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[Shriprakash Sinha](#) *

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Article

Machine Learning Discoveries of ANT XR2-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

[†] Current address: Aspects of unpublished work were presented in a poster session at (1) the recently concluded first ever Wnt Gordon Conference, from 6-11 August 2017, held in Stowe, VT 05672, USA.

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 ANT XR cell adhesion molecule 2 (ANT XR2)-X (X, a particular gene/protein) at 2nd order level after drug administration. These rankings reveal which ANT XR2-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 ANT XR2 and X. In this research work, we cover combinations of ANT XR2 with Collagen (COL), Integrin (ITG), Matrix metalloproteinases (MMP), WNT, TNF and Interleukin (IL).

Keywords: ANT XR2, 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In the unpublished preprint Sinha [1], a frame work of a search engine was developed which can rank combinations of factors (genes/proteins) in a signaling pathway.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 Interested readers are advised to go through unpublished preprints Sinha [1] and Sinha [2] for details regarding the search engine and the discoveries mentioned in there.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. Anthrax Toxin Receptor Related Synergies

3.1.1. ANT XR2 - Collagen Cross Family Analysis

Anthrax toxin receptor ANTRX is known to capture the Bacillus anthracis toxin and form the cause of the anthrax disease. Regulatory mechanism of the ANT XR1 has been demonstrated essential component in the fibrosis processes in fibroproliferative diseases. Loss of ANT XR1 (a.k.a TEM8) in fibroblasts leads to increased rates of synthesis of fiber-forming collagens, resulting in progressive fibrosis in skin and other organs Besschetnova *et al.* [5]. TEM8 interacts with the cleaved C5 domain of collagen 3(VI) Nanda *et al.* [6]. Hotchkiss *et al.* [7] also indicate the interaction of TEM8 and collagens. Bell *et al.* [8] indicate that a recombinant portion of ANT XR2 (a.k.a CMG2) was found to bind collagen type IV and laminin, suggesting a potential role in basement membrane matrix synthesis and assembly. Bürgi *et al.* [9] show that CMG2/ANT XR2 regulates extracellular collagen VI which accumulates in hyaline fibromatosis syndrome. A distinctive early childhood-onset disorder, systemic hyalinosis, is characterized by mutations in the anthrax toxin receptor 2 gene (ANTRX2) as shown by Shieh *et al.* [10]. Not much is known about the behaviour of ANTRX2 with collagens in colorectal cancer. In CRC cells treated with ETC-1922159 these were up regulated. Our search engine was able to rank the 2nd order combinations between these two to see if there is a possible existing synergy based on the already explored pathological functionality in the above works. At in silico level we found possible high numerical valued ranks pointed to some of the combinations of ANT XR2 and collagen family.

On the left side of Table 1 we found **ANT XR2** to be up regulated w.r.t COL-5A3/28A1 (probably COL-7A1 also). These are reflected in rankings of 2006 (laplace) and 2217 (rbf) for COL5A3-ANT XR2; 2259 (linear) and 2296 (rbf) for COL28A1-ANT XR2 and probably 2119 (linear) and 1690 (rbf) for COL7A1-ANT XR2. On the right side, we found, **COL-7A1/28A1** up regulated w.r.t ANT XR2. These are reflected in rankings of 1722 (laplace) and 2121 (rbf) for COL7A1-ANT XR2 and 1731 (linear) and 2362 (rbf) for COL28A1-ANT XR2. Table 2 shows the derived influences which can be represented graphically, with the following influences - • ANT XR2 w.r.t COL with COL5A3 – > ANT XR2 and COL28A1 – > ANT XR2; and • COL w.r.t ANT XR2 with COL7A1 < – ANT XR2 and COL28A1 < – ANT XR2.

Table 1. 2nd order combinatorial hypotheses between ANTRX2 and COL family

RANKING ANTRX2 VS COL FAMILY							
RANKING OF ANTRX2 W.R.T COL FAMILY				RANKING OF COL FAMILY W.R.T ANTRX2			
	laplace	linear	rbf		laplace	linear	rbf
COL5A3-ANTXR2	2006	1473	2217	COL5A3-ANTXR2	984	1782	933
COL6A1-ANTXR2	1935	1061	1366	COL6A1-ANTXR2	324	2211	398
COL7A1-ANTXR2	1002	2119	1690	COL7A1-ANTXR2	1722	956	2121
COL9A2-ANTXR2	1498	552	1361	COL9A2-ANTXR2	2391	80	135
COL17A1-ANTXR2	1906	1086	780	COL17A1-ANTXR2	576	2504	229
COL28A1-ANTXR2	1409	2259	2296	COL28A1-ANTXR2	478	1731	2362

Table 2. 2nd order combinatorial hypotheses between ANTRX2 and COL family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t COL	
COL5A3	ANTXR2
COL28A1	ANTXR2
COL w.r.t ANTXR2	
COL7A1	ANTXR2
COL28A1	ANTXR2

3.1.2. ANTXR2 - Integrin Cross Family Analysis

To define whether there is a possible functional cooperation between TEM8/ANTXR1 and integrins to accomplish cell spreading, Werner *et al.* [11] sought to disrupt the function of collagen binding integrins, which mostly belong to $\beta 1$ family of integrins, with $\beta 1$ integrin-blocking antibodies. Their experiments suggest that the participation of $\beta 1$ integrins can be excluded in TEM8-mediated cell spreading on collagen in primary fibroblasts. However, collectively, they indicate that the participation of these integrins in TEM8 spreading on collagen vary with cell type. The cytoplasmic domain of ANTXR1 affects binding of the protective antigen which is similar to integrin I domains that convert between open and closed conformations that bind ligand with high and low affinities, respectively. These findings are observed by Go *et al.* [12]. Scobie *et al.* [13] observe similar behaviour. Abnormal clustering of TEM8/ANTXR1 with integrin $\beta 1$ and vascular endothelial growth factor receptor 2 (VEGFR2) occurs in endothelial cells within cutaneous infantile hemangiomas, the most common vascular anomaly in childhood Besschetnova *et al.* [5]. These findings suggest the possibility to synergy between ANTXR2 and Integrin family. In CRC cells treated with ETC-1922159, ANTXR2 and integrin families were up regulated. The search engine allotted high numerical valued ranks to some of the 2nd order combinations of ANTXR2 and integrin family members thus pointing to possible synergy in CRC cells. Table 3 shows the rankings of ANTRX2 along with integrin family members.

Table 3. 2nd order combinatorial hypotheses between ANTRX2 and ITG family

RANKING ANTRX2 VS ITG FAMILY							
RANKING OF ANTRX2 W.R.T ITG FAMILY				RANKING OF ITG FAMILY W.R.T ANTXR2			
	laplace	linear	rbf		laplace	linear	rbf
ITGA2-ANTXR2	2261	1129	2444	ITGA2-ANTXR2	657	1662	215
ITGA3-ANTXR2	2027	2134	179	ITGA3-ANTXR2	305	1402	278
ITGA6-ANTXR2	1065	1850	1660	ITGA6-ANTXR2	352	2029	583
ITGB1-ANTXR2	2192	1273	1431	ITGB1-ANTXR2	1538	987	1593
ITGB1BP1-ANTXR2	2444	498	2128	ITGB1BP1-ANTXR2	1743	152	534
ITGB4-ANTXR2	1484	699	249	ITGB4-ANTXR2	123	1420	2116
ITGB5-ANTXR2	1318	1860	2315	ITGB5-ANTXR2	2216	718	1182
ITGB6-ANTXR2	1205	1262	1244	ITGB6-ANTXR2	1200	2000	1896
ITGB8-ANTXR2	1710	2354	2136	ITGB8-ANTXR2	296	1724	1485

On the left side, we found **ANTRX2** to be up regulated w.r.t ITG-A2/A3/B1BP1/B5/B8. These are reflected in the rankings of 2261 (laplace) and 2444 (rbf) for ITGA2-ANTXR2; 2027 (laplace) and 2134 (linear) for ITGA3-ANTXR2; 2444 (laplace) and 2128 (rbf) for ITGB1BP1-ANTXR2; 1860 (linear)and 2315 (rbf) for ITGB5-ANTXR2; and 2354 (linear)and 2136 (rbf) for ITGB8-ANTXR2. On the right side, we found **ITGB6** to be up regulated w.r.t ANTRX2. These are reflected in the rankings of 2000 (linear) and 1896 (rbf) for ITGB6-ANTXR2; Table 4 shows the derived influences which can be represented graphically, with the following influences - • ANTXR2 w.r.t ITG with ITG-A2/A3 – > ANTXR2 and ITG-B1BP1/B5/B8 – > ANTXR2; and • ITG w.r.t ANTXR2 with ITGB6 < – ANTXR2.

Table 4. 2nd order combinatorial hypotheses between ANTRX2 and ITG family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t ITG	
ANTXR2	ITG-A2/A3
ANTXR2	ITG-B1BP1/B5/B8
ITG w.r.t ANTRX2	
ITGB6	ANTXR2

3.1.3. ANTRX2 - MMP Cross Family Analysis

Compromised interactions between TEM8/ANTXR1-deficient endothelial and fibroblastic cells cause dramatic reduction in the activity of the matrix-degrading enzyme MMP2 Besschetnova *et al.* [5]. They observe experimentally that loss of MMP2 activity requires loss of TEM8/ANTXR1 function in both endothelial and fibroblastic cells. Matrix metalloproteinases (MMP) are members of the metzincin group of proteases which share the conserved zinc-binding motif in their catalytic active site Löffek *et al.* [14]. these enzymes are capable of degrading all kinds of extracellular matrix proteins, but also can process a number of bioactive molecules as well as play a major role in cell behaviors such as proliferation, migration, differentiation, apoptosis and host defense, Wikipedia contributors [15]. In CRC cells treated with ETC-1922159, ANTRX2 and integrin families were up regulated. The search engine allotted high numerical valued ranks to some of the 2nd order combinations of ANTRX2 and integrin family members thus pointing to possible synergy in CRC cells. Table 5 shows the rankings of ANTRX2 along with MMP family members.

Table 5. 2nd order combinatorial hypotheses between ANTRX2 and MMP family

RANKING ANTRX2 VS MMP FAMILY							
RANKING OF ANTRX2 W.R.T MMP FAMILY				RANKING OF MMP FAMILY W.R.T ANTRX2			
	laplace	linear	rbf		laplace	linear	rbf
MMP1-ANTXR2	1428	1407	1620	MMP1-ANTXR2	244	2009	2142
MMP14-ANTXR2	1067	1141	900	MMP14-ANTXR2	866	971	443
MMP15-ANTXR2	1457	740	1881	MMP15-ANTXR2	121	2219	1926
MMP28-ANTXR2	2468	1765	1202	MMP28-ANTXR2	11	1857	2092

On the left side, we found **ANTXR2** to be upregulated w.r.t MMP28. This is reflected in rankings of 2468 (laplace) and 1765 (linear) for MMP28-ANTXR2. On the right side we found **MMP-1/15/28** up regulated w.r.t ANTRX2. These are reflected in rankings of 2009 (linear) and 2142 (rbf) for MMP1-ANTXR2; 2219 (linear) and 1926 (rbf) for MMP15-ANTXR2; and 1857 (linear) and 2092 (rbf) for MMP28-ANTXR2. Table 6 shows the derived influences which can be represented graphically, with the following influences - • ANTRX2 w.r.t MMP with ANTRX2 < – MMP28 and • MMP w.r.t ANTRX2 with MMP-1/15/28 < – ANTRX2.

Table 6. 2nd order combinatorial hypotheses between ANTRX2 and MMP family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t MMP	
ANTXR2	MMP28
MMP w.r.t ANTRX2	
MMP-1/15/28	ANTXR2

3.1.4. ANTRX2 - WNT Cross Family Analysis

Abrami *et al.* [16] show that LRP6 can indeed form a complex with ATRs (anthrax toxin receptors), and that this interaction plays a role both in Wnt signalling and in anthrax toxin endocytosis. Through

the ATR-LRP6 interaction, adhesion to the extracellular matrix could locally control Wnt signalling. The authors demonstrated that physical and functional interaction between CMG2/ANTXR2 and LRP6 also raised the possibility that the complex clinical manifestation of Systemic Hyalinosis might be due in part to defects in Wnt signalling. Fluorescence microscopy and biochemical analyses showed that LRP6 enables toxin internalization by interacting at the cell surface with PA receptors TEM8/ATR and/or CMG2/ANTXR2 to form a multi-component complex that enters cells upon PA binding (Wei *et al.* [17]). Verma *et al.* [18] postulate that the developmentally controlled expression of TEM8 modulates endothelial cell response to canonical Wnt signaling to regulate vessel patterning and density. These findings definitely indicate the synergy of ANTRX with Wnts. In CRC cells treated with ETC-1922159, ANTXR2 and WNT families were up regulated. The search engine allotted high numerical valued ranks to some of the 2nd order combinations of ANTXR2 and WNT family members thus pointing to possible synergy in CRC cells. Table 7 shows the rankings of ANTRX2 along with WNT family members.

Table 7. 2nd order combinatorial hypotheses between ANTRX2 and WNT family

RANKING ANTRX2 VS WNT FAMILY							
RANKING OF ANTRX2 W.R.T WNT FAMILY				RANKING OF WNT FAMILY W.R.T ANTRX2			
	laplace	linear	rbf		laplace	linear	rbf
WNT2B-ANTXR2	1160	1013	2286	WNT2B-ANTXR2	1577	1367	944
WNT4-ANTXR2	1735	1833	2341	WNT4-ANTXR2	175	1643	97
WNT7B-ANTXR2	2453	304	1196	WNT7B-ANTXR2	2106	242	1144
WNT9A-ANTXR2	1618	487	1766	WNT9A-ANTXR2	2317	162	845

On the left side, we found **ANTXR2** to be upregulated w.r.t WNT4. This is reflected in rankings of 1833 (linear) and 2341 (rbf) for WNT4-ANTXR2. Table 8 shows the derived influences which can be represented graphically, with the following influences - • ANTXR2 w.r.t WNT with ANTXR2 < – WNT4. This synergistic upregulation of the WNT4 with ANTXR2 might indicate possible control over the signalling in CRC cells treated with ETC-1922159.

Table 8. 2nd order combinatorial hypotheses between ANTRX2 and WNT family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t WNT	
ANTXR2	WNT4

3.1.5. ANTXR2 - TNF Cross Family Analysis

The author could not find much about TNF-ANTXR2 combinations in pathological cases in existing literature, however, Lee *et al.* [19] report the "both LeTx and EdTx markedly inhibited LPS-induced transcription of tumour necrosis factor alpha (TNF- α), interleukin (IL)-1 β , and IL-6 in J774A.1 cells. In contrast, EdTx synergised with LPS to increase the transcription of IL-6 and IL-8 in HAECs. We showed that HAECs are suitable for anthrax toxin research and express higher levels of the two anthrax toxin receptors - tumour endothelial marker 8 (TEM8/ANTXR1) and capillary morphogenesis protein 2 (CMG2/ANTXR2) - than do J774A.1 cells". The high expression of the ANTXR-1/2 is shown, however, the possible synergy between ANTXR and TNFs in not shown. Our search engine pointed to some of the combinations in CRC cells treated with ETC-1922159 treatment. In Table 9, on the left we found **ANTXR2** to be up regulated w.r.t TNF-AIP1/RSF10B. These are reflected in rankings of 1769 (linear) and 1946 (rbf) for TNFAIP1-ANTXR2 and 2278 (linear) and 2218 (rbf) for TNFRSF10B-ANTXR2. On the right we found, **TNF-RSF10A/RSF10D/RSF12A/RSF14** was up regulated w.r.t ANTXR2. These are reflected in rankings of 2260 (laplace) and 2377 (rbf) for TNFRSF10A-ANTXR2, 2258 (laplace)

and 2363 (rbf) for TNFRSF10D-ANTXR2, 2190 (laplace) and 2061 (rbf) for TNFRSF12A-ANTXR2 and 2370(laplace) and 1777 (linear) for TNFRSF14-ANTXR2.

Table 9. 2nd order combinatorial hypotheses between ANTRX2 and TNF family

RANKING ANTRX2 VS TNF FAMILY							
RANKING OF ANTRX2 W.R.T TNF FAMILY				RANKING OF TNF FAMILY W.R.T ANTRX2			
	laplace	linear	rbf		laplace	linear	rbf
TNF-ANTXR2	1439	1568	1285	TNF-ANTXR2	709	1758	1479
TNFAIP1-ANTXR2	1552	1769	1946	TNFAIP1-ANTXR2	1252	2177	218
TNFAIP2-ANTXR2	125	962	2134	TNFAIP2-ANTXR2	659	1156	2109
TNFAIP3-ANTXR2	1184	1253	1558	TNFAIP3-ANTXR2	1429	2485	1731
TNFRSF1A-ANTXR2	1063	310	2145	TNFRSF1A-ANTXR2	1557	2471	935
TNFRSF10A-ANTXR2	351	1358	1280	TNFRSF10A-ANTXR2	2260	32	2377
TNFRSF10B-ANTXR2	2278	2218	982	TNFRSF10B-ANTXR2	852	715	216
TNFRSF10D-ANTXR2	1352	891	1685	TNFRSF10D-ANTXR2	2258	454	2363
TNFRSF12A-ANTXR2	551	1283	1794	TNFRSF12A-ANTXR2	2190	1150	2061
TNFRSF14-ANTXR2	999	442	498	TNFRSF14-ANTXR2	2370	1777	1014
TNFRSF21-ANTXR2	897	997	298	TNFRSF21-ANTXR2	1474	343	510
TNFRSF10-ANTXR2	2151	966	324	TNFRSF10-ANTXR2	2065	112	339
TNFRSF15-ANTXR2	868	967	1590	TNFRSF15-ANTXR2	664	1211	1669

Table 10 shows the derived influences which can be represented graphically, with the following influences - • ANTRX2 w.r.t TNF with ANTRX2 < – TNFAIP1 and ANTRX2 < – TNFRSF10B and • TNF w.r.t ANTRX2 with TNFRSF10A < – ANTRX2; TNFRSF10D < – ANTRX2; TNFRSF12A < – ANTRX2 and TNFRSF14 < – ANTRX2. This synergistic upregulation of the TNF with ANTRX2 might indicate possible control over the signalling in CRC cells treated with ETC-1922159.

Table 10. 2nd order combinatorial hypotheses between ANTRX2 and TNF family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t TNF	
ANTXR2	TNFAIP1
ANTXR2	TNFRSF10B
TNF w.r.t ANTRX2	
TNFRSF10A	ANTXR2
TNFRSF10D	ANTXR2
TNFRSF12A	ANTXR2
TNFRSF14	ANTXR2

3.1.6. ANTRX2 - IL Cross Family Analysis

The author could not find much about TNF-ANTXR2 combinations in pathological cases in existing literature, however, Lee *et al.* [19] report the "both LeTx and EdTx markedly inhibited LPS-induced transcription of tumour necrosis factor alpha (TNF- α), interleukin (IL)-1 β , and IL-6 in J774A.1 cells. In contrast, EdTx synergised with LPS to increase the transcription of IL-6 and IL-8 in HAECs. We showed that HAECs are suitable for anthrax toxin research and express higher levels of the two anthrax toxin receptors - tumour endothelial marker 8 (TEM8/ANTXR1) and capillary morphogenesis protein 2 (CMG2/ANTXR2) – than do J774A.1 cells". The high expression of the ANTRX-1/2 is shown, however, the possible synergy between ANTRX and IL in not shown. Our search engine pointed to some of the combinations in CRC cells treated with ETC-1922159 treatment. In Table 11, on the left we found ANTRX2 to be up regulated w.r.t IL-1RN/6ST/17C/17REL. These are reflected in rankings of 1914 (linear) and 1894 (rbf) for IL1RN-ANTXR2; 1944 (laplace), 2219 (linear) and 1914 (rbf) for IL6ST-ANTXR2; 1832 (laplace) and 2334 (linear) for IL17C-ANTXR2 and 1889 (linear) and 2303 (rbf)

for IL17REL-ANTXR2. On the right we found, **IL-1A/1B/6ST/17C** was up regulated w.r.t ANTXR2. These are reflected in rankings of 2356 (linear) and 1859 (rbf) for IL1A-ANTXR2; 1780 (linear) and 1865 (rbf) for IL6ST-ANTXR2; 1924 (laplace) and 1901 (rbf) for IL15RA-ANTXR2; and 2121 (linear) and 2437 (rbf) for IL17C-ANTXR2.

Table 11. 2nd order combinatorial hypotheses between ANTRX2 and IL family

RANKING ANTRX2 VS IL FAMILY							
RANKING OF ANTRX2 W.R.T IL FAMILY				RANKING OF IL FAMILY W.R.T ANTXR2			
	laplace	linear	rbf		laplace	linear	rbf
IL1A-ANTXR2	1733	454	2253	IL1A-ANTXR2	275	2356	1859
IL1B-ANTXR2	1222	1302	714	IL1B-ANTXR2	330	2011	1762
IL1RAP-ANTXR2	1288	367	80	IL1RAP-ANTXR2	2339	442	747
IL1RN-ANTXR2	1389	1914	1894	IL1RN-ANTXR2	349	1031	1919
IL2RG-ANTXR2	1897	25	432	IL2RG-ANTXR2	368	1867	450
IL6ST-ANTXR2	1944	2219	1914	IL6ST-ANTXR2	46	1780	1865
IL8-ANTXR2	1169	1281	1398	IL8-ANTXR2	1343	2002	434
IL10RB-ANTXR2	1737	496	1545	IL10RB-ANTXR2	1403	800	754
IL15-ANTXR2	787	1812	927	IL15-ANTXR2	1002	1340	481
IL15RA-ANTXR2	840	800	1695	IL15RA-ANTXR2	1924	636	1901
IL17C-ANTXR2	1832	2334	1191	IL17C-ANTXR2	339	2121	2437
IL17REL-ANTXR2	29	1889	2303	IL17REL-ANTXR2	2406	111	960

Table 12 shows the derived influences which can be represented graphically, with the following influences - ● ANTXR2 w.r.t IL with ANTXR2 < – IL1RN; ANTXR2 < – IL6ST; ANTXR2 < – IL17C and ANTXR2 < – IL17REL; and ● IL w.r.t ANTXR2 with IL1A < – ANTXR2; IL1B < – ANTXR2; IL6ST < – ANTXR2; and IL17C < – ANTXR2.

Table 12. 2nd order combinatorial hypotheses between ANTRX2 and IL family.

UNEXPLORED COMBINATORIAL HYPOTHESES	
ANTXR2 w.r.t IL	
ANTXR2	IL1RN
ANTXR2	IL6ST
ANTXR2	IL17C
ANTXR2	IL17REL
IL w.r.t ANTXR2	
IL1A	ANTXR2
IL1B	ANTXR2
IL6ST	ANTXR2
IL17C	ANTXR2

Conclusion

Presented here are a range of multiple synergistic ANTXR2 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.

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.* [20]. 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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Conflicts of Interest: There are no conflicts to declare

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