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Article

Machine Learning Discoveries of TNF-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 Tumor necrosis factor (TNF)-X (X, a particular gene/protein) at 2nd order level after drug administration. These rankings reveal which TNF-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 TNF and X. In this research work, we cover combinations of TNF with Wnt, mucin (MUC), six transmembrane epithelial antigen of prostate 4 (STEAP4), ubiquitin conjugating enzyme E2 (UBE2) family and BCL family.

Keywords: TNF; 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In the unpublished preprint Sinha [1], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway.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 Interested readers are advised to go through unpublished preprints Sinha [1] and Sinha [2] for details regarding the search engine and the discoveries mentioned in there.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 pipeline works by computing sensitivity indices 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.

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. Tumor Necrosis Factor Related Synergies

3.1.1. TNF - WNT Cross Family Analysis

Brooks *et al.* [5] observed TNF- α induced alterations in the Wnt signaling cascade as a potential mechanism for obesity-associated colorectal tumorigenesis. Effects of TNF inhibitors on parathyroid hormone and Wnt signaling antagonists in rheumatoid arthritis have been studied in Adami *et al.* [6]. A complex interaction between Wnt signaling and TNF- α in nucleus pulposus cells has been studied by Hiyama *et al.* [7]. Ma and Hottiger [8] study the crosstalk between Wnt/ β -catenin and NF- κ B signaling pathway during inflammation. Roubert *et al.* [9] study the influence of tumor necrosis factor- α on the tumorigenic Wnt-signaling pathway in human mammary tissue from obese women. Jang *et al.* [10] observe that WNT/ β -catenin pathway modulates the TNF- α -induced inflammatory response in bronchial epithelial cells. These studies suggest already existing synergistic roles of WNTs and TNFs. In CRC cells affected with ETC-1922159, members of TNF and WNT family were found to be up regulated. Our search engine allotted high numerical valued ranks to some of the combinations between WNTs and TNFs. Table 1 shows rankings of TNF w.r.t to WNTs on the left half and vice versa on the right half.

On the left half, we found **TNF-RSF1A/RSF10A/RSF10B/SF15** to be up regulated w.r.t WNT2B. These were reflected in rankings of 2170 (laplace) and 2127 (linear) for TNFRSF1A - WNT2B; 1861 (laplace), 2367 (linear) and 1800 (rbf) for TNFRSF10A - WNT2B; 2020 (laplace) and 1881 (rbf) for TNFRSF10B - WNT2B and 2476 (laplace) and 2073 (rbf) for TNFSF15 - WNT2B. **TNF-RSF10A/RSF10D/RSF12A/SF10** were found to be up regulated w.r.t WNT4. These were reflected in rankings of 2509 (laplace) and 2460 (linear) for TNFRSF10A - WNT4; 2233 (linear) and 2126 (rbf) for TNFRSF10D - WNT4; 2294 (linear), 1775 (linear) and 2384 (rbf) for TNFRSF12A - WNT4 and 2451 (linear) and 1782 (rbf) for TNFSF10 - WNT4. **TNF-RSF12A/SF10** were found to be up regulated w.r.t WNT7B. These were reflected in rankings of 2100 (laplace) and 1983 (rbf) for TNFRSF12A - WNT7B and 2462 (laplace) and 2179 (rbf) for TNFSF10 - WNT7B. **TNFRSF21** were found to be up regulated w.r.t WNT9A. These were reflected in rankings of 1805 (laplace) and 1999 (linear) for TNFRSF21 - WNT9A.

On the left half, we found **WNT2B** to be up regulated w.r.t **TNF-RSF10B/RSF10D/RSF14**. These were reflected in rankings of 1797 (laplace) and 2056 (rbf) for TNFRSF10B - WNT2B; 1989 (linear) and 2130 (rbf) for TNFRSF10D - WNT2B and 1932 (laplace) and 2399 (rbf) for TNFRSF14 - WNT2B. **WNT4** was upregulated w.r.t **TNF-AIP3/RSF10B**. These are reflected in rankings of 2336 (laplace), 2511 (linear) and 2342 (rbf) for TNFAIP3 - WNT4 and 2105 (linear) and 2264 (rbf) for TNFRSF10B - WNT4. **WNT7B** was upregulated w.r.t **TNF, TNF-RSF1A/RSF14**. These are reflected in rankings of 2511 (linear) and 2210 (rbf) for TNF - WNT7B; 2084 (laplace), 1975 (linear) and 2154 (rbf) for TNFRSF1A - WNT7B and 2079 (laplace) and 1928 (rbf) for TNFRSF14 - WNT7B. **WNT9A** was upregulated w.r.t **TNF-AIP2/AIP3/RSF10A/RSF12A/SF10**. These are reflected in rankings of 2125 (laplace) and 2437 (linear) for TNFAIP2 - WNT9A; 1764 (laplace) and 2460 (linear) for TNFAIP3 - WNT9A; 2259 (laplace) and 2413 (linear) for TNFRSF10A - WNT9A; 2345 (laplace) and 2466 (rbf) for TNFRSF12A - WNT9A and 2054 (laplace) and 2338 (linear) for TNFSF10 - WNT9A.

Table 1. 2nd order combinatorial hypotheses between ABC and IL

RANKING TNF FAMILY VS WNT FAMILY							
RANKING OF TNF FAMILY W.R.T WNT2B				RANKING OF WNT2B W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TNF - WNT2B	503	893	1656	TNF - WNT2B	1341	808	1366
TNFAIP1 - WNT2B	235	156	1811	TNFAIP1 - WNT2B	1671	1434	1404
TNFAIP2 - WNT2B	868	527	2439	TNFAIP2 - WNT2B	1130	218	1105
TNFAIP3 - WNT2B	1135	2381	1688	TNFAIP3 - WNT2B	997	1280	1902
TNFRSF1A - WNT2B	2170	2127	1628	TNFRSF1A - WNT2B	1747	1857	1550
TNFRSF10A - WNT2B	1861	2367	1800	TNFRSF10A - WNT2B	100	464	1162
TNFRSF10B - WNT2B	2020	615	1881	TNFRSF10B - WNT2B	1797	120	2056
TNFRSF10D - WNT2B	29	2515	1174	TNFRSF10D - WNT2B	1348	1989	2130
TNFRSF12A - WNT2B	1072	2061	1109	TNFRSF12A - WNT2B	1595	298	1432
TNFRSF14 - WNT2B	333	1585	1247	TNFRSF14 - WNT2B	1932	277	2399
TNFRSF21 - WNT2B	1275	648	1114	TNFRSF21 - WNT2B	1396	620	2136
TNFSF10 - WNT2B	1204	2287	1396	TNFSF10 - WNT2B	1732	738	1751
TNFSF15 - WNT2B	2476	359	2073	TNFSF15 - WNT2B	402	128	1875
RANKING OF TNF FAMILY W.R.T WNT4				RANKING OF WNT4 W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TNF - WNT4	1982	1301	928	TNF - WNT4	1021	420	864
TNFAIP1 - WNT4	1434	1078	804	TNFAIP1 - WNT4	1114	337	1015
TNFAIP2 - WNT4	1810	1047	330	TNFAIP2 - WNT4	1611	1341	423
TNFAIP3 - WNT4	646	1955	1534	TNFAIP3 - WNT4	2336	2511	2342
TNFRSF1A - WNT4	915	545	829	TNFRSF1A - WNT4	132	333	1321
TNFRSF10A - WNT4	2509	2460	897	TNFRSF10A - WNT4	535	202	582
TNFRSF10B - WNT4	517	875	1365	TNFRSF10B - WNT4	320	2105	2264
TNFRSF10D - WNT4	1719	2233	2126	TNFRSF10D - WNT4	660	49	341
TNFRSF12A - WNT4	2294	1775	2384	TNFRSF12A - WNT4	649	1756	780
TNFRSF14 - WNT4	1608	2284	1436	TNFRSF14 - WNT4	61	519	1542
TNFRSF21 - WNT4	1915	1596	93	TNFRSF21 - WNT4	201	533	657
TNFSF10 - WNT4	1747	2451	1782	TNFSF10 - WNT4	904	1511	2280
TNFSF15 - WNT4	1542	806	2439	TNFSF15 - WNT4	64	709	793

Table 1. Cont.

RANKING TNF FAMILY VS WNT FAMILY				RANKING TNF FAMILY VS WNT FAMILY			
RANKING OF TNF FAMILY W.R.T WNT7				RANKING OF WNT7 W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TNF - WNT7B	815	381	47	TNF - WNT7B	1530	2511	2210
TNFAIP1 - WNT7B	313	1438	992	TNFAIP1 - WNT7B	2196	519	1058
TNFAIP2 - WNT7B	1897	85	631	TNFAIP2 - WNT7B	2121	599	1313
TNFAIP3 - WNT7B	577	1807	1251	TNFAIP3 - WNT7B	1901	1357	830
TNFRSF1A - WNT7B	165	844	353	TNFRSF1A - WNT7B	2084	1975	2154
TNFRSF10A - WNT7B	1084	1341	2119	TNFRSF10A - WNT7B	1301	1120	1663
TNFRSF10B - WNT7B	1274	1980	744	TNFRSF10B - WNT7B	1209	908	1075
TNFRSF10D - WNT7B	1314	774	1928	TNFRSF10D - WNT7B	1252	2301	1250
TNFRSF12A - WNT7B	2100	1332	1983	TNFRSF12A - WNT7B	1104	22	1879
TNFRSF14 - WNT7B	1576	981	1811	TNFRSF14 - WNT7B	2079	1028	1928
TNFRSF21 - WNT7B	1565	798	720	TNFRSF21 - WNT7B	2114	1219	737
TNFSF10 - WNT7B	1598	2462	2179	TNFSF10 - WNT7B	2129	763	204
TNFSF15 - WNT7B	1026	756	621	TNFSF15 - WNT7B	130	1599	2504
RANKING OF TNF FAMILY W.R.T WNT9A				RANKING OF WNT9A W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TNF - WNT9A	1624	2025	799	TNF - WNT9A	1121	1930	1400
TNFAIP1 - WNT9A	1433	839	465	TNFAIP1 - WNT9A	1254	569	394
TNFAIP2 - WNT9A	40	2167	397	TNFAIP2 - WNT9A	2125	2437	1605
TNFAIP3 - WNT9A	1427	1109	2040	TNFAIP3 - WNT9A	1764	2460	1032
TNFRSF1A - WNT9A	1470	719	1933	TNFRSF1A - WNT9A	1645	58	1419
TNFRSF10A - WNT9A	2272	1234	918	TNFRSF10A - WNT9A	2259	2413	1204
TNFRSF10B - WNT9A	2249	1222	1071	TNFRSF10B - WNT9A	882	566	813
TNFRSF10D - WNT9A	410	2132	968	TNFRSF10D - WNT9A	1808	1055	568
TNFRSF12A - WNT9A	1080	373	1120	TNFRSF12A - WNT9A	2345	1211	2466
TNFRSF14 - WNT9A	1106	2166	198	TNFRSF14 - WNT9A	1127	1147	1191
TNFRSF21 - WNT9A	1805	1999	986	TNFRSF21 - WNT9A	1265	832	1098
TNFSF10 - WNT9A	1258	864	1839	TNFSF10 - WNT9A	2054	2338	1523
TNFSF15 - WNT9A	1621	1129	1139	TNFSF15 - WNT9A	37	1105	1076

Table 2 shows the derived influences which can be represented graphically, with the following influences - • TNF w.r.t WNT with TNF-RSF1A/RSF10A/RSF10B/SF15 < - WNT2B; TNF-RSF10A/RSF10D/RSF12A/SF10 < - WNT4; TNF-RSF12A/SF10 < - WNT7B and TNF-RSF21 < - WNT9A; and • WNT w.r.t TNF with TNF-RSF10B/RSF10D/RSF14 - > WNT2B; TNF-AIP3/RSF10B - > WNT4; TNF, TNF-RSF1A/RSF14 - > WNT7B; and TNF-AIP2/AIP3/RSF10A/RSF12A/SF10 - > WNT9A.

Table 2. 2nd order combinatorial hypotheses between TNF and WNT family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TNF w.r.t WNT	
TNF-RSF1A/RSF10A/RSF10B/SF15	WNT2B
TNF-RSF10A/RSF10D/RSF12A/SF10	WNT4
TNF-RSF12A/SF10	WNT7B
TNF-RSF21	WNT9A
WNT w.r.t TNF	
TNF-RSF10B/RSF10D/RSF14	WNT2B
TNF-AIP3/RSF10B	WNT4
TNF, TNF-RSF1A/RSF14	WNT7B
TNF-AIP2/AIP3/RSF10A/RSF12A/SF10	WNT9A

3.1.2. MUC - TNF Cross Family Analysis

In a recent development in Sheng *et al.* [11] MUC13 promoted tumor necrosis factor (TNF)-induced NFκB activation by interacting with TNFR1 and the E3 ligase, cIAP1, to increase ubiquitination of RIPK1. Dharmani *et al.* [12] show that TNF-α and MUC2 (Mucin 2) play major roles in disease onset and progression in dextran sodium sulphate-induced colitis. TNF-α is also shown to induce mucin hypersecretion and MUC2 gene expression by human airway epithelial cells by Levine *et al.* [13]. Also, inhibition of TNF-α induced MUC5AC expression and production by wogonin through the inactivation of NF-κB signaling in airway epithelial cells, as shown by Sikder *et al.* [14]. Similarly, neutrophil elastase induces MUC5AC production in human airway epithelial cells via a cascade involving protein kinase-C, reactive oxygen species, and TNF-α-converting enzyme, as shown by Shao and Nadel [15]. TNF-α or transforming growth factor-α stimulation of human epithelial cells resulted in mucus secretion as measured by MUC5AC mRNA and protein (Lora *et al.* [16]). In earlier experiments by Fischer *et al.* [17], TNF-α was found to stimulate mucin secretion and cyclic GMP production by guinea pig tracheal epithelial cells in vitro. Similar earlier experiments by Lin *et al.* [18], induction of mucin gene expression in middle ear of rats by TNF-α was the potential cause for mucoid otitis media. Effects of TNF-α and IL-1β on mucin, lysozyme, IL-6 and IL-8 in passage-2 normal human nasal epithelial cells have been studied by Yoon *et al.* [19]. Also, Mercogliano *et al.* [20] show that TNF-α induced MUC4 expression elicits trastuzumab resistance in HER2-+ive breast cancer. These findings suggest deep synergy between Mucin family and TNF family members. However, not all synergies might have been explored till now. A set of family members of MUC and TNFs were found to be UP regulated after ETC-1922159 treatment in CRC cells.

Tables 2 and 3 show the additional range of TNFs and MUCs that might be engaged in CRC through the NFκB pathway, in the light of the recent findings of MUC13 and TNFRSF1A in Sheng *et al.* [11]. Table 2 shows the rankings of the TNF family w.r.t to MUCIN family and Table 3 shows the rankings of the MUCIN family w.r.t to TNF family. Followed by this are the derived influences from the majority votings of the rankings in the foregoing tables, which are depicted in Table 4.

RANKING TNF FAMILY W.R.T MUC FAMILY

RANKING OF TNF FAMILY W.R.T MUC1				RANKING OF TNF FAMILY W.R.T MUC3A			
	laplace	linear	rbf		laplace	linear	rbf
MUC1 - TNF	112	72	88	MUC3A - TNF	1353	1659	1479
MUC1 - TNFAIP1	1193	1603	997	MUC3A - TNFAIP1	2178	1209	1347
MUC1 - TNFAIP2	716	405	2340	MUC3A - TNFAIP2	1075	1614	1158
MUC1 - TNFAIP3	2115	1636	1882	MUC3A - TNFAIP3	962	1020	2491
MUC1 - TNFRSF1A	1380	422	1390	MUC3A - TNFRSF1A	461	1708	189
MUC1 - TNFRSF10A	1009	2180	1095	MUC3A - TNFRSF10A	2237	1910	335
MUC1 - TNFRSF10B	1923	732	88	MUC3A - TNFRSF10B	450	1443	2040
MUC1 - TNFRSF10D	2303	2154	376	MUC3A - TNFRSF10D	1678	2049	102
MUC1 - TNFRSF12A	2019	2009	1700	MUC3A - TNFRSF12A	2349	1315	382
MUC1 - TNFRSF14	1955	1899	1429	MUC3A - TNFRSF14	956	1442	1953
MUC1 - TNFRSF21	337	477	968	MUC3A - TNFRSF21	1297	1492	1959
MUC1 - TNFSF10	1111	1592	1198	MUC3A - TNFSF10	891	257	798
MUC1 - TNFSF15	936	986	2391	MUC3A - TNFSF15	2285	795	1164
RANKING OF TNF FAMILY W.R.T MUC4				RANKING OF TNF FAMILY W.R.T MUC12			
	laplace	linear	rbf		laplace	linear	rbf
MUC4 - TNF	1896	231	1355	MUC12 - TNF	1862	102	135
MUC4 - TNFAIP1	864	397	987	MUC12 - TNFAIP1	1386	479	942
MUC4 - TNFAIP2	73	1011	1087	MUC12 - TNFAIP2	1056	303	1587
MUC4 - TNFAIP3	1159	1751	179	MUC12 - TNFAIP3	2493	1259	1330
MUC4 - TNFRSF1A	179	71	16	MUC12 - TNFRSF1A	1709	1440	837
MUC4 - TNFRSF10A	1668	1892	1652	MUC12 - TNFRSF10A	598	531	363
MUC4 - TNFRSF10B	2024	1396	331	MUC12 - TNFRSF10B	409	1572	1297
MUC4 - TNFRSF10D	2503	2403	2356	MUC12 - TNFRSF10D	30	102	149
MUC4 - TNFRSF12A	1684	700	745	MUC12 - TNFRSF12A	298	882	153
MUC4 - TNFRSF14	1675	2029	1146	MUC12 - TNFRSF14	1749	2237	135
MUC4 - TNFRSF21	647	326	323	MUC12 - TNFRSF21	1795	607	2438
MUC4 - TNFSF10	936	2134	1957	MUC12 - TNFSF10	801	1795	2435
MUC4 - TNFSF15	1440	1180	1627	MUC12 - TNFSF15	1741	889	1098

Table 2. Cont.

RANKING TNF FAMILY W.R.T MUC FAMILY							
RANKING OF TNF FAMILY W.R.T MUC13				RANKING OF TNF FAMILY W.R.T MUC17			
	laplace	linear	rbf		laplace	linear	rbf
MUC13 - TNF	2282	220	127	MUC17 - TNF	683	362	515
MUC13 - TNFAIP1	378	230	1935	MUC17 - TNFAIP1	117	188	272
MUC13 - TNFAIP2	2464	220	697	MUC17 - TNFAIP2	1311	414	351
MUC13 - TNFAIP3	2274	1233	1446	MUC17 - TNFAIP3	1589	1547	1539
MUC13 - TNFRSF1A	274	2152	514	MUC17 - TNFRSF1A	428	205	329
MUC13 - TNFRSF10A	2500	938	1844	MUC17 - TNFRSF10A	2269	2364	2005
MUC13 - TNFRSF10B	1891	1497	225	MUC17 - TNFRSF10B	1199	1323	2120
MUC13 - TNFRSF10D	1191	2263	2294	MUC17 - TNFRSF10D	1798	1378	2302
MUC13 - TNFRSF12A	460	1753	1704	MUC17 - TNFRSF12A	2041	2303	1049
MUC13 - TNFRSF14	2220	1602	1359	MUC17 - TNFRSF14	2043	825	1700
MUC13 - TNFRSF21	1612	1673	127	MUC17 - TNFRSF21	2013	393	119
MUC13 - TNFSF10	2236	1598	1495	MUC17 - TNFSF10	280	1025	817
MUC13 - TNFSF15	2423	1488	1292	MUC17 - TNFSF15	833	967	950
RANKING OF TNF FAMILY W.R.T MUC20							
	laplace	linear	rbf		laplace	linear	rbf
MUC20 - TNF	2267	262	145				
MUC20 - TNFAIP1	1273	2296	178				
MUC20 - TNFAIP2	1062	598	339				
MUC20 - TNFAIP3	2205	435	2136				
MUC20 - TNFRSF1A	483	2346	145				
MUC20 - TNFRSF10A	100	2305	917				
MUC20 - TNFRSF10B	775	1578	1556				
MUC20 - TNFRSF10D	200	1487	799				
MUC20 - TNFRSF12A	318	1607	2258				
MUC20 - TNFRSF14	410	1832	745				
MUC20 - TNFRSF21	1686	2259	164				
MUC20 - TNFSF10	1005	2139	1548				
MUC20 - TNFSF15	2493	387	2108				

Table 3. 2nd order interaction ranking between MUC w.r.t TNF family members.

RANKING MUC FAMILY W.R.T TNF FAMILY							
RANKING OF MUC1 W.R.T TNF FAMILY				RANKING OF MUC3A W.R.T TNF FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
MUC1 - TNF	368	142	21	MUC3A - TNF	1478	985	2373
MUC1 - TNFAIP1	692	91	1591	MUC3A - TNFAIP1	1485	536	1698
MUC1 - TNFAIP2	2290	476	398	MUC3A - TNFAIP2	1254	1265	75
MUC1 - TNFAIP3	810	492	748	MUC3A - TNFAIP3	1844	960	243
MUC1 - TNFRSF1A	1089	2344	2312	MUC3A - TNFRSF1A	496	574	792
MUC1 - TNFRSF10A	1263	351	826	MUC3A - TNFRSF10A	1315	1525	1815
MUC1 - TNFRSF10B	1630	1604	2103	MUC3A - TNFRSF10B	351	1920	1489
MUC1 - TNFRSF10D	975	1026	984	MUC3A - TNFRSF10D	596	950	1016
MUC1 - TNFRSF12A	1597	1811	1078	MUC3A - TNFRSF12A	436	595	2124
MUC1 - TNFRSF14	739	2119	938	MUC3A - TNFRSF14	1612	1383	329
MUC1 - TNFRSF21	766	1495	2322	MUC3A - TNFRSF21	1254	1357	1162
MUC1 - TNFSF10	1360	1969	477	MUC3A - TNFSF10	774	980	2053
MUC1 - TNFSF15	424	1183	542	MUC3A - TNFSF15	75	1261	624
RANKING OF MUC4 W.R.T TNF FAMILY				RANKING OF MUC12 W.R.T TNF FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
MUC4 - TNF	1656	777	565	MUC12 - TNF	266	1223	628
MUC4 - TNFAIP1	2483	895	390	MUC12 - TNFAIP1	2321	668	2457
MUC4 - TNFAIP2	1875	1792	180	MUC12 - TNFAIP2	281	1829	1913
MUC4 - TNFAIP3	54	498	464	MUC12 - TNFAIP3	2353	153	576
MUC4 - TNFRSF1A	1074	753	68	MUC12 - TNFRSF1A	1481	1952	1406
MUC4 - TNFRSF10A	683	311	997	MUC12 - TNFRSF10A	445	337	888
MUC4 - TNFRSF10B	98	1413	704	MUC12 - TNFRSF10B	792	164	133
MUC4 - TNFRSF10D	1916	230	80	MUC12 - TNFRSF10D	167	193	521
MUC4 - TNFRSF12A	1321	2190	150	MUC12 - TNFRSF12A	216	2093	302
MUC4 - TNFRSF14	606	704	1493	MUC12 - TNFRSF14	105	59	69
MUC4 - TNFRSF21	1225	1967	1093	MUC12 - TNFRSF21	1471	1975	1769
MUC4 - TNFSF10	815	1108	1906	MUC12 - TNFSF10	662	2135	2255
MUC4 - TNFSF15	1141	1841	920	MUC12 - TNFSF15	1619	2204	1257

Table 3. Cont.

RANKING MUC FAMILY W.R.T TNF FAMILY							
RANKING OF MUC13 W.R.T TNF FAMILY				RANKING OF MUC17 W.R.T TNF FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
MUC13 - TNF	623	292	295	MUC17 - TNF	203	811	57
MUC13 - TNFAIP1	823	755	81	MUC17 - TNFAIP1	381	193	118
MUC13 - TNFAIP2	1118	2464	116	MUC17 - TNFAIP2	1069	822	136
MUC13 - TNFAIP3	1189	546	541	MUC17 - TNFAIP3	2132	47	937
MUC13 - TNFRSF1A	978	1506	490	MUC17 - TNFRSF1A	120	497	864
MUC13 - TNFRSF10A	1180	540	1926	MUC17 - TNFRSF10A	852	218	346
MUC13 - TNFRSF10B	280	1105	190	MUC17 - TNFRSF10B	933	1667	1166
MUC13 - TNFRSF10D	655	725	1668	MUC17 - TNFRSF10D	546	133	304
MUC13 - TNFRSF12A	401	1242	999	MUC17 - TNFRSF12A	18	1675	86
MUC13 - TNFRSF14	1324	374	389	MUC17 - TNFRSF14	819	296	1014
MUC13 - TNFRSF21	690	2337	107	MUC17 - TNFRSF21	1659	814	889
MUC13 - TNFSF10	1146	1208	2159	MUC17 - TNFSF10	387	1542	156
MUC13 - TNFSF15	1633	314	155	MUC17 - TNFSF15	1207	1040	522
RANKING OF MUC20 W.R.T TNF FAMILY							
	laplace	linear	rbf		laplace	linear	rbf
MUC20 - TNF	57	216	903				
MUC20 - TNFAIP1	1265	2266	2057				
MUC20 - TNFAIP2	241	2404	2157				
MUC20 - TNFAIP3	484	1012	513				
MUC20 - TNFRSF1A	748	173	2193				
MUC20 - TNFRSF10A	620	427	1054				
MUC20 - TNFRSF10B	765	1563	790				
MUC20 - TNFRSF10D	509	2185	794				
MUC20 - TNFRSF12A	216	2093	302				
MUC20 - TNFRSF14	2298	651	1368				
MUC20 - TNFRSF21	2374	1611	1140				
MUC20 - TNFSF10	1257	1088	1031				
MUC20 - TNFSF15	142	2159	7				

Table 4. 2nd order combinatorial hypotheses between MUC and TNF.

UNEXPLORED COMBINATORIAL HYPOTHESES	
TNF w.r.t MUC	
MUC1	TNFAIP3/TNFRSF10D/TNFRSF12A/TNFRSF14
MUC3A	TNFRSF10A/TNFRSF10D
MUC4	TNFRSF10D/TNFSF10
MUC12	TNFRSF21/TNFSF10
MUC13	TNFRSF10A/TNFRSF10D
MUC17	TNFRSF10A/TNFRSF10D/TNFRSF12A
MUC20	TNFAIP3/TNFSF15
MUC w.r.t TNF	
MUC1	TNFRSF1A
MUC4	TNFAIP2
MUC12	TNFAIP1/TNFAIP2/TNFRSF21/TNFSF10
MUC13	TNFAIP1/TNFAIP2

Considering Table 2, **TNF family w.r.t MUC1**, we find TNFAIP3, TNFRSF-10D/12A/14 to be highly up regulated. These are reflected in the rankings of 2115 (laplace) and 1882 (rbf) for MUC1 - TNFAIP3; 2303 (laplace) and 2154 (linear) for MUC1 - TNFRSF10D; 2019 (laplace) and 2009 (linear) for MUC1 - TNFRSF12A; and 1955 (laplace) and 1899 (linear) for MUC1 - TNFRSF14. **TNF family w.r.t MUC3A**, we find TNFRSF-10A/10D to be highly up regulated. These are reflected in the rankings of 2237 (laplace) and 1910 (linear) for MUC3 - TNFRSF10A; 1678 (laplace) and 2049 (linear) for MUC3 - TNFRSF10D. **TNF family w.r.t MUC4** we find TNFRSF10D/TNFSF10 to be highly up regulated. These are reflected in the rankings of 2503 (laplace), 2403 (linear) and 2356 (rbf) for MUC4 - TNFRSF10D and 2134 (laplace) and 1957 (linear) for MUC4 - TNFSF10. **TNF family w.r.t MUC12** we find TNFRSF21/TNFSF10 to be highly up regulated. These are reflected in the rankings of 1795 (laplace) and 2438 (linear) for MUC12 - TNFRSF21 and 1795 (linear) 2435 (rbf) for MUC12 - TNFSF10. **TNF family w.r.t MUC13** we find TNFRSF10A/TNFRSF10D to be highly up regulated. These are reflected in the rankings of 2500 (laplace) and 1844 (rbf) for MUC13 - TNFRSF10A and 2263 (linear) and 2294 (rbf) for MUC13 - TNFRSF10D. **TNF family w.r.t MUC17** we find TNFRSF-10A/10D/12A to be highly up regulated. These are reflected in the rankings of 2269 (laplace) 2364 (linear) and 2005 (rbf) for MUC17 - TNFRSF10A; 1798 (laplace) and 2302 (rbf) for MUC17 - TNFRSF10D and 2041 (laplace) and 2303 (linear) for MUC17 - TNFRSF12A. **TNF family w.r.t MUC20** we find TNFAIP3/TNFSF15 to be highly up regulated. These are reflected in the rankings of 2205 (laplace) and 2136 (rbf) for MUC20 - TNFAIP3 and 2493 (laplace) and 2108 (rbf) for MUC20 - TNFSF15.

Considering Table 3, **MUC1 w.r.t TNF family**, we find TNFRSF1A to be highly up regulated. These are reflected in the rankings of 2344 (linear) and 2312 (rbf) for MUC1 - TNFRSF1A. **MUC4 w.r.t TNF family**, we find TNFAIP2 to be highly up regulated. These are reflected in the rankings of 1875 (laplace) and 1792 (linear) for MUC4 - TNFAIP2. **MUC12 w.r.t TNF family** we find TNFAIP1/TNFAIP2/TNFRSF21/TNFSF10 to be highly up regulated. These are reflected in the rankings of 2321 (laplace) and 2457 (rbf) for MUC12 - TNFAIP1; 1829 (linear) and 1913 (rbf) for MUC12 - TNFAIP2; 1975 (linear) and 1769 (rbf) for MUC12 - TNFRSF21; 2135 (linear) and 2255 (rbf) for MUC12 - TNFSF10. **MUC12 w.r.t TNF family** we find TNFRSF21/TNFSF10 to be highly up regulated. These are reflected in the rankings of 1795 (laplace) and 2438 (linear) for MUC12 - TNFRSF21 and 1795 (linear) and 2435 (rbf) for MUC12 - TNFSF10. **MUC20 w.r.t TNF family** we find TNFAIP1/TNFAIP2 to be highly up regulated. These are reflected in the rankings of 2266 (laplace) and 2057 (rbf) for MUC20 - TNFAIP1 and 2404 (linear) and 2157 (rbf) for MUC20 - TNFAIP2.

One can also interpret the results of the Table 4 graphically, with the following influences - • TNF family w.r.t MUC family with MUC1 – > TNFAIP3/TNFRSF-10D/12A/14; MUC3A – > TNFRSF-10A/10D; MUC4 – > TNFRSF10D/TNFSF10; MUC12 – > TNFRSF21/TNFSF10; MUC13 – > TNFRSF-10A/10D; MUC17 – > TNFRSF-10A/10D/12A; MUC20 – > TNFAIP3/TNFSF15 and

- MUC family w.r.t TNF family with MUC1 < – TNFRSF1A; MUC4 < – TNFAIP2; MUC12 < – TNFAIP1/TNFAIP2/TNFRSF21/TNFSF10 and MUC13 < – TNFAIP1/TNFAIP2.

3.1.3. STEAP4 - TNF Cross Family Analysis

STEAP4 or six transmembrane epithelial antigen of prostate 4, resides in the golgi apparatus and functions as a metalloredutase with the capacity to reduce insoluble ferric ions Fe^{3+} to soluble ferrous ions Fe^{2+} . Emerging role of STEAP4 in metabolism and homeostasis of cellular iron and copper in metabolism and homeostasis of cellular iron and copper has been studied in Scarl *et al.* [21]. STEAP4 was first identified as a novel gene induced by TNF- α during adipose differentiation by Moldes *et al.* [22]. Zhang *et al.* [23] observe that STEAP4 was up-regulated by LPS at a very early time point, consistent with reports that STEAP4 could be up-regulated by tumor necrosis factor-alpha. Tanaka *et al.* [24] show that STEAP4 is expressed on monocytes/neutrophils, and is regulated by TNF antagonist in patients with rheumatoid arthritis. Also, Tanaka *et al.* [25] show STEAP4 is a tumor necrosis factor alpha-induced protein that regulates IL-6, IL-8, and cell proliferation in synovium from patients with rheumatoid arthritis. Gauss *et al.* [26] observe that the STEAP4 expression in adipocytes is normally induced by nutritional stress, leptin, and proinflammatory cytokines, including TNF- α , interleukin-1 β , and interleukin-6. ZHANG *et al.* [27] show that the downregulation of STEAP4, a highly-expressed TNF- α -inducible gene in adipose tissue, is associated with obesity in humans 1. Liang *et al.* [28] show that STEAP comprises a novel inflammatory nexus in patients with pustular skin disorders. They show that in primary human keratinocytes STEAP4 expression was induced by TNF- α , IL-1 β , IL-36 α , IL-36 γ , IL-17A, and IL-17A combined with TNF- α or IL-22. Gomes *et al.* [29] further show the TNF STEAP interactions while studying the structure of STEAP proteins and its applications to cancer therapy. Such interactions point to the existing synergy between STEAP4 and TNF- α . In CRC cells treated with ETC-1922159, both TNF members and STEAP4 were found to be up regulated. Our search engine alloted the dual combinations with numerically high ranked values thus pointing to the possible synergies that might be existing in the cells and may not have been explored. Table 6 shows the rankings of each with the other. On the left we found, **TNF, TNF-AIP1/AIP2/AIP3/RSF10B/RSF10D/SF10** to be up regulated w.r.t STEAP4. These are reflected in rankings of 1914 (linear) and 2130 (rbf) for TNF - STEAP4; 2189 (laplace) and 1910 (rbf) for TNFAIP1 - STEAP4; 2002 (linear) and 1840 (rbf) for TNFAIP2 - STEAP4; 1882 (linear) and 2197 (rbf) for TNFAIP3 - STEAP4; 2210 (laplace), 1717 (linear) and 1827 (rbf) for TNFRSF10B - STEAP4; 2192 (linear) and 1797 (rbf) for TNFRSF10D - STEAP4; and 2083 (laplace) and 1773 (rbf) for TNFSF10 - STEAP4. On the right we found, **STEAP4** to be up regulated w.r.t TNF-RSF10A/RSF10D/RSF14. These are reflected in rankings of 1796 (linear) and 2026 (rbf) for TNFRSF10A - STEAP4; 2339 (linear) and 2405 (rbf) for TNFRSF10D - STEAP4; and 1956 (linear) and 2256 (rbf) for TNFRSF14 - STEAP4.

Table 5. 2nd order combinatorial hypotheses between TNF and STEAP4 family.

RANKING TNF FAMILY VS STEAP4 FAMILY							
RANKING OF TNF FAMILY W.R.T STEAP4				RANKING OF STEAP4 W.R.T IL FAMILY			
	laplace	linear	rbf		laplace	linear	rbf
TNF - STEAP4	1579	1914	2130	TNF - STEAP4	1116	1482	999
TNFAIP1 - STEAP4	2189	1293	1910	TNFAIP1 - STEAP4	691	611	1105
TNFAIP2 - STEAP4	1172	2002	1840	TNFAIP2 - STEAP4	228	1747	2463
TNFAIP3 - STEAP4	1458	1882	2197	TNFAIP3 - STEAP4	159	727	219
TNFRSF1A - STEAP4	803	75	1086	TNFRSF1A - STEAP4	1483	408	1966
TNFRSF10A - STEAP4	239	1949	339	TNFRSF10A - STEAP4	1512	1796	2026
TNFRSF10B - STEAP4	2210	1717	1827	TNFRSF10B - STEAP4	565	571	248
TNFRSF10D - STEAP4	510	2192	1797	TNFRSF10D - STEAP4	1018	2339	2405
TNFRSF12A - STEAP4	757	338	1497	TNFRSF12A - STEAP4	1495	1430	581
TNFRSF14 - STEAP4	1323	1512	792	TNFRSF14 - STEAP4	1363	1956	2256
TNFRSF21 - STEAP4	1643	1920	165	TNFRSF21 - STEAP4	1646	802	160
TNFSF10 - STEAP4	2083	544	1773	TNFSF10 - STEAP4	845	675	2468
TNFSF15 - STEAP4	631	1296	1020	TNFSF15 - STEAP4	1558	600	784

Table 6. 2nd order combinatorial hypotheses between STEAP4 and TNF

One can also interpret the results of the Table 4 graphically, with the following influences - • TNF w.r.t STEAP4 with TNF, TNF-AIP1/AIP2/AIP3/RSF10B/RSF10D/SF10 < – STEAP4 and • STEAP4 w.r.t TNF with TNF-RSF10A/RSF10D/RSF14 – > STEAP4.

UNEXPLORED COMBINATORIAL HYPOTHESES

TNF w.r.t STEAP4	
TNF,	STEAP4
TNF-AIP1/AIP2/AIP3/RSF10B/RSF10D/SF10	
STEAP4 w.r.t TNF	
TNF-RSF10A/RSF10D/RSF14	STEAP4

3.1.4. TNF - UBE2 Cross Family Analysis

Fu *et al.* [30] show that the ubiquitin conjugating enzyme UBE2L3 regulates TNF α -induced linear ubiquitination. They show by western blotting of HOIL-1L immunoprecipitates demonstrates that endogenous HOIL-1L interacts with endogenous UBE2L3 in vivo and these associations are stable following TNF α stimulation. Through various hypotheses, the authors show the interaction of UBE2L3 with TNF. In conclusion, the authors state that increased UBE2L3 expression enhances NF- κ B activation, and increased levels of NF- κ B activity are linked to inflammatory and autoimmune diseases. Li *et al.* [31] show that TNF- α increases ubiquitin-conjugating activity in skeletal muscle by up-regulating UBCH2/E2_{20k}. Shembade *et al.* [32] show that IL-1 β or TNF induce late depletion of UBE2D3 (UBCH5C) and UBE2N (UBC13) in mouse embryonic fibroblasts. These studies show a

definite synergy between UBE family and TNFs. In CRC cells treated with ETC-1922159, both TNF members and UBE2 were found to be up regulated. Our search engine allotted the dual combinations with numerically high ranked values thus pointing to the possible synergies that might be existing in the cells and may not have been explored. Tables 7 and 8 shows the rankings of each with the other.

Table 7. 2nd order combinatorial hypotheses between UBE2 and TNF

RANKING TNF FAMILY VS UBE2 FAMILY							
RANKING OF UBE2A W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T UBE2A			
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2A	1360	2307	1720	TNF - UBE2A	499	1379	750
TNFAIP1 - UBE2A	498	2357	2455	TNFAIP1 - UBE2A	1340	2494	578
TNFAIP2 - UBE2A	524	1161	2385	TNFAIP2 - UBE2A	441	1852	691
TNFAIP3 - UBE2A	855	1642	812	TNFAIP3 - UBE2A	1157	1048	207
TNFRSF1A - UBE2A	2457	1087	2020	TNFRSF1A - UBE2A	1066	655	1701
TNFRSF10A - UBE2A	2164	2126	621	TNFRSF10A - UBE2A	2116	858	2376
TNFRSF10B - UBE2A	2284	1901	1203	TNFRSF10B - UBE2A	362	1083	756
TNFRSF10D - UBE2A	1989	2291	677	TNFRSF10D - UBE2A	1848	1336	903
TNFRSF12A - UBE2A	2484	2427	339	TNFRSF12A - UBE2A	1537	1304	629
TNFRSF14 - UBE2A	2301	2180	2323	TNFRSF14 - UBE2A	908	1519	1945
TNFRSF21 - UBE2A	2419	2035	1169	TNFRSF21 - UBE2A	605	2245	60
TNFSF10 - UBE2A	832	2202	1036	TNFSF10 - UBE2A	1520	44	2125
TNFSF15 - UBE2A	1768	1184	1942	TNFSF15 - UBE2A	545	580	1448

Table 7. Cont.

RANKING TNF FAMILY VS UBE2 FAMILY							
RANKING OF UBE2B W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T UBE2B			
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2B	1072	2046	1316	TNF - UBE2B	1719	218	346
TNFAIP1 - UBE2B	1097	744	1295	TNFAIP1 - UBE2B	920	90	1028
TNFAIP2 - UBE2B	669	1158	2407	TNFAIP2 - UBE2B	1680	147	45
TNFAIP3 - UBE2B	470	1528	1388	TNFAIP3 - UBE2B	2259	742	1610
TNFRSF1A - UBE2B	937	1473	2390	TNFRSF1A - UBE2B	1277	1454	1258
TNFRSF10A - UBE2B	2132	1128	2184	TNFRSF10A - UBE2B	551	2318	2265
TNFRSF10B - UBE2B	2399	2000	402	TNFRSF10B - UBE2B	2272	1268	1080
TNFRSF10D - UBE2B	1959	1562	2232	TNFRSF10D - UBE2B	1157	207	1729
TNFRSF12A - UBE2B	1632	12	2259	TNFRSF12A - UBE2B	1940	1868	1758
TNFRSF14 - UBE2B	1137	2297	2373	TNFRSF14 - UBE2B	1143	1657	1507
TNFRSF21 - UBE2B	1986	1439	1754	TNFRSF21 - UBE2B	1291	569	17
TNFSF10 - UBE2B	2265	1488	769	TNFSF10 - UBE2B	2208	2326	2470
TNFSF15 - UBE2B	1432	2460	1655	TNFSF15 - UBE2B	2055	1964	183
RANKING OF UBE2F W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T UBE2F			
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2F	2162	2484	2500	TNF - UBE2F	638	435	1471
TNFAIP1 - UBE2F	1732	2239	2003	TNFAIP1 - UBE2F	447	1376	1357
TNFAIP2 - UBE2F	693	1446	1706	TNFAIP2 - UBE2F	900	208	883
TNFAIP3 - UBE2F	498	2265	1264	TNFAIP3 - UBE2F	1881	113	1185
TNFRSF1A - UBE2F	1980	2255	1872	TNFRSF1A - UBE2F	368	1756	266
TNFRSF10A - UBE2F	2085	2218	179	TNFRSF10A - UBE2F	1767	1599	781
TNFRSF10B - UBE2F	2432	2011	2144	TNFRSF10B - UBE2F	1413	1157	1510
TNFRSF10D - UBE2F	1164	1400	2150	TNFRSF10D - UBE2F	389	206	2481
TNFRSF12A - UBE2F	2458	2336	531	TNFRSF12A - UBE2F	581	2022	630
TNFRSF14 - UBE2F	1757	574	1070	TNFRSF14 - UBE2F	2324	1924	1954
TNFRSF21 - UBE2F	1056	2498	1418	TNFRSF21 - UBE2F	718	2123	1022
TNFSF10 - UBE2F	1710	2365	1691	TNFSF10 - UBE2F	1656	1584	810
TNFSF15 - UBE2F	1910	1171	2353	TNFSF15 - UBE2F	1224	1637	394

Table 8. 2nd order combinatorial hypotheses between UBE2 and TNF

RANKING TNF FAMILY VS UBE2 FAMILY							
RANKING OF UBE2H W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T UBE2H			
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2H	967	1966	1018	TNF - UBE2H	2277	770	640
TNFAIP1 - UBE2H	1235	1484	817	TNFAIP1 - UBE2H	883	2396	608
TNFAIP2 - UBE2H	1251	978	2517	TNFAIP2 - UBE2H	762	1362	593
TNFAIP3 - UBE2H	889	1055	1837	TNFAIP3 - UBE2H	1942	1421	1467
TNFRSF1A - UBE2H	589	1498	1428	TNFRSF1A - UBE2H	1134	2154	182
TNFRSF10A - UBE2H	1317	905	2229	TNFRSF10A - UBE2H	1139	202	1061
TNFRSF10B - UBE2H	33	2128	1725	TNFRSF10B - UBE2H	1053	539	1207
TNFRSF10D - UBE2H	1326	1814	1657	TNFRSF10D - UBE2H	2227	926	791
TNFRSF12A - UBE2H	1950	1793	1851	TNFRSF12A - UBE2H	1347	776	1899
TNFRSF14 - UBE2H	2297	1601	2385	TNFRSF14 - UBE2H	2244	703	1208
TNFRSF21 - UBE2H	2022	1131	2231	TNFRSF21 - UBE2H	827	880	672
TNFSF10 - UBE2H	2387	7	760	TNFSF10 - UBE2H	1313	1169	2002
TNFSF15 - UBE2H	125	58	96	TNFSF15 - UBE2H	350	2416	1960
RANKING OF UBE2J1 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T UBE2J1			
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2J1	1289	2308	2336	TNF - UBE2J1	1101	1549	105
TNFAIP1 - UBE2J1	1109	2292	1756	TNFAIP1 - UBE2J1	329	1971	252
TNFAIP2 - UBE2J1	1379	1516	1696	TNFAIP2 - UBE2J1	112	22	969
TNFAIP3 - UBE2J1	187	1261	1065	TNFAIP3 - UBE2J1	289	891	1202
TNFRSF1A - UBE2J1	1992	326	2268	TNFRSF1A - UBE2J1	1422	624	73
TNFRSF10A - UBE2J1	1893	2090	2363	TNFRSF10A - UBE2J1	2379	2213	2135
TNFRSF10B - UBE2J1	1913	1299	1838	TNFRSF10B - UBE2J1	807	1793	1231
TNFRSF10D - UBE2J1	325	1500	588	TNFRSF10D - UBE2J1	2393	1360	2102
TNFRSF12A - UBE2J1	2401	1901	437	TNFRSF12A - UBE2J1	380	1284	650
TNFRSF14 - UBE2J1	2277	2347	1943	TNFRSF14 - UBE2J1	1614	2133	2313
TNFRSF21 - UBE2J1	1976	2333	1681	TNFRSF21 - UBE2J1	1315	1266	1070
TNFSF10 - UBE2J1	511	2508	506	TNFSF10 - UBE2J1	1322	203	1148
TNFSF15 - UBE2J1	2021	2013	2515	TNFSF15 - UBE2J1	678	886	1128

Table 8. Cont.

RANKING TNF FAMILY VS UBE2 FAMILY							
RANKING OF UBE2Z W.R.T TNF FAMILY	RANKING OF UBE2Z W.R.T TNF FAMILY			RANKING OF TNF FAMILY W.R.T UBE2Z	RANKING OF TNF FAMILY W.R.T UBE2Z		
	laplace	linear	rbf		laplace	linear	rbf
TNF - UBE2Z	2264	2505	2479	TNF - UBE2Z	739	701	1241
TNFAIP1 - UBE2Z	1283	2055	2332	TNFAIP1 - UBE2Z	1198	1213	226
TNFAIP2 - UBE2Z	2404	1625	2139	TNFAIP2 - UBE2Z	1281	1431	492
TNFAIP3 - UBE2Z	1066	1152	1627	TNFAIP3 - UBE2Z	530	51	972
TNFRSF1A - UBE2Z	2473	2194	2405	TNFRSF1A - UBE2Z	692	43	1382
TNFRSF10A - UBE2Z	2234	2152	713	TNFRSF10A - UBE2Z	2410	2103	1513
TNFRSF10B - UBE2Z	1501	451	2081	TNFRSF10B - UBE2Z	948	1369	403
TNFRSF10D - UBE2Z	2264	2360	2278	TNFRSF10D - UBE2Z	1786	661	1746
TNFRSF12A - UBE2Z	2207	2149	353	TNFRSF12A - UBE2Z	1621	2010	1448
TNFRSF14 - UBE2Z	1683	1983	705	TNFRSF14 - UBE2Z	1779	1360	2100
TNFRSF21 - UBE2Z	994	604	219	TNFRSF21 - UBE2Z	459	1030	584
TNFSF10 - UBE2Z	516	2374	2235	TNFSF10 - UBE2Z	1100	2047	168
TNFSF15 - UBE2Z	2081	1037	2102	TNFSF15 - UBE2Z	1342	1180	536

On the left side is the ranking of UBE2 family w.r.t TNF family. We found **UBE2A** to be up regulated w.r.t TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF10D/RSF12A/RSF14/RSF21/SF15. These are reflected in rankings of 2357 (linear) and 2455 (rbf) for TNFAIP1 - UBE2A; 2457 (laplace) and 2020 (rbf) for TNFRSF1A - UBE2A; 2164 (laplace) and 2126 (linear) for TNFRSF10A - UBE2A; 2284 (laplace) and 1901 (linear) for TNFRSF10B - UBE2A; 1989 (laplace) and 2291 (linear) for TNFRSF10D - UBE2A; 2484 (laplace) and 2427 (linear) for TNFRSF12A - UBE2A; 2301 (laplace), 2180 (linear) and 2323 (rbf) for TNFRSF14 - UBE2A; 2419 (laplace) and 2035 (linear) for TNFRSF21 - UBE2A; 1768 (laplace) and 1942 (rbf) for TNFSF15 - UBE2A. **UBE2B** to be up regulated w.r.t TNF-RSF10A/RSF10B/RSF10D/RSF14/RSF21. These are reflected in rankings of 2132 (laplace) and 2184 (rbf) for TNFRSF10A - UBE2B; 2399 (laplace) and 2000 (linear) for TNFRSF10B - UBE2B; 1959 (laplace) and 2232 (rbf) for TNFRSF10D - UBE2B; 2297 (linear) and 2373 (rbf) for TNFRSF14 - UBE2B; and 1986 (laplace) and 1754 (rbf) for TNFRSF21 - UBE2B. **UBE2F** to be up regulated w.r.t TNF, TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/SF15. These are reflected in rankings of 2162 (laplace), 2484 (linear) and 2500 (rbf) for TNF - UBE2F; 1732 (laplace), 2239 (linear) and 2003 (rbf) for TNFAIP1 - UBE2F; 1980 (laplace), 2255 (linear) and 1872 (rbf) for TNFRSF1A - UBE2F; 2085 (laplace), 2218 (linear) for TNFRSF10A - UBE2F; 2432 (laplace), 2011 (linear) and 2144 (rbf) for TNFRSF10B - UBE2F; 2458 (laplace) and 2336 (linear) for TNFRSF12A - UBE2F; 1910 (laplace) and 2353 (rbf) for TNFSF15 - UBE2F; **UBE2H** to be up regulated w.r.t TNF-RSF12A/RSF14/RSF21. These are reflected in rankings of 1950 (laplace), 1793 (linear) and 1851 (rbf) for TNFRSF12A - UBE2H; 2297 (laplace) and 2385 (rbf) for TNFRSF14 - UBE2H; and 2022 (laplace) and 2231 (rbf) for TNFRSF21 - UBE2H; **UBE2J1** to be up regulated w.r.t TNF, TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/RSF14/RSF21/SF15. These are reflected in rankings of 2308 (linear) and 2336 (rbf) for TNF - UBE2J1; 2292 (linear) and 1756

(rbf) for TNFAIP1 - UBE2J1; 1992 (laplace) and 2268 (rbf) for TNFRSF1A - UBE2J1; 1893 (laplace), 2090 (linear) and 2363 (rbf) for TNFRSF10A - UBE2J1; 1913 (laplace) and 1838 (rbf) for TNFRSF10B - UBE2J1; 2401 (laplace) and 1901 (linear) for TNFRSF12A - UBE2J1; 2277 (laplace), 2347 (linear) and 1943 (rbf) for TNFRSF14 - UBE2J1; 1976 (laplace), 2333 (linear) for TNFRSF21 - UBE2J1; and 2021 (laplace), 2013 (linear) and 2515 (rbf) for TNFSF15 - UBE2J1; **UBE2Z** to be up regulated w.r.t TNF, TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF12A/SF10/SF15. These are reflected in rankings of 2264 (laplace), 2505 (linear) and 2479 (rbf) for TNF - UBE2Z; 2055 (linear) and 2332 (rbf) for TNFAIP1 - UBE2Z; 2404 (laplace) and 2139 (rbf) for TNFAIP2 - UBE2Z; 2473 (laplace), 2194 (linear) and 2405 (rbf) for TNFRSF1A - UBE2Z; 2234 (laplace) and 2152 (linear) for TNFRSF10A - UBE2Z; 2264 (laplace), 2360 (linear) and 2278 (rbf) for TNFRSF10D - UBE2Z; 2207 (laplace) and 2149 (linear) for TNFRSF12A - UBE2Z; 2374 (linear) and 2235 (rbf) for TNFSF10 - UBE2Z; and 2081 (laplace) and 2102 (rbf) for TNFSF15 - UBE2Z;

One the right side is the ranking of TNF family w.r.t UBE2 family. We found **TNF-RSF10A** to be up regulated w.r.t UBE2A. This is reflected in rankings of 2116 (laplace) and 2376 (rbf) for TNFRSF10A - UBE2A. **TNF-RSF10A/RSF12A/SF10/SF15** were up regulated w.r.t UBE2B. These are reflected in rankings of 2318 (linear) and 2265 (linear) for TNFRSF10A - UBE2B; 1940 (laplace); 1868 (linear) and 1758 (linear) for TNFRSF12A - UBE2B; 2208 (laplace); 2326 (linear) and 2470 (linear) for TNFSF10 - UBE2B; and 2055 (laplace) and 1964 (linear) for TNFSF15 - UBE2B. **TNF-RSF14** were up regulated w.r.t UBE2F. These is reflected in rankings of 2324 (laplace) and 1924 (linear) for TNF-RSF14 - UBE2F. **TNF-SF15** were up regulated w.r.t UBE2H. These is reflected in rankings of 2416 (linear) and 1960 (rbf) for TNF-SF15 - UBE2H. **TNF-RSF10A/RSF10D/RSF14** were up regulated w.r.t UBE2J1. These are reflected in rankings of 2379 (laplace), 2213 (linear) and 2135 (rbf) for TNFRSF10A - UBE2J1; 2393 (laplace) and 2102 (rbf) for TNFRSF10D - UBE2J1; and 2133 (linear) and 2313 (rbf) for TNFRSF14 - UBE2J1. **TNF-RSF10A/RSF14** were up regulated w.r.t UBE2Z. These are reflected in rankings of 2410 (laplace) and 2103 (laplace) for TNFRSF10A - UBE2Z and 1779 (laplace) and 2100 (rbf) for TNFRSF14 - UBE2Z.

One can also interpret the results of the Table 9 graphically, with the following influences - • UBE2 w.r.t TNF with TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF10D/RSF12A/RSF14/RSF21/SF15 - > UBE2A; TNF-RSF10A/RSF10B/RSF10D/RSF14/RSF21 - > UBE2B; TNF, TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/SF15 - > UBE2F; TNF-RSF12A/RSF14/RSF21 - > UBE2H; TNF, TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/RSF14/RSF21/SF15 - > UBE2J1; and TNF, TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF12A/SF10/SF15 - > UBE2Z • TNF w.r.t UBE2 with TNF-RSF10A < - UBE2A; TNF-RSF10A/RSF12A/SF10/SF15 < - UBE2B; TNF-RSF14 < - UBE2F; TNF-SF15 < - UBE2H; TNF-RSF10A/RSF10D/RSF14 < - UBE2J1; TNF-RSF10A/RSF14 < - UBE2Z.

Table 9. 2nd order combinatorial hypotheses between TNF and UBE2 family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
UBE2 w.r.t TNF	
TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF10D/RSF10E/RSF14/RSF21/SF15	UBE2A
TNF-RSF10A/RSF10B/RSF10D/RSF14/RSF21	UBE2B
TNF,	UBE2F
TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/SF15	
TNF-RSF12A/RSF14/RSF21	UBE2H
TNF,	UBE2J1
TNF-AIP1/RSF1A/RSF10A/RSF10B/RSF12A/RSF14/RSF21/SF15	
TNF,	UBE2Z
TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF12A/SF10/SF15	
TNF w.r.t UBE2	
TNF-RSF10A	UBE2A
TNF-RSF10A/RSF12A/SF10/SF15	UBE2B
TNF-RSF14	UBE2F
TNF-SF15	UBE2H
TNF-RSF10A/RSF10D/RSF14	UBE2J1
TNF-RSF10A/RSF14	UBE2Z

3.1.5. TNF - BCL Cross Family Analysis

Tamatani *et al.* [33] observe that tumor necrosis factor induces BCL-2 and BCL-x expression through NF κ B activation in primary hippocampal neurons. The role of Bcl-2 Expression in EGF Inhibition of TNF- α /IFN- γ -induced Villous Trophoblast Apoptosis has been studied by Ho *et al.* [34]. Genestier *et al.* [35] show that tumor necrosis factor- α up-regulates BCL-2 expression and decreases calcium-dependent apoptosis in human B cell lines. In breast carcinoma cells, Bcl-x and Bcl-2 inhibit TNF and FAS-induced apoptosis and activation of phospholipase A2 (Jäättelä *et al.* [36]). Kim *et al.* [37] show that TNF- α -induced ROS production triggering apoptosis is directly linked to Romo1 and BCL-X_L. Kuwata *et al.* [38] showed that IL-10-inducible BCL-3 negatively regulates LPS-induced TNF- α production in macrophages. Esche *et al.* [39] showed that tumor necrosis factor- α -promoted expression of BCL-2 and inhibition of mitochondrial cytochrome c release mediate resistance of mature dendritic cells to melanoma-induced apoptosis. These studies show a definite synergy between BCL family and TNFs. In CRC cells treated with ETC-1922159, both TNF members and BCL were found to be up regulated. Our search engine allotted the dual combinations with numerically high ranked values thus pointing to the possible synergies that might be existing in the cells and may not have been explored. Table 10 and 11 show the rankings of each with the other.

Table 10. 2nd order combinatorial hypotheses between BCL and TNF

RANKING TNF FAMILY VS BCL FAMILY							
RANKING OF BCL2L1 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL2L1			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL2L1	174	14	235	TNF - BCL2L1	56	1101	294
TNFAIP1 - BCL2L1	1527	435	791	TNFAIP1 - BCL2L1	1497	1150	74
TNFAIP2 - BCL2L1	2142	1735	798	TNFAIP2 - BCL2L1	1485	1735	400
TNFAIP3 - BCL2L1	2467	842	867	TNFAIP3 - BCL2L1	1109	1939	1553
TNFRSF1A - BCL2L1	1004	1558	383	TNFRSF1A - BCL2L1	492	376	1016
TNFRSF10A - BCL2L1	1906	1270	1222	TNFRSF10A - BCL2L1	2273	1928	508
TNFRSF10B - BCL2L1	1506	2235	589	TNFRSF10B - BCL2L1	1003	2252	2217
TNFRSF10D - BCL2L1	1920	1555	1787	TNFRSF10D - BCL2L1	1868	2420	2392
TNFRSF12A - BCL2L1	1254	1388	1319	TNFRSF12A - BCL2L1	1923	53	1936
TNFRSF14 - BCL2L1	688	237	2009	TNFRSF14 - BCL2L1	340	2350	2414
TNFRSF21 - BCL2L1	1465	1269	100	TNFRSF21 - BCL2L1	2139	718	289
TNFSF10 - BCL2L1	532	560	2332	TNFSF10 - BCL2L1	2115	2299	1307
TNFSF15 - BCL2L1	1026	1551	1134	TNFSF15 - BCL2L1	453	423	25
RANKING OF BCL2L2 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL2L2			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL2L2	1822	1926	2359	TNF - BCL2L2	2140	109	1062
TNFAIP1 - BCL2L2	2266	2478	1847	TNFAIP1 - BCL2L2	2235	1607	712
TNFAIP2 - BCL2L2	823	535	1117	TNFAIP2 - BCL2L2	109	1002	54
TNFAIP3 - BCL2L2	1201	1103	1511	TNFAIP3 - BCL2L2	1470	1696	1276
TNFRSF1A - BCL2L2	1124	2311	1920	TNFRSF1A - BCL2L2	1912	169	1531
TNFRSF10A - BCL2L2	1063	1532	2458	TNFRSF10A - BCL2L2	1643	1095	953
TNFRSF10B - BCL2L2	2478	739	2239	TNFRSF10B - BCL2L2	2153	1164	1983
TNFRSF10D - BCL2L2	910	2278	2237	TNFRSF10D - BCL2L2	35	1012	1905
TNFRSF12A - BCL2L2	1945	240	2484	TNFRSF12A - BCL2L2	1971	1633	975
TNFRSF14 - BCL2L2	2358	1648	2310	TNFRSF14 - BCL2L2	1027	825	1228
TNFRSF21 - BCL2L2	2292	1850	1014	TNFRSF21 - BCL2L2	1138	486	554
TNFSF10 - BCL2L2	2438	547	2013	TNFSF10 - BCL2L2	2212	902	169
TNFSF15 - BCL2L2	1196	2443	2350	TNFSF15 - BCL2L2	2285	165	1330

Table 10. Cont.

RANKING TNF FAMILY VS BCL FAMILY				RANKING TNF FAMILY VS BCL FAMILY			
RANKING OF BCL2L13 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL2L13			
	laplace	linear	rbf		laplace	linear	rbf
TNF -	2437	2482	2482	TNF -	1162	103	462
BCL2L13				BCL2L13			
TNFAIP1	1863	2386	989	TNFAIP1	852	606	787
-				-			
BCL2L13				BCL2L13			
TNFAIP2	793	293	1846	TNFAIP2	438	479	742
-				-			
BCL2L13				BCL2L13			
TNFAIP3	1350	1030	2129	TNFAIP3	1804	879	626
-				-			
BCL2L13				BCL2L13			
TNFRSF1A	1173	1962	2489	TNFRSF1A	1577	1512	476
-				-			
BCL2L13				BCL2L13			
TNFRSF10A	737	2055	2499	TNFRSF10A	1534	2360	1105
-				-			
BCL2L13				BCL2L13			
TNFRSF10B	1992	885	906	TNFRSF10B	2177	960	1053
-				-			
BCL2L13				BCL2L13			
TNFRSF10D	2204	2159	2343	TNFRSF10D	171	1983	960
-				-			
BCL2L13				BCL2L13			
TNFRSF12A	2183	2509	241	TNFRSF12A	59	1706	2046
-				-			
BCL2L13				BCL2L13			
TNFRSF14	1852	1974	2339	TNFRSF14	2459	2381	1187
-				-			
BCL2L13				BCL2L13			
TNFRSF21	2280	2424	2301	TNFRSF21	52	1054	394
-				-			
BCL2L13				BCL2L13			
TNFSF10	1088	2429	1803	TNFSF10	1764	1186	1227
-				-			
BCL2L13				BCL2L13			
TNFSF15	1286	2438	2252	TNFSF15	638	1962	814
-				-			
BCL2L13				BCL2L13			

Table 11. 2nd order combinatorial hypotheses between BCL and TNF

RANKING TNF FAMILY VS BCL FAMILY							
RANKING OF BCL3 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL3			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL3	652	370	642	TNF - BCL3	598	311	2473
TNFAIP1 - BCL3	168	723	124	TNFAIP1 - BCL3	596	500	158
TNFAIP2 - BCL3	642	856	1098	TNFAIP2 - BCL3	59	776	323
TNFAIP3 - BCL3	2377	534	567	TNFAIP3 - BCL3	300	940	1527
TNFRSF1A - BCL3	163	206	740	TNFRSF1A - BCL3	83	476	1355
TNFRSF10A - BCL3	799	865	1044	TNFRSF10A - BCL3	2388	2493	88
TNFRSF10B - BCL3	1632	2427	1868	TNFRSF10B - BCL3	757	1508	1062
TNFRSF10D - BCL3	1110	858	714	TNFRSF10D - BCL3	2213	1091	1972
TNFRSF12A - BCL3	273	931	623	TNFRSF12A - BCL3	671	1869	1286
TNFRSF14 - BCL3	232	85	1422	TNFRSF14 - BCL3	2149	1311	1650
TNFRSF21 - BCL3	340	1384	2474	TNFRSF21 - BCL3	411	729	998
TNFSF10 - BCL3	1537	1753	1638	TNFSF10 - BCL3	1926	1523	2107
TNFSF15 - BCL3	129	284	729	TNFSF15 - BCL3	1649	1032	2122
RANKING OF BCL6 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL6			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL6	2271	2071	1810	TNF - BCL6	806	437	1411
TNFAIP1 - BCL6	2135	2158	1330	TNFAIP1 - BCL6	1089	850	372
TNFAIP2 - BCL6	2340	1428	1808	TNFAIP2 - BCL6	152	334	703
TNFAIP3 - BCL6	267	1336	1219	TNFAIP3 - BCL6	1884	1935	855
TNFRSF1A - BCL6	1598	1771	2503	TNFRSF1A - BCL6	788	741	1130
TNFRSF10A - BCL6	1327	1831	2096	TNFRSF10A - BCL6	607	1249	2360
TNFRSF10B - BCL6	1373	1873	1264	TNFRSF10B - BCL6	1746	1282	361
TNFRSF10D - BCL6	2213	2188	788	TNFRSF10D - BCL6	1540	1301	2008
TNFRSF12A - BCL6	1867	99	2261	TNFRSF12A - BCL6	545	2200	1910
TNFRSF14 - BCL6	1409	1337	2028	TNFRSF14 - BCL6	731	1302	1902
TNFRSF21 - BCL6	645	2071	2335	TNFRSF21 - BCL6	40	1850	50
TNFSF10 - BCL6	919	445	99	TNFSF10 - BCL6	2119	1102	1626
TNFSF15 - BCL6	2106	1692	1451	TNFSF15 - BCL6	969	1475	226

Table 11. Cont.

RANKING TNF FAMILY VS BCL FAMILY							
RANKING OF BCL9L W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL9L			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL9L	1964	1172	1478	TNF - BCL9L	2218	98	425
TNFAIP1 - BCL9L	439	1445	264	TNFAIP1 - BCL9L	766	2470	1802
TNFAIP2 - BCL9L	1250	1473	696	TNFAIP2 - BCL9L	646	567	85
TNFAIP3 - BCL9L	534	630	618	TNFAIP3 - BCL9L	1046	1223	2296
TNFRSF1A - BCL9L	2050	1096	978	TNFRSF1A - BCL9L	863	500	825
TNFRSF10A - BCL9L	212	1682	980	TNFRSF10A - BCL9L	2140	241	1547
TNFRSF10B - BCL9L	952	698	685	TNFRSF10B - BCL9L	286	414	2046
TNFRSF10D - BCL9L	1315	181	1423	TNFRSF10D - BCL9L	1956	112	990
TNFRSF12A - BCL9L	430	1167	1470	TNFRSF12A - BCL9L	1797	1280	1699
TNFRSF14 - BCL9L	1433	635	1497	TNFRSF14 - BCL9L	670	1055	1540
TNFRSF21 - BCL9L	495	2326	468	TNFRSF21 - BCL9L	1291	1378	246
TNFSF10 - BCL9L	1889	974	183	TNFSF10 - BCL9L	1812	1796	2095
TNFSF15 - BCL9L	878	2389	71	TNFSF15 - BCL9L	1939	2114	2405
RANKING OF BCL10 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T BCL10			
	laplace	linear	rbf		laplace	linear	rbf
TNF - BCL10	708	79	979	TNF - BCL10	1931	114	1573
TNFAIP1 - BCL10	1657	805	1298	TNFAIP1 - BCL10	690	1941	7
TNFAIP2 - BCL10	1101	2197	312	TNFAIP2 - BCL10	523	2099	339
TNFAIP3 - BCL10	985	813	767	TNFAIP3 - BCL10	935	595	1870
TNFRSF1A - BCL10	745	1191	1288	TNFRSF1A - BCL10	362	173	448
TNFRSF10A - BCL10	451	819	954	TNFRSF10A - BCL10	1547	415	2426
TNFRSF10B - BCL10	791	537	1446	TNFRSF10B - BCL10	582	658	1464
TNFRSF10D - BCL10	1831	1694	2040	TNFRSF10D - BCL10	302	19	2497
TNFRSF12A - BCL10	2015	1072	1883	TNFRSF12A - BCL10	1865	1234	1540
TNFRSF14 - BCL10	254	1400	847	TNFRSF14 - BCL10	1175	1894	2227
TNFRSF21 - BCL10	1912	571	958	TNFRSF21 - BCL10	848	1943	418
TNFSF10 - BCL10	1743	931	1657	TNFSF10 - BCL10	2020	1522	1054
TNFSF15 - BCL10	254	1469	577	TNFSF15 - BCL10	1256	188	1074

On the left side is the ranking of BCL family w.r.t TNF family. We found **BCL2L2** to be up regulated w.r.t TNF, TNF-AIP1/RSF1A/RSF10B/RSF10D/RSF12A/RSF14/RSF21/ SF10/SF15. These are reflected in rankings of 1822 (laplace), 1926 (linear) and 2359 (rbf) for TNF - BCL2L2; 2266 (laplace), 2478 (linear) and 1847 (rbf) for TNFAIP1 - BCL2L2; 2311 (linear) and 1920 (rbf) for TNFRSF1A - BCL2L2; 2478 (laplace) and 2239 (rbf) for TNFRSF10B - BCL2L2; 2278 (linear) and 2237 (rbf) for TNFRSF10D - BCL2L2; 1945 (laplace) and 2484 (rbf) for TNFRSF12A - BCL2L2; 2358 (laplace) and 2310 (rbf) for TNFRSF14 - BCL2L2; 2292 (laplace) and 1850 (linear) for TNFRSF21 - BCL2L2; 2438 (laplace) and 2013 (rbf) for TNFSF10 - BCL2L2 and 2443 (linear) and 2350 (rbf) for TNFSF15 - BCL2L2; **BCL2L3** was up regulated w.r.t TNF, TNF-AIP1/RSF1A/RSF10A/RSF10D/RSF12A/RSF14/RSF21/ SF10/SF15. These are reflected in rankings of 2437 (laplace), 2482 (linear) and 2482 (rbf) for TNF - BCL2L13; 1863 (laplace) and 2386 (linear) for TNFAIP1 - BCL2L13; 1962 (linear) and 2489 (rbf) for TNFRSF1A - BCL2L13; 2055 (linear) and 2499 (rbf) for TNFRSF10A - BCL2L13; 2204 (laplace), 2159 (linear) and 2343 (rbf) for TNFRSF10D - BCL2L13; 2183 (laplace), 2509 (linear) for TNFRSF12A - BCL2L13; 1852 (laplace), 1974 (linear) and 2339 (rbf) for TNFRSF14 - BCL2L13; 2280 (laplace), 2424 (linear) and 2301 (rbf) for TNFRSF21 - BCL2L13; 2429 (linear) and 1803 (rbf) for TNFSF10 - BCL2L13; and 2438 (linear) and 2252 (rbf) for TNFSF15 - BCL2L13; **BCL3** was up regulated w.r.t TNFRSF10B. This is reflected in rankings of 2427 (laplace) and 1868 (rbf). **BCL6** was up regulated w.r.t TNF, TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF21. These are reflected in rankings of 2271 (laplace), 2071 (linear) and 1810 (rbf) for TNF - BCL6; 2135 (laplace) and 2158 (linear) for TNFAIP1 - BCL6; 2340 (laplace) 1808 (rbf) for TNFAIP2 - BCL6; 1771 (linear) and 2503 (rbf) for TNFRSF1A - BCL6; and 1831 (linear) and 2096 (rbf) for TNFRSF10A - BCL6; and 2213 (laplace) and 2188 (rbf) for TNFRSF10D - BCL6; and 2071 (linear) and 2335 (rbf) for TNFRSF21 - BCL6; **BCL10** was up regulated w.r.t TNF-RSF10D/RSF12A. These are reflected in rankings of 1831 (laplace) and 2040 (rbf) for TNFRSF10D - BCL10; and 2015 (laplace) and 1883 (rbf) for TNFRSF12A - BCL10;

On the right side is the ranking of TNF family w.r.t BCL family. We found **TNF-RSF10A/RSF10B/RSF10D/RSF12A/RSF14/SF10** to be up regulated w.r.t BCL2L1. These are reflected in rankings of 2273 (laplace) and 1928 (linear) for TNFRSF10A - BCL2L1; 2252 (linear) and 2217 (rbf) for TNFRSF10B - BCL2L1; 1868 (laplace), 2420 (linear) and 2392 (rbf) for TNFRSF10D - BCL2L1; 1923 (laplace) and 1936 (rbf) for TNFRSF12A - BCL2L1; 2350 (linear) and 2414 (rbf) for TNFRSF14 - BCL2L1 and 2115 (laplace) and 2299 (linear) for TNFSF10 - BCL2L1; **TNFRSF10B** was up regulated w.r.t BCL2L2. This is reflected in rankings of 2153 (laplace) and 1983 (rbf) for TNFRSF10B - BCL2L2; **TNFRSF14** was up regulated w.r.t BCL2L13. This is reflected in rankings of 2459 (laplace) and 2381 (linear) for TNFRSF14 - BCL2L13; **TNF-RSF10A/SF10D/SF10** were up regulated w.r.t BCL3. These are reflected in rankings of 2388 (laplace) and 2493 (linear) for TNFRSF10A - BCL3; 2213 (laplace) and 1972 (rbf) for TNFRSF10D - BCL3 and 1926 (laplace) and 2107 (rbf) for TNFSF10 - BCL3; **TNFRSF12A** was up regulated w.r.t BCL6. This is reflected in rankings of 2200 (linear) and 1910 (rbf) for TNFRSF12A - BCL6; **TNF-AIP1/SF10/SF15** was up regulated w.r.t BCL9L. This is reflected in rankings of 2470 (linear) and 1802 (rbf) for TNFAIP1 - BCL9L; 1812 (laplace), 1796 (linear) and 2095 (rbf) for TNFSF10 - BCL9L; and 1939 (laplace), 2114 (linear) and 2405 (rbf) for TNFSF15 - BCL9L; **TNFRSF14** was up regulated w.r.t BCL10. This is reflected in rankings of 1894 (linear) and 2227 (rbf) for TNFRSF14 - BCL10;

One can also interpret the results of the Table 12 graphically, with the following influences - ● BCL w.r.t TNF with TNF, TNF-AIP1/RSF1A/RSF10B/RSF10D/RSF12A/RSF14/RSF21/SF10/SF15 - > BCL2L2; TNF, TNF-AIP1/RSF1A/RSF10A/RSF10D/RSF12A/ RSF14/RSF21/SF10/SF15 - > BCL2L13; TNFRSF10B - > BCL3; TNF, TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF21 - > BCL6; TNF-RSF10D/RSF12A - > BCL10; ● TNF w.r.t BCL with TNF-RSF10A/RSF10B/RSF10D/RSF12A/RSF14/SF10 < - BCL2L1; TNF-RSF10B < - BCL2L2; TNF-RSF14 < - BCL2L13; TNF-RSF10A/SF10D/SF10 < - BCL3; TNF-RSF12A < - BCL6; TNF-AIP1 < - BCL9L; TNF-SF10/SF15 < - BCL9L and TNF-RSF14 < - BCL10.

Table 12. 2nd order combinatorial hypotheses between TNF and BCL family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
BCL w.r.t TNF	
TNF,	BCL2L2
TNF-AIP1/RSF1A/RSF10B/RSF10D/RSF12A/RSF14/RSF21/SF10/SF15	
TNF,	BCL2L13
TNF-AIP1/RSF1A/RSF10A/RSF10D/RSF12A/RSF14/RSF21/SF10/SF15	
TNFRSF10B	BCL3
TNF,	BCL6
TNF-AIP1/AIP2/RSF1A/RSF10A/RSF10D/RSF21	
TNF-RSF10D/RSF12A	BCL10
TNF w.r.t BCL	
TNF-RSF10A/RSF10B/RSF10D/RSF12A/RSF14/SF10/SF15	BCL2L1
TNF-RSF10B	BCL2L2
TNF-RSF14	BCL2L13
TNF-RSF10A/SF10D/SF10	BCL3
TNF-RSF12A	BCL6
TNF-AIP1	BCL9L
TNF-SF10/SF15	BCL9L
TNF-RSF14	BCL10

Conclusions

Presented here are a range of multiple synergistic TNF 2nd 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 Madan *et al.* [40]. 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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