	1503
MUC13 - RIPK2	824	2483	227	MUC17 - RIPK2	248	934	37
MUC13 - RIPK3	1687	19	24	MUC17 - RIPK3	342	64	329
MUC13 - RIPK4	562	532	184	MUC17 - RIPK4	209	2335	1080
Ranking of MUC20 w.r.t RIPK family							
	laplace	linear	rbf				
MUC20 - RIPK1	1419	760	1794				
MUC20 - RIPK2	948	2482	137				
MUC20 - RIPK3	2192	2288	1796				
MUC20 - RIPK4	1564	1619	2179				

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Table 5. 2^{nd} order interaction ranking between RIPK w.r.t MUC family members.

RANKING RIPK FAMILY W.R.T MUC FAMILY							
RANKING OF RIPK FAMILY W.R.T MUC1				RANKING OF RIPK FAMILY W.R.T MUC3A			
	laplace	linear	rbf		laplace	linear	rbf
MUC1 - RIPK1	1839	58	2421	MUC3A - RIPK1	783	1668	1842
MUC1 - RIPK2	1913	2091	954	MUC3A - RIPK2	758	2301	459
MUC1 - RIPK3	1038	268	295	MUC3A - RIPK3	268	1595	1893
MUC1 - RIPK4	1385	2246	1298	MUC3A - RIPK4	1770	1109	1461
RANKING OF RIPK FAMILY W.R.T MUC4				RANKING OF RIPK FAMILY W.R.T MUC12			
	laplace	linear	rbf		laplace	linear	rbf
MUC4 - RIPK1	562	1621	2216	MUC12 - RIPK1	1462	682	2351
MUC4 - RIPK2	383	924	494	MUC12 - RIPK2	989	597	1798
MUC4 - RIPK3	541	43	129	MUC12 - RIPK3	2158	1286	1636
MUC4 - RIPK4	1981	1949	2028	MUC12 - RIPK4	1577	975	976
RANKING OF RIPK FAMILY W.R.T MUC13				RANKING OF RIPK FAMILY W.R.T MUC17			
	laplace	linear	rbf		laplace	linear	rbf
MUC13 - RIPK1	1961	1535	32	MUC17 - RIPK1	260	446	260
MUC13 - RIPK2	784	494	1467	MUC17 - RIPK2	1021	1114	2355
MUC13 - RIPK3	860	1514	1425	MUC17 - RIPK3	427	223	128
MUC13 - RIPK4	107	1387	1972	MUC17 - RIPK4	1567	2225	2048
RANKING OF RIPK FAMILY W.R.T MUC20							
	laplace	linear	rbf				
MUC20 - RIPK1	514	2042	420				
MUC20 - RIPK2	1039	1751	1950				
MUC20 - RIPK3	303	2504	280				
MUC20 - RIPK4	794	1193	989				

In table 5, RIPK-1/2 was found to be highly upregulated with MUC1. This is reflected in the rankings of 1839 (laplace) and 2421 (rbf) for MUC1 - RIPK1; and 1913 (laplace) and 2091 (linear) for MUC1 - RIPK2. RIPK4 was found to be highly upregulated with MUC4. This is reflected in the rankings of 1981 (laplace), 1949 (linear) and 2028 for MUC4 - RIPK4. RIPK4 was found to be highly up regulated with MUC17. This is reflected in the rankings of 2225 (linear) and 2048 (rbf) for MUC17 - RIPK4. RIPK2 was found to be highly up regulated with MUC20. This is reflected in the rankings of 1751 (linear) and 1950 (rbf) for MUC20 - RIPK2.

One can also interpret the results of the table 6 graphically, with the following influences - • MUC w.r.t RIKP family with MUC1 $<-$ RIPK1; MUC3A $<-$ RIPK3; MUC12 $<-$ RIPK4; MUC20 $<-$ RIPK3 and • RIPK w.r.t MUC family with MUC1 $->$ RIPK-1/2; MUC4 $->$ RIPK4; MUC17 $->$ RIPK4; MUC20 $->$ RIPK2.

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Table 6. 2^{nd} order combinatorial hypotheses between MUC and RIPK.

UNEXPLORED COMBINATORIAL HYPOTHESES	
MUC w.r.t RIKP family	
MUC1	RIPK1
MUC3A	RIPK3
MUC12	RIPK4
MUC20	RIPK3
RIPK w.r.t MUC family	
MUC1	RIPK1/RIPK2
MUC4	RIPK4
MUC17	RIPK4
MUC20	RIPK2

3.1.3. TNF - NF-κB-2/I Cross Family Analysis

The NF-κB family and NF-κB-Inhibitor i.e NF-κB-I play a significant role in immune response to infection. Problems in its functioning leads to cancer, infections, inflammatory and autoimmune diseases. The discovery and seminal work by Sen and Baltimore [13] on NF-κB lead to range of research on immune responses and study of related pathological cases. Tanaka and Nakano [14] have shown that NF-κB2 limits TNF-α induced osteoclastogenesis. Recently, in Japanese population, Imamura *et al.* [15] show that the impaired NF-κBIE gene function decreases cellular uptake of methotrexate by down-regulating SLC19A1 expression in a human rheumatoid arthritis cell line. They postulate that NF-κBIE could be closely related to NF-κB activity. Also, Lee *et al.* [16] show through deep study of fold-change analysis of the inter-relation between NF-κB and TNFs. However, the synergy between these members has yet not been explored completely. We found some interesting combinations that were allocated high numerical ranking (in silico) to indicate synergistic up regulation in CRC cells after ETC-1922159 treatment, apart from the individual up regulation that was observed in wet experiements.

Tables 7 and 8 depict the rankings of TNF family w.r.t to NF-κB-2/I and vice versa, respectively. Followed by this is table 9 that contains the derived influences via majority voting of the rankings in the tables containing two-way cross family rankings.

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Table 7. 2^{nd} order interaction ranking between TNF w.r.t NFkB-2/I family members.

Ranking TNF family w.r.t NFkB-2/I family						
Ranking of TNF family w.r.t NFkB2				Ranking of TNF family w.r.t NFkBI-A			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - TNF	1620	615	1897	NFkBI-A - TNF	820	1495	1109
NFkB2 - TNF-AIP1	324	649	1387	NFkBI-A - TNF-AIP1	1779	1904	1400
NFkB2 - TNF-AIP2	1437	715	1986	NFkBI-A - TNF-AIP2	1247	217	766
NFkB2 - TNF-AIP3	1272	1574	441	NFkBI-A - TNF-AIP3	776	981	212
NFkB2 - TNF-RSF1A	30	2465	575	NFkBI-A - TNF-RSF1A	1580	1422	43
NFkB2 - TNF-RSF10A	2095	817	2509	NFkBI-A - TNF-RSF10A	2499	1438	2191
NFkB2 - TNF-RSF10B	37	1411	250	NFkBI-A - TNF-RSF10B	2075	1555	1401
NFkB2 - TNF-RSF10D	2473	12	1499	NFkBI-A - TNF-RSF10D	2498	2344	2501
NFkB2 - TNF-RSF12A	1813	824	1893	NFkBI-A - TNF-RSF12A	2337	1101	1491
NFkB2 - TNF-RSF14	1799	834	302	NFkBI-A - TNF-RSF14	1974	2045	1136
NFkB2 - TNF-RSF21	332	1973	1719	NFkBI-A - TNF-RSF21	1119	951	903
NFkB2 - TNF-SF10	1627	1614	1299	NFkBI-A - TNF-SF10	2185	499	2316
NFkB2 - TNF-SF15	564	2437	1064	NFkBI-A - TNF-SF15	564	1684	1473
Ranking of TNF family w.r.t NFkBI-E				Ranking of TNF family w.r.t NFkBI-Z			
	laplace	linear	rbf		laplace	linear	rbf
NFkBI-E - TNF	2443	925	228	NFkBI-Z - TNF	851	776	850
NFkBI-E - TNF-AIP1	1720	685	971	NFkBI-Z - TNF-AIP1	153	397	621
NFkBI-E - TNF-AIP2	2347	1863	964	NFkBI-Z - TNF-AIP2	2188	432	566
NFkBI-E - TNF-AIP3	559	1663	280	NFkBI-Z - TNF-AIP3	775	10	2362
NFkBI-E - TNF-RSF1A	846	1624	176	NFkBI-Z - TNF-RSF1A	399	2006	93
NFkBI-E - TNF-RSF10A	840	359	952	NFkBI-Z - TNF-RSF10A	1380	2004	1540
NFkBI-E - TNF-RSF10B	835	2257	1294	NFkBI-Z - TNF-RSF10B	2204	1438	1991
NFkBI-E - TNF-RSF10D	2454	1018	1566	NFkBI-Z - TNF-RSF10D	2214	2033	2514
NFkBI-E - TNF-RSF12A	383	166	1464	NFkBI-Z - TNF-RSF12A	1638	2370	1841
NFkBI-E - TNF-RSF14	1877	2282	1426	NFkBI-Z - TNF-RSF14	1120	1505	1899
NFkBI-E - TNF-RSF21	2129	1293	831	NFkBI-Z - TNF-RSF21	207	804	344
NFkBI-E - TNF-SF10	890	1096	1816	NFkBI-Z - TNF-SF10	609	1088	1344
NFkBI-E - TNF-SF15	523	1957	32	NFkBI-Z - TNF-SF15	1237	1375	2196

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Table 8. 2^{nd} order interaction ranking between NFkB-2/I family w.r.t TNF family members.

Ranking NFkB-2/I family w.r.t TNF family							
Ranking of NFkB-2/I family w.r.t TNF				Ranking of NFkB-2/I family w.r.t TNF-AIP1			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF	1632	989	1453	NFkB-2 - TNF-AIP1	2027	1807	1140
NFkBI-A - TNF	904	561	658	NFkBI-A - TNF-AIP1	2072	349	1218
NFkBI-E - TNF	2116	1247	803	NFkBI-E - TNF-AIP1	56	420	1551
NFkBI-Z - TNF	691	51	265	NFkBI-Z - TNF-AIP1	499	1648	646
Ranking of NFkB-2/I family w.r.t TNF-AIP2				Ranking of NFkB-2/I family w.r.t TNF-AIP3			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF-AIP2	2077	1027	2224	NFkB-2 - TNF-AIP3	1042	2336	2130
NFkBI-A - TNF-AIP2	499	22	1192	NFkBI-A - TNF-AIP3	1452	411	637
NFkBI-E - TNF-AIP2	526	1755	338	NFkBI-E - TNF-AIP3	711	1686	2041
NFkBI-Z - TNF-AIP2	452	988	1617	NFkBI-Z - TNF-AIP3	1979	886	278
Ranking of NFkB-2/I family w.r.t TNF-RSF1A				Ranking of NFkB-2/I family w.r.t TNF-RSF10A			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF-RSF1A	648	164	990	NFkB-2 - TNF-RSF10A	611	1007	454
NFkBI-A - TNF-RSF1A	435	1454	130	NFkBI-A - TNF-RSF10A	458	190	1412
NFkBI-E - TNF-RSF1A	431	980	1417	NFkBI-E - TNF-RSF10A	1719	263	374
NFkBI-Z - TNF-RSF1A	550	2213	1447	NFkBI-Z - TNF-RSF10A	342	742	732
Ranking of NFkB-2/I w.r.t TNF-RSF10B				Ranking of NFkB-2/I w.r.t TNF-RSF10D			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF-RSF10B	713	1408	2397	NFkB-2 - TNF-RSF10D	123	1939	543
NFkBI-A - TNF-RSF10B	1237	1054	562	NFkBI-A - TNF-RSF10D	371	948	584
NFkBI-E - TNF-RSF10B	1352	931	2142	NFkBI-E - TNF-RSF10D	2136	621	1811
NFkBI-Z - TNF-RSF10B	165	2407	361	NFkBI-Z - TNF-RSF10D	259	400	1341
Ranking of NFkB-2/I family w.r.t TNF-RSF12A				Ranking of NFkB-2/I family w.r.t TNF-RSF14			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF-RSF12A	250	341	1232	NFkB-2 - TNF-RSF14	299	1253	543
NFkBI-A - TNF-RSF12A	689	2225	17	NFkBI-A - TNF-RSF14	280	1126	277
NFkBI-E - TNF-RSF12A	1188	1133	765	NFkBI-E - TNF-RSF14	278	2025	1557
NFkBI-Z - TNF-RSF12A	973	1590	2298	NFkBI-Z - TNF-RSF14	131	893	1953
Ranking of NFkB-2/I family w.r.t TNF-RSF21				Ranking of NFkB-2/I family w.r.t TNF-SF10			
	laplace	linear	rbf		laplace	linear	rbf
NFkB-2 - TNF-RSF21	250	341	1232	NFkB-2 - TNF-SF10	1643	496	743
NFkBI-A - TNF-RSF21	689	2225	17	NFkBI-A - TNF-SF10	262	1238	1352
NFkBI-E - TNF-RSF21	1188	1133	765	NFkBI-E - TNF-SF10	985	1090	158
NFkBI-Z - TNF-RSF21	973	1590	2298	NFkBI-Z - TNF-SF10	537	1557	2104
Ranking of NFkB-2/I family w.r.t TNF-SF15							
	laplace	linear	rbf				
NFkB-2 - TNF-SF15	1521	786	1211				
NFkBI-A - TNF-SF15	2367	325	1079				
NFkBI-E - TNF-SF15	97	1868	1195				
NFkBI-Z - TNF-SF15	774	407	372				

Table 9. 2^{nd} order combinatorial hypotheses between NFkB-2/I and TNF.

Unexplored combinatorial hypotheses	
TNF w.r.t NFkB-2/I	
NFkB2	TNF-RSF10A/RSF12A
NFkBI-A	TNF-AIP1/RSF10A/RSF10D/RSF14/SF10
NFkBI-E	TNF-AIP2/RSF14
NFkBI-Z	TNF-RSF10B/RSF10D/RSF12A
NFkB-2/I w.r.t TNF	
NFkB-2	TNF-AIP1/AIP2/AIP3
NFkBI-E	TNF-RSF10D

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In table 7 we find TNF-RSF10A/RSF12A up regulated with NFkB2. These are reflected in rankings of 2095 (laplace) and 2509 (rbf) for NFkB2 - TNFRSF10A; and 1813 (laplace) and 1893 (rbf) for NFkB2 - TNFRSF12A. TNF-AIP1/RSF10A/RSF10D/RSF14/SF10 were found to be up regulated with NFkBI-A. These are reflected in rankings of 1779 (laplace) and 1904 (linear) for NFkBI-A - TNF-AIP1; 2499 (laplace) and 2191 (rbf) for NFkBI-A - TNFRSF10A; 2498 (laplace), 2344 (linear) and 2501 (rbf) for NFkBI-A - TNFRSF10D; 1974 (laplace) and 2045 (linear) for NFkBI-A - TNFRSF14; and 2185 (laplace) and 2316 (rbf) for NFkBI-A - TNFSF10, respectively. TNF-AIP2/RSF14 were found to be up regulated with NFkBI-E. These are reflected in rankings of 2347 (laplace) and 1863 (linear) for NFkBI-E - TNFAIP2; and 1877 (laplace) and 2282 (linear) for NFkBI-E - TNFRSF14, respectively. Finally, TNF-RSF10B/RSF10D/RSF12A were found to be up regulated with NFkBI-Z. These are reflected in rankings of 2204 (laplace) and 1991 (rbf) for NFkBI-Z - TNFRSF10B; 2214 (laplace), 2033 (linear) and 2514 (rbf) for NFkBI-Z - TNFRSF10D; and 2370 (linear) and 1841 (rbf) for NFkBI-Z - TNFRSF12A, respectively. In table 8 we find NFkB-2 to be up regulated along with TNF-AIP1/AIP2/AIP3. These are reflected in rankings of 2027 (linear) and 1807 (rbf) for NFkB2 - TNFAIP1; 2077 (laplace) and 2224 (rbf) for NFkB2 - TNFAIP2; and 2336 (linear) and 2130 (rbf) for NFkB2 - TNFAIP3, respectively. Finally, NFkBI-E was found to be up regulated with TNFRSF10D. These are reflected in rankings of 2136 (laplace) and 1811 (rbf) for NFkBI-E - TNFRSF10D.

One can also interpret the results of the table 9 graphically, with the following influences - • TNF w.r.t NFkB family with NFkB2 $->$ TNF-RSF10A/RSF12A; NFkBI-A $->$ TNF-AIP1/RSF10A/RSF10D /RSF14/SF10; NFkBI-E $->$ TNF-AIP2/RSF14; NFkBI-Z $->$ TNF-RSF10B/RSF10D/RSF12A; and • NFkB w.r.t TNF family with NFkB-2 $<-$ TNF-AIP1/AIP2/AIP3 and NFkBI-E $<-$ TNF-RSF10D.

3.1.4. NFκB-2/I - STAT Cross Family Analysis

Grivennikov and Karin [17] show the potent collaboration and cross talk of STAT3 and NF-κB in cancer. In chronic lymphocytic leukemia cells, Liu *et al.* [18] observe that STAT-3 activates NF-κB. Co-opertion between STAT3 and NF-κB pathways has been observed in subtypes of diffuse large B Cell Lymphoma by Lam *et al.* [19]. Lee *et al.* [20] also shows a signal network involving coactivated NF-κB and STAT3 and altered p53 modulates BAX/BCL-XL expression and promotes cell survival of head and neck squamous cell carcinomas. These observations show a definite, concommitent functioning of the two pathways and we further found that some of them were up regulated synergistically in CRC cells after ETC-1922159 treatment, via in silico ranking of the combinations. Tables 10 and 11 show ranking of STAT family w.r.t NFkB-2/I and vice versa, respectively. Followed by this is the derived influences from majority voting of rankings in the two foregoing tables, which is shown in table 12.

Table 10. 2^{nd} order interaction ranking between STAT w.r.t NFkB-2/I family members.

Ranking STAT family w.r.t NFκB-2/I family							
Ranking of STAT2 w.r.t NFκB-2/I family				Ranking of STAT3 w.r.t NFκB-2/I family			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - STAT2	2220	1068	1207	NFkB2 - STAT3	2125	252	1453
NFkBIA - STAT2	2211	1253	2402	NFkBIA - STAT3	1614	702	1333
NFkBIE - STAT2	1809	512	1207	NFkBIE - STAT3	1493	211	1850
NFkBIZ - STAT2	802	2121	1862	NFkBIZ - STAT3	1633	1679	2122
Ranking of NFκB-2/I family w.r.t STAT5A							
	laplace	linear	rbf				
NFkB2 - STAT5A	2034	1321	1502				
NFkBIA - STAT5A	490	2215	283				
NFkBIE - STAT5A	578	1969	2485				
NFkBIZ - STAT5A	2286	473	1409				

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Table 11. 2^{nd} order interaction ranking between NFkB-2/I family w.r.t STAT members.

RANKING NFKB-2/I FAMILY W.R.T STAT FAMILY							
RANKING OF NFKB-2/I FAMILY W.R.T STAT2				RANKING OF NFKB-2/I FAMILY W.R.T STAT3			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - STAT2	935	952	86	NFkB2 - STAT3	858	606	162
NFkBIA - STAT2	543	36	1180	NFkBIA - STAT3	1547	88	476
NFkBIE - STAT2	1449	1861	1262	NFkBIE - STAT3	1731	1063	509
NFkBIZ - STAT2	483	1150	262	NFkBIZ - STAT3	1262	489	1145
RANKING OF NFKB-2/I FAMILY W.R.T STAT3							
	laplace	linear	rbf				
NFkB2 - STAT5A	558	1070	670				
NFkBIA - STAT5A	1509	1020	81				
NFkBIE - STAT5A	18	854	1052				
NFkBIZ - STAT5A	83	1208	240				

Table 12. 2^{nd} order combinatorial hypotheses between NFkB-2/I and TNF

UNEXPLORED COMBINATORIAL HYPOTHESES	
STAT w.r.t NFkB-2/I	
NFkBIA	STAT2
NFkBIZ	STAT2
NFkBIE	STAT5A

Tables 10 and 11 show the rankings of STAT family w.r.t NFkB-2/I and vice versa, respectively. Followed by this is the influence between the components in table 12, via majority voting of the rankings. In the drug treated CRC cells, we found members of the STAT family to be up regulated with NFkB-2/I. These are reflected with rankings of 2211 (laplace) and 2402 (rbf) for NFkBIA $->$ STAT2; 2121 (linear) and 1862 (rbf) for NFkBIZ $->$ STAT2; and 1969 (linear) and 2485 (rbf) for NFkBIE $->$ STAT5A, respectively. One can also interpret the results of the table 12 graphically, with the following influences - • STAT w.r.t NFkB-2/I with NFkBIA $->$ STAT2; NFkBIZ $->$ STAT2; and NFkBIE $->$ STAT5A;

3.1.5. IKBKE and STAT Cross Family Analysis

Ng *et al.* [21] show that phosphorylation of STAT1 by IκB kinase ε (IKBKE) inhibits STAT1 homodimerization, and thus assembly of GAF, but does not disrupt ISGF3 formation. Furthermore, Guo *et al.* [22] show that IKBKE is induced by STAT3 and tobacco carcinogen and determines chemosensitivity in non-small cell lung cancer. It has already been established in some cases that IKBKE has a confirmed role with one of the STAT members. Here we found that both IKBKE and STAT were up regulated after ETC-1922159 treatment of CRC cells. Table 13 shows ranking of STAT family vs IKBKE and vice versa. Table 14 shows the dervied influences from majority voting of the rankings. On the left half of table 13 we find STAT2 to be up regulated w.r.t IKBKE. This is reflected with the rankings of 2033 (linear) and 1892 (rbf) for STAT2 - IKBKE. On the right half of the same table we find IKBKE being up regulated w.r.t STAT-3/5A. These are reflected in rankings of 2179 (linear) and 1976 (rbf) for STAT3 - IKBKE; and 2085 (laplace), 2409 (linear) and 2277 (rbf) for STAT5A - IKBKE, respectively. One can also interpret the results of the table 14 graphically, with the following influences - • STAT w.r.t IKBKE with STAT2 $<-$ IKBKE; and • IKBKE w.r.t STAT with STAT3 $->$ IKBKE and STAT5A $->$ IKBKE;

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Table 13. 2^{nd} order interaction ranking between STAT family w.r.t IKBKE.

RANKING STAT FAMILY VS IKBKE							
RANKING OF STAT FAMILY W.R.T IKBKE FAMILY				RANKING OF IKBKE W.R.T STAT FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
STAT2 - IKBKE	1267	2033	1892	STAT2 - IKBKE	1604	554	2108
STAT3 - IKBKE	1055	2144	1672	STAT3 - IKBKE	1442	2179	1976
STAT5A - IKBKE	178	1687	1183	STAT5A - IKBKE	2085	2409	2277

Table 14. 2^{nd} order combinatorial hypotheses between NFkB-2/I and TNF

UNEXPLORED COMBINATORIAL HYPOTHESES	
STAT w.r.t IKBKE	
STAT2	IKBKE
IKBKE w.r.t STAT	
STAT3	IKBKE
STAT5A	IKBKE

3.1.6. IKBKE - TRAF Cross Family Analysis

Shen *et al.* [23] show interaction of IKBKE with TRAF2, by observing that IκB kinase ε phosphorylates TRAF2 to promote mammary epithelial cell transformation. Zhou *et al.* [24] observe IKKε-mediated tumorigenesis requires K63-linked polyubiquitination by a cIAP1/cIAP2/TRAF2 E3 ubiquitin ligase complex. Also, Nakanishi and Akira [25] show NF-κB activation through IKK-i-dependent I-TRAF/TANK phosphorylation. These findings suggest interaction between IKBKE - TRAF family members. IKBKE and TRAF members were found to be up regulated in CRC cells treated with ETC-1922159. Their combinations were allocated with high numerical ranks indicating synergistic up regulation. Table 15 rankings between TRAF and IKBKE, both ways. TRAF4 was found to up regulated with IKBKE and the rankings reflect the same with 2158 (linear) and 2416 (rbf). Also IKBKE was found to be up regulated with TRAF6 and the rankings reflect the same with 2105 (laplace) and 1819 (rbf). Table 16 reflects the derived influences graphically for - • TRAF w.r.t IKBKE with TRAF6 $<-$ IKBKE and • IKBKE w.r.t TRAF with TRAF4 $->$ IKBKE.

Table 15. 2^{nd} order interaction ranking between STAT family w.r.t IKBKE.

RANKING TRAF FAMILY VS IKBKE							
RANKING OF IKBKE W.R.T TRAF FAMILY				RANKING OF TRAF FAMILY W.R.T IKBKE			
	laplace	linear	rbf		laplace	linear	rbf
TRAF4 - IKBKE	1235	2158	2416	TRAF4 - IKBKE	1606	461	1330
TRAF6 - IKBKE	1694	389	1554	TRAF6 - IKBKE	2105	1376	1819
TRAFD1 - IKBKE	1687	532	1793	TRAFD1 - IKBKE	866	733	496
TRAF3IP2 - IKBKE	1349	738	1987	TRAF3IP2 - IKBKE	924	1966	334

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Table 16. 2^{nd} order combinatorial hypotheses between NFkB-2/I and TNF

UNEXPLORED COMBINATORIAL HYPOTHESES	
TRAF w.r.t IKBKE	
TRAF6	IKBKE
IKBKE w.r.t TRAF	
TRAF4	IKBKE

3.1.7. ABC Transporters - NFκB Cross Family Analysis

Gerbod-Giannone *et al.* [26] observe that TNFα induces ABCA1 through NF-κB in macrophages and in phagocytes ingesting apoptotic cells. ABCA1 has also been found to be a key regulator in cholesterol related problems. Van Eck *et al.* [27] report leukocyte ABCA1 controls susceptibility to atherosclerosis and macrophage recruitment into tissues. The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor, as shown by Tang *et al.* [28]. Furthermore, macrophage ABCA1 reduces MyD88-dependent Toll-like receptor trafficking to lipid rafts by reduction of lipid raft cholesterol, as shown by Zhu *et al.* [29]. These findings suggest the intricate role of NFκB family components play with ABC transporters. Both were up regulated in CRC cells after treatment with ETC-1922159. Our search engine allocated numerically high rank to several of the combinations in silico. These have been tabulated in tables 17 and 18, i.e rankings of ABC transporters w.r.t NFkB members and vice versa, respectively. Table 19 shows the un explored hypotheses between the two in the form of the derived influences after majority voting of the two-way cross family the rankings.

Table 17. 2^{nd} order interaction ranking between ABC w.r.t NFkB-2/I family members.

RANKING ABC FAMILY W.R.T NFKB-2/I FAMILY							
RANKING OF ABC FAMILY W.R.T NFKB2				RANKING OF ABC FAMILY W.R.T NFKBI-A			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - ABC-A5	851	1517	350	ABC-A5 - NFkBIA	398	365	1660
ABC-B11 - NFkB2	1684	400	412	ABC-B11 - NFkBIA	1079	566	104
NFkB2 - ABC-C3	127	2031	6	ABC-C3 - NFkBIA	601	1048	1760
NFkB2 - ABC-C5	1035	1431	889	NFkBIA - ABC-C5	1683	2404	1341
NFkB2 - ABC-C13	1399	1951	747	NFkBIA - ABC-C13	200	886	1275
NFkB2 - ABC-D1	1317	1133	1773	ABC-D1 - NFkBIA	1361	1361	1432
NFkB2 - ABC-G1	1983	1343	1140	ABC-G1 - NFkBIA	21	313	461
NFkB2 - ABC-G2	1322	955	1292	ABC-G2 - NFkBIA	809	613	48
RANKING OF ABC FAMILY W.R.T NFKBI-E				RANKING OF ABC FAMILY W.R.T NFKBI-Z			
	laplace	linear	rbf		laplace	linear	rbf
ABC-A5 - NFkBIE	1445	1662	679	ABC-A5 - NFkBIZ	699	1806	1290
ABC-B11 - NFkBIE	2285	1154	54	ABC-B11 - NFkBIZ	1240	37	803
ABC-C3 - NFkBIE	1547	2168	355	ABC-C3 - NFkBIZ	468	1366	1571
NFkBIE - ABC-C5	876	2048	1735	ABC-C5 - NFkBIZ	1278	1714	1065
NFkBIE - ABC-C13	623	1992	2351	ABC-C13 - NFkBIZ	1083	1063	1386
ABC-D1 - NFkBIE	2380	1795	861	ABC-D1 - NFkBIZ	1677	1688	794
ABC-G1 - NFkBIE	2193	251	208	ABC-G1 - NFkBIZ	979	2373	590
ABC-G2 - NFkBIE	2124	383	766	ABC-G2 - NFkBIZ	86	77	845

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Table 18. 2^{nd} order interaction ranking between NFkB-2/I w.r.t ABC family members.

Ranking NFkB-2/I family w.r.t ABC family							
Ranking of NFkB-2/I family w.r.t ABC-A5				Ranking of NFkB-2/I family w.r.t ABC-B11			
	laplace	linear	rbf		laplace	linear	rbf
ABC-A5 - NFkB2	2097	1772	2086	NFkB2 - ABC-B11	1916	1955	1020
ABC-A5 - NFkBIA	827	1142	379	NFkBIA - ABC-B11	365	1702	602
ABC-A5 - NFkBIE	1276	1749	1795	NFkBIE - ABC-B11	893	1285	1173
ABC-A5 - NFkBIZ	778	272	930	NFkBIZ - ABC-B11	683	254	421
Ranking of NFkB-2/I family w.r.t ABC-C3				Ranking of NFkB-2/I family w.r.t ABC-C5			
	laplace	linear	rbf		laplace	linear	rbf
ABC-C3 - NFkB2	1225	936	281	NFkB2 - ABC-C5	1510	1712	939
ABC-C3 - NFkBIA	782	271	1996	NFkBIA - ABC-C5	2017	953	1649
ABC-C3 - NFkBIE	1071	1094	308	NFkBIE - ABC-C5	567	615	1600
ABC-C3 - NFkBIZ	546	653	841	ABC-C5 - NFkBIZ	1978	943	160
Ranking of NFkB-2/I family w.r.t ABC-C13				Ranking of NFkB-2/I family w.r.t TNF-ABC-D1			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - ABC-C13	618	1423	1550	NFkB2 - ABC-D1	2094	1655	318
NFkBIA - ABC-C13	1499	1092	456	NFkBIA - ABC-D1	613	1812	1581
NFkBIE - ABC-C13	2318	586	2513	NFkBIE - ABC-D1	806	2204	410
NFkBIZ - ABC-C13	1799	2175	1068	NFkBIZ - ABC-D1	16	1723	955
Ranking of NFkB-2/I family w.r.t ABC-G1				Ranking of NFkB-2/I family w.r.t ABC-G2			
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - ABC-G1	1951	2240	2215	NFkB2 - ABC-G2	957	1427	788
NFkBIA - ABC-G1	1155	258	238	NFKBIA - ABC-G2	508	417	686
NFkBIE - ABC-G1	2034	612	490	NFKBIE - ABC-G2	2223	806	685
NFkBIZ - ABC-G1	1146	324	900	NFkBIZ - ABC-G2	229	221	1196

Table 19. 2^{nd} order combinatorial hypotheses between NFkB-2/I and ABC

Unexplored combinatorial hypotheses	
ABC w.r.t NFkB-2/I family	
NFkIBE	ABC-C13/ABC-D1
NFkB-2/I w.r.t ABC family	
NFkB2	ABC-A5/ABC-B11
NFkBIE	ABC-C13
NFkBIZ	ABC-C13
NFkB2	ABC-G1

In table 17, we find ABC-C13/ABC-D1 to be up regulated w.r.t. NFkBIE. These are reflected in rankings of 2048 (linear) and 1735 (rbf) for ABC-C13 - NFkBIE and 2380 (laplace) and 1795 (linear) for ABC-D1 - NFkBIE, respectively. In table 18, we find NFkB2 to be up regulated w.r.t ABC-A5/ABC-B11. These are reflected in rankings of 2097 (laplace), 1772 (linear) and 2086 (rbf) for NFkB2 - ABC-A5; and 1916 (linear) and 1955 (rbf) for NFkB2 - ABC-B11, respectively. NFkBIE was up regulated with ABC-C13 and the rankings for the same are reflected in 2318 (laplace) and 2513 (rbf). Also, NFkBIZ was up regulated with ABC-C13 and the rankings for the same are reflected in 1799 (laplace) and 2175 (linear). NFkB2 was up regulated with ABC-G1 and the rankings for the same are reflected in 1951 (laplace), 2240 (linear) and 2215 (rbf).

Finally, 19 shows derived influences which can be represented graphically, with the following influences - • ABC w.r.t NFkB-2/I family with NFkIBE $->$ ABC-C13/ABC-D1 and • NFkB-2/I w.r.t ABC family with NFkB2 $<-$ ABC-A5/ABC-B11; NFkBIE $<-$ ABC-C13; NFkBIZ $<-$ ABC-C13 and NFkB2 $<-$ ABC-G1;

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3.1.8. IKBKE - UBA/UBE Cross Family Analysis

Not much is known about IKBKE and Ubiquitination modifier enzyme and ubiquitination conjugating enzymes interaction. They were found them to be up regulated in CRC cells after ETC-1922159 treatment. Our search engine allocated high ranks to some of the combinations between IKBKE and UBA/UBE family members. These combinations might be worth exploring if it is of interest. Tables 20 shows the rankings of UBE/A w.r.t to IKBKE and vice versa. We find IKBKE to be up regulated w.r.t UBA/E2 family. These are reflected with rankings of 2327 (laplace), 1807 (linear) and 2066 (rbf) for IKBKE - UBA-1; 2326 (linear) and 2456 (rbf) IKBKE - UBA-7; 2162 (laplace) and 1817 (linear) for IKBKE - UBA-P1; 2422 (laplace) and 2328 (rbf) for IKBKE - UBE2-A; 2367 (linear) and 2427 (rbf) for IKBKE - UBE2-B; and finally 2366 (laplace) and 1909 (rbf) for IKBKE - UBE2-Z; We also find UBA/E2 family to be up regulated w.r.t IKBKE also. This is reflected in rankings of 2189 (laplace) and 2271 (linear) for IKBKE - UBA-7; 2262 (laplace), 1901 (linear) and 2341 (rbf) for IKBKE - UBA-P1; 2293 (laplace), 2319 (linear) and 2396 (rbf) for IKBKE - UBE2-A; 2129 (laplace) and 1795 (linear) for IKBKE - UBE2-B; 2494 (laplace), 2233 (linear) and 1896 (rbf) for IKBKE - UBE2-F; 2016 (laplace) and 2103 (linear) for IKBKE - UBE2-Z;

Table 20. 2^{nd} order interaction ranking between UBA/E2 family w.r.t IKBKE.

Ranking UBA/E2 family vs IKBKE							
Ranking of UBA/E2 family w.r.t IKBKE				Ranking of IKBKE w.r.t UBA/E2 family			
	laplace	linear	rbf		laplace	linear	rbf
IKBKE - UBA-1	1752	785	966	UBA-1 - IKBKE	2327	1807	2066
IKBKE - UBA-7	2189	2271	1335	IKBKE - UBA-7	1134	2326	2456
IKBKE - UBA-P1	2262	1901	2341	IKBKE - UBA-P1	2162	1817	1407
IKBKE - UBA-LD2	2034	1773	1409	IKBKE - UBA-LD2	1381	1647	556
IKBKE - UBE2-A	2293	2319	2396	IKBKE - UBE2-A	2422	536	2328
IKBKE - UBE2-B	2129	1516	1795	IKBKE - UBE2-B	680	2367	2427
IKBKE - UBE2-F	2494	2233	1896	IKBKE - UBE2-F	2309	181	24
IKBKE - UBE2-H	1265	1666	1257	IKBKE - UBE2-H	385	710	746
IKBKE - UBE2-J1	905	1936	1046	IKBKE - UBE2-J1	903	1729	2215
IKBKE - UBE2-Z	2016	2103	481	IKBKE - UBE2-Z	783	2366	1909

Table 21 shows the derived influences which can be represented graphically, with the following influences - • UBA/E2 w.r.t IKBKE with IKBKE $->$ UBA-1; IKBKE $->$ UBA-7; IKBKE $->$ UBA-P1; and IKBKE $->$ UBE2-A; IKBKE $->$ UBE2-B; IKBKE $->$ UBE2-Z •; IKBKE w.r.t UBE/A2 with IKBKE $<-$ UBA-7; IKBKE $<-$ UBA-P1; IKBKE $<-$ UBA-LD2; and IKBKE $<-$ UBE2-A; IKBKE $<-$ UBE2-B; IKBKE $<-$ UBE2-F; IKBKE $<-$ UBE2-Z;

Table 21. 2^{nd} order combinatorial hypotheses between NFkB-2/I and TNF

Unexplored combinatorial hypotheses	
UBA/E2 w.r.t IKBKE	
IKBKE	UBA-1/7/P1
IKBKE	UBE2-A/B/Z
IKBKE w.r.t UBE/A2	
IKBKE	UBA-7/P1/LD2
IKBKE	UBE2-A/B/F/Z

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3.1.9. REL-A/B - NFκB Cross Family Analysis

REL-A is known to be associated with NF-κB and most deeply studied member of the NF-κB. Tian *et al.* [30] observe that the NFkB subunit RELA is a master transcriptional regulator of the committed epithelial-mesenchymal transition in airway epithelial cells. Ke *et al.* [31] observe that inactivation of NF-κB p65 (RelA) in liver improves insulin sensitivity and inhibits cAMP/PKA pathway. Weichert *et al.* [32] observe that high expression of RelA/p65 is associated with activation of NF-κB-dependent signaling in pancreatic cancer. These findings and many others not cited here show the deep interaction between REL and NF-κB members. Table 22 shows rankings of RELA w.r.t NFkB members and vice versa. Table 23 shows rankings of RELB w.r.t NFkB members and vice versa. Finally, table 24 shows the hypotheses generated from majority voting of the ranks. In table 22 we find RELA to be up regulated w.r.t NFKB2. This is reflected in rankings of 2454 (laplace) and 2307 (rbf) for NFkB2 - RELA. Similarly, NFKBIA was found to be up regulated w.r.t RELA. This is reflected in rankings of 2106 (laplace) and 2305 (linear) for NFKBIA - RELA. In table 23 we find NFkB2 to be up regulated RELB. This is reflected in 2146 (laplace) and 1788 (rbf) for NFkB2 - RELB. Similarly, we find NFKBIZ to be 1776 (laplace), 2244 (linear) and 1869 (rbf) for NFKBIZ - RELB. Table 24 shows the derived influences which can be represented graphically, with the following influences - • NFkB-2/I family w.r.t REL-B with NFkB2 $<-$ REL-B and NFKBIZ $<-$ RELB and • REL-A w.r.t NFkB-2/I family with NFkB2 $->$ RELA and NFKBIA $->$ RELA.

Table 22. 2^{nd} order interaction ranking between NFkB-2/I VS REL-A family members.

Ranking NFkB-2/I VS REL-A						
Ranking of NFkB-2/I family w.r.t REL-A				Ranking of REL-A w.r.t NFkB-2/I family		
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - RELA	664	420	271	NFkB2 - RELA	2454	794	2307
NFKBIA - RELA	198	205	190	NFKBIA - RELA	2106	2305	1153
NFKBIE - RELA	1503	2321	331	NFKBIE - RELA	1664	456	1926
NFKBIZ - RELA	323	1714	619	NFKBIZ - RELA	1924	1687	1584

Table 23. 2^{nd} order interaction ranking between NFkB-2/I VS REL-B family members.

Ranking REL-B VS NFkB-2/I family						
Ranking of NFKB-2/I w.r.t REL-B				Ranking of REL-B w.r.t NFKB-2/I		
	laplace	linear	rbf		laplace	linear	rbf
NFkB2 - RELB	503	2146	1788	NFkB2 - RELB	1156	1346	2184
NFKBIA - RELB	239	1576	924	NFKBIA - RELB	968	424	1725
NFKBIE - RELB	1203	714	2200	NFKBIE - RELB	1414	2228	800
NFKBIZ - RELB	1776	2244	1869	NFKBIZ - RELB	746	1281	1055

Table 24. 2^{nd} order combinatorial hypotheses between NFkB-2/I and ABC

Unexplored combinatorial hypotheses	
NFkB-2/I family w.r.t REL-B	
NFkB2	RELB
NFKBIZ	RELB
REL-A w.r.t NFkB-2/I family	
NFkB2	RELA
NFKBIA	RELA

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Conclusion

Presented here are a range of multiple synergistic NFκB 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.

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.* [33]. 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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