Theatre for *In Silico* Systems Oncology

Version 1.0

A Next-Generation Multi-Scale Modeling and Simulation Platform for *In Silico* Systems Oncology

Therapeutics Editor User Manual

Version 1.0.20220606

Biomedical Informatics & Engineering Research Laboratory,
Lahore University of Management Sciences (LUMS), Pakistan

This page has been intentionally left blank

Contents

Table of Figures

List of Tables

2. Therapeutics Editor

Therapeutics Editor (TE) allows the user to create therapeutic interventions for the networks created using Networks Editor (NE); it enables the creation of therapies using information about drugs and their targets. Constructing a therapy involves designing and testing a single drug or multi-drug cocktails on a network model by allowing the user to integrate modifications in the network nodes or interactions. The resulting therapy models can be analyzed to predict cell fate outcomes, which may be visualized as three-dimensional landscapes. Network analysis for therapies can be viewed as an 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape', 'Probability Landscape', and 'ODE Landscape'. Details of TE's Graphical User Interface (GUI), analysis techniques, and result visualization have been outlined in the proceeding text.

2.1 Graphical User Interface

In **Figure 2.1.1,** various sections and toolbars of Therapeutics Editor's intuitive GUI have been labeled, and the functionality of each feature has been explained below.

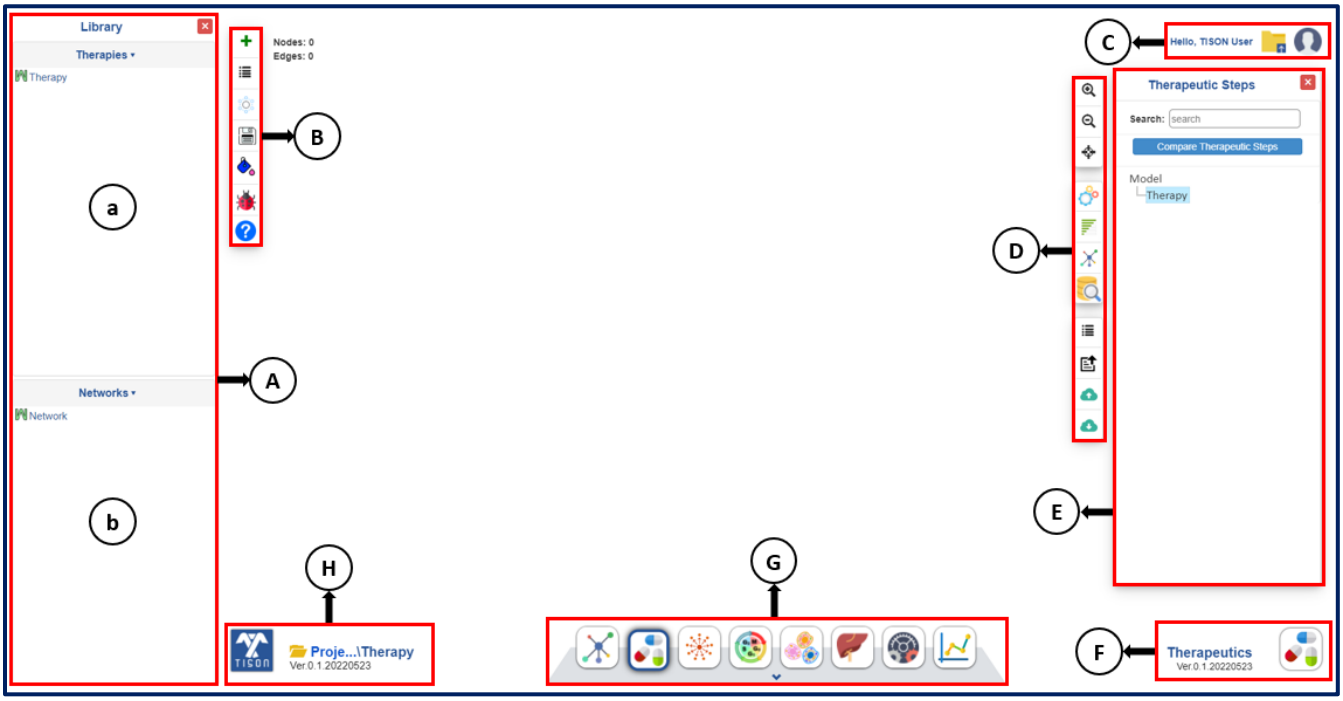


Figure 2.1.1 - The main GUI of TISON's TE.

2.1.1 The Library Section

In **Figure 2.1.1 (A)**,

- The panel on the left of the GUI provides access to the library of user-constructed and saved therapies. A user can 'Edit Properties', 'Duplicate Therapy', and 'Delete Therapy'.
- The 'Networks' panel displays the networks constructed in NE. To create a therapy, the user can select a network from this panel by double-clicking on the network's name (**Figure 2.1.1-b**).

2.1.2 Canvas Toolbar (Left)

In **Figure 2.1.1 (B)**,

- 'Create Therapy' button  in the toolbar creates a new therapy by allowing the user to choose from preconstructed networks against which a therapy needs to be created.
- 'Show Therapies Library' button  displays and hides the library section.
- 'Change Layout' button  allows the user to alter the network layout by opting for either one of the following options: grid, circle, concentric, cose and BFL (Breadthfirst Layout).
- 'Save Therapy' button  in the toolbar saves the current therapy in TISON's database against the user's current project.
- 'Change Background Color' button  allows the user to recolor the canvas.
- 'Report a Bug' button  opens https://github.com/BIRL/TISON/issues and allows the user to report any bug/issue in TISON.
- 'Help' button  opens the 'Therapeutics Editor Manual' located at https://tison.lums.edu.pk/Manuals/TherapeuticsManual.pdf .

2.1.3 Account

In **Figure 2.1.1 (C)**,

- Text at the top right corner 

of the canvas displays the username saved during the registration process.
- 'Return to Project Explorer' icon  in the account settings panel takes the user back to the project explorer and shows the list of projects.
- User icon  allows the user to either return to TISON's home page via 'Main' or sign out from TISON via 'Sign Out'.

2.1.4 Canvas Toolbar (Right)

In **Figure 2.1.1 (D)**,

- 'Zoom In' button  zooms in on the canvas and repositions the network to the center of the screen.
- 'Zoom Out' button  zooms out of the canvas and repositions the network to the center of the screen.
- 'Reset to Default' button  resets the zoom level/canvas size to default.
- 'Perform Network Analysis for Therapy' button  allows the user to perform network analyses on therapies using Deterministic Analysis (DA) and Probabilistic Analysis (PA).
- 'Running Analyses' button  allows the user to check the status of analyses running in the Therapeutics Editor.
- 'View Network' button  allows the user to switch the view back to the network after viewing the landscapes (visual plots include Attractor Landscape, Potential Energy Landscape, and Probability Landscape).
- 'Search Database' button  allows the user to search databases (e.g. Drug Gene Interaction Database (DGIdb)) integrated into TE for drugs against a gene and their scores.
- 'Show Therapeutic Steps' button  displays and hides the therapeutic steps panel.
- 'Upload Case Study'  button allows the user to upload template therapies from the database.
- 'Import Therapy' button  allows the user to upload a network and a drug file.
- 'Export Therapy' button  downloads a (.zip) folder containing input files provided by the user and output files produced after analysis. Folder may include network file, parameters file, custom states file, fixed nodes file, cell fate logic file, and the result files.

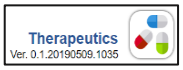
2.1.5 Therapeutic Steps Panel

In **Figure 2.1.1 (E)**,

- The panel on the right of the GUI provides access to user-specified therapeutic interventions.
- The search bar provides the option to search for specific therapies, drugs and node modifications created in the therapeutic steps panel.

- The user can compare the results of any two or more therapies by clicking on the 'Compare Therapeutic Steps' option; the comparison can be displayed as a simple bar chart or a stack chart.

2.1.6 Editor Logo

- In **Figure 2.1.1 (F)**, the editor logo and text 

at the bottom right corner of the canvas display the editor being used.

2.1.7 Canvas Toolbar (Bottom)

- In **Figure 2.1.1 (G)**, the toolbar at the bottom of the canvas allows the user to switch between different editors in TISON.

1. Networks button  navigates to "Networks Editor."
2. Therapeutics button  navigates to "Therapeutics Editor."
3. Environments button  navigates to "Environments Editor."
4. Cell Circuits button  navigates to "Cell Circuits Editor."
5. Cell Lines button  navigates to "Cell Lines Editor."
6. Organoids button  navigates to "Organoids Editor."
7. Simulations button  navigates to "Simulations Editor."
8. Analytics button  navigates to "Analytics Editor."

2.1.8 Project Panel

In **Figure 2.1.1 (H)**,

- 'TISON Home' button  on the bottom left corner of the canvas takes the user back to TISON's home page.
- 'Project Information' button 

displays the project name at the bottom left corner of the canvas and takes the user back to the 'Project Explorer' page.

2.2 Features List

A guide to the functionality of each GUI button and the parameters required to create a therapy is provided in the following sections.

2.2.1 'Create Therapy' Button

The 'Create Therapy' button in the left canvas toolbar (highlighted in **Figure 2.2.1.1**) allows the user to create a new therapy by selecting a network from a list of preconstructed networks in NE.

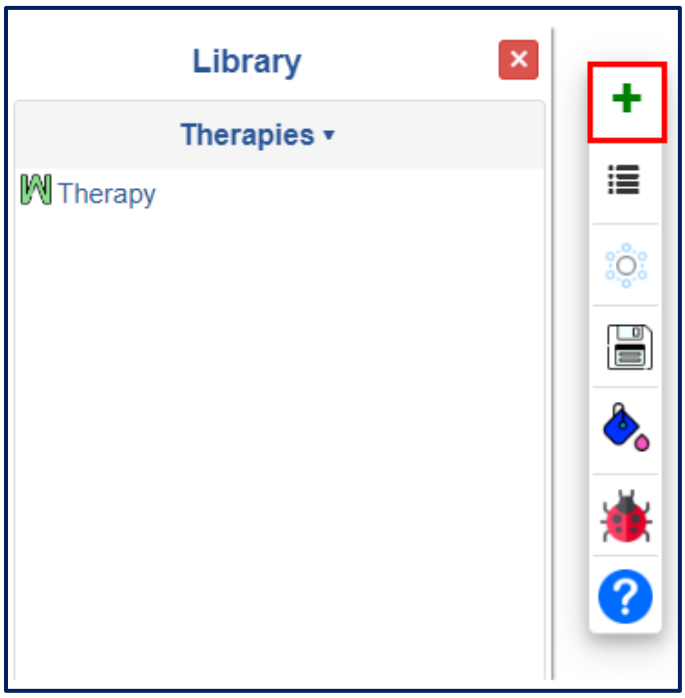


Figure 2.2.1.1 - 'Create Therapy' button in TE.

Once the user clicks this button, the following window will appear (**Figure 2.2.1.2**).

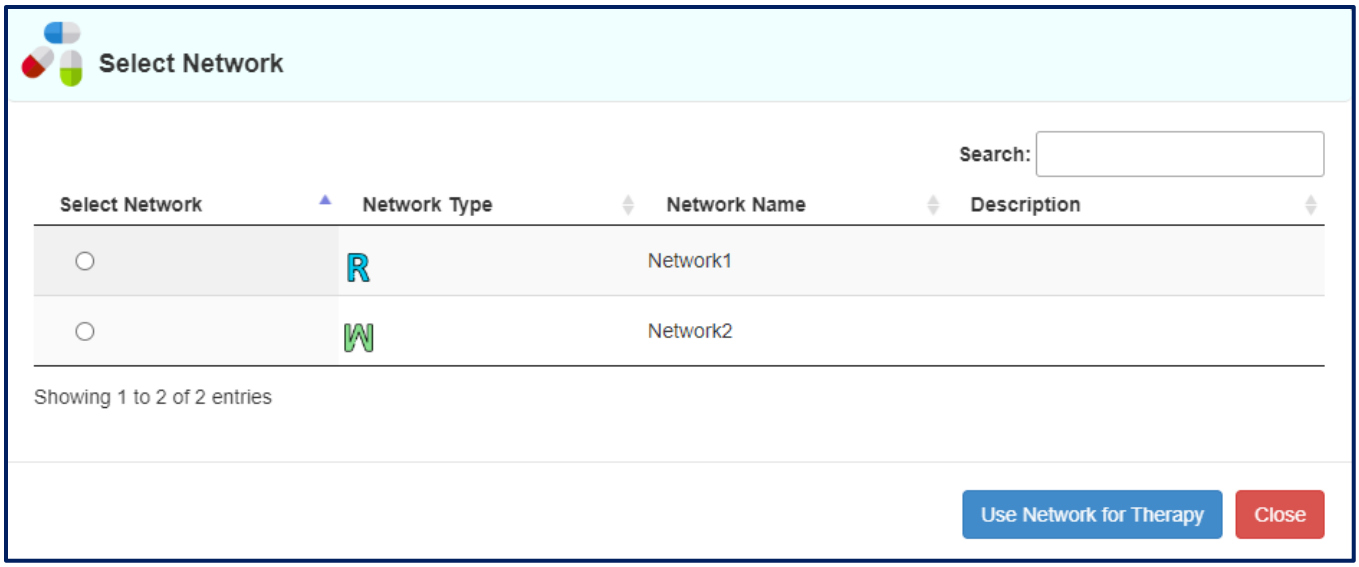


Figure 2.2.1.2 - Window for selecting a network to create a therapy.

Selecting a network and clicking on 'Use Network for Therapy' button will allow the user to develop a therapeutic intervention for that network.

2.2.2 'Show Therapies Library' Button

The 'Show Therapies Library' button (highlighted in **Figure** 2.2.2.1) has a toggling action that provides the option to display or hide the library panel (i.e., if the library is open, it will close it and vice versa).

Figure 2.2.2.1 - 'Show Therapies Library' button in TE.

The library panel allows users to view a previously constructed and saved therapy from the database. Any of the user's pre-constructed networks or therapies can be loaded from the library section by double-clicking on their respective names. The selected therapy (or network) gets highlighted to help the user locate the therapy they are working on; moreover, options to 'Edit Properties', 'Duplicate Therapy' and 'Delete Therapy' are also displayed (**Figure 2.2.2.2**).

Figure 2.2.2.2 - Options to 'Edit Properties', 'Duplicate Therapy', and 'Delete Therapy'.

2.2.3 'Change Layout' Button

The network's layout in TE can be changed using the button highlighted in **Figure 2.2.3.1.**

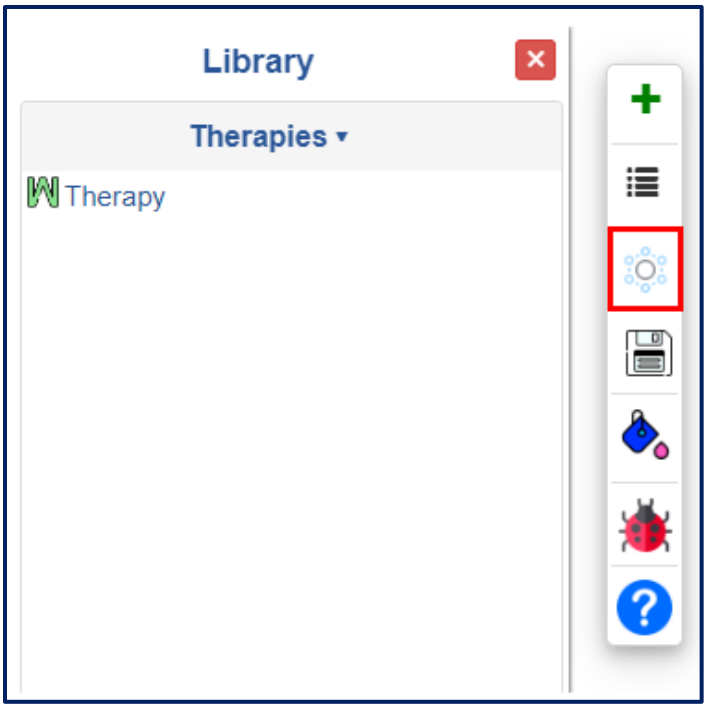


Figure 2.2.3.1 - 'Change Layout' button in TE.

The user can change the network into any of the following conformations upon clicking the button: grid, circle, concentric, cose and BFL (Breadthfirst Layout) (**Figure 2.2.3.2**).

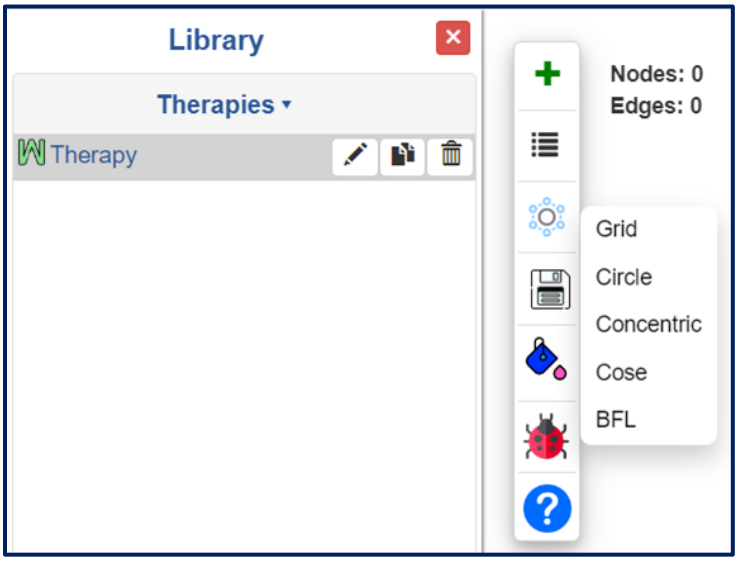


Figure 2.2.3.2 - Various network conformations.

2.2.4 'Save Therapy' Button

After constructing a therapy, the user may save it in TISON's database through the 'Save therapy' button (highlighted in **Figure 2.2.4.1**).

Figure 2.2.4.1 - 'Save Therapy' button in TE.

If the changes are not saved in the database, the user will be asked to enter the name and description of the therapy (**Figure 2.2.4.2**). The therapy will only be saved if its name is not greater than 50 characters and has not already been used for a previously saved therapy; moreover, the description of the therapy cannot exceed 250 characters.

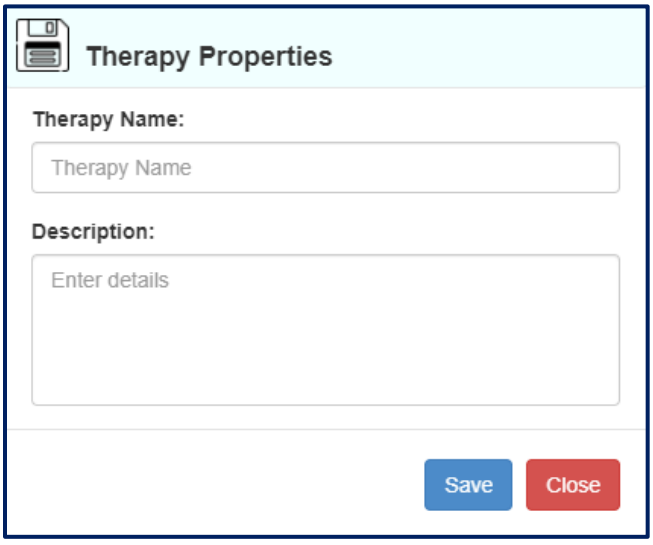


Figure 2.2.4.2 - Save therapy window for a newly created therapy.

If the user tries to switch to another therapy, the prompt shown in **Figure 2.2.4.3** will appear to ensure that any unsaved changes are saved in the database.

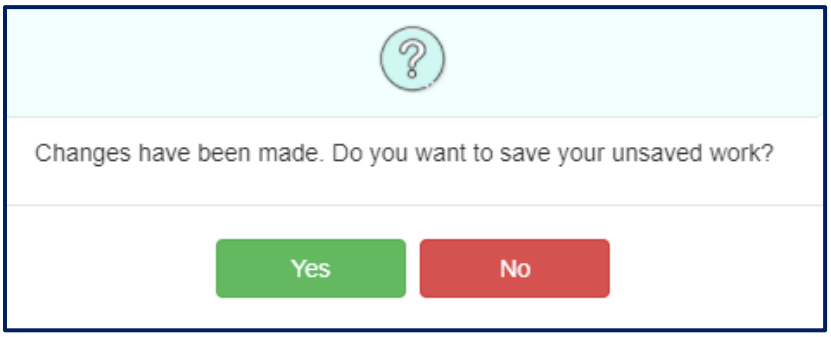


Figure 2.2.4.3 - Save network prompt.

If the user switches to another editor after creating a new therapy, the following prompt will appear to allow the user to save the unsaved therapeutic steps (**Figure 2.2.4.4)**.

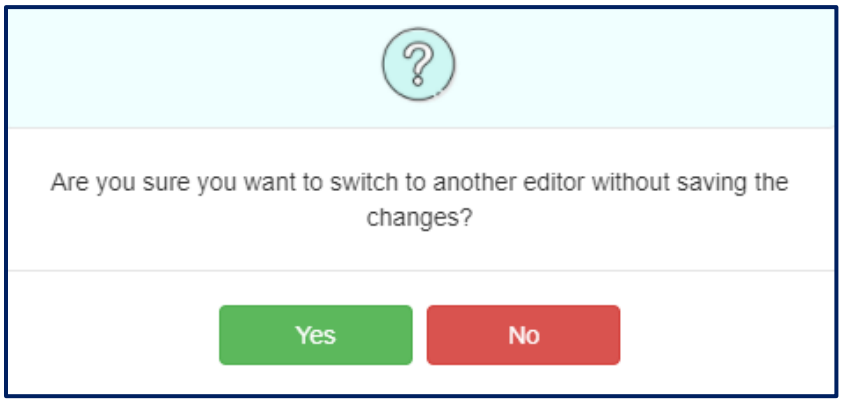


Figure 2.2.4.4 - Switching editors without saving therapy prompt.

2.2.5 'Change Background Color' Button

The canvas color can be changed according to the user's choice by selecting the 'Change Background Color' button, as shown below (**Figure 2.2.5.1**).

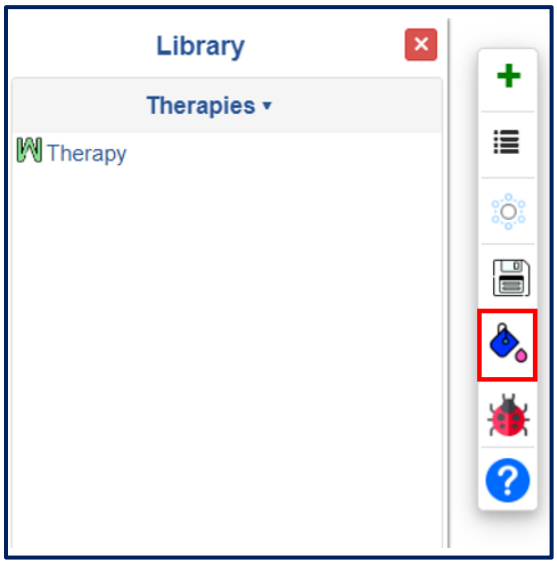


Figure 2.2.5.1 - 'Change Background Color' button in TE.

A window will appear next to the button, allowing the user to select a specific color for the background (**Figure 2.2.5.2**).

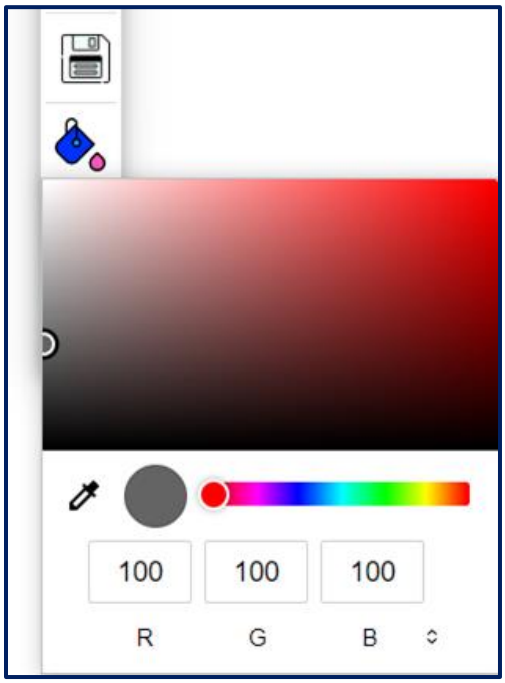


Figure 2.2.5.2 - 'Change Background Color' display.

2.2.6 'Report a Bug' Button

Upon encountering any problem in the software, the user can report the bug by clicking on the 'Report a Bug' button highlighted in **Figure 2.2.6.1.**

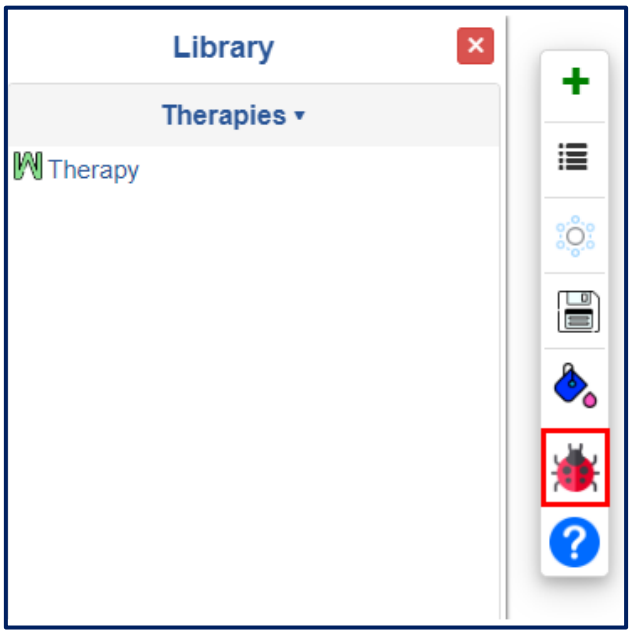


Figure 2.2.6.1 - 'Report a Bug' button in TE.

This icon directs the user to a page on GitHub (Issues - BIRL/TISON - GitHub), where the user can report their issue (**Figure 2.2.6.2**).

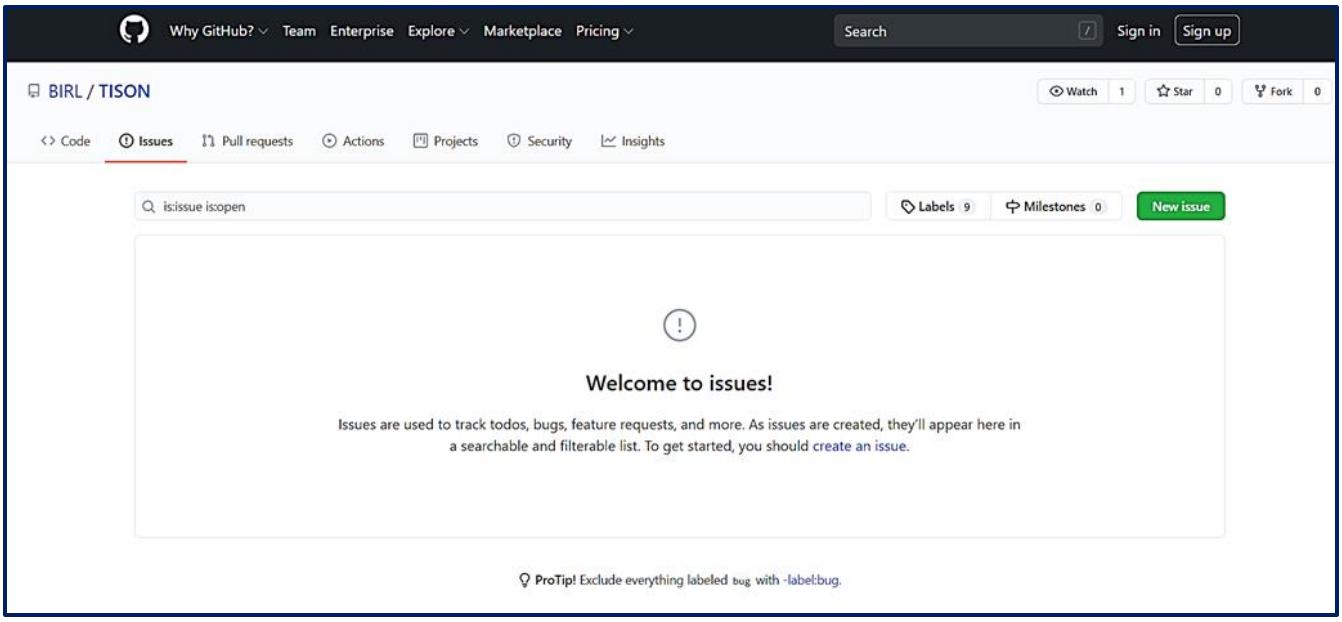


Figure 2.2.6.2 - GitHub page for reporting TISON's bugs.

2.2.7 'Help' Button

Clicking on the 'Help' button highlighted in **Figure 2.2.7.1** opens a user guide manual of the editor currently in use (https://tison.lums.edu.pk/Manuals/TherapeuticsManual.pdf).

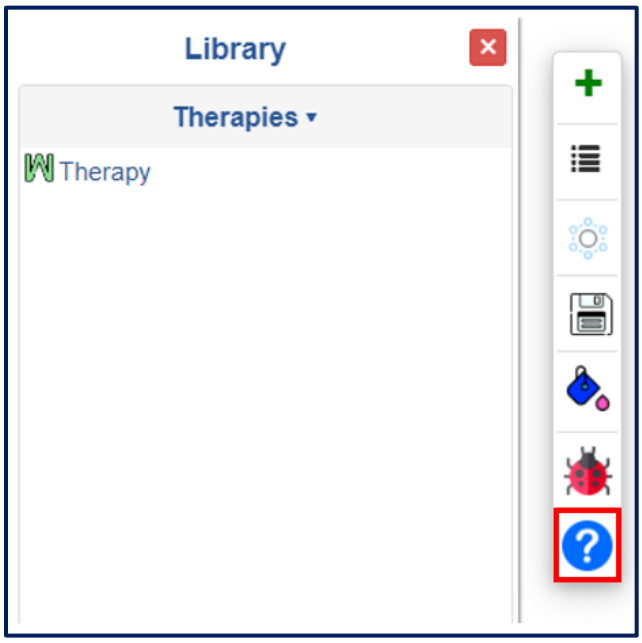


Figure 2.2.7.1 - 'Help' button in TE.

2.2.8 Username

The username entered by the user during the registration process appears in the highlighted area in **Figure 2.2.8.1.**

Figure 2.2.8.1 - Username displayed in TE.

2.2.9 Project Folder

The 'Return to Project Explorer' icon highlighted in **Figure 2.2.9.1** allows the user to return to the project explorer page and view the list of created and saved projects in TISON's database.

Figure 2.2.9.1 - 'Project Folder' icon in TE.

2.2.10 Return to TISON Home Page or Sign Out

The highlighted icon in **Figure 2.2.10.1** displays the current TISON user. Upon clicking it, the user is given the option to either return to the TISON home page by selecting 'Main' or sign out from TISON by selecting 'Sign Out'.

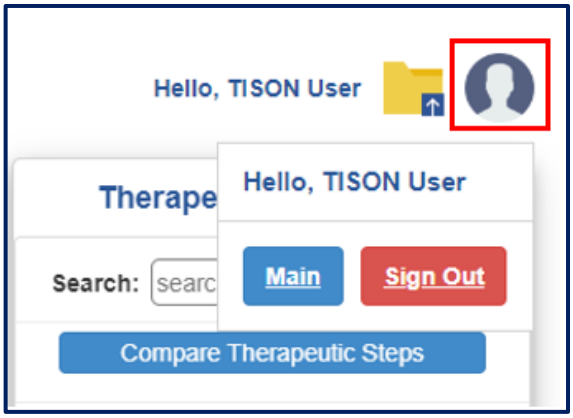


Figure 2.2.10.1 - Return to TISON home page 'Main' from TE or 'Sign Out'.

2.2.11 'Zoom In', 'Zoom Out' and 'Reset to Default' Buttons

The highlighted icon bar (**Figure 2.2.11.1**) allows the user to zoom in on the canvas **(a)**, zoom out of the canvas **(b),** and reset the canvas to default size **(c)**. Moreover, the zoom level can also be adjusted with the mouse scroll wheel.

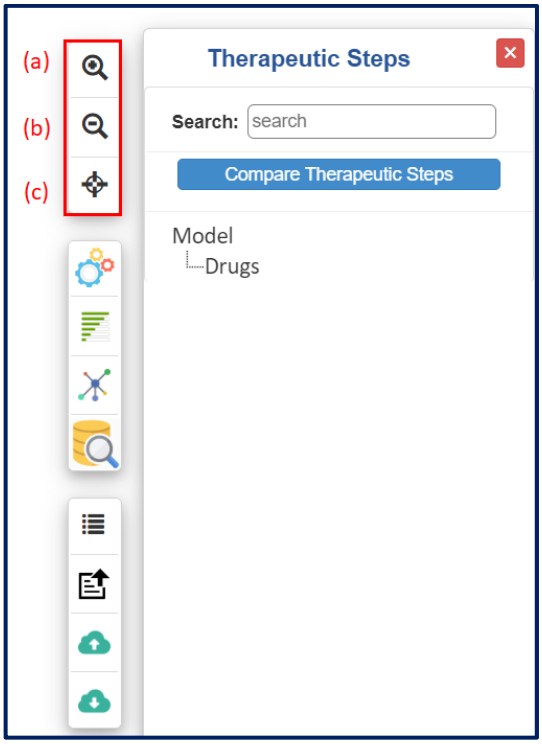


Figure 2.2.11.1 - 'Zoom control buttons': *(a) 'Zoom In' (b) 'Zoom Out' (c) 'Reset to Default'.*

2.3 Network Analysis for Therapy

The highlighted toolbar (**Figure 2.3.1**) provides access to the various features of network analysis for therapy. The 'Perform Network Analysis for Therapy' button (**Figure 2.3.1(d)**) provides options to perform Deterministic Analysis (DA) and Probabilistic Analysis (PA) on constructed therapies. The 'Analysis Progress and Status' button (**Figure 2.3.1(e)**) shows the different analyses running simultaneously. The 'View Network' button (**Figure 2.3.1(f)**) switches the view to the network after viewing the landscapes. The 'Search Database' button (**Figure 2.3.1(g)**) allows the user to search databases (e.g., Drug Gene Interaction Database (DGIdb)) integrated into TE for drugs and their corresponding scores against a gene.

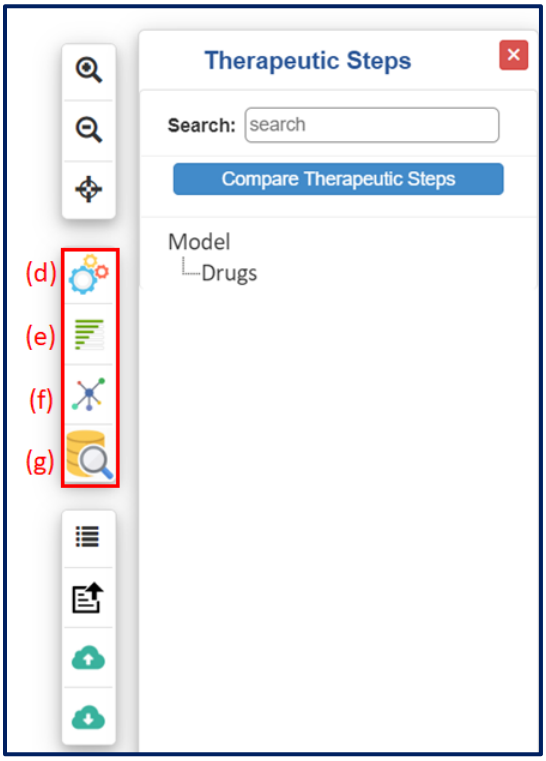


Figure 2.3.1 - Network analysis for therapy toolbar in TE: *(d) 'Perform Network Analysis for Therapy' button, (e) 'Running Analysis' button, (f) 'View Network' button, and (g) 'Search Database' button.*

2.3.1 'Perform Network Analysis for Therapy' Button

For a *rules-based therapy*, the user can perform Deterministic Analysis (DA) only, while for a *weight-based therapy*, DA and Probabilistic Analysis (PA) can be performed by clicking on the button highlighted in **Figure 2.3.1.1**

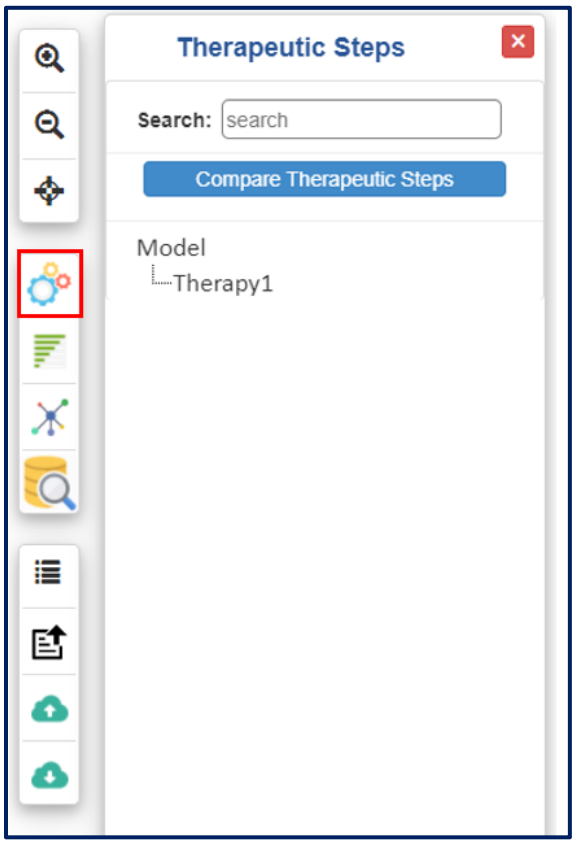


Figure 2.3.1.1 - 'Perform Network Analysis for Therapy' button.

a) Deterministic Analysis:

In Deterministic Analysis (DA), a biomolecular network is represented as a closed system, although a node's basal value can incorporate external influence. The DA pipeline assumes a lack of external noise or extracellular signaling perturbations and makes use of a basal value vector, $B = [b_1, b_2, \dots, b_n]$, provided by the user or obtained from integrated databases. This vector contains biomolecular expression values that indicate the basal activity of each node, where the basal activity values signify the node expressions without any inputs from the network. The interaction weight matrix, $I_w = [w_{ij}]_{n\times n}$ represents the nature and effective mass of interaction between two participating nodes. The node-interaction model can simulate the node state transition function.

In order to update the node states using DA, a transition function is used; this integrates each model component, including basal values, interaction weights, and node states[2], as given by the piecewise equation below:

$$x_i(t+1) = \begin{cases} 1 & if \sum_j \left(w_{ji}x_j(t)\right) + b_i > 0 \\ 0 & else\ if \sum_j \left(w_{ji}x_j(t)\right) + b_i < 0 \\ x_i & else\ if \sum_j \left(w_{ji}x_j(t)\right) + b_i = 0 \end{cases}$$

The equation above defines the node (x_i) state transition from time step t to $t+1$. The summation, $\sum_j \left(w_{ji}x_j(t)\right) + b_i$, adds weighted interactions and the basal value. The result from the expression is translated using a *sign* (*Signum* function) (**Figure 2.3.1.2**)which is defined as follows[3]:

$$x_i(t+1) = f(\sum_j \left(w_{ji}x_j(t)\right) + b_i)$$

where, f is *Signum* (or *Sign*) function defined over any input variable, z , as follows:[3]

$$f(z) = \begin{cases} 1 & if\ z > 0 \\ 0 & if\ z = 0 \\ -1 & if\ z < 0 \end{cases}$$

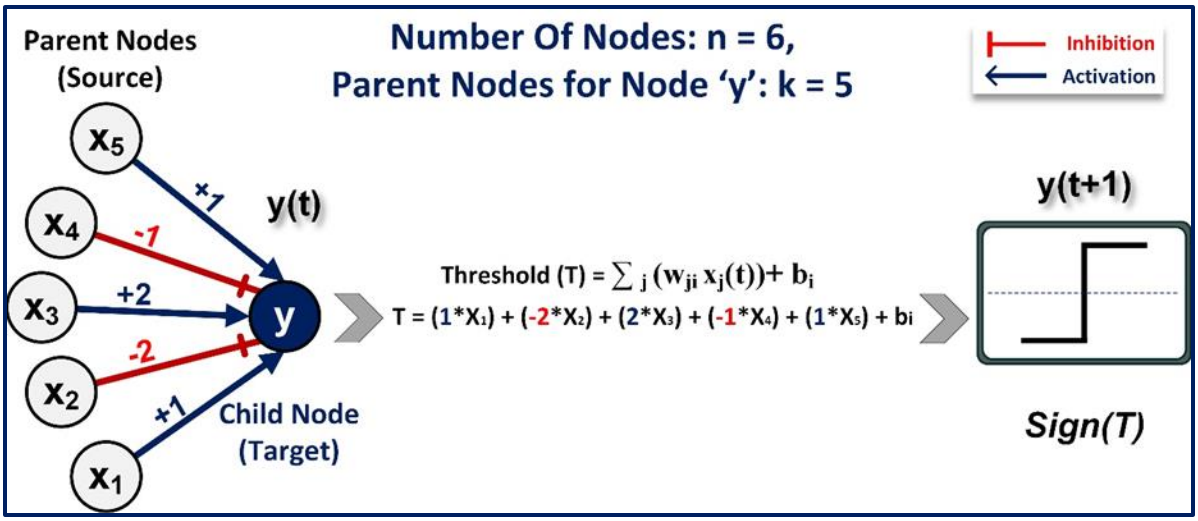


Figure 2.3.1.2 - Node update for DA in TISON*. The example network has one target node, y, with five parent nodes (k=5) regulating it. Three of these parents activate the node, while the remaining two inhibit its activity.*

Figure 2.3.1.3 outlines the workflow of the DA pipeline implemented in TISON. The DA pipeline starts with a set of initial network states that can be generated by (1) exhaustive sampling (ES) of the state-space, (2) random sampling (RS) of the state-space, or (3) providing a custom state-space (CS) file. ES generates all (2^n) possible states for onward analysis. RS selects a user-specified number of states from the complete state-space, thereby making the analysis of more extensive networks feasible. Using the CS option, TISON's user can selectively incorporate specific network states for further analysis. To achieve a steady-state, network states and transition functions are used. Once the system attains a steady state, the frequently recurring states (or attractors) are identified for attractor landscape plotting (discussed later).

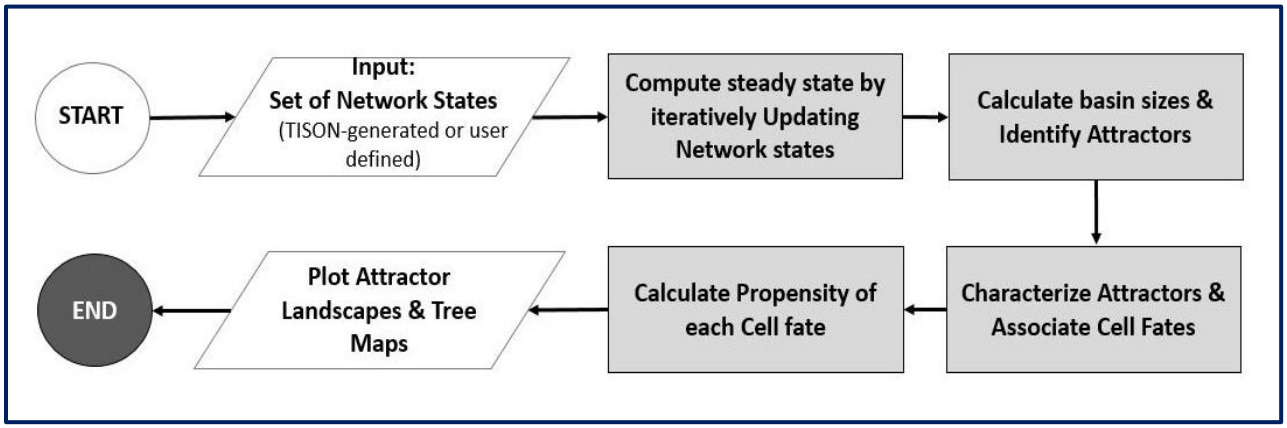


Figure 2.3.1.3 - DA pipeline implemented in TISON.

To assist in the cell fate discovery process, TISON's user can incorporate specific node update rules into the DA pipeline; node update rules are employed during the network update and stored in the form of truth tables. State transition rules are constructed from the exhaustive state space, and only the biologically plausible node state transitions are employed onwards. Therefore, each node state gets updated from the rules table. An example of rules-based DA is given below in **Figure 2.3.1.4.** The child node 'C' with parent nodes 'A' and 'B' is updated to state '0' by applying the rule '0 1 0' (in the red box).

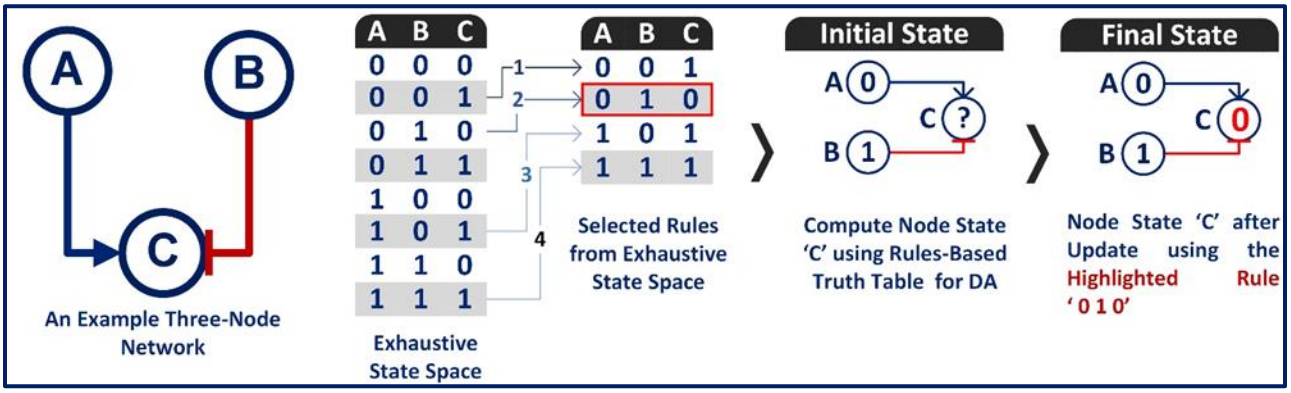


Figure 2.3.1.4 - Rules-based DA methodology. *A three-node network with two-parent nodes, 'A' and 'B,' and one child node, 'C', is considered for rules-based DA. The state of node 'C' ('0') is computed by using rules-based logic defined (red box) from within the exhaustive state space.*

The following window (**Figure 2.3.1.5**) will open when the user selects the 'Perform Network Analysis for Therapy' icon for a *rules-based therapy*, for which the 'Deterministic Analysis' tab is selected by default (For visual reference, see Section 2.5, Table 2.5.1 - Video 1).

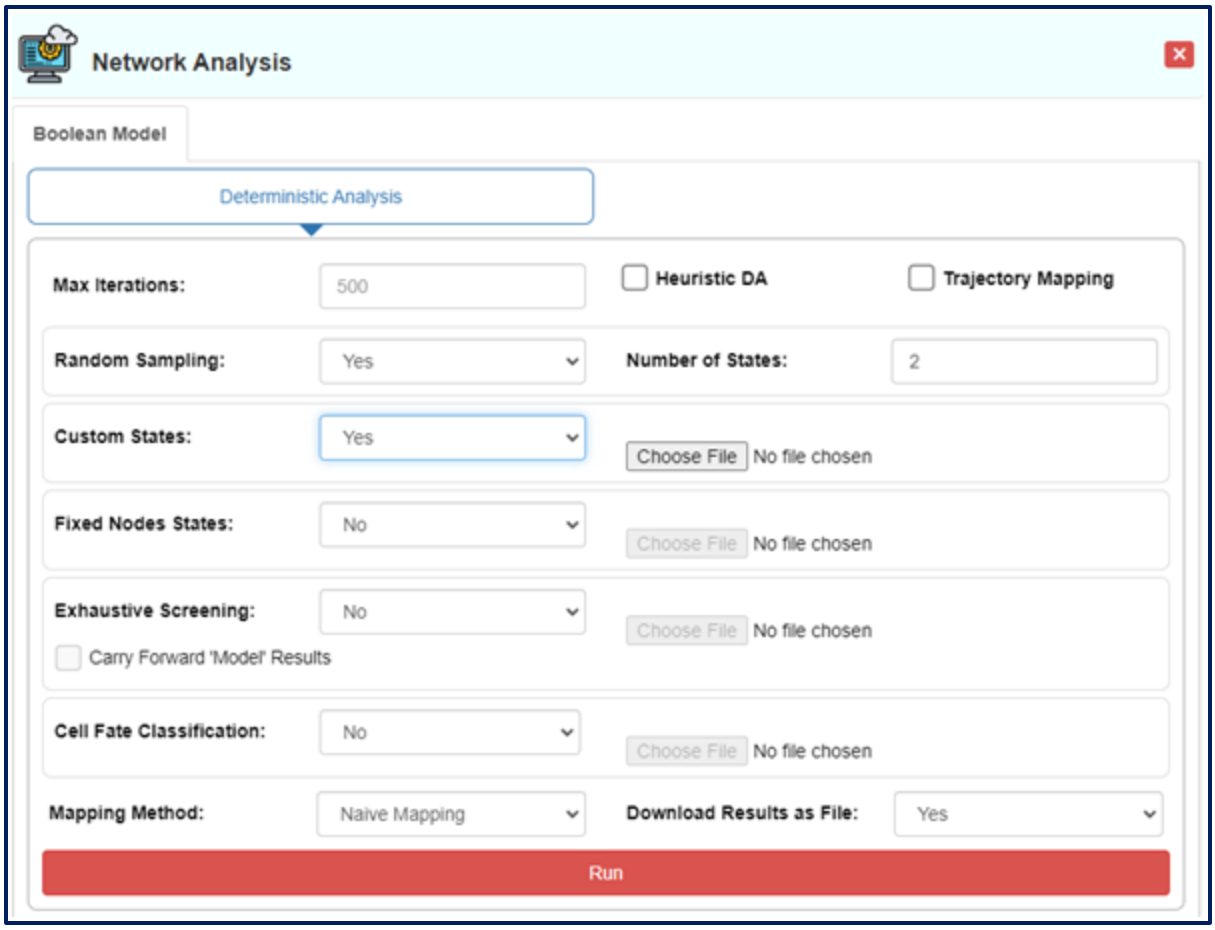


Figure 2.3.1.5 - Network analysis for therapy window for DA.

- **Steps for average node activity/propensity calculation:**

The result file provides the average node activity, which is computed by the following steps.

Step 1: Multiply the basin size ratio with its corresponding node state for all attractors (shown in **Figure 2.3.1.6**).

Step 2: Perform Step 1 for all attractors (shown in **Figure 2.3.1.7**).

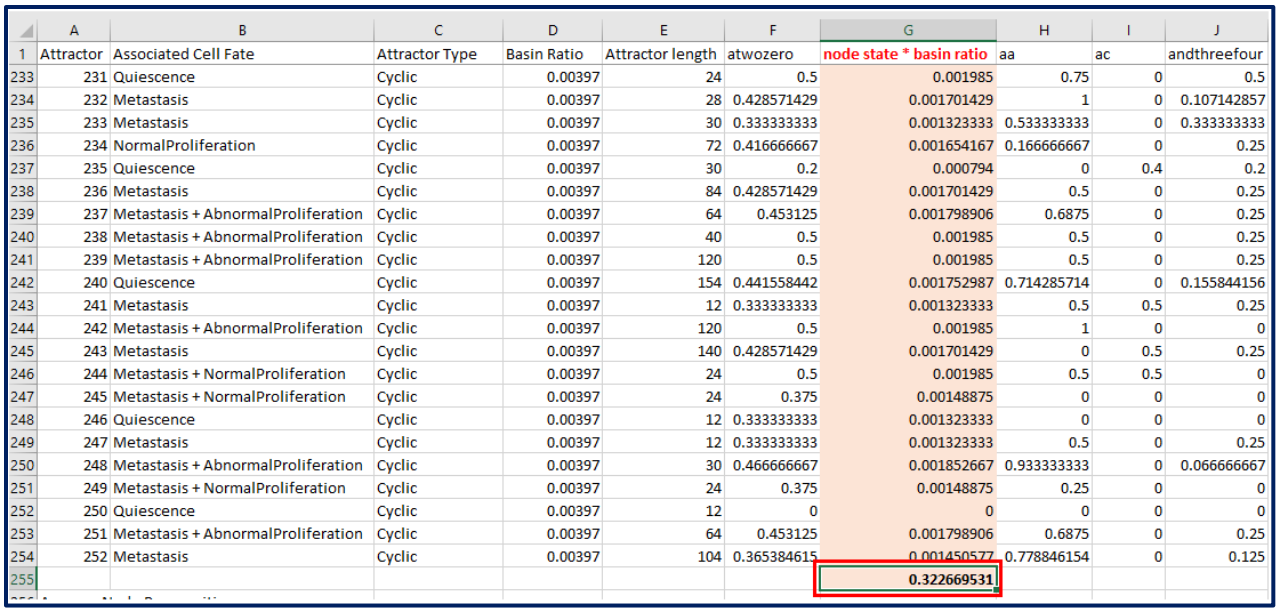
Step 3: Sum the entire column (shown in **Figure 2.3.1.8**).

	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
2										
3	1	Quiescence	Cyclic	0.00397	8	0.375	=F3*D3	0.5	0	0
4	2	Quiescence	Cyclic	0.00397	12	0.333333333		0.5	0	0.25
5	3	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5		0.5	0	0.25
6	4	Metastasis	Cyclic	0.00397	28	0.428571429		1	0	0.25
7	5	Quiescence	Cyclic	0.00397	84	0		0	0	0
8	6	Quiescence	Cyclic	0.00397	12	0		0	0	0
9	7	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5		1	0	0
10	8	Quiescence	Cyclic	0.00397	58	0.137931034		0.413793103	0	0.172413793
11	9	Quiescence	Cyclic	0.00397	24	0.5		0.5	0.5	0
12	10	Metastasis	Cyclic	0.00397	30	0		0	0	0
13	11	Quiescence	Cyclic	0.00397	84	0		0	0	0
14	12	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5		0.5	0	0.25
15	13	Metastasis	Cyclic	0.00397	12	0.333333333		0	0	0
16	14	Quiescence	Cyclic	0.00397	12	0		0	0	0
17	15	Quiescence	Cyclic	0.00397	60	0.1		0	0	0.2
18	16	Quiescence	Cyclic	0.00397	8	0.5		0	0	0
19	17	Metastasis	Cyclic	0.00397	28	0.428571429		1	0	0.107142857
20	18	NormalProliferation	Cyclic	0.00397	40	0.5		0	0	0

Figure 2.3.1.6 - Step 1 for average node activity calculation.

	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
233	231	Quiescence	Cyclic	0.00397	24	0.5	0.001985	0.75	0	0.5
234	232	Metastasis	Cyclic	0.00397	28	0.428571429	0.001701429	1	0	0.107142857
235	233	Metastasis	Cyclic	0.00397	30	0.333333333	0.001323333	0.533333333	0	0.333333333
236	234	NormalProliferation	Cyclic	0.00397	72	0.416666667	0.001654167	0.166666667	0	0.25
237	235	Quiescence	Cyclic	0.00397	30	0.2	0.000794	0	0.4	0.2
238	236	Metastasis	Cyclic	0.00397	84	0.428571429	0.001701429	0.5	0	0.25
239	237	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
240	238	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5	0.001985	0.5	0	0.25
241	239	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	0.5	0	0.25
242	240	Quiescence	Cyclic	0.00397	154	0.441558442	0.001752987	0.714285714	0	0.155844156
243	241	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0.5	0.25
244	242	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	1	0	0
245	243	Metastasis	Cyclic	0.00397	140	0.428571429	0.001701429	0	0.5	0.25
246	244	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.5	0.001985	0.5	0.5	0
247	245	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0	0	0
248	246	Quiescence	Cyclic	0.00397	12	0.333333333	0.001323333	0	0	0
249	247	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0	0.25
250	248	Metastasis + AbnormalProliferation	Cyclic	0.00397	30	0.466666667	0.001852667	0.933333333	0	0.066666667
251	249	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0.25	0	0
252	250	Quiescence	Cyclic	0.00397	12	0	0	0	0	0
253	251	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
254	252	Metastasis	Cyclic	0.00397	104	0.365384615	0.001450577	0.778846154	0	0.125
255										

Figure 2.3.1.7 - Step 2 for average node activity calculation.



	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
233	231	Quiescence	Cyclic	0.00397	24	0.5	0.001985	0.75	0	0.5
234	232	Metastasis	Cyclic	0.00397	28	0.428571429	0.001701429	1	0	0.107142857
235	233	Metastasis	Cyclic	0.00397	30	0.333333333	0.001323333	0.533333333	0	0.333333333
236	234	NormalProliferation	Cyclic	0.00397	72	0.416666667	0.001654167	0.166666667	0	0.25
237	235	Quiescence	Cyclic	0.00397	30	0.2	0.000794	0	0.4	0.2
238	236	Metastasis	Cyclic	0.00397	84	0.428571429	0.001701429	0.5	0	0.25
239	237	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
240	238	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5	0.001985	0.5	0	0.25
241	239	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	0.5	0	0.25
242	240	Quiescence	Cyclic	0.00397	154	0.441558442	0.001752987	0.714285714	0	0.155844156
243	241	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0.5	0.25
244	242	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	1	0	0
245	243	Metastasis	Cyclic	0.00397	140	0.428571429	0.001701429	0	0.5	0.25
246	244	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.5	0.001985	0.5	0.5	0
247	245	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0	0	0
248	246	Quiescence	Cyclic	0.00397	12	0.333333333	0.001323333	0	0	0
249	247	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0	0.25
250	248	Metastasis + AbnormalProliferation	Cyclic	0.00397	30	0.466666667	0.001852667	0.933333333	0	0.066666667
251	249	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0.25	0	0
252	250	Quiescence	Cyclic	0.00397	12	0	0	0	0	0
253	251	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
254	252	Metastasis	Cyclic	0.00397	104	0.365384615	0.001450577	0.778846154	0	0.125
255							0.322669531			

Figure 2.3.1.8 - Step 3 for average node activity calculation.

To perform DA, the user will have to enter various parameters, each of which is explained below.

- **Max iterations:** Number of iterations for which the algorithm searches the network for an attractor.
- **Heuristic DA:** The network sample can be considerably large for extensive networks and may require a large system memory by TISON. To address this issue, we have implemented a heuristic DA pipeline that works seamlessly on low-memory and large-memory systems. It is implemented by performing a one-step DA and retaining the unique network, after which an n-step DA is performed on these unique states.
- **Trajectory Mapping:** Trajectory mapping traces the route from the initial to the final boundary state, passing through all the intermediate transient states. Users can select “Trajectory Mapping” from the analysis modal allowing TISON to store and then download the trajectory mapping file. This file shows the complete state transition trajectory from initial network states at iteration 0 to the final iteration number.
- **Random Sampling:** This option caters to insufficient system memory (if the system cannot store 2^n states). Exhaustive sampling, in which ‘Random Sampling’ is turned *OFF*, generates all (2^n) possible states for onward analysis. However, when ‘Random Sampling’ is turned *ON*, a user-specified number of states is selected from the complete state-space, thus, making the analysis of more extensive networks feasible. Biological systems tend to be robust, so the final basin size ratio remains accurate within a small error range for a large sampling rate.
- **Number of States:** This parameter is needed when ‘Random Sampling’ has been selected; it refers to the user-specified number of states from the complete state-space, thus making the analysis of larger networks feasible. The default number of states is 256, while the maximum number of states is 10,000. If any state, i.e., exhaustive, random, or user-specified custom states, is not converged, then result file will indicate the number of unconverged states and their values in binary form.

- **Custom States:** The Custom States file (format .csv) contains the network nodes which have been logically defined in the network file (intermediate nodes). TISON's user can selectively incorporate specific network states of certain intermediate nodes for network analysis through the Custom State option. Selecting the 'Heuristic DA' option along with this allows the user to prune the network state-space using one-step DA, which is especially useful in large networks as it enables traceable computation.

  To exemplify, if a user has a network of ten nodes consisting of input, processing, and output nodes, they must provide a customized state space for each node; the values must be in binary (0 or 1). This will allow the analysis of the network towards the generation of steady space outcomes. Assuming that the user has opted for '2' custom states (**Figure 2.3.1.9**), their custom state file of ten nodes will resemble the sample shown in **Figure 2.3.1.10**.

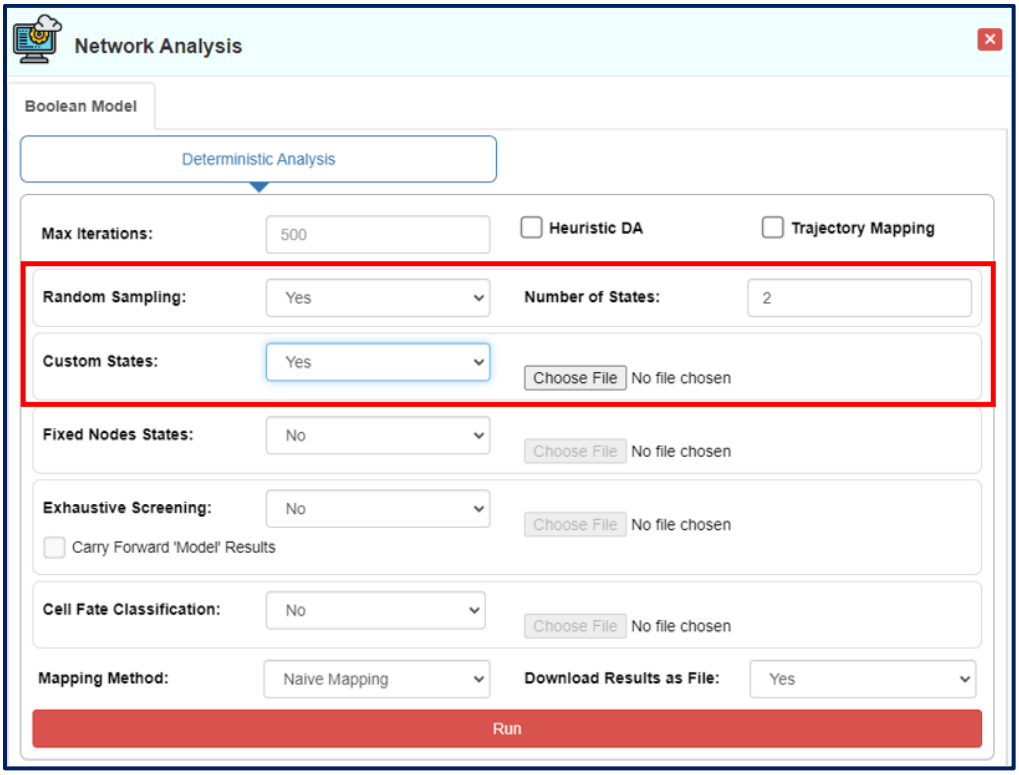


Figure 2.3.1.9 - Setting up the input parameters.

	A	B	C	D	E	F	G	H	I	J	K
1	a	b	c	d	e	f	g	h	i	j	
2	0	0	1	0	0	0	0	0	0	0	
3	1	0	1	0	0	0	0	0	0	1	
4											

Figure 2.3.1.10 - Custom states (.csv) file: *User specifies the names of intermediate nodes in the first row, followed by their custom node values in the second row.*
The user gives a single row of inputs, and analysis is run only once.

- **Fixed Nodes States:** Nodes are returned to the specified value provided by the user after each iteration, i.e., time step; this is to imitate the effect of a drug, biological mutation, input condition, or other external conditions on a network. Hence, the value of that input node is fixed throughout the analysis. For instance, a value of 0.2 means that, after each iteration, 20% of the fixed nodes will be in the *ON* state (set to 1), and 80% of the nodes will be in the *OFF* state (set to 0). The option is shown in the **Figure 2.3.1.11.**

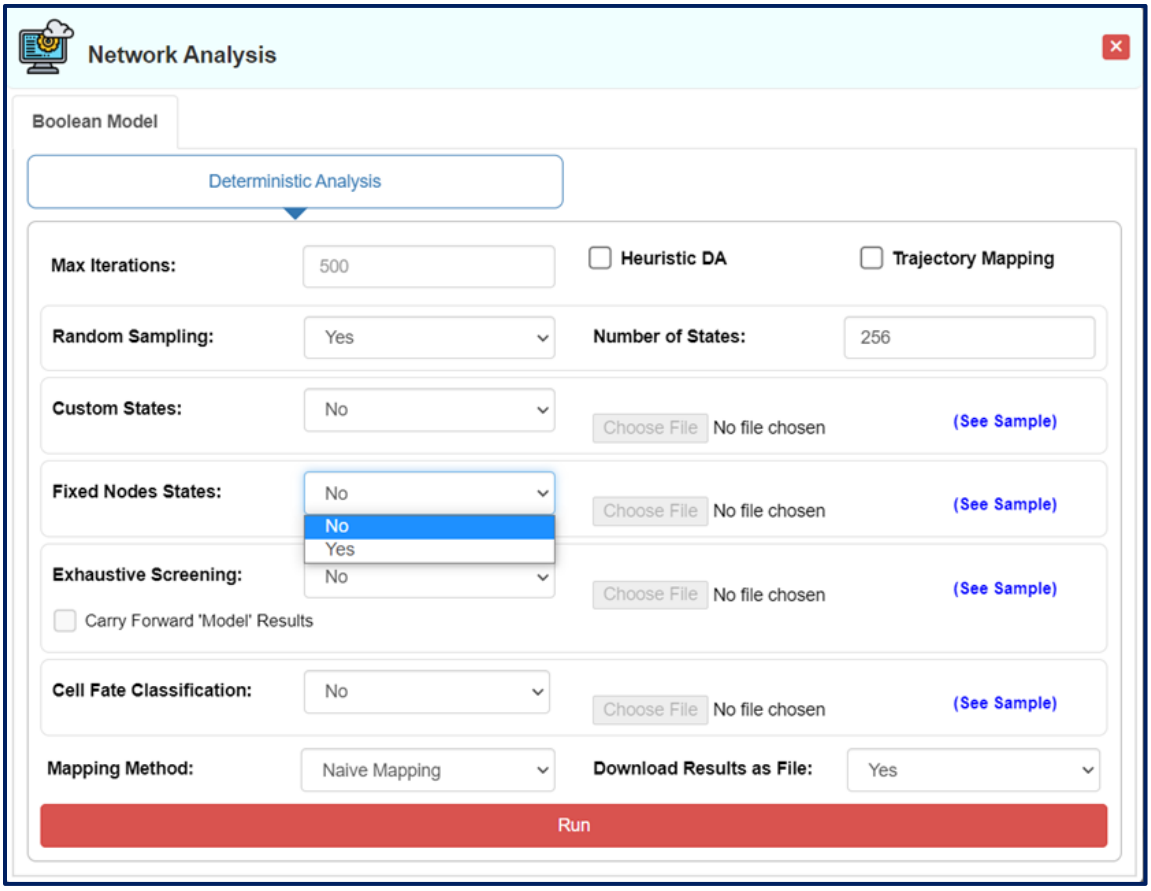


Figure 2.3.1.11 - Fixed nodes states options.

The format for a fixed nodes states file is shown in the figure below (**Figure 2.3.1.12**). The input file (format.csv) should contain only one excel sheet.

	A	B	C	D	E	F
1	EGF	ECM	Fas	Wnt	Tgf	
2	1	1	0	1	0	
3						
4						
5						

Figure 2.3.1.12 - Fixed nodes (.csv) file.

- **Exhaustive Screening:** The 'exhaustive screening feature' (**Figure 2.3.1.13**) can be used to evaluate different nodes (in a single analysis) towards identifying more efficacious target nodes for therapies. It imitates the effect of a second drug, i.e., taking the effect of the previous drug for onwards analysis.

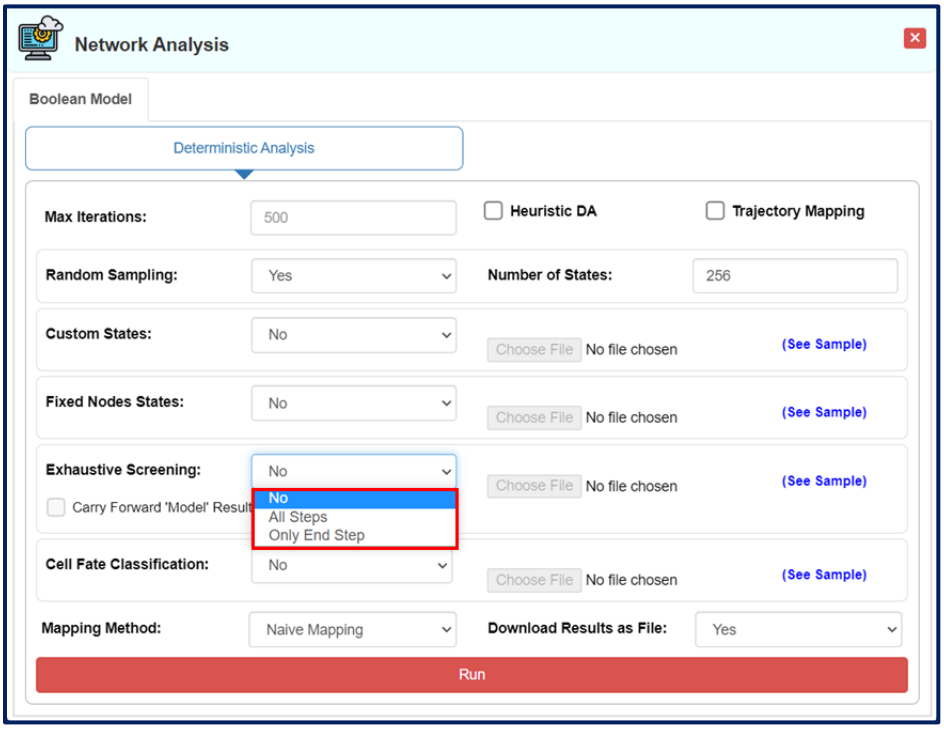
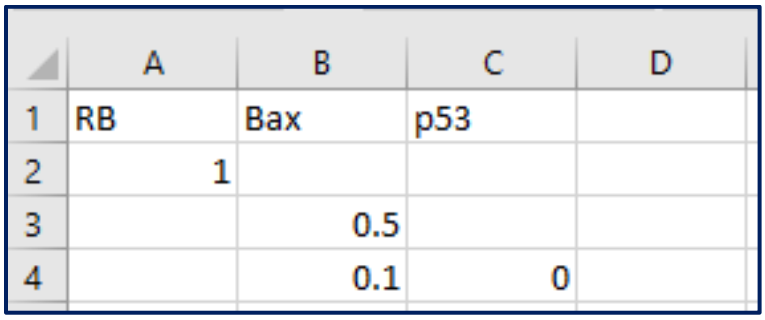


Figure 2.3.1.13 - Exhaustive screening options.

The user must provide an exhaustive screening file (format .csv) (**Figure 2.3.1.14**), wherein the network node names must be defined in the first row of proceeding columns, followed by the 'node state (between 0 and 1)' in the rows corresponding to each fixed node value. Not all network nodes need to

be added in an exhaustive screening file; users can choose to give a selective node for exhaustive evaluation. Each row indicates a separate analysis. As a result, the user will receive the same number of summary and detailed result files as the number of rows in the exhaustive screening file, along with a combined result file with individual cell fates. The users can select 'All Steps' or 'Only End Step' option for exhaustive screening. Using 'All Steps', exhaustive screening is performed on all therapeutic steps of the therapy, whereas with 'Only End Step' it is only undertaken at the last therapeutic step.



	A	B	C	D
1	RB	Bax	p53	
2	1			
3		0.5		
4		0.1	0	

Figure 2.3.1.14 - Exhaustive Screening (.csv) file. *Users can give single or multiple rows of inputs, and the analysis is performed for each of the rows.*

When the user chooses the option to 'Carry Forward Model Results' (**Figure 2.3.1.15**), the results from the model analysis, i.e., the network analysis results without any therapeutic intervention, are incorporated for onward analysis in successive therapeutic steps. If 'Carry Forward Model Results' option is not selected by the user, then the results of network analysis are not incorporated into the therapy for onward analyses.

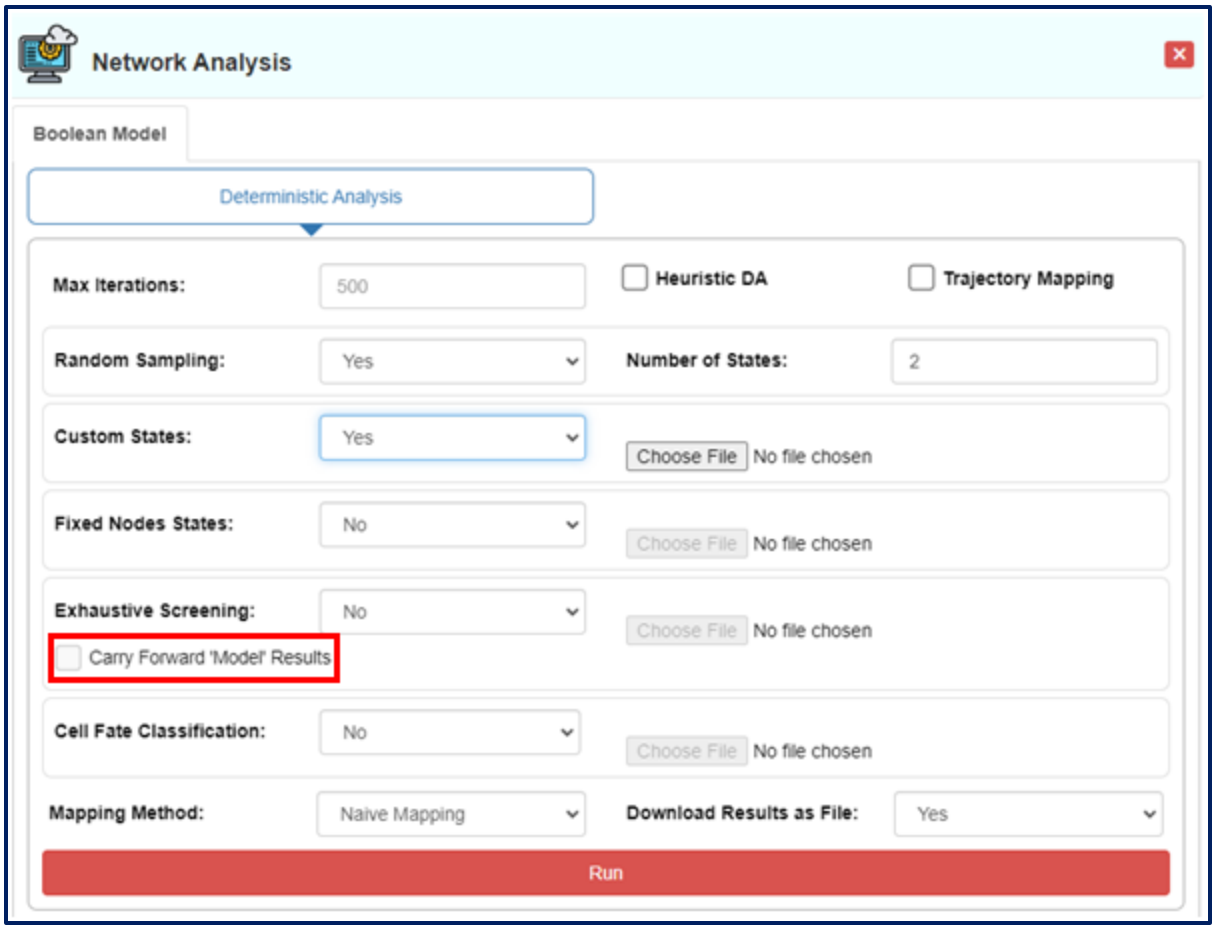


Figure 2.3.1.15 - Option to 'Carry Forward 'Model' Results'.

- **Cell Fate Classification:** Attractors are mapped to user-defined cell fates such as apoptosis, cell cycle arrest, uncontrolled proliferation, etc. The user must provide a cell fate classification file (format .csv), wherein the first column carries cell fate names in each cell (the first cell of column 1 is left empty). The node names must be defined in the first row of proceeding columns, followed by the "node state" in the rows corresponding to each cell fate. The 'node state' in a specific cell fate can be one of the following:

i. '0' includes the node expressions from 0.00 to 0.20. It represents that the node is turned "OFF", and its biological interpretation can either be an absence of expression or minimal and ineffective expression.
ii. '2' includes the node expressions from 0.21 to 0.80. It represents that the node is in a transient state, and its biological interpretation can be a cyclic or fluctuating form of expression.
iii. '1' includes the node expressions from 0.81 to 1.00. It represents that the node is turned "ON", and its biological interpretation can be a high and effective form of expression.

If separated by a semi-colon, multiple node states can be defined in the same cell. The cell fate classification file format is shown in the figure below (**Figure 2.3.1.16**). Please note that no white spaces should be left in the cell fates or node names, i.e., in first column and first row.

	A	B	C	D	E	F	G	H	I
1		Rho	CyclinD	CyclinE	CyclinA	CyclinB	MMP	Ecad	
2	Metastasis	2					1	0	
3	NormalProliferation		2	2	2	2			
4	AbnormalProliferation		1	1;2	1;2	1;2			
5	Quiescence		0					1;2	
6	Quiescence			0				1;2	
7	Quiescence				0			1;2	
8	Quiescence					0		1;2	
9	Quiescence		0				0;2		
10	Quiescence			0			0;2		
11	Quiescence				0		0;2		
12	Quiescence					0	0;2		
13	Quiescence	0;1	0						
14	Quiescence	0;1		0					
15	Quiescence	0;1			0				
16	Quiescence	0;1				0			

Figure 2.3.1.16 - Cell fate classification file (.csv): *Cell fates names are defined in the first column and node names in the first row of proceeding columns. Each cell fate contains specific nodes states in the rows corresponding to each node, being '0', '1', and '2'. Multiple node states can be defined as separated by a semi-colon in the same cell.*

- **Mapping Method:** Mapping methods employed to map a hyper-dimensional space to a three-dimensional space include 'Naive Bayes mapping' (**Figure 2.3.1.17**) and 'Sammon mapping' (**Figure 2.3.1.18**). 'Naive mapping' maps the state-space onto a Cartesian plane without considering the spatial distance between different steady-states. 'Sammon mapping', on the other hand, clusters the related network states before their projection onto a Cartesian plane.
- **Download Results as File:** This allows the user to download the analysis results as a '.zip' file.

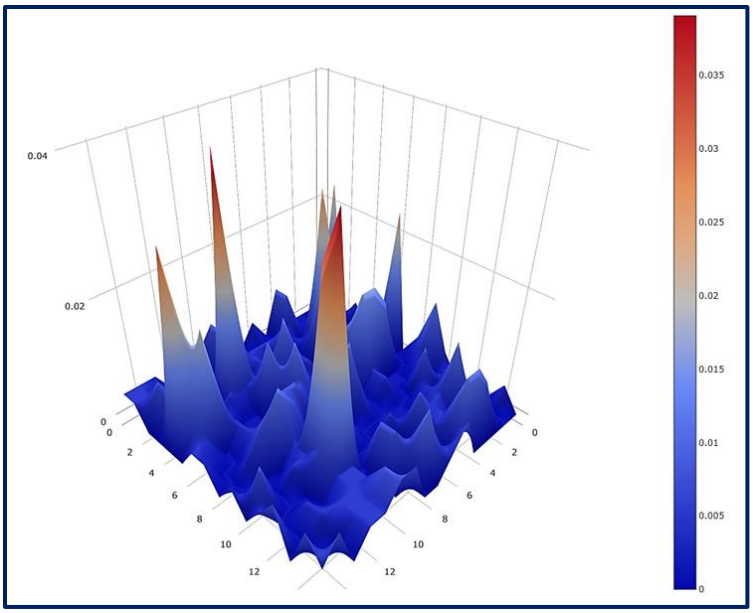


Figure 2.3.1.17 - Naive Bayes mapping basin ratio landscape.

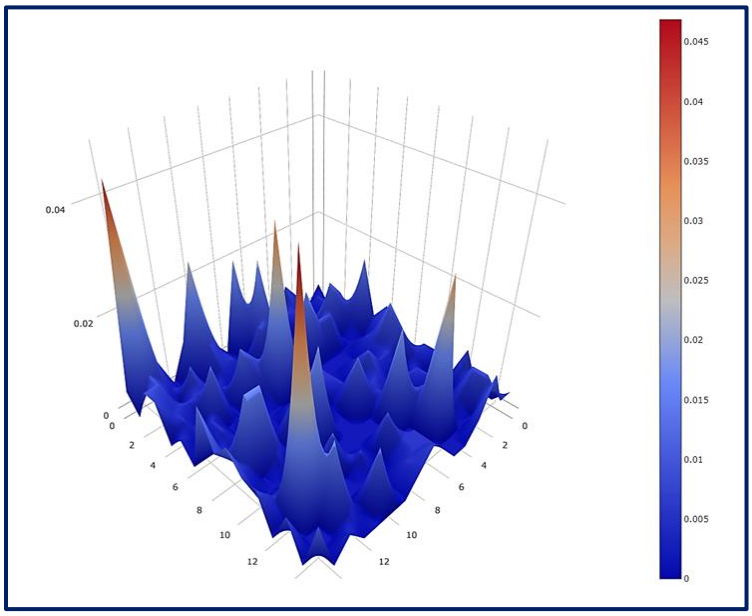


Figure 2.3.1.18 - Sammon mapping basin ratio landscape.

b) Probabilistic Analysis:

The probabilistic analysis (PA) pipeline of TISON (**Figure 2.3.1.19**) works off a biomolecular network input with nodes and interaction weights. As in the case of DA, PA can also employ exhaustive, randomly sampled, or user-defined custom state-space for onwards analysis. However, unlike DA, the transitions between network states follow a *Markovian* model in PA. Network state at the next time step, $t+1$, is determined only by its state at the current time step, t, with no dependence on earlier time steps. As a result, the state transition probability from t to $t+1$ is a product of individual node transition probabilities from t to $t+1$ [2,4]:

$$\mathrm{T}\{x_i(t+1), x_2(t+1), \dots x_n(t+1) | x_1(t), x_2(t), \dots x_n(t)\} = \prod_{i=1}^{n} T\{x_i(t+1) | x_1(t), x_2(t), \dots x_n(t)\}$$

To integrate the effect of background basal-level activity of nodes, ($B = [b_1, b_2, \dots, b_n]$), in the absence of any input, the cumulative inputs to each node are added with its basal expression. Intrinsic and extrinsic noise is incorporated with the use of noise parameter[4], μ. An additional parameter, *c,* is used to quantify the self-degradation of a node[2]. Given that the nodes lie within a dynamic system, the output from a single node is computed using the cumulative effect of its interaction with the parent nodes, noise, basal values, and self-degradation constant. The input can be either positive, negative, or zero, according to which the node transition probability is updated. The transition matrix *T* for each node[5] is defined as follows:

$$T\{x_i(t+1) = 1 | x_1(t), x_2(t), \dots x_n(t)\} = \frac{1}{2} + \frac{1}{2} \tanh\left[\mu\left(\sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i\right)\right] \quad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i > 0$$

$$T\{x_i(t+1) = 0 | x_1(t), x_2(t), \dots x_n(t)\} = \frac{1}{2} - \frac{1}{2} \tanh\left[\mu\left(\sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i\right)\right] \quad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i < 0$$

$$T\{x_i(t+1) = x_i(t) | x_1(t), x_2(t), \dots x_n(t)\} = 1 - c \qquad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i = 0$$

If the input $\sum_{j=1}^{n}\left(w_{ji}S_j(t)\right) + b_i$ received by a node *i* is positive (>0), the probability of transitioning to an ON state is high, and its probability of transitioning to an OFF state is low. Conversely, if the input $\sum_{j=1}^{n}\left(w_{ji}S_j(t)\right) + b_i$ is negative (<0), the node will transition to an OFF state with a high probability, but its transition to an ON state will have a low probability. The last condition defines the probability of a node staying in the same state at the next time step, as there is essentially no change in the input coming into the node. In such a case, the transition probability is dependent on the self-degradation of the node with a value equal to 1 – *c*.

Following the computation of network transition probabilities, it is important to see how the states evolve to reach a *steady-state*, such that there is no further change in their probabilities. This requires the use of kinetic master equations to update state probabilities over time[5] and is given below:

$$\frac{dP_i}{dt} = -\sum_j \mathrm{T}_{ij} P_i + \sum_j \mathrm{T}_{ji} P_j$$

The state transition probabilities change over time due to the effect of neighboring network states according to transitions in both directions from *i* to *j*, and *j* to *i*. T_{ij} is the probability of a network state transitioning from a network state *i* to *j*, and *vice versa* for T_{ji}. *i* and *j* iterate over all the states (i.e., from 1 to 2^n). The steady-state probabilities are stored in variables, P_i and P_j; wherein all states are initially assigned equal probabilities[6] ($P_i = \frac{1}{2^n}$). After that, each P_i is dynamically updated until a steady-state, i.e., $P_i(t+1)$ equals $P_i(t)$ to a good approximation. For tractable computation in large networks, the state-space can also be pruned using one-step DA (termed Heuristic PA). Finally, a potential energy (*PE*) value is assigned to each state to represent its stability, which is then used to construct PE landscapes for visualizing PA results.

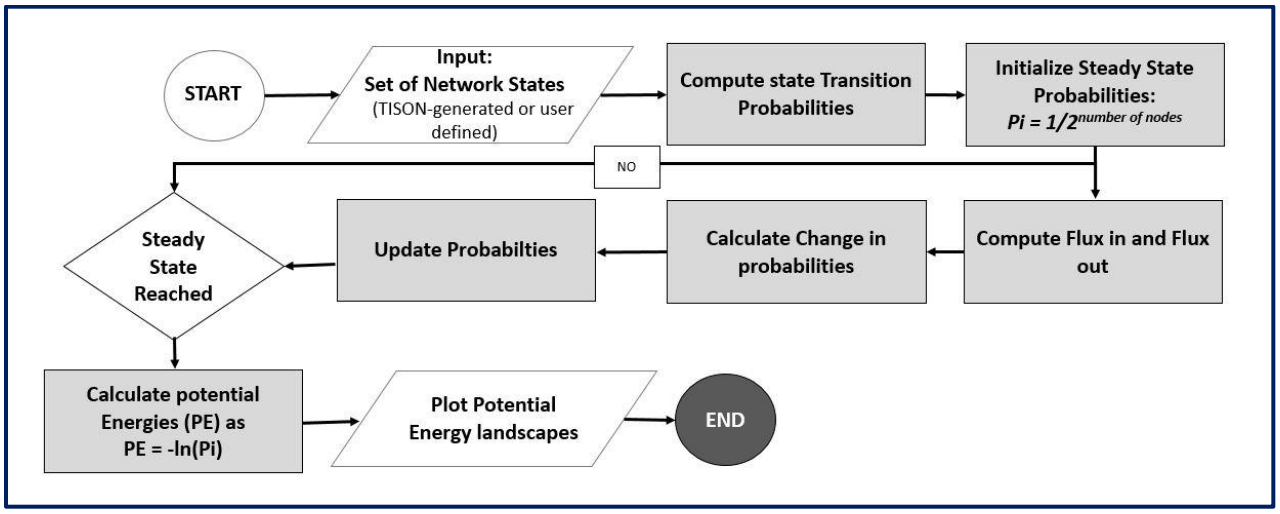


Figure 2.3.1.19 - PA pipeline implemented in TISON.

The window for PA is shown in the **Figure 2.3.1.20** below.

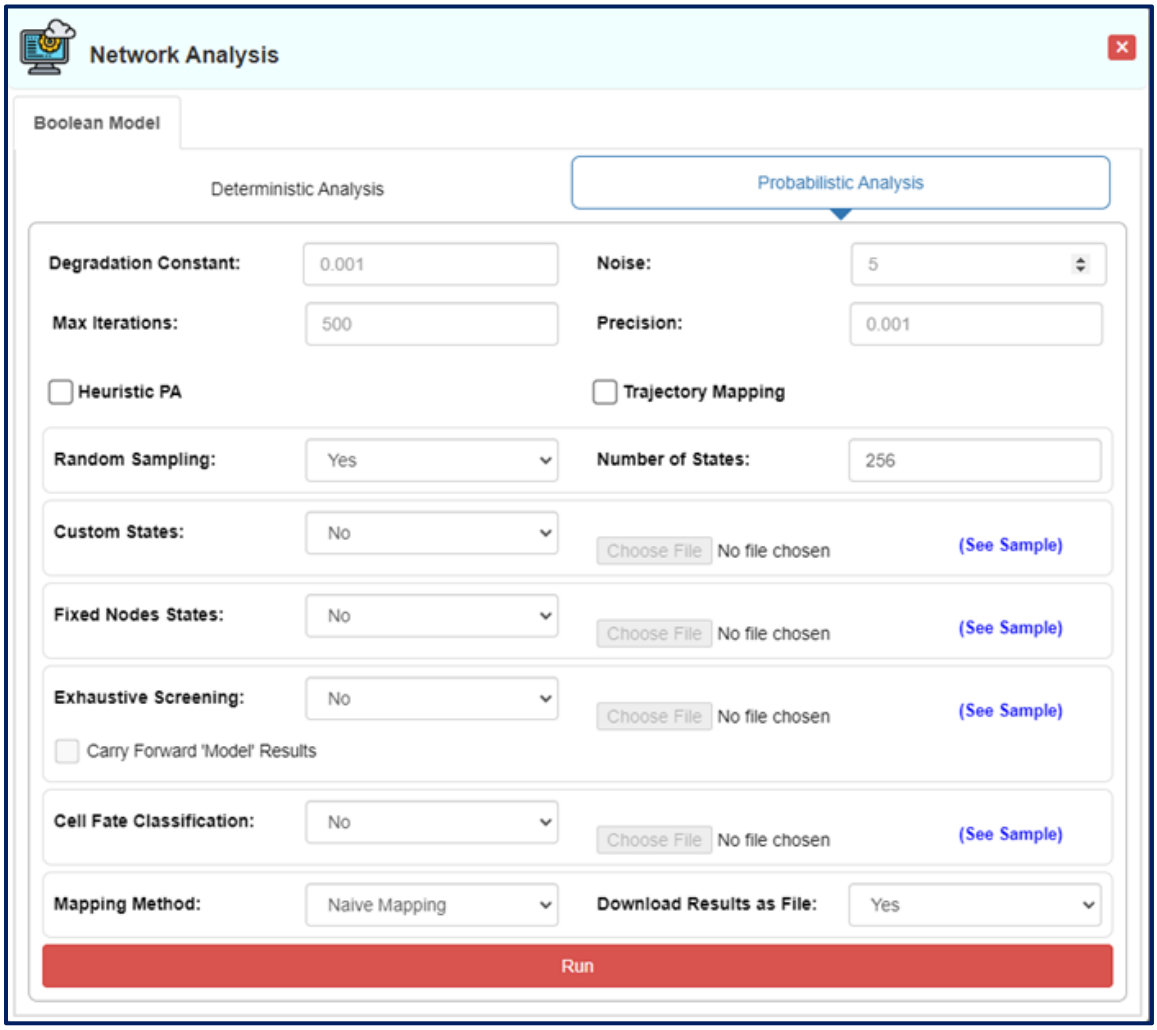


Figure 2.3.1.20 - Network analysis for therapy window for PA.

Parameters for Probabilistic Analysis are as follows:

- **Degradation Constant:** Used to quantify the self-degradation of the node.
- **Noise**: Intrinsic and extrinsic noise is incorporated with the use of noise parameter 'Environment Induced Noise' or 'μ'.
- **Max Iterations**: Number of iterations when solving the master equations to obtain steady-state transition probabilities.
- **Precision**: Maximum difference in transition probabilities of nodes between each iteration, for which a steady state is declared.
- **Heuristic PA**: The network sample can be considerably large for extensive networks and may require a large system memory by TISON. To address this issue, we have implemented a heuristic PA pipeline that

works seamlessly on low-memory and large-memory systems. It is implemented by performing a one-step DA and retaining the unique network, after which an n-step PA is performed on these unique states.

- **Trajectory Mapping:** Trajectory mapping traces the route from the initial to the final boundary state, passing through all the intermediate transient states. Users can select "Trajectory Mapping" from the analysis modal allowing TISON to store and then download the trajectory mapping file. This file shows the complete state transition trajectory from initial network states at iteration 0 to the final iteration number.
- **Random Sampling**: This option caters to insufficient system memory (if the system cannot store 2n states). Exhaustive sampling, in which 'Random Sampling' is turned OFF, generates all (2n) possible states for onward analysis. However, when 'Random Sampling' is turned ON, a user-specified number of states is selected from the complete state-space, thus, making the analysis of more extensive networks feasible.
- **Number of States**: User-specified number of states from the complete state-space; this makes the analysis of more extensive networks feasible. The default number of states is 256 whereas the maximum number allowed is 10,000. If any state, i.e., exhaustive, random, or user-specified custom states, is not converged, then result file will indicate the number of unconverged states and their values in binary form.
- **Custom States**: The Custom States file (format .csv) contains those network nodes which have been logically defined in the network file (intermediate nodes). Using the Custom State option, TISON's user can selectively incorporate specific network states of certain intermediate nodes for network analysis. Checking the 'Heuristic PA' option allows a user to prune network state-space using heuristic PA, which is especially useful in large networks to enable traceable computations.
- **Fixed Nodes States**: States in the transition matrix are selected based on the fixed nodes. This is to mimic the effect of a drug, biological mutations, input conditions, or other external conditions upon a network. A user can choose 'No' to inactivate fixed nodes states.
- **Exhaustive Screening:** The exhaustive screening feature can be used to evaluate different nodes (in a single analysis) towards identifying more efficacious node targets for therapies. It mimics the effect of a second drug, i.e., taking the effect of the previous drug for onwards analysis. The user must provide an exhaustive screening file (format .csv) wherein the network node names must be defined in the first row of proceeding columns, followed by the 'node state (between 0 and 1)' in the rows corresponding to each fixed node value. Not all network nodes need to be added in an exhaustive screening file; users can choose to give a selective node for exhaustive evaluation. Each row indicates a separate analysis. As a result, the user will receive the same number of summary and detailed result files as the number of rows in the exhaustive screening file, along with a combined result file with individual cell fates. The users can select 'All Steps' or 'Only End Step' option for exhaustive screening. Using 'All Steps', exhaustive screening is performed on all therapeutic steps of the therapy, whereas with 'Only End Step' it is only undertaken at the last therapeutic step.
- **Cell Fate Classification**: Attractors are mapped to user-defined cell fates such as apoptosis, cell cycle arrest, uncontrolled proliferation, etc. The user must provide a cell fate classification file (format .csv) in

Figure 2.3.1.16, wherein the first column carries cell fate names in each cell (the first cell of column 1 is left empty). The node names must be defined in the first row of proceeding columns, followed by the 'node state' in the rows corresponding to each cell fate. The "node state" in a specific cell fate can be one of the following:

i. '0' includes the node expressions from 0.00 to 0.20. It represents that the node is turned "OFF", and its biological interpretation can either be an absence of expression or minimal and ineffective expression.

ii. '2' includes the node expressions from 0.21 to 0.80. It represents that the node is in a transient state, and its biological interpretation can be a cyclic or fluctuating form of expression.

iii. '1' includes the node expressions from 0.81 to 1.00. It represents that the node is turned "ON", and its biological interpretation can be a high and effective form of expression.

If separated by a semi-colon, multiple node states can be defined in the same cell. The cell fate classification file format is shown in the figure (**Figure 2.3.1.16)**. Please note that no white spaces should be left in the cell fates or node names, i.e., in first column and first row.

- **Mapping Method:** Mapping methods employed to map a hyper-dimensional space to a three-dimensional space include 'Naive Bayes mapping' (**Figure 2.3.1.17**) and 'Sammon mapping' (**Figure 2.3.1.18**). 'Naive mapping' maps the state-space onto a Cartesian plane without considering the spatial distance between different steady-states. 'Sammon mapping', on the other hand, clusters the related network states before their projection onto a Cartesian plane.
- **Download Results as File:** This allows the user to download the analysis results as a '.zip' file.

Table 2.3.1 - Network types with their associated analyses

Network Type	Analysis Type	Applicable	Associated Results	Output Result File	Components of Output Result File
Rules-based Network	Deterministic Analysis	✓	Attractor Landscape	Summary and RA Files	1) One output result file with all cell fates, their basin size ratios, and each network node's steady state value, etc. Along with average node propensities, individual cell fates, and total converged and unconverged states. 2) A robustness analysis (RA) results file with separated cell fates and mean basin size ratios along with their standard deviations and standard error of means.
		✓	Cell Fate Landscape		
Weight-based Network	Deterministic Analysis	✓	Attractor Landscape	Detail, Summary, and RA File	1) If the user provides cell fates, they will receive a detailed file with each fate's individual network node propensities in 0s and 1s. 2) Summary file with mean of each network node plotted with the cell fate for cyclic attractors, along with average node propensities, individual cell fates, and total converged and unconverged states. 3) Robustness Analysis (RA) results file with separated cell fates and mean basin size ratios along with their standard deviations and standard error of means.
		✓	Cell Fate Landscape		
	Probabilistic Analysis	✓	Potential Energy Landscape	Detail and RA Files	1) File containing cell fate and probability along with network node value. 2) A robustness analysis (RA) results file with separated cell fates and mean basin size ratios along with their standard deviations and standard error of means.
		✓	Probability Landscape		
		✓	Cell Fate Landscape		

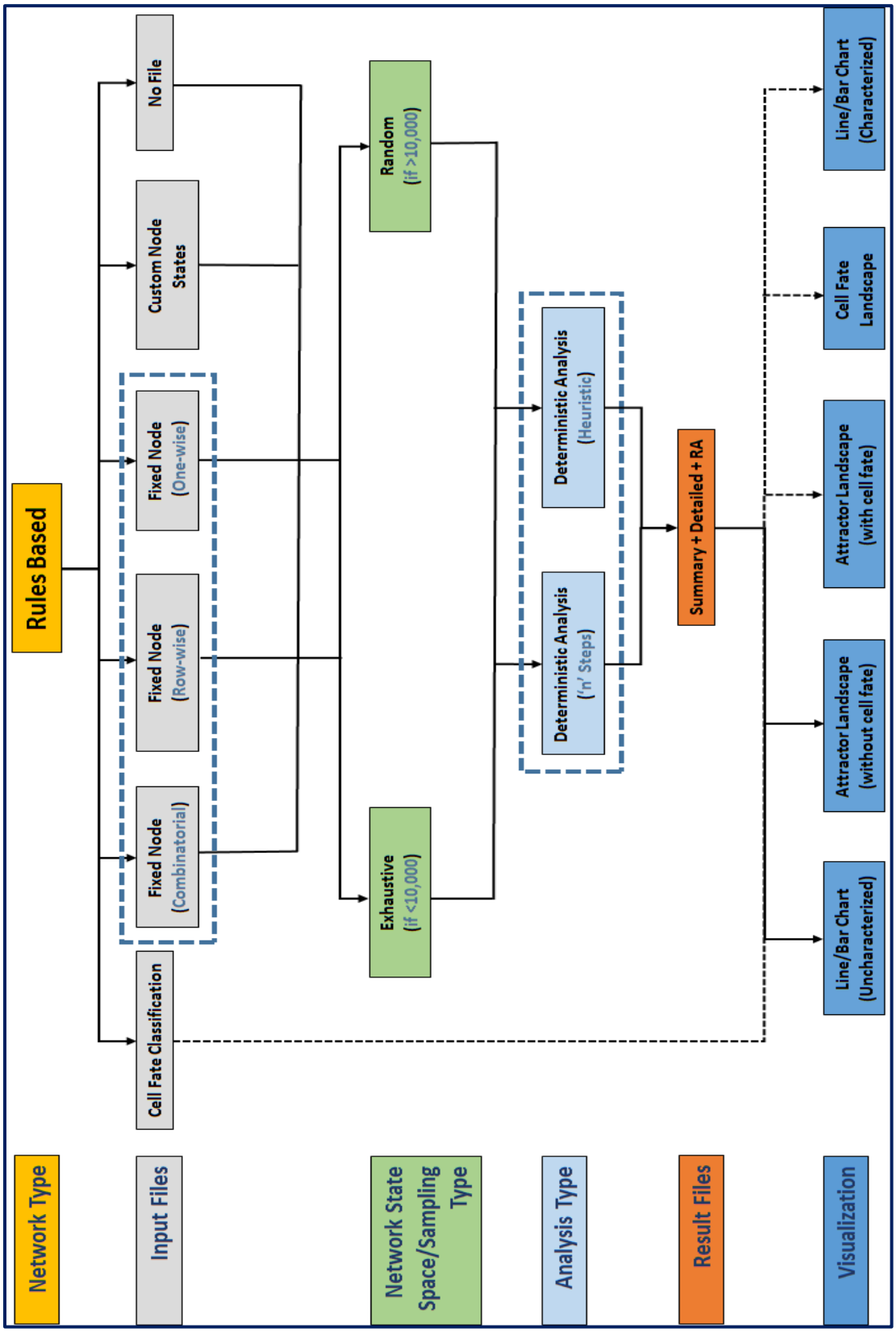


Figure 2.3.1.21 - TISON's Rule-based networks pipeline.

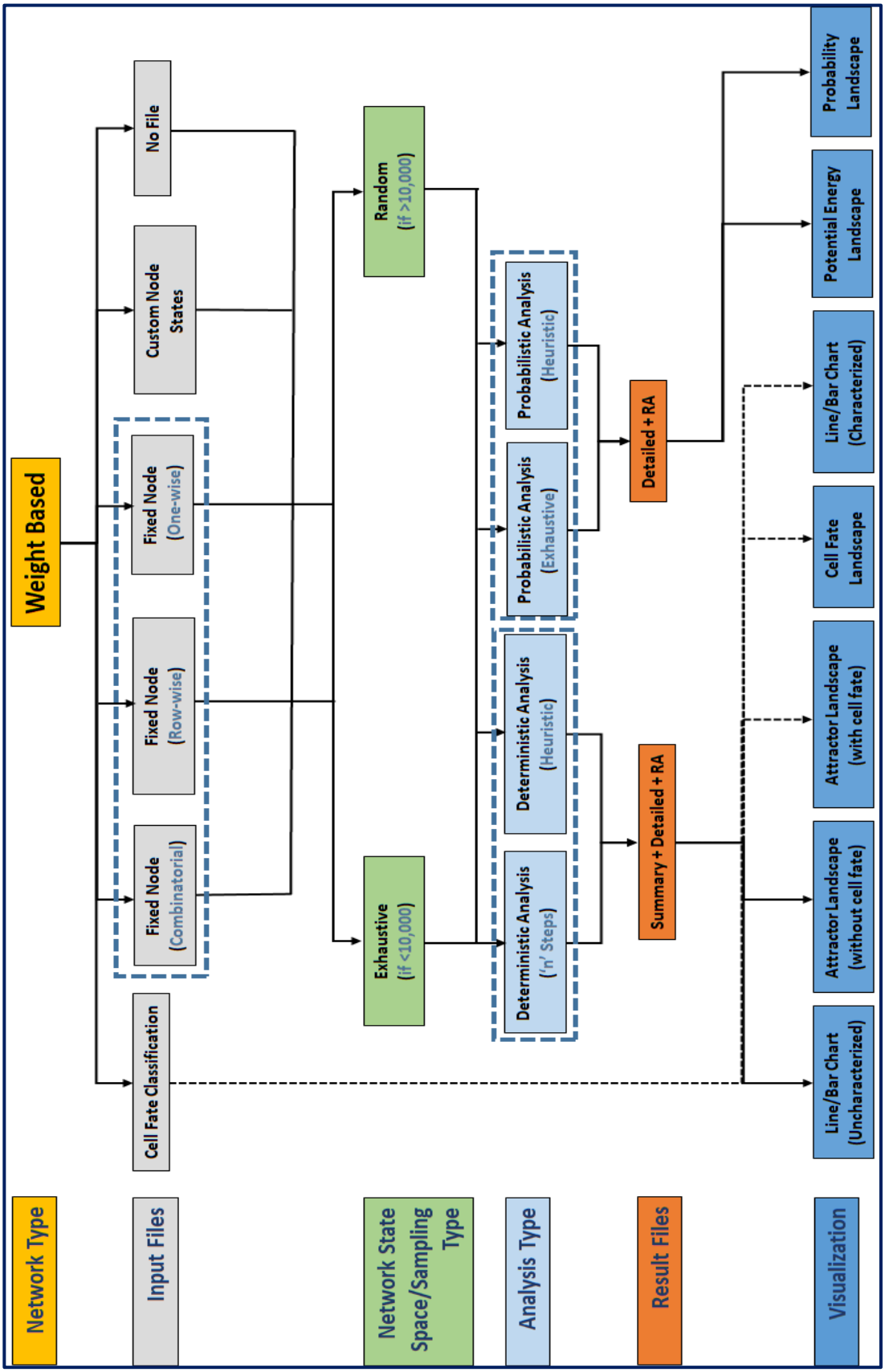


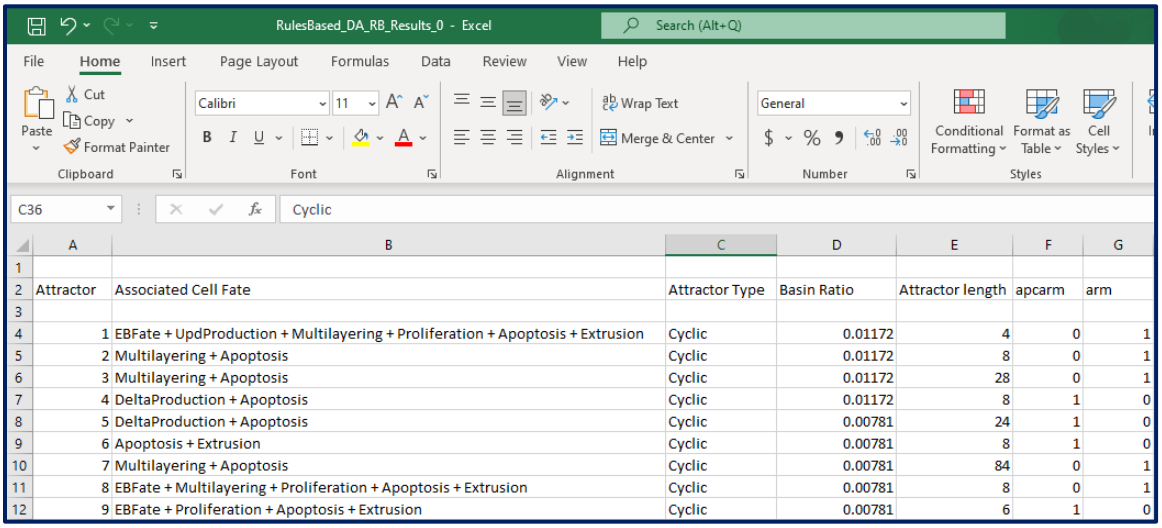
Figure 2.3.1.22 - TISON's Weight-based networks pipeline.

Downloaded Files Format:

i. Deterministic Analysis (DA)

Performing DA will allow the user to download the different files for multi-input analyses.

For a *rules-based network*, a DA file (**Figure 2.3.1.23**) and an RA file (**Figure 2.3.1.24)** are generated.

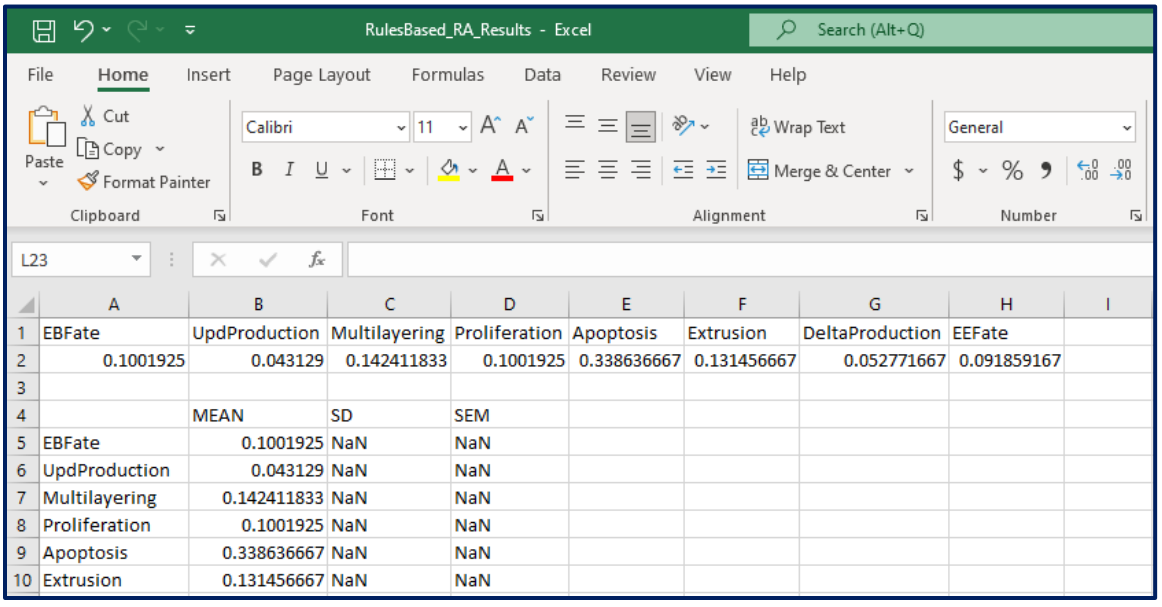


RulesBased_DA_RB_Results_0 - Excel

C36 | Cyclic

	A	B	C	D	E	F	G
1							
2	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	apcarm	arm
3							
4	1	EBFate + UpdProduction + Multilayering + Proliferation + Apoptosis + Extrusion	Cyclic	0.01172	4	0	1
5	2	Multilayering + Apoptosis	Cyclic	0.01172	8	0	1
6	3	Multilayering + Apoptosis	Cyclic	0.01172	28	0	1
7	4	DeltaProduction + Apoptosis	Cyclic	0.01172	8	1	0
8	5	DeltaProduction + Apoptosis	Cyclic	0.00781	24	1	0
9	6	Apoptosis + Extrusion	Cyclic	0.00781	8	1	0
10	7	Multilayering + Apoptosis	Cyclic	0.00781	84	0	1
11	8	EBFate + Multilayering + Proliferation + Apoptosis + Extrusion	Cyclic	0.00781	8	0	1
12	9	EBFate + Proliferation + Apoptosis + Extrusion	Cyclic	0.00781	6	1	0

Figure 2.3.1.23 - DA results file for a rules-based DA.



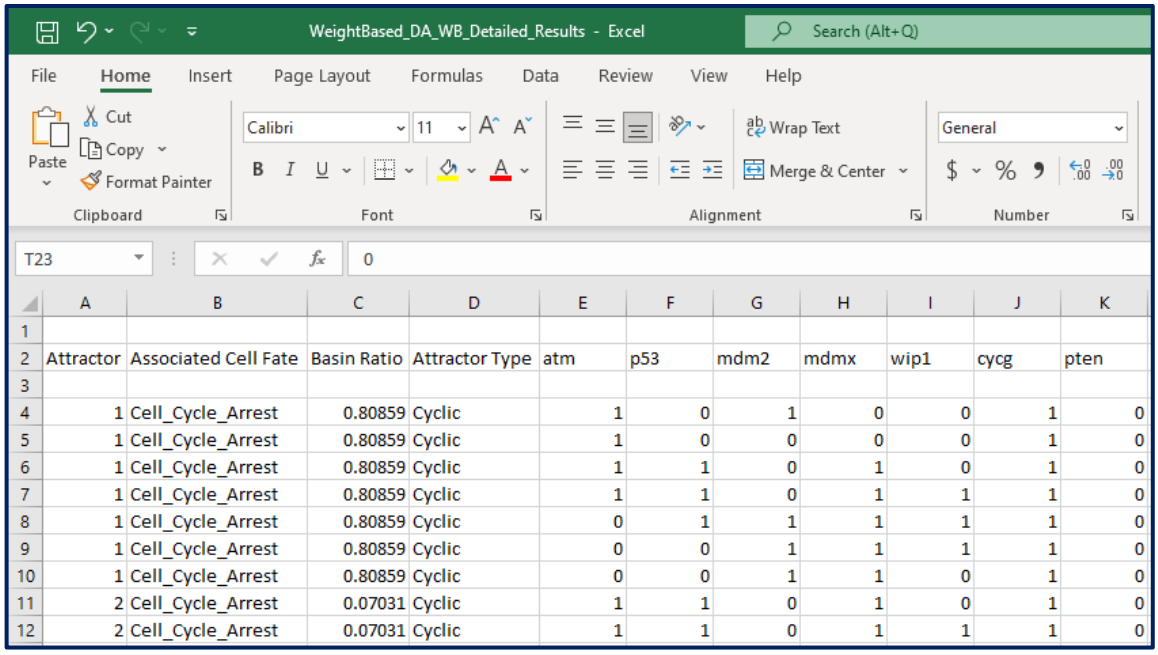
RulesBased_RA_Results - Excel

L23

	A	B	C	D	E	F	G	H	I
1	EBFate	UpdProduction	Multilayering	Proliferation	Apoptosis	Extrusion	DeltaProduction	EEFate	
2	0.1001925	0.043129	0.142411833	0.1001925	0.338636667	0.131456667	0.052771667	0.091859167	
3									
4		MEAN	SD	SEM					
5	EBFate	0.1001925	NaN	NaN					
6	UpdProduction	0.043129	NaN	NaN					
7	Multilayering	0.142411833	NaN	NaN					
8	Proliferation	0.1001925	NaN	NaN					
9	Apoptosis	0.338636667	NaN	NaN					
10	Extrusion	0.131456667	NaN	NaN					

Figure 2.3.1.24 - RA results file for a rules-based DA.

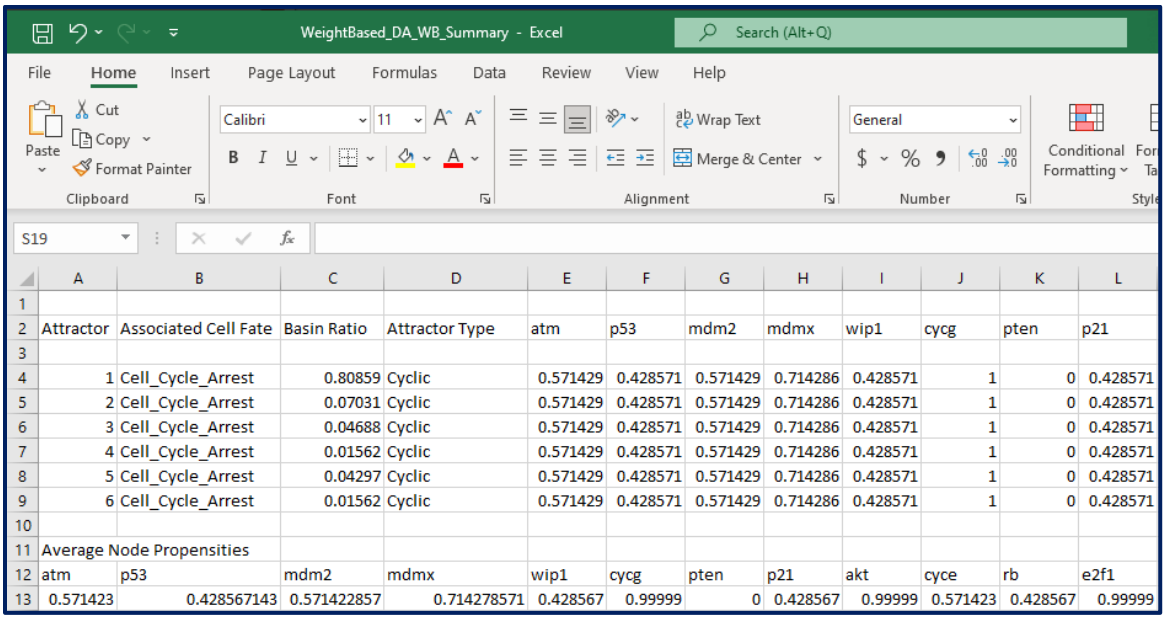
For a *weight-based network*, a detailed result file (**Figure 2.3.1.25**), a summary file (**Figure 2.3.1.26**), and a RA file (**Figure 2.3.1.27**) can be downloaded after performing DA. The detailed file displays every node state in each attractor in terms of 0 or 1, whereas the summary file contains the average node value for each node if the attractor is the same.



WeightBased_DA_WB_Detailed_Results - Excel

	A	B	C	D	E	F	G	H	I	J	K
1											
2	Attractor	Associated Cell Fate	Basin Ratio	Attractor Type	atm	p53	mdm2	mdmx	wip1	cycg	pten
3											
4	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	0	1	0	0	1	0
5	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	0	0	0	0	1	0
6	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	1	0	1	0	1	0
7	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	1	0	1	1	1	0
8	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	1	1	1	1	1	0
9	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	0	1	1	1	1	0
10	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	0	1	1	0	1	0
11	2	Cell_Cycle_Arrest	0.07031	Cyclic	1	1	0	1	0	1	0
12	2	Cell_Cycle_Arrest	0.07031	Cyclic	1	1	0	1	1	1	0

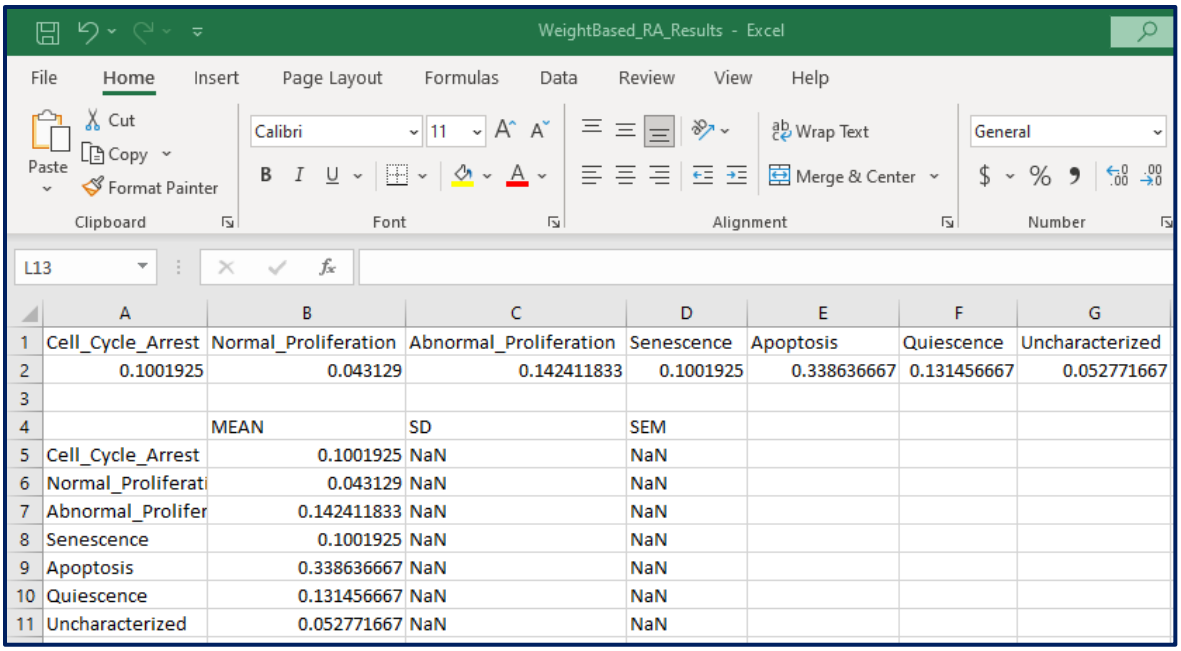
Figure 2.3.1.25 - Detailed results file for a weight-based DA.



WeightBased_DA_WB_Summary - Excel

	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Attractor	Associated Cell Fate	Basin Ratio	Attractor Type	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21
3												
4	1	Cell_Cycle_Arrest	0.80859	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
5	2	Cell_Cycle_Arrest	0.07031	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
6	3	Cell_Cycle_Arrest	0.04688	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
7	4	Cell_Cycle_Arrest	0.01562	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
8	5	Cell_Cycle_Arrest	0.04297	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
9	6	Cell_Cycle_Arrest	0.01562	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
10												
11	Average Node Propensities											
12	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21	akt	cyce	rb	e2f1
13	0.571423	0.428567143	0.571422857	0.714278571	0.428567	0.99999	0	0.428567	0.99999	0.571423	0.428567	0.99999

Figure 2.3.1.26 - Summary file for a weight-based DA.



	A	B	C	D	E	F	G
1	Cell_Cycle_Arrest	Normal_Proliferation	Abnormal_Proliferation	Senescence	Apoptosis	Quiescence	Uncharacterized
2	0.1001925	0.043129	0.142411833	0.1001925	0.338636667	0.131456667	0.052771667
3							
4		MEAN	SD	SEM			
5	Cell_Cycle_Arrest	0.1001925	NaN	NaN			
6	Normal_Proliferati	0.043129	NaN	NaN			
7	Abnormal_Prolifer	0.142411833	NaN	NaN			
8	Senescence	0.1001925	NaN	NaN			
9	Apoptosis	0.338636667	NaN	NaN			
10	Quiescence	0.131456667	NaN	NaN			
11	Uncharacterized	0.052771667	NaN	NaN			

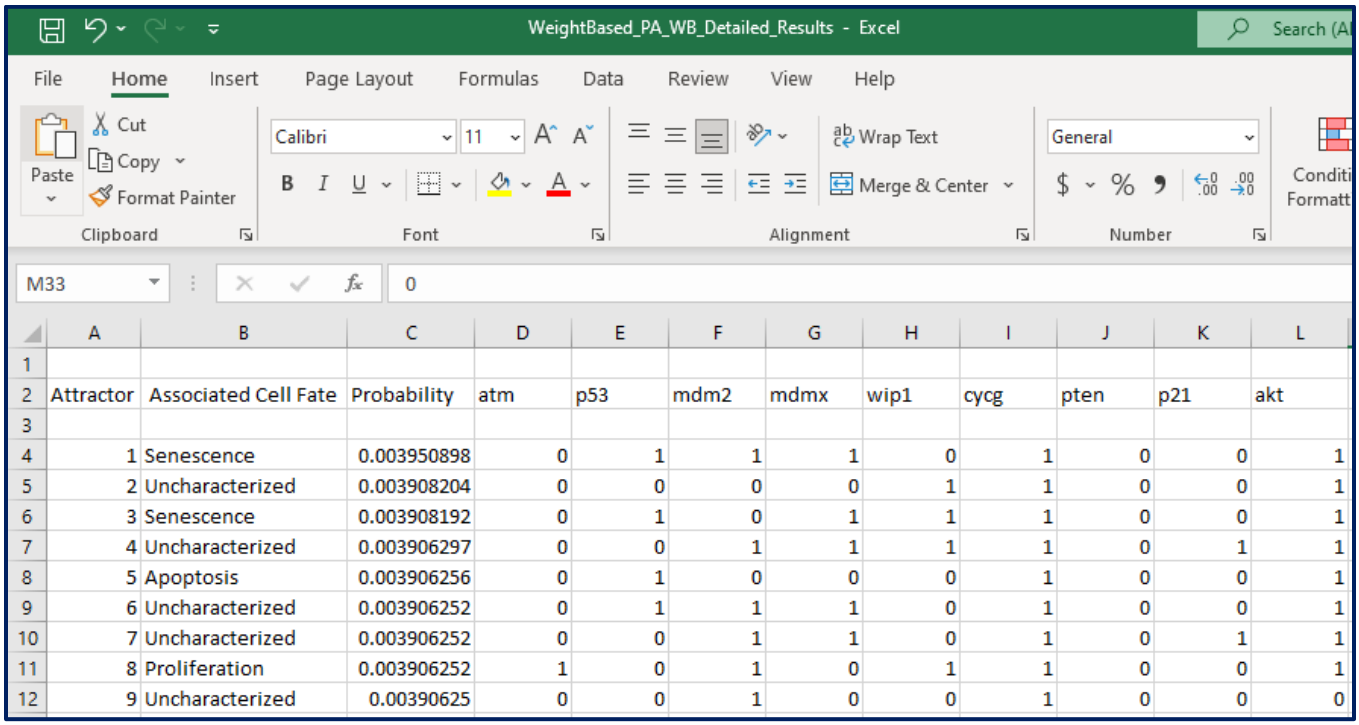
Figure 2.3.1.27 - RA result file for a weight-based DA.

The robustness result file (**Figure 2.3.1.24**) displays the occurrence of each phenotype with respect to the inputs as well as the overall mean, standard deviation (SD), and standard error of means (SEM) of cell fates. The column headers in the files are as explained below:

- **Attractor:** Serial number and rank (in terms of highest basin size ratio) of an attractor.
- **Associated Cell Fate:** The fate assigned to an attractor using its node values and cell fate logic file. If fate is not categorized or if cell fate logic is not assigned, the respective states will be 'Uncharacterized'.
- **Basin Ratio:** The ratio of initial states which converge to an attractor.
- **Attractor Type:** The attractor type can be either cyclic or point. This signifies whether the attractor continuously moves through a finite set of states *(cyclic*) or remains in a single state (*point)*.
- **Attractor Length:** The number of states of an attractor. Point attractors always have an attractor length 1, while cyclic attractors toggle between the Boolean states having attractor length > 1 (only for rules-based network).
- **Average Node Propensities:** The average node activity after analysis is calculated by multiplying node activity for each attractor with its respective basin size ratio and then summing them over all attractors.
- **Individual Cell Fates:** Each cell fate with its associated cell fate propensities.
- **Total Sample Size:** The sample size which the user has input as a random sample or the exhaustive network state space.
- **Total States Converged:** The number of state space which converged towards forming attractors.

ii. Probabilistic Analysis (PA)

For PA, the following *weight-based* probabilistic result file is produced (**Figure 2.3.1.28**) and an RA file, once the analysis is completed (**Figure 2.3.1.29**).

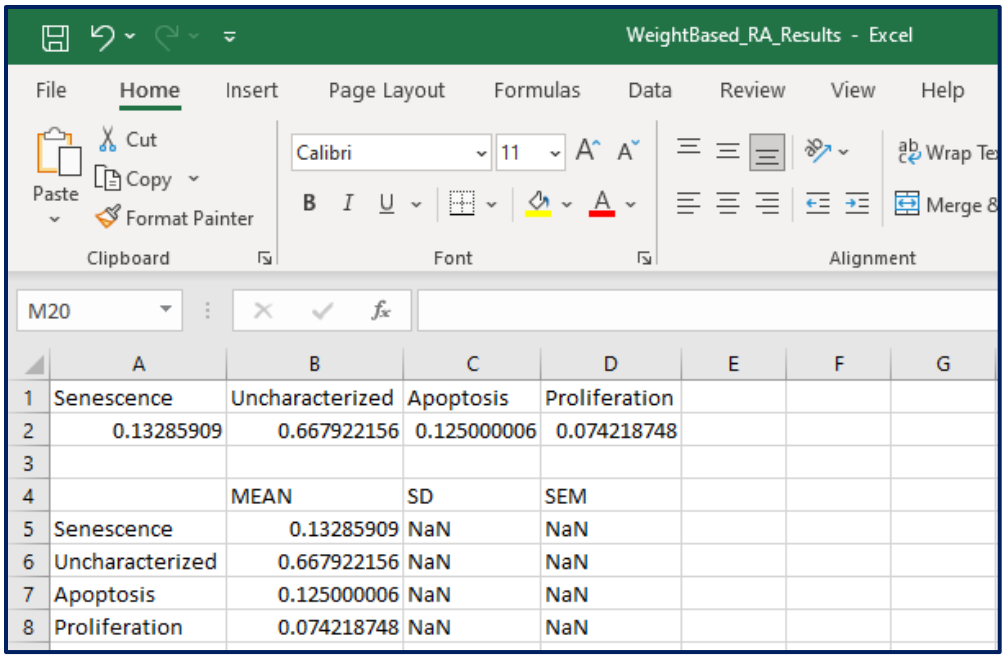


	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Attractor	Associated Cell Fate	Probability	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21	akt
3												
4	1	Senescence	0.003950898	0	1	1	1	0	1	0	0	1
5	2	Uncharacterized	0.003908204	0	0	0	0	1	1	0	0	1
6	3	Senescence	0.003908192	0	1	0	1	1	1	0	0	1
7	4	Uncharacterized	0.003906297	0	0	1	1	1	1	0	1	1
8	5	Apoptosis	0.003906256	0	1	0	0	0	1	0	0	1
9	6	Uncharacterized	0.003906252	0	1	1	1	0	1	0	0	1
10	7	Uncharacterized	0.003906252	0	0	1	1	0	1	0	1	1
11	8	Proliferation	0.003906252	1	0	1	0	1	1	0	0	1
12	9	Uncharacterized	0.00390625	0	0	1	0	0	1	0	0	0

Figure 2.3.1.28 - Result file for weight-based PA.

In addition to the parameters like **i. Deterministic Analysis (DA)** described above, the PA result file has the following:

- **Probability:** Steady-state probabilities of the respective state (probability of finding system in state on random observation).



WeightBased_RA_Results - Excel

File Home Insert Page Layout Formulas Data Review View Help

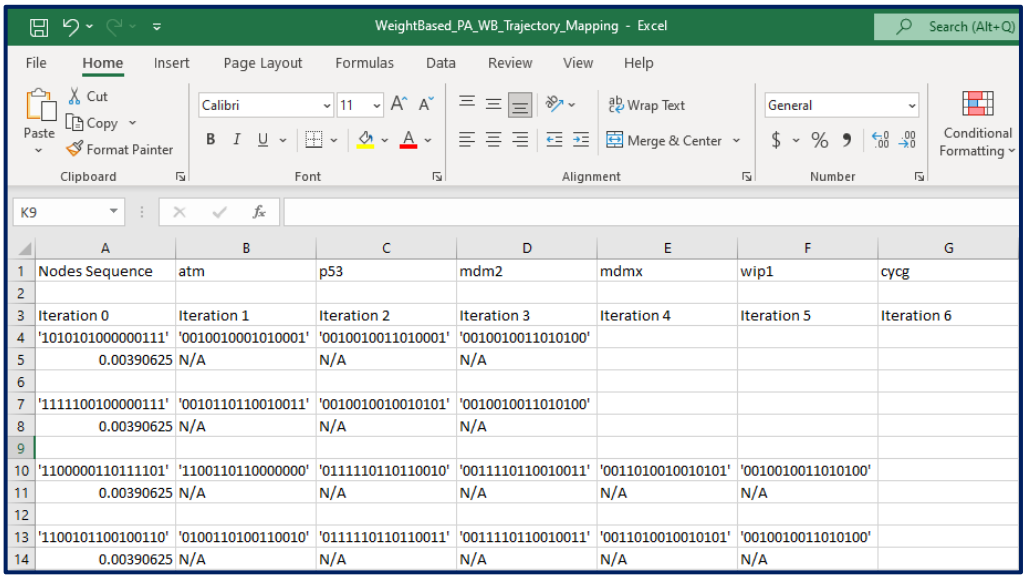
Paste Cut Copy Format Painter Clipboard | Calibri 11 Font | Wrap Te Merge & Alignment

M20

	A	B	C	D	E	F	G
1	Senescence	Uncharacterized	Apoptosis	Proliferation			
2	0.13285909	0.667922156	0.125000006	0.074218748			
3							
4		MEAN	SD	SEM			
5	Senescence	0.13285909	NaN	NaN			
6	Uncharacterized	0.667922156	NaN	NaN			
7	Apoptosis	0.125000006	NaN	NaN			
8	Proliferation	0.074218748	NaN	NaN			

Figure 2.3.1.29 - RA Result file for weight-based PA.

- **Trajectory Mapping**: In case user selects 'Trajectory Mapping' in the analysis modal in **Figure 2.3.1.30**, the following network trajectory file is also downloaded.



WeightBased_PA_WB_Trajectory_Mapping - Excel

File Home Insert Page Layout Formulas Data Review View Help

Paste Cut Copy Format Painter Clipboard | Calibri 11 Font | Wrap Text Merge & Center Alignment | General Number | Conditional Formatting

K9

	A	B	C	D	E	F	G
1	Nodes Sequence	atm	p53	mdm2	mdmx	wip1	cycg
2							
3	Iteration 0	Iteration 1	Iteration 2	Iteration 3	Iteration 4	Iteration 5	Iteration 6
4	'1010101000000111'	'0010010001010001'	'0010010011010001'	'0010010011010100'			
5	0.00390625	N/A	N/A	N/A			
6							
7	'1111100100000111'	'0010110110010011'	'0010010010010101'	'0010010011010100'			
8	0.00390625	N/A	N/A	N/A			
9							
10	'1100000110111101'	'1100110110000000'	'0111110110110010'	'0011110110010011'	'0011010010010101'	'0010010011010100'	
11	0.00390625	N/A	N/A	N/A	N/A	N/A	
12							
13	'1100101100100110'	'0100110100110010'	'0111110110110011'	'0011110110010011'	'0011010010010101'	'0010010011010100'	
14	0.00390625	N/A	N/A	N/A	N/A	N/A	

Figure 2.3.1.30 - Output result file for 'Trajectory Mapping'.

2.3.2 'Analysis Progress and Status' Button

'Analysis Progress and Status' button in the canvas toolbar (highlighted in **Figure 2.3.2.1**) allows the user to analyze, monitor and manage multiple therapies (**Figure 2.3.2.2**).

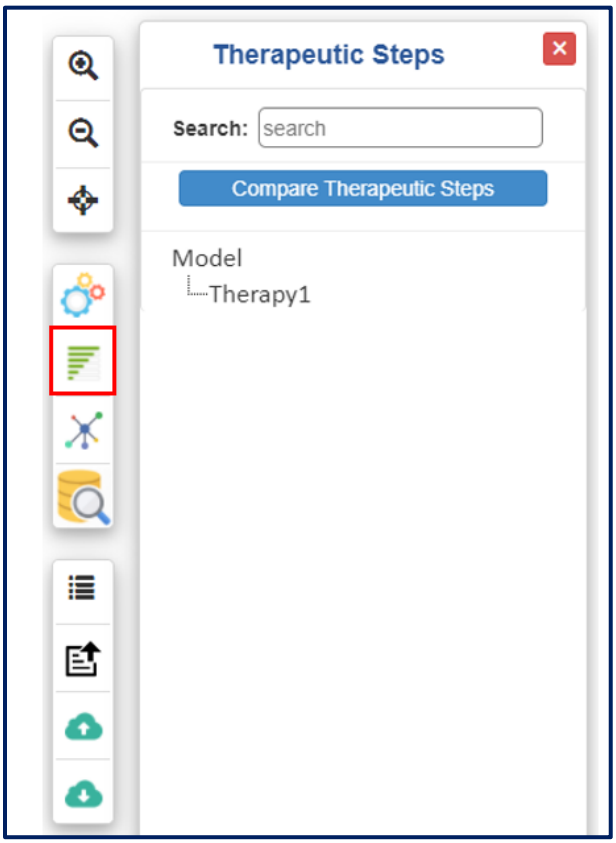


Figure 2.3.2.1 - Analysis Progress and Status' button in TE.

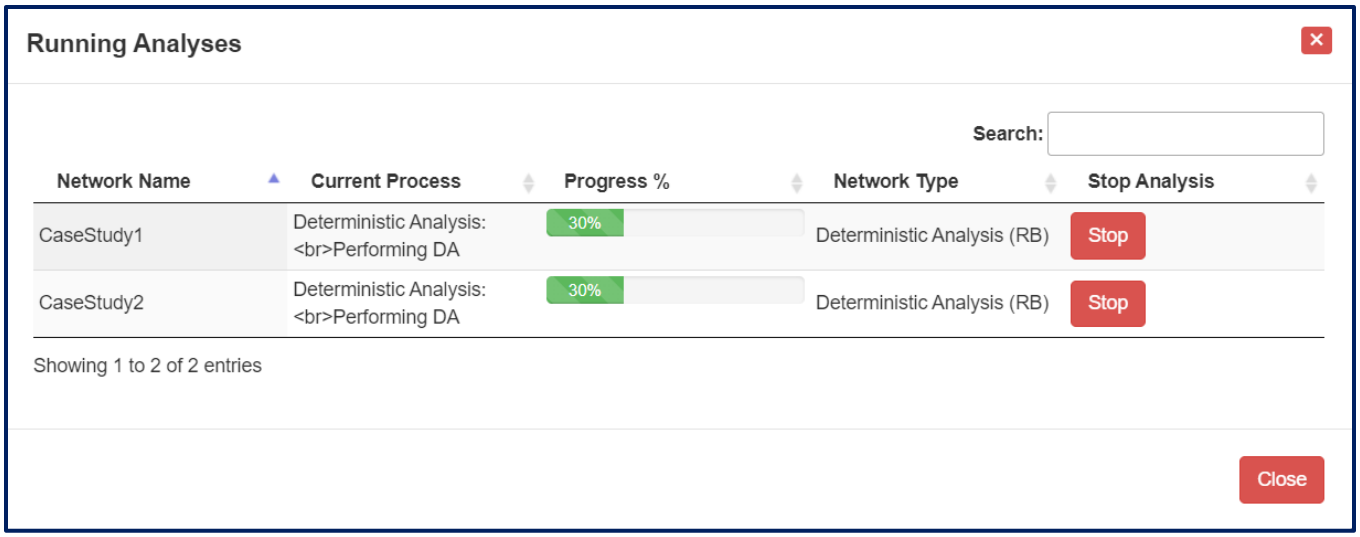


Figure 2.3.2.2 - 'Analysis Progress and Status' window.

2.3.3 'View Network' Button

After performing an analysis, the results can be viewed 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape', 'Probability Landscape'. The button highlighted in **Figure 2.3.3.1** will help the user switch back to the network after viewing the landscapes.

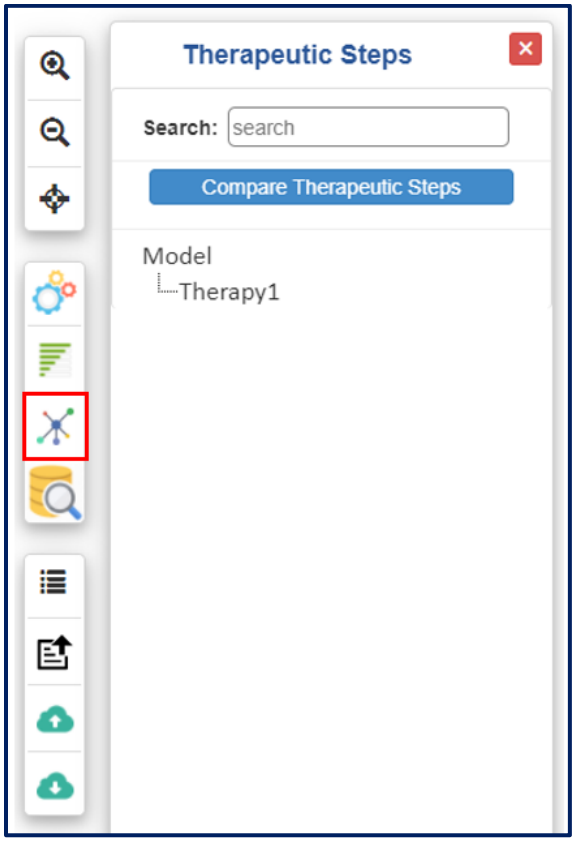


Figure 2.3.3.1 - View Network' button in TE.

2.3.4 'Search Database' Button

'Search Database' button shown in **Figure 2.3.4.1** allows the user to search a drug along with its score against a particular gene from the Drug Gene Interaction Database (DGIdb) integrated into TE.

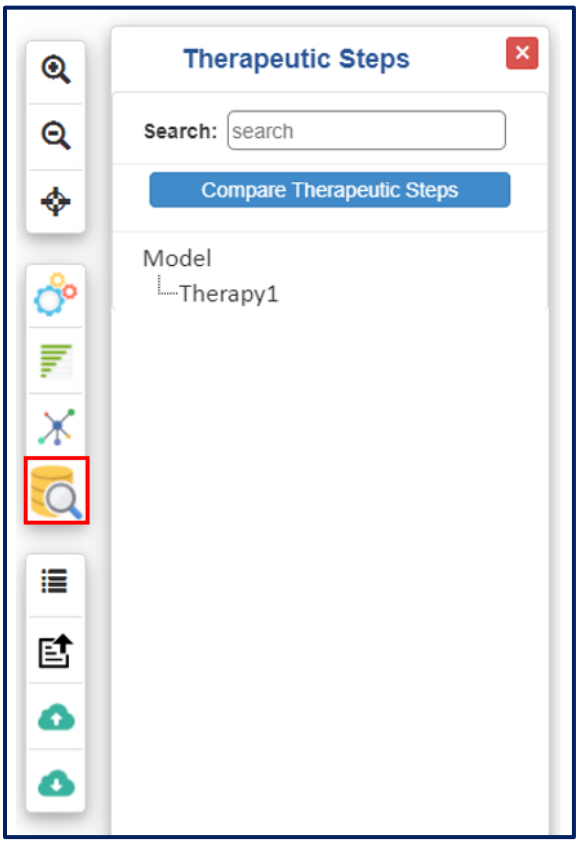


Figure 2.3.4.1 - 'Search Database' button in TE.

The window shown in **Figure 2.3.4.2** appears upon a single click on the 'Search Database' button.

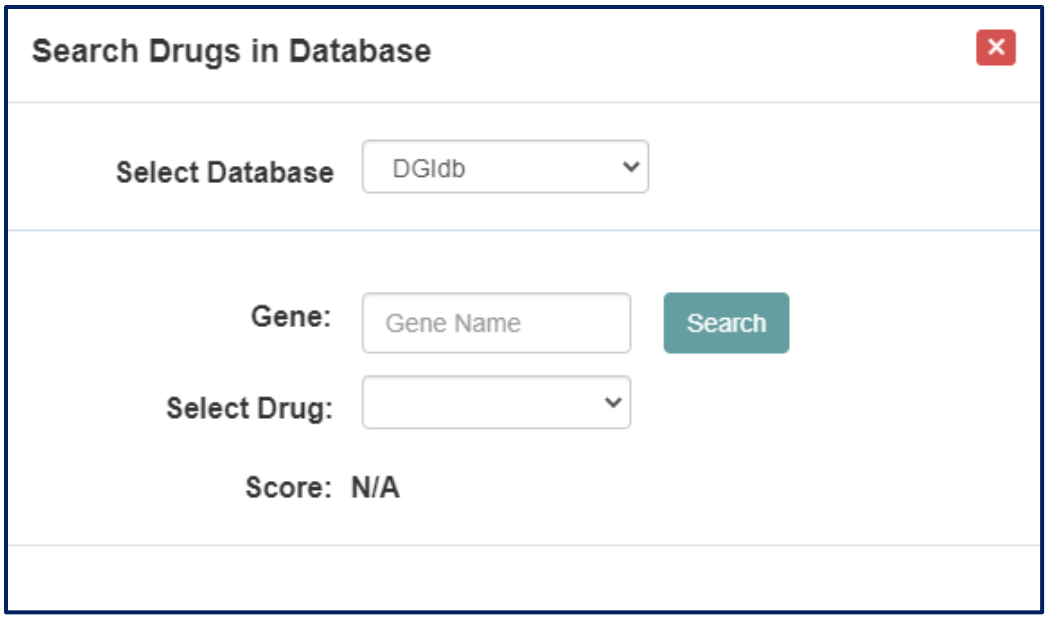


Figure 2.3.4.2 - 'Search drugs in database' window.

The user can enter a gene name and click 'Search' to retrieve the all the available drugs (**Figure 2.3.4.4**) against the target gene, using a drop-down list in 'Select Drug' option, to obtain the respective drug-gene interaction score (**Figure 2.3.4.3**).

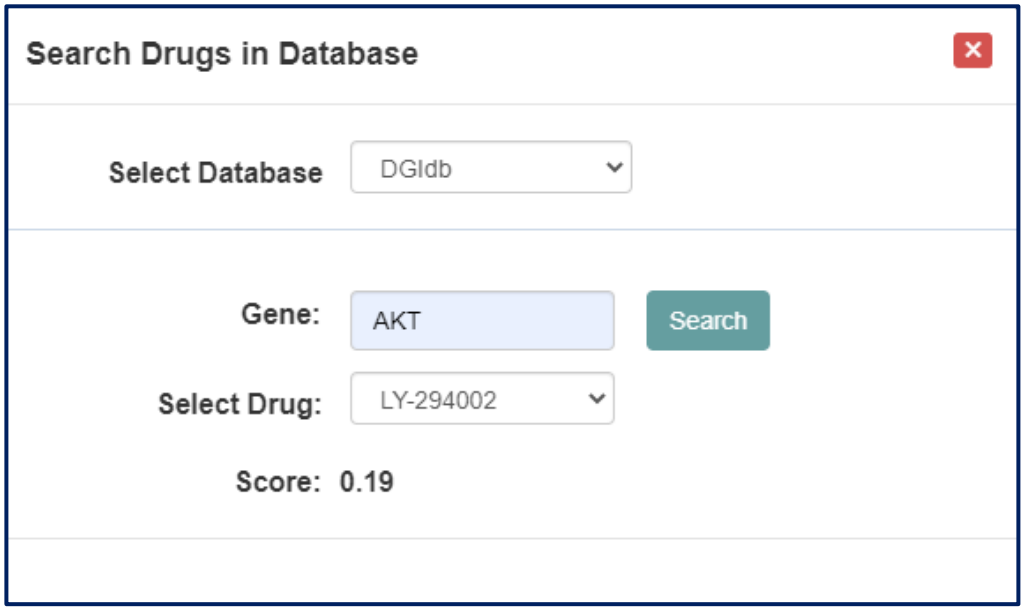


Figure 2.3.4.3 - Enter gene name and click search to retrieve the available drugs.

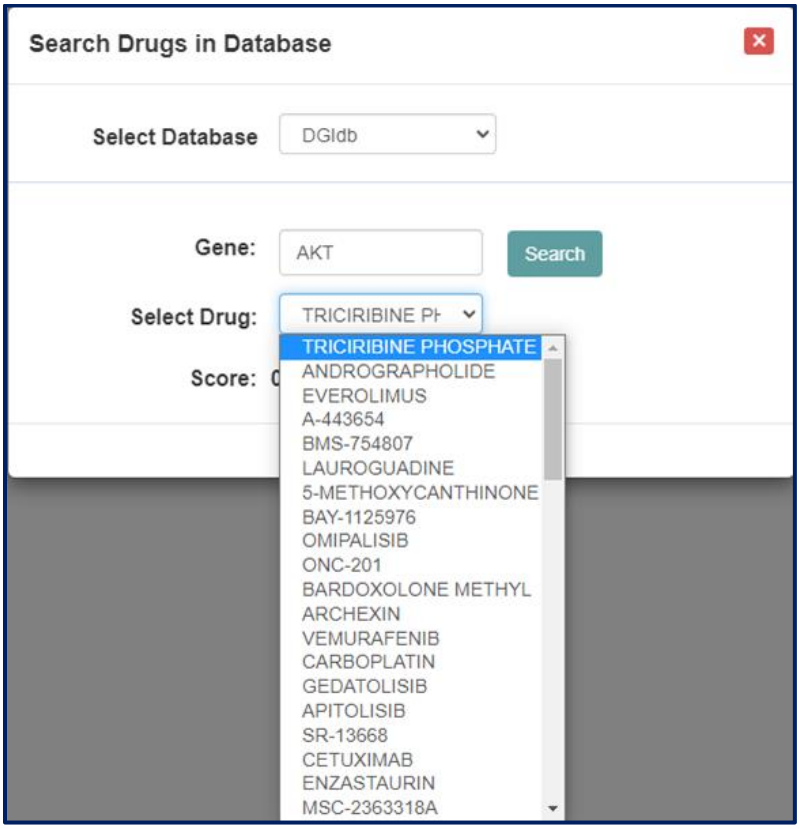


Figure 2.3.4.4 - List of available drugs in DGIdb against the searched gene.

2.3.5 'Show Therapeutic Steps' Button

'Show Therapeutic Steps' button (highlighted in **Figure 2.3.5.1**) has a toggling action that provides the option to display or hide the Therapeutic Steps panel (i.e., if the panel is open, it will close it and vice versa). The panel allows the user to view all the therapeutic steps performed on a network.

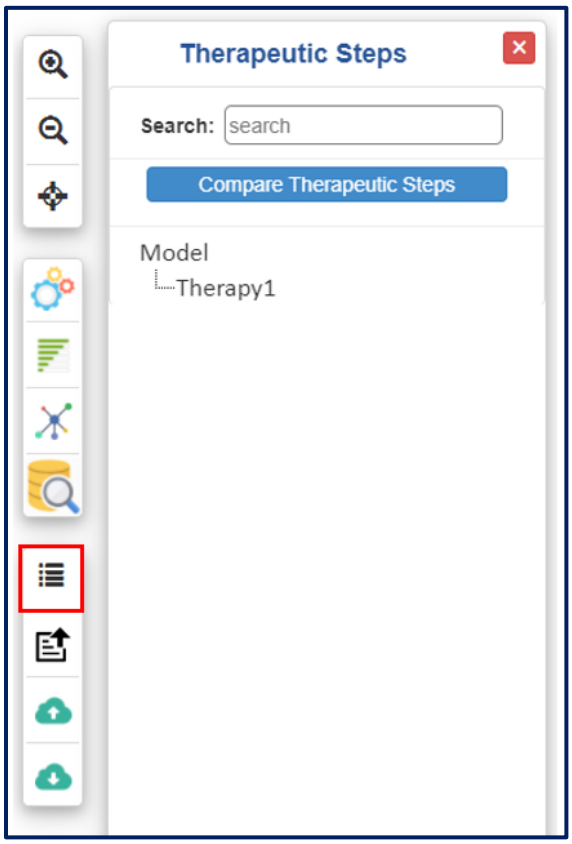


Figure 2.3.5.1 - 'Show Therapeutic Steps' button.

2.3.6 'Upload Case Study', 'Import therapy' and 'Export therapy' Buttons

The user can upload a case study or import and export therapeutic interventions using the buttons shown below (**Figure 2.3.6.1**).

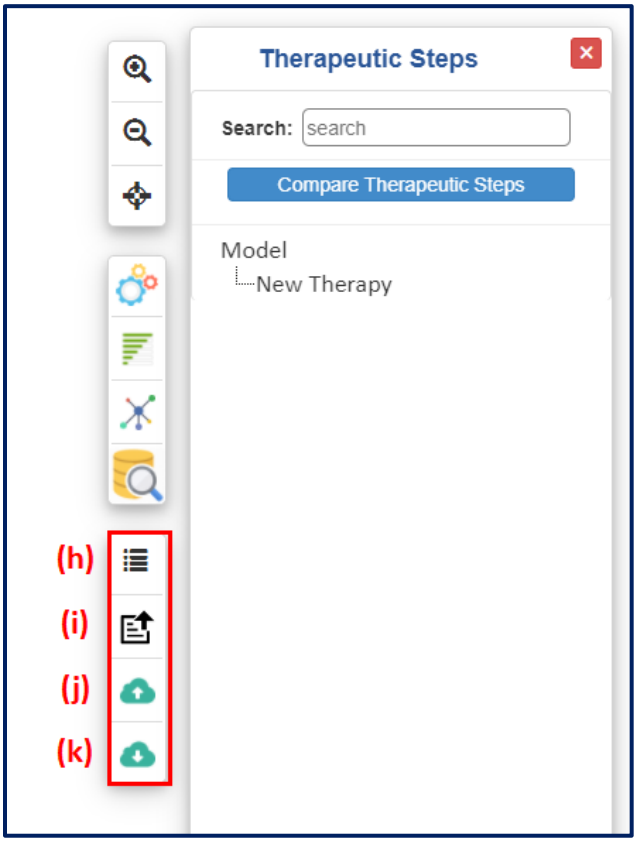


Figure 2.3.6.1 – Therapeutic step panel and case study related buttons: *(h) 'Show Therapeutic Steps', (i) 'Upload Case Study', (j) 'Import Therapy', (k) 'Export Therapy'.*

Refer to **Section 2.3.5** for an explanation on the 'Show Therapeutic Steps' button (**Figure 2.3.6.1 (h)**). The 'Upload Case Study' button (**Figure 2.3.6.1 (i)**) allows the user to upload a template case study, as shown in **Figure 2.3.6.2.**

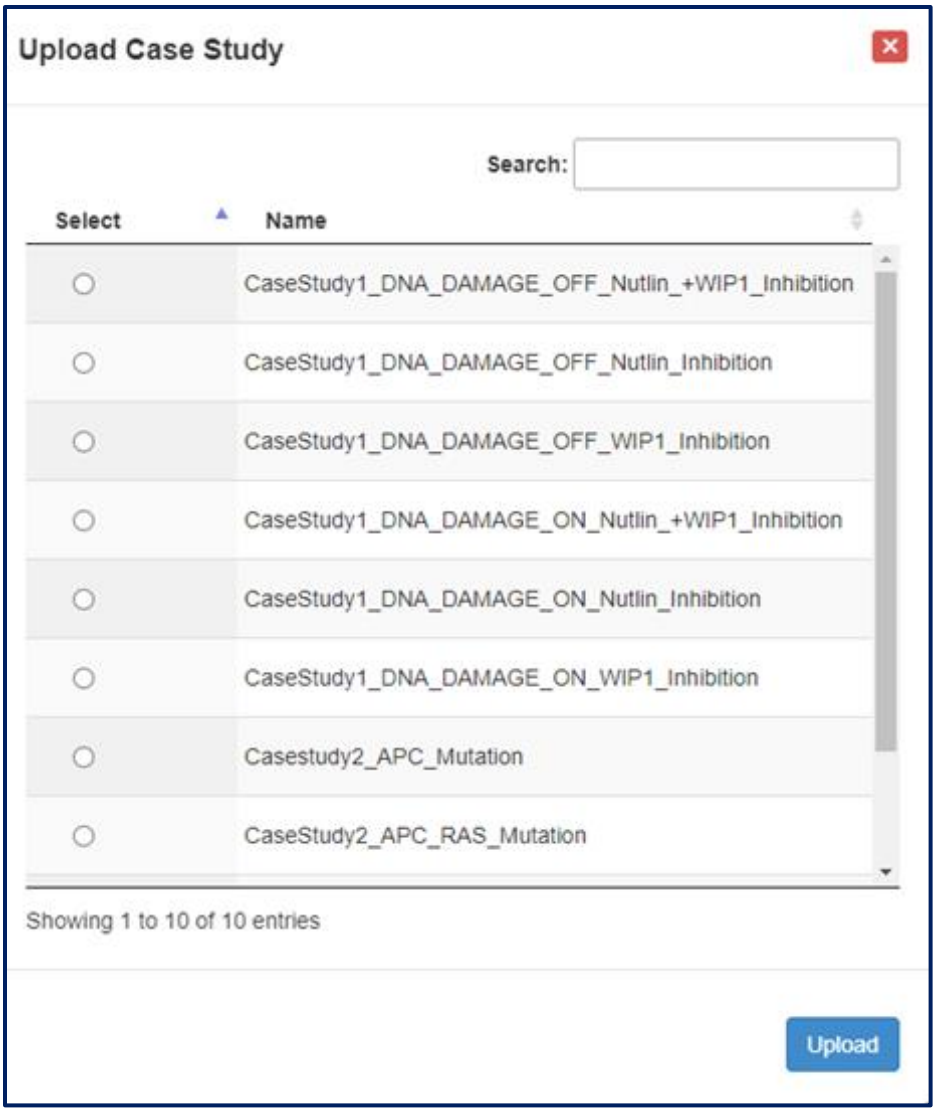


Figure 2.3.6.2 - 'Upload Case Study' prompt.

'Import Therapy' button (**Figure 2.3.6.1 (j)**) allows the user to upload a network with the files mentioned in **Figure 2.3.6.3** and **Figure 2.3.6.4.**

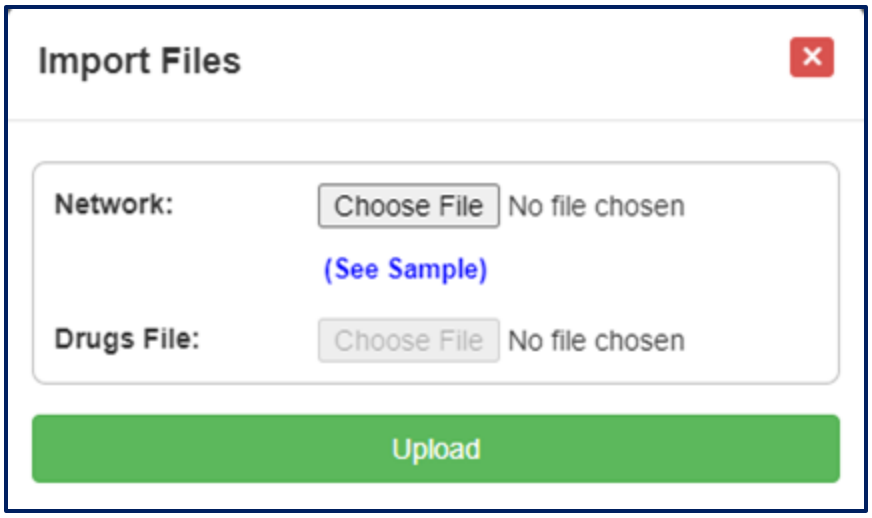


Figure 2.3.6.3 - Upload files window for importing network and drugs file.

The format for a 'Drugs File' ('.txt') to import a therapeutic intervention is illustrated below:

Figure 2.3.6.4 - Drugs file format (.txt) for importing therapeutic intervention.

The format for a ‘Parameters File’ must be ‘.csv' to import a therapeutic intervention, as is illustrated below (**Figure 2.3.6.5**).

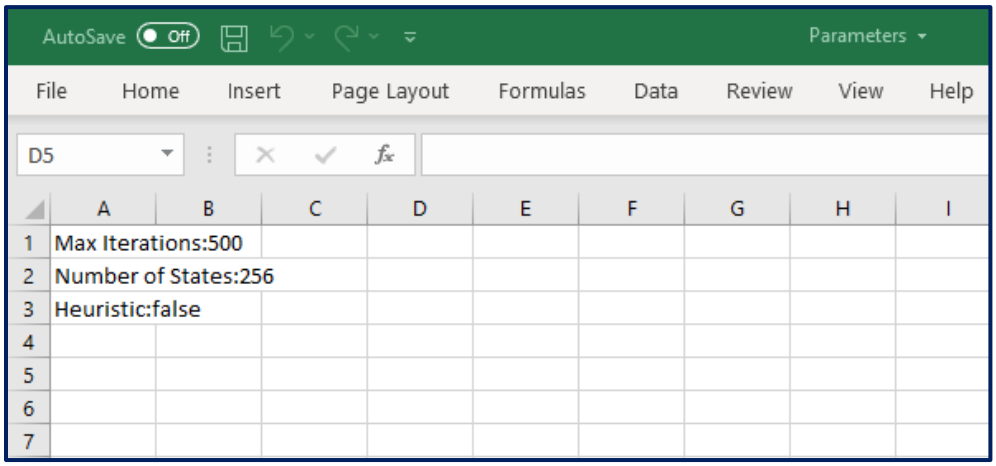


Figure 2.3.6.5 - Parameters file format (.csv) for importing therapeutic intervention.

‘Export Therapy’ button (**Figure 2.3.6.1 (k)**) downloads a ‘.zip’ file containing the uploaded network along with the result files (detailed and summary files for control and therapy case), parameters file, fixed nodes state file, cell fate logic file, and drug data after network analysis for a therapy has been performed.

2.4 Therapeutic Steps

The panel on the right side of the canvas (**Figure 2.4.1**) helps the user to add drugs/therapies in a network and keep track of the changes made in an organized manner, that is, the sequence in which the drugs are added. The search bar provides the option to search for specific therapies, drugs and node modifications created in the 'Therapeutic Steps' panel.

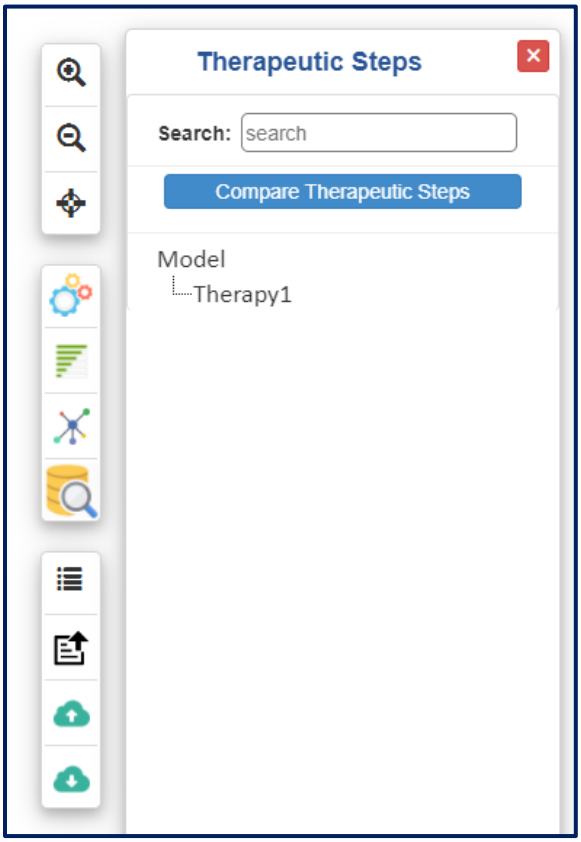


Figure 2.4.1 - 'Therapeutic Steps' panel.

The 'Compare Therapeutic Steps' feature (**Figure 2.4.2**) will only be activated if cell fate classification has been provided with the network analysis. In this feature, the cell fates from the network analysis results of each therapeutic step are displayed in a graphical format. This allows the user to graphically compare the results between different controls and therapies or within therapies in the form of a graph. These graphs are constructed with discrete cell fate basin ratios (for a rules-based network) or cell fate probabilities (for a weight-based network), both of which are extracted from the results of a therapeutic analysis.

The discrete basin size ratio or probability for a cell fate is the summation of its occurrence in each stable attractor. If an attractor exhibits multiple fates, it is segregated into discrete cell fates by dividing the basin size ratio or probability of the attractor with the total number of fates contained within it. The resultant value is assigned to each of the cell fates within the attractor before the final summation. The final stack chart or bar chart contains a summation of discrete cell fate basin size ratios or probabilities on the y-axis and the various therapies under comparison on the x-axis. The graph legend illustrates the color codes assigned to each cell fate.

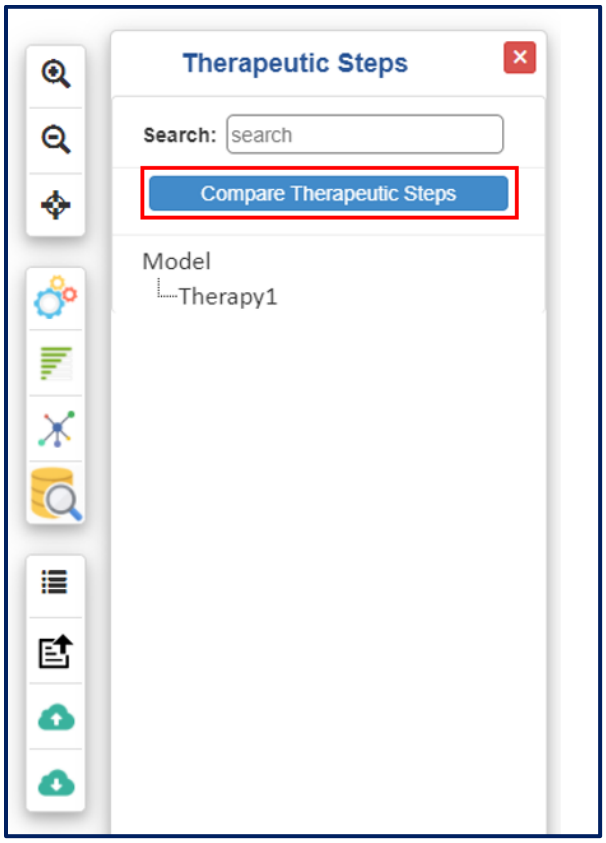


Figure 2.4.2 - 'Compare Therapeutic Steps' option in 'Therapeutic Steps' panel.

'Compare Therapeutic Steps' option provides the user with two types of charts, Bar Chart and Stack Chart, to compare the basin size ratios of resultant cell fates between the user-selected therapies or between a control and a therapy (For visual reference, see Section 2.5**,** Table 2.5.1 - Video 2).

Selecting the 'Compare Therapeutic Steps' button will open the following window (**Figure 2.4.3**)

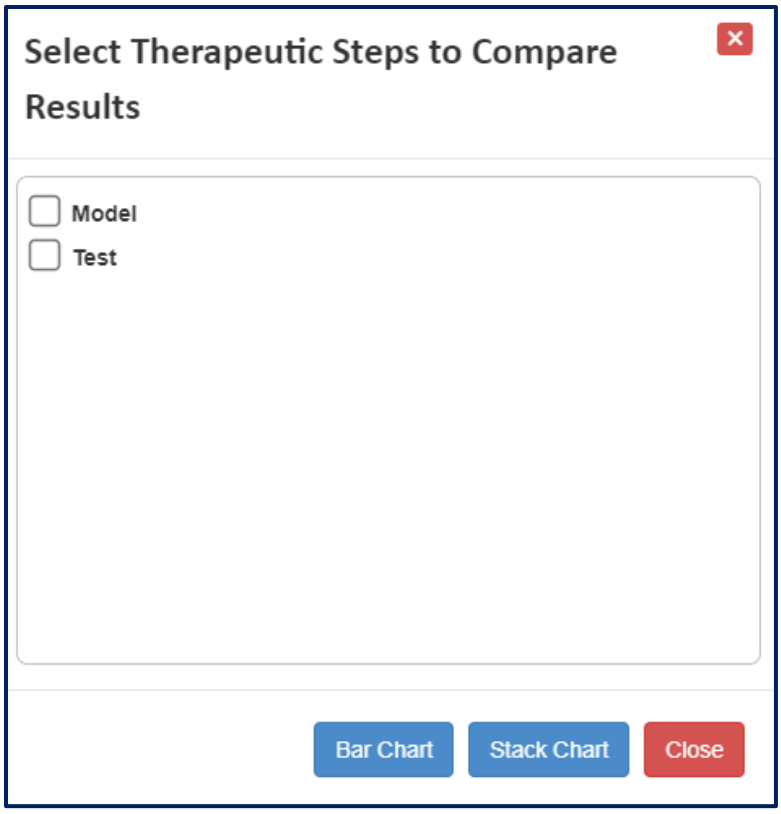


Figure 2.4.3 - Selecting therapies to compare the resultant cell fates.

Selecting the 'Bar Chart' option will display comparative therapeutic evaluation results of the selected therapies as a bar chart (shown in **Figure 2.4.4)**.

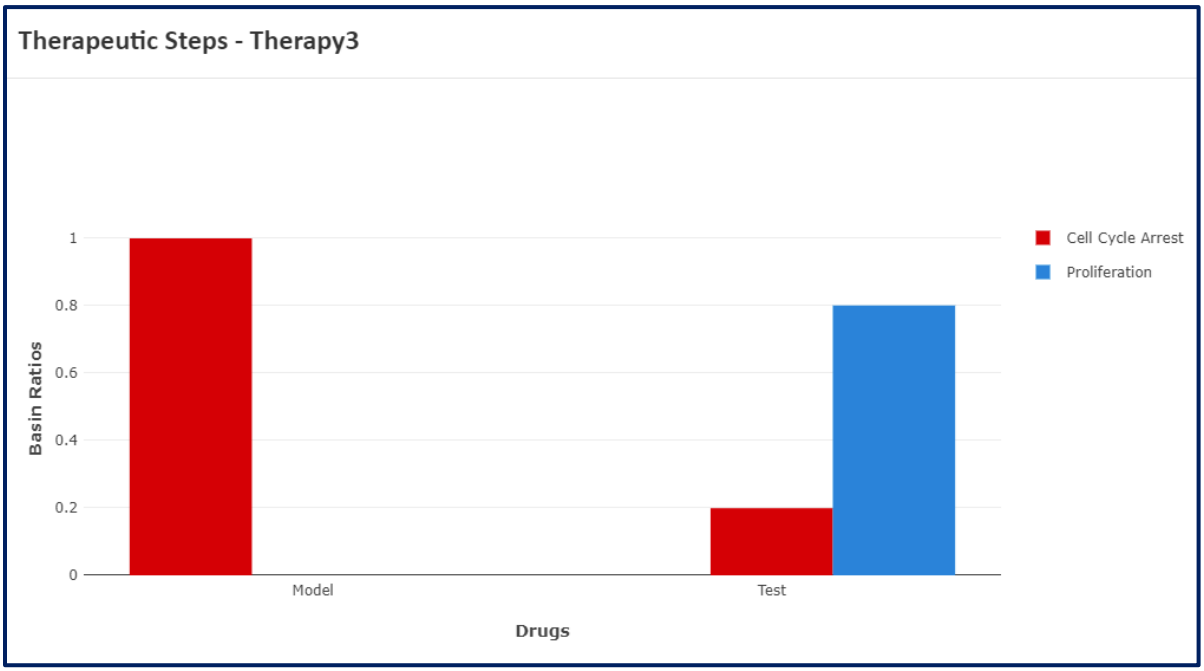


Figure 2.4.4 - Bar chart for comparative therapeutic evaluation of the selected therapies.

Selecting the 'Stack Chart' option will display comparative therapeutic evaluation results of the selected therapies as a stack chart (shown in **Figure 2.4.5**).

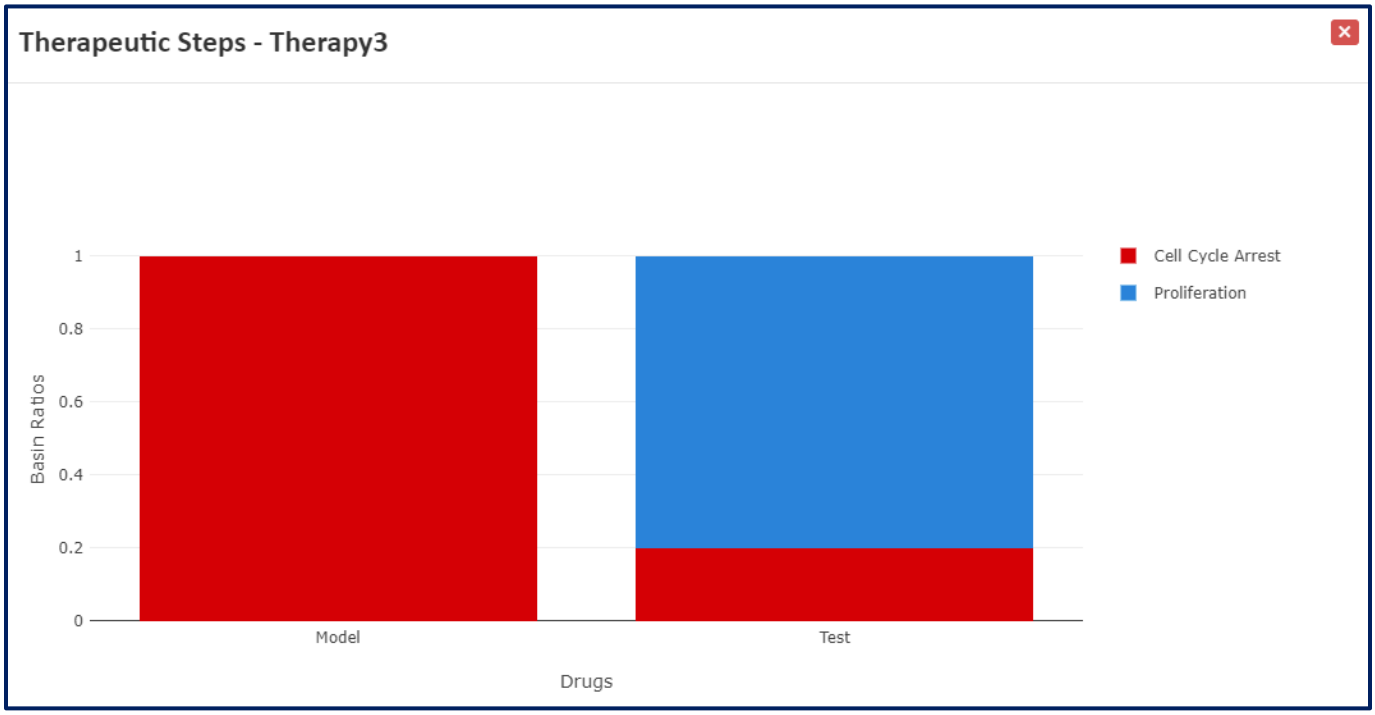


Figure 2.4.5 - Stack chart for comparative therapeutic evaluation of the selected therapies.

2.4.1 Creating a Therapy

After selecting a network for creating a therapeutic intervention and saving the therapy name, that particular therapy will appear in the 'Therapeutic Steps' panel under 'Model' (**Figure 2.4.1.1)** (For visual reference, see Section 2.5, Table 2.5.1 - Video 2).

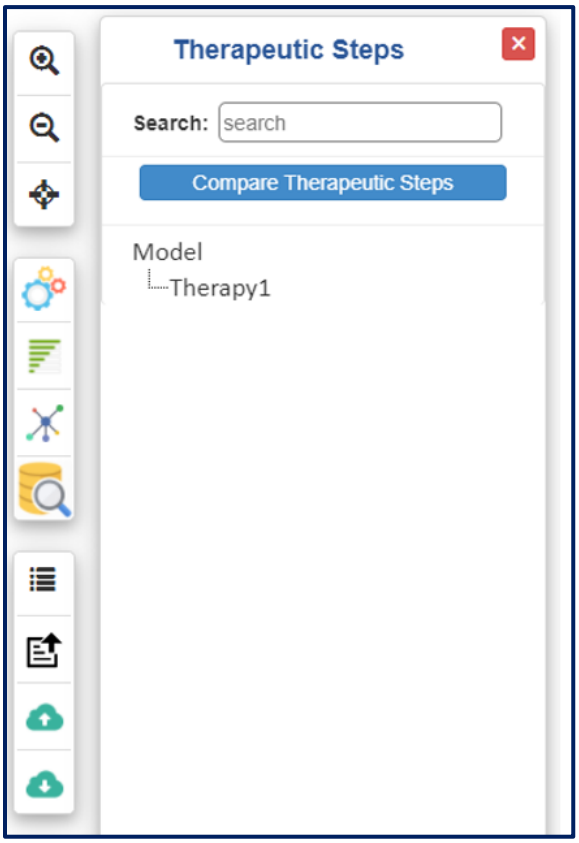


Figure 2.4.1.1 - 'Therapeutic Steps' panel after creating a therapeutic intervention termed 'Therapy1'.

Right-clicking on the newly created therapy appearing in the 'Therapeutic Steps' panel will allow the user to add or delete 'Therapeutic Steps', that is, a drug/mutation as shown below (**Figure 2.4.1.2**).

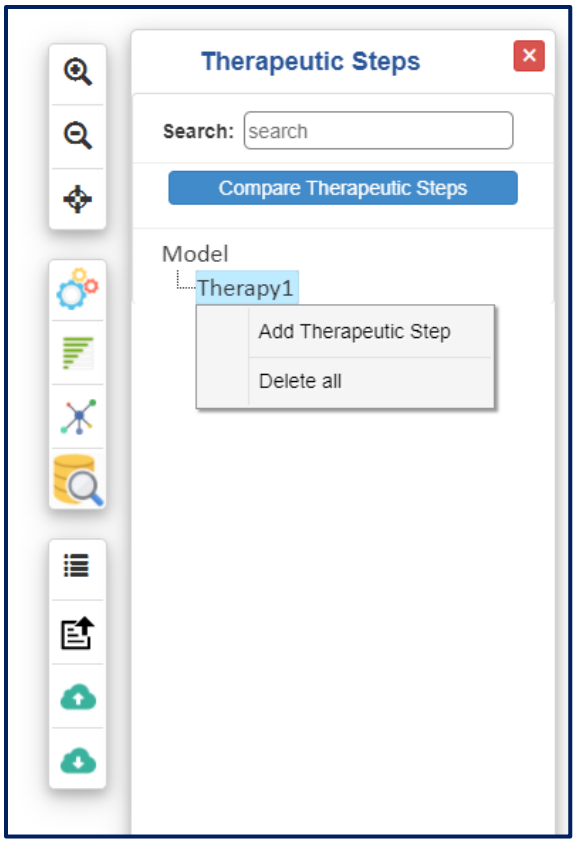


Figure 2.4.1.2 - 'Add or delete drug option' after right-clicking on the therapy displayed in the 'Therapeutic Steps' panel.

Clicking the 'Add Therapeutic Step' option will open a 'Therapeutic Step Name' modal in which the user must input a unique therapy name (**Figure 2.4.1.3**); once the user clicks on 'Add', a new therapeutic step will be created in the Therapeutic Steps panel (**Figure 2.4.1.4).**

Users can specify the mode of therapy, for example, 'knock-up', 'knock-down', 'knock-out' and 'knock-in', for onward targeting of nodes for each therapeutic intervention. Moreover, if the user wants to delete all the mutations/drugs created in the 'Therapeutic Steps', the 'Delete all' option can be used (**Figure 2.4.1.2**).

Figure 2.4.1.3 - Therapeutic Step' name window.

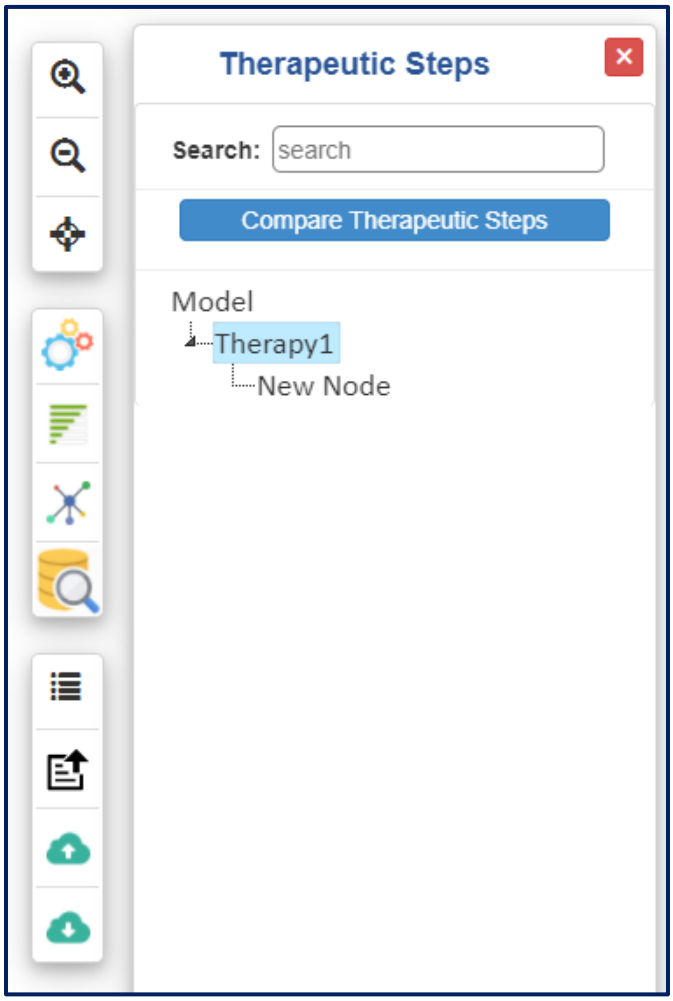


Figure 2.4.1.4 - Creating a new mutation/drug within a therapy.

a. Rules-based Therapy

In the case of *rules-based therapy*, right-clicking on the 'New Node' will open the window shown in **Figure 2.4.1.5** which allows the user to incorporate therapy through four different options: (i) Knock Up, (ii) Knock Down, (iii) Knock Out, and (iv) Knock In. Additionally, users can perform 'Edge Knock In', 'Edge Knock Out', and can also 'Delete', 'Rename' as well as 'View Results' of therapy. The user can also perform network analysis for the therapeutic step/s created in therapeutics panel.

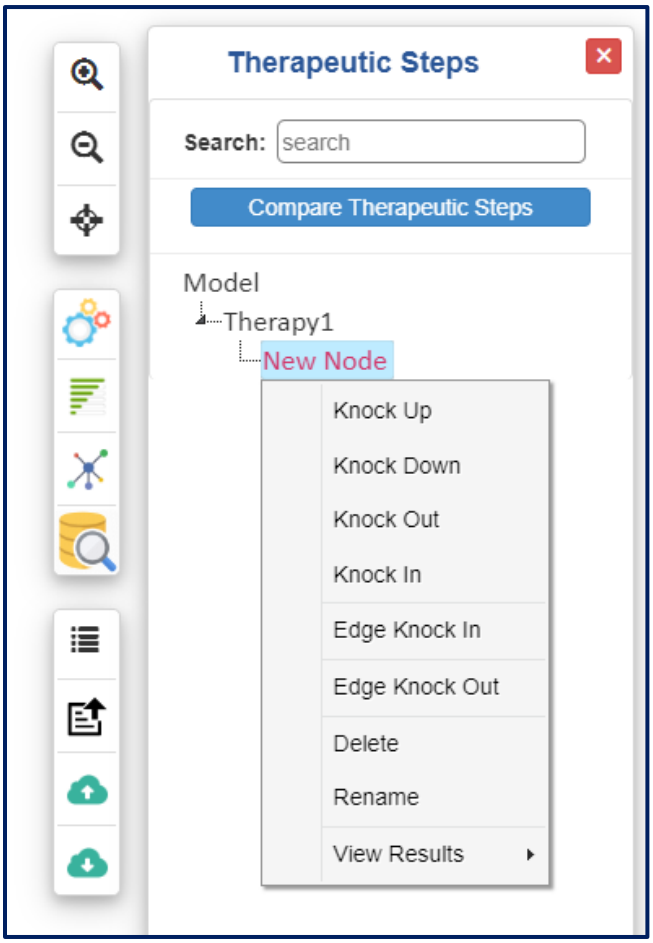


Figure 2.4.1.5 - Modes of therapy along with available modifications.

i. Knock Up/Down

In case the user chooses 'Knock Up' or 'Knock Down', the modals shown in **Figure 2.4.1.6** and **Figure 2.4.1.7** will appear respectively.

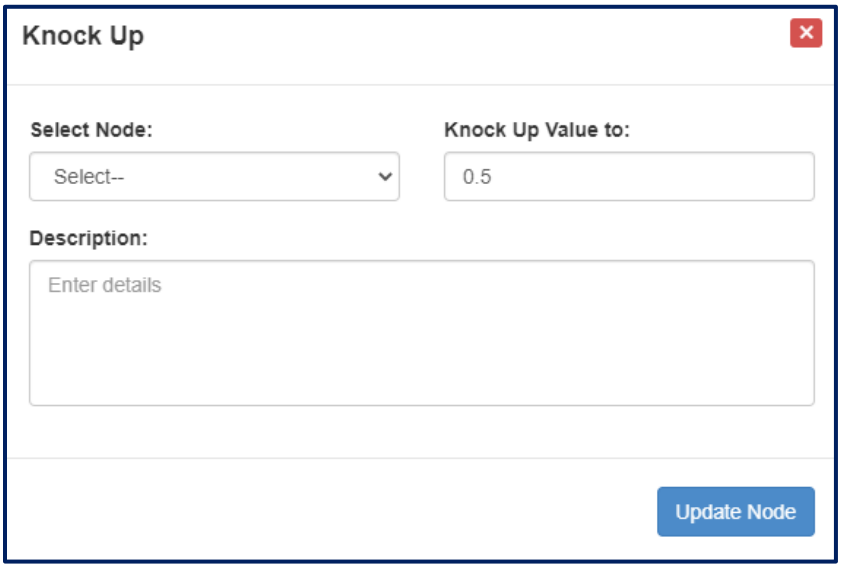


Figure 2.4.1.6 - 'Knock Up' window for a rules-based network.

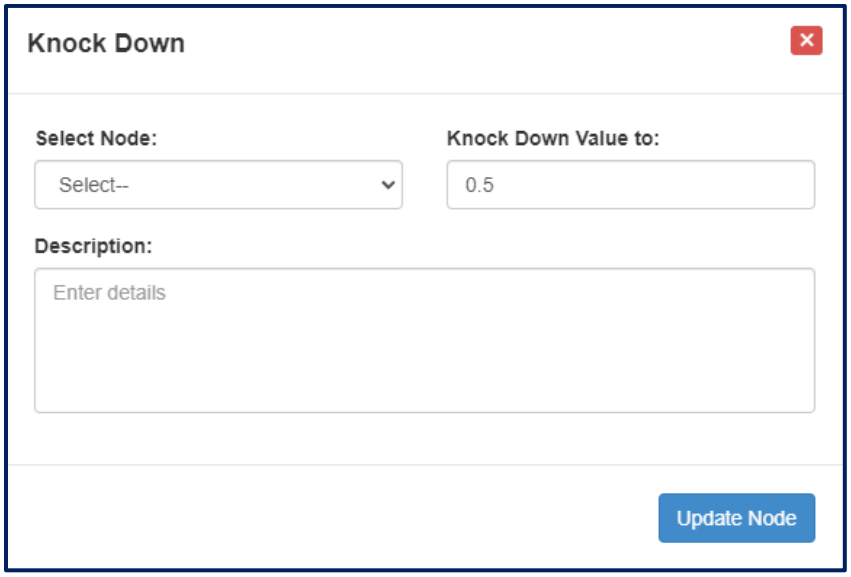


Figure 2.4.1.7 - 'Knock Down' window for a rules-based network.

Clicking on 'Select Node' will reveal a drop-down containing all the nodes in the network/therapy. The user then selects a node from the drop-down list and specifies a knock up or knock down value. As a result, the rules of the network/therapy will be updated: (i) the in-coming regulation will be deleted and (ii) the new user-defined value will be assigned to it as a 'fixed node' value. Additionally, the user can add a 'Description' for each node while updating its rule for ease. Once 'Update Node' is clicked, nodes which are knocked up will be shown in green and the nodes which are knocked down will be shown in red underneath the therapeutic step in the therapeutics panel (**Figure 2.4.1.16**). The implementation of these features is summarized below in **Figure 2.4.1.8.**

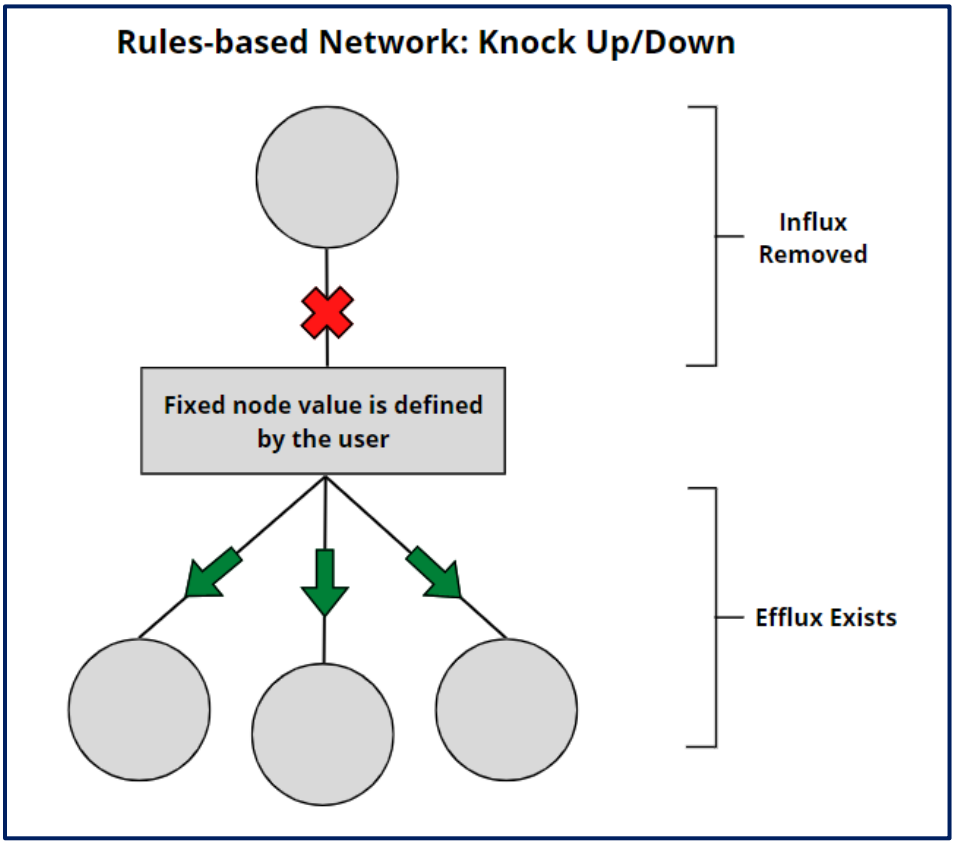


Figure 2.4.1.8 - Implementation of 'Knock Up/Down' in a rules-based network.

ii. Knock Out

Selecting 'Knock Out' will open the window shown in **Figure 2.4.1.9.** The user can choose a node from the drop-down menu upon clicking 'Select Node'. The node to be knocked out will (i) be assigned '0' as a node value and (ii) its own rule will be deleted and the any other regulations, elicited by this node, is removed as well. On clicking 'Update Node,' this mutation will be shown in yellow underneath the therapeutic step **(Figure 2.4.1.16**). The implementation of this feature has been summarized in **Figure 2.4.1.10 .**

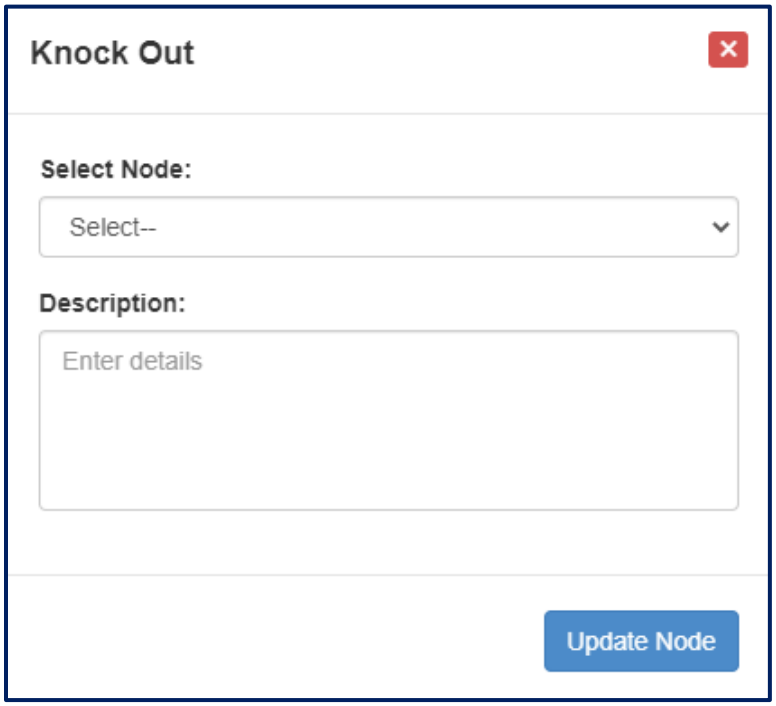


Figure 2.4.1.9 - 'Knock Out' window for a rules-based network.

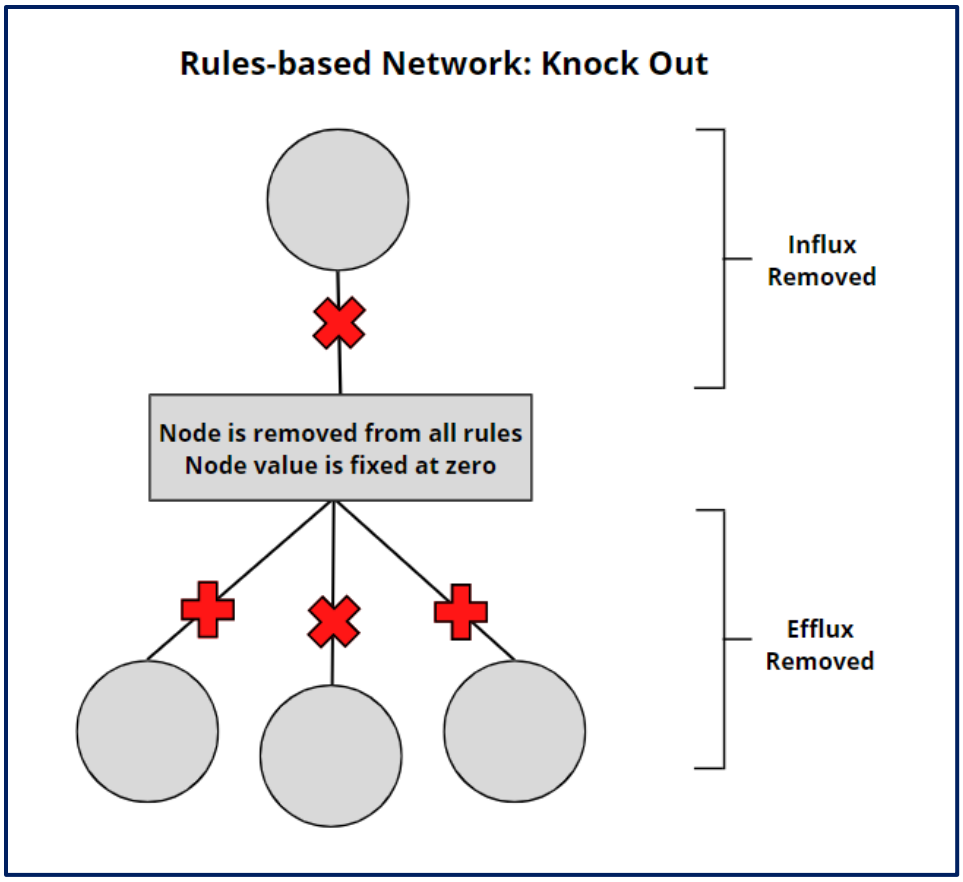


Figure 2.4.1.10 - Implementation of 'Knock Out' in a rules-based network.

iii. Knock In

Selecting 'Knock In' option, opens the window shown in **Figure 2.4.1.11** and allows the user to add a new node and its corresponding rule to the network. First, the user must provide the name of the new node followed by the definition of 'Inward Regulations' either by selecting the 'New Node Rule' or 'Fixed Node Value' option. In the presence of an inward regulation, the user must define the node rule whereas in the absence of any inwards regulation, a fixed value must be assigned to the new node. For defining the 'Outward Regulations' of a new node, the user must select an outward node from the drop-down menu. Once an existing node from the network is chosen from the list, pressing 'Add New Rule' tab will open another window (**Figure 2.4.1.12**) containing all the rules associated with the selected outward node. The user can choose which rules to change and update by selecting an operator (&& or ||) and filling in the 'Addition to Rule' box. Clicking 'Add Rules' will close this window and insert this updated rule into the 'Outward Regulations' table. Once 'Update Node' is clicked, this 'Knock In' drug/mutation will be shown in blue underneath the therapeutic step **(Figure 2.4.1.16**).The implementation of this feature has been summarized in **Figure 2.4.1.13.**

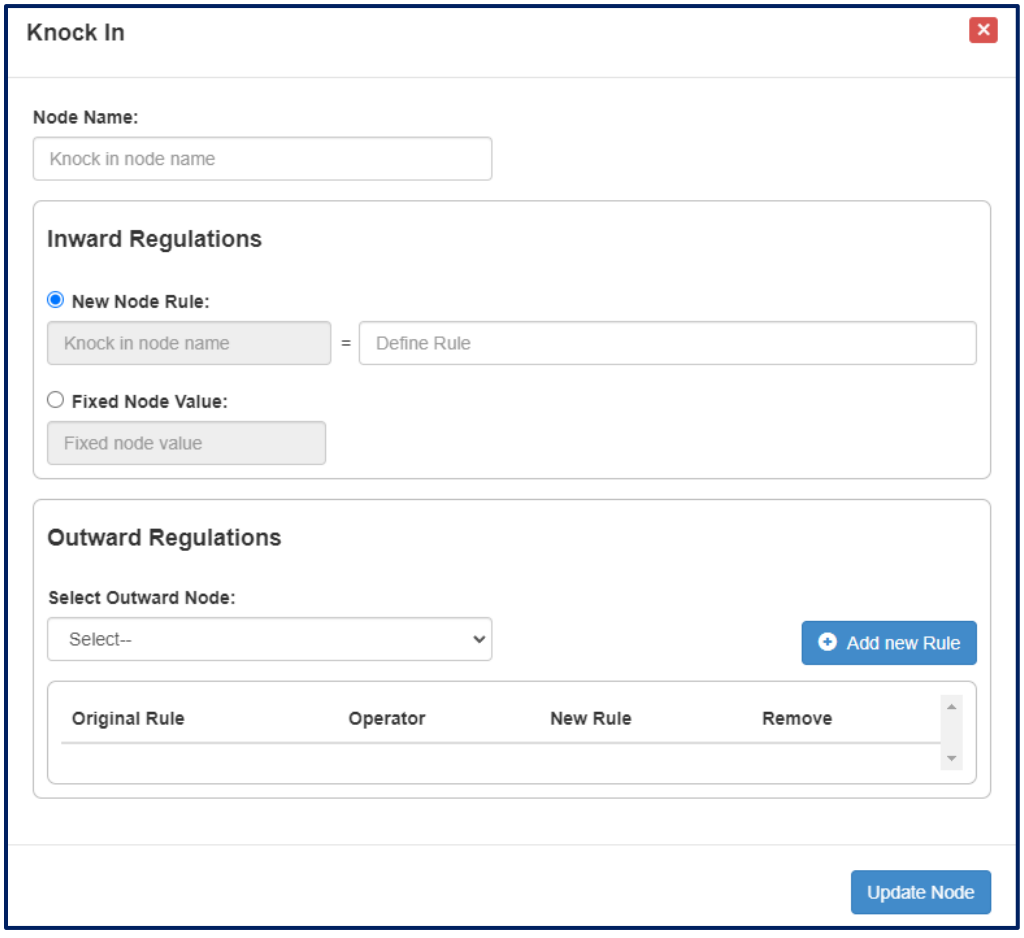


Figure 2.4.1.11 - 'Knock In' window for a rules-based network.

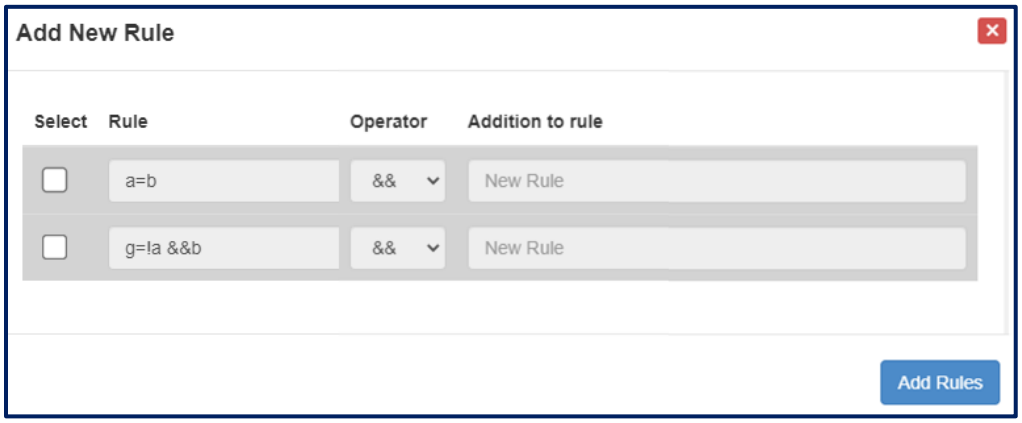


Figure 2.4.1.12 - 'Add New Rule' window.

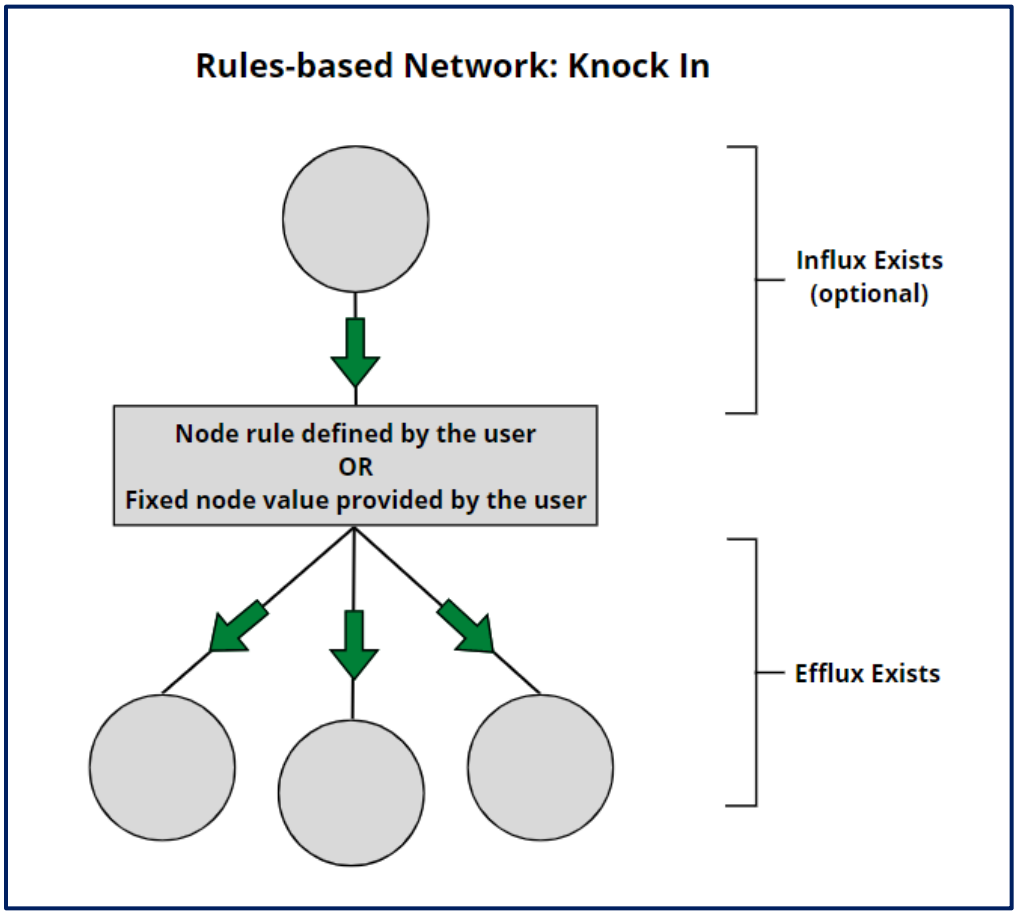


Figure 2.4.1.13 - Implementation of 'Knock In' in a rules-based network.

iv. Edge Knock In

Selecting the 'Edge Knock In' option will open the modal shown in **Figure 2.4.1.14.** The user must define the source and target node, the new edge will connect, as well as define the link type (either activation or inhibition.) The user must update the rule of the target node to contain the source node, with either the 'or (II)' or 'and (&&)' operators. An optional description can also be given to the new edge. Clicking 'Add Link' will update the therapy, and this drug/mutation will be displayed in blue in the therapeutic steps panel (**Figure 2.4.1.16**).

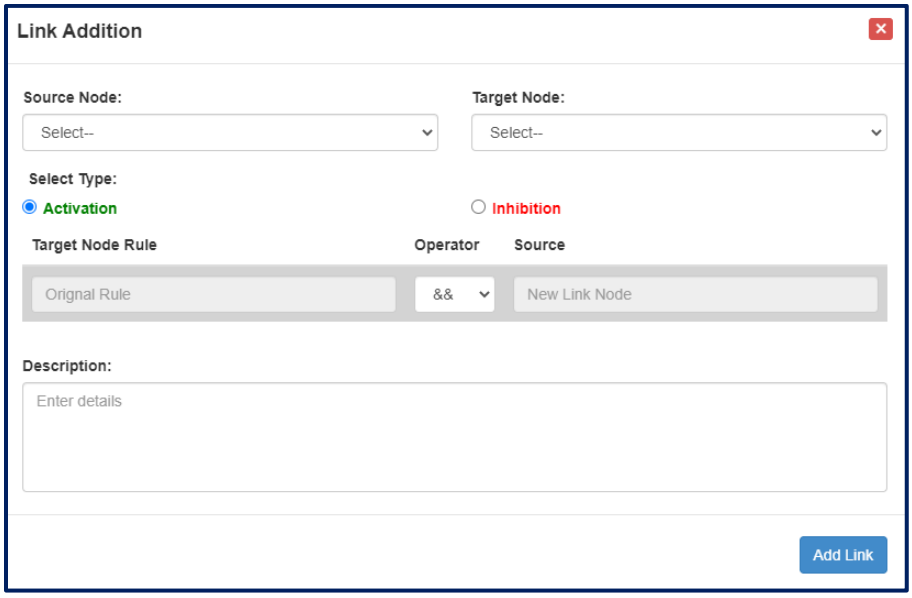


Figure 2.4.1.14 - 'Edge Knock In' window for a rules-based network.

v. Edge Knock Out

Selecting the 'Edge Knock Out' option will open the modal shown in **Figure 2.4.1.15.** To remove an edge, the user must choose the source and target node from the drop-down list. An optional description can also be provided. Selecting 'Remove Link' will delete the edge from the network. This drug/mutation will be displayed in light blue underneath the therapeutic step (**Figure 2.4.1.16).**

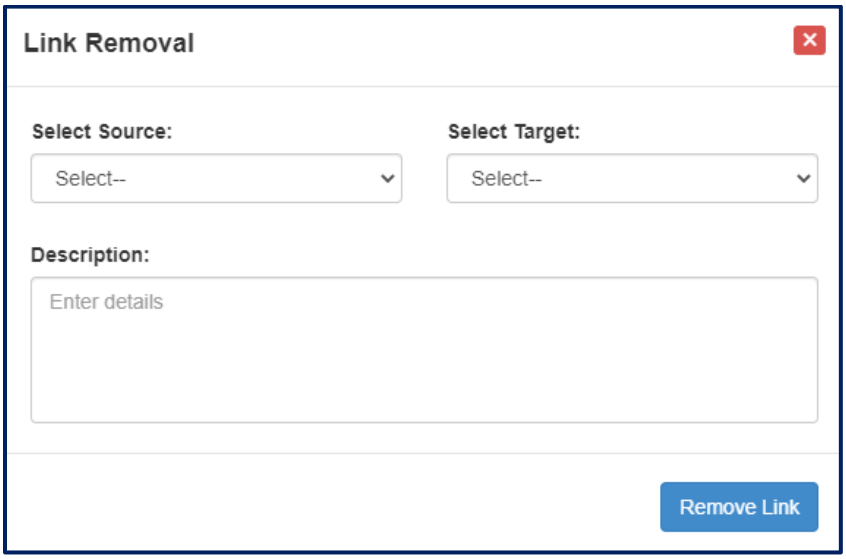


Figure 2.4.1.15 - 'Edge Knock Out' window for a rules-based network.

All the modified nodes, along with their assigned values, appear under the therapeutic step.

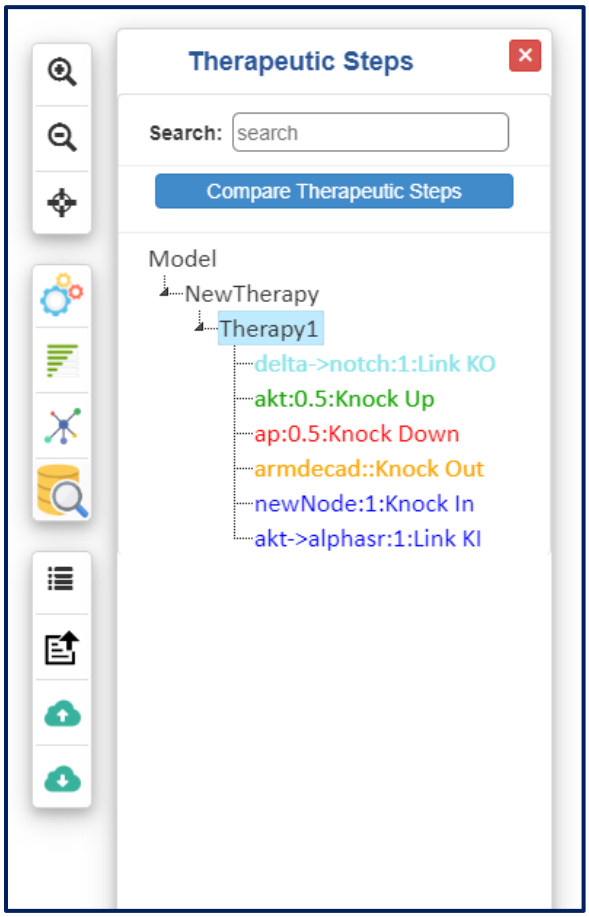


Figure 2.4.1.16 - Target nodes along with their assigned custom values appear under the therapeutic step in a rules-based therapy.

b. Weight-based Therapy

In the case of a *weight-based therapy*, right-clicking on the 'New node' (**Figure 2.4.1.17**) will allow the user to incorporate therapy from four different options: (i) Knock Up, (ii) Knock Down, (iii) Knock Out, and (iv) Knock In. It also enables the user to update node 'Target Link' and allows for 'Edge Knock In'. Users can add patient-specific data using the 'Add/Remove Expression File' option. Additionally, users can also 'Delete' or 'Rename' a node and 'View Results' after performing network analysis for the therapy.

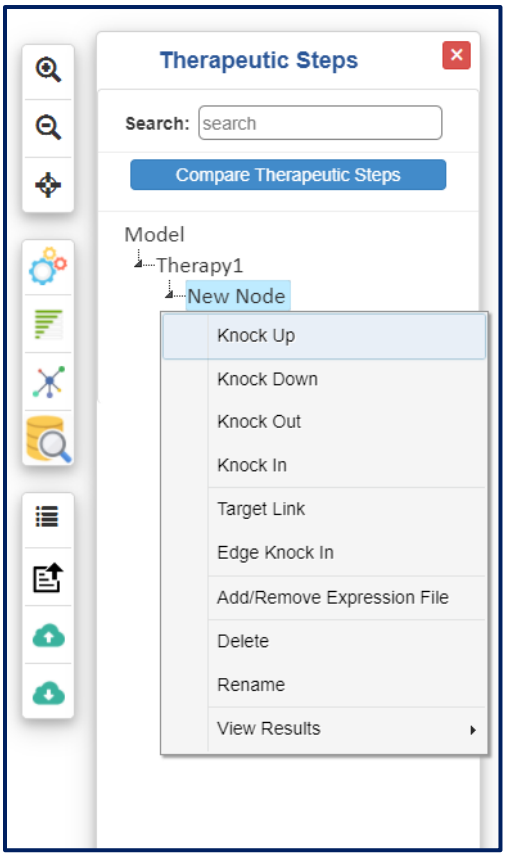


Figure 2.4.1.17 - Right-click on 'New node' options for a weight-based therapy.

i. Knock Up/Down

Upon clicking the 'Knock Up' option, the window shown in **Figure 2.4.1.18** appears. Clicking 'Select Node' opens a drop-down menu containing all the nodes present in the network. User can then select the node to be targeted and assigns it a value, which is incorporated as a fixed node that stays constant throughout the analysis. Additionally, all incoming edge weights are removed, and '0' is assigned as the basal value of the node. In contrast to this, the user can choose the 'Knock Down' option (**Figure 2.4.1.19**) if they would like to decrease the node value; regardless, the implementation will be the same for both. Once 'Update Node' is clicked, 'Knock Up' is shown in green, and 'Knock Down' will be shown in red underneath the therapeutic step (**Figure 2.4.1.27**). The implementation behind these two features is summarized below in **Figure 2.4.1.20.**

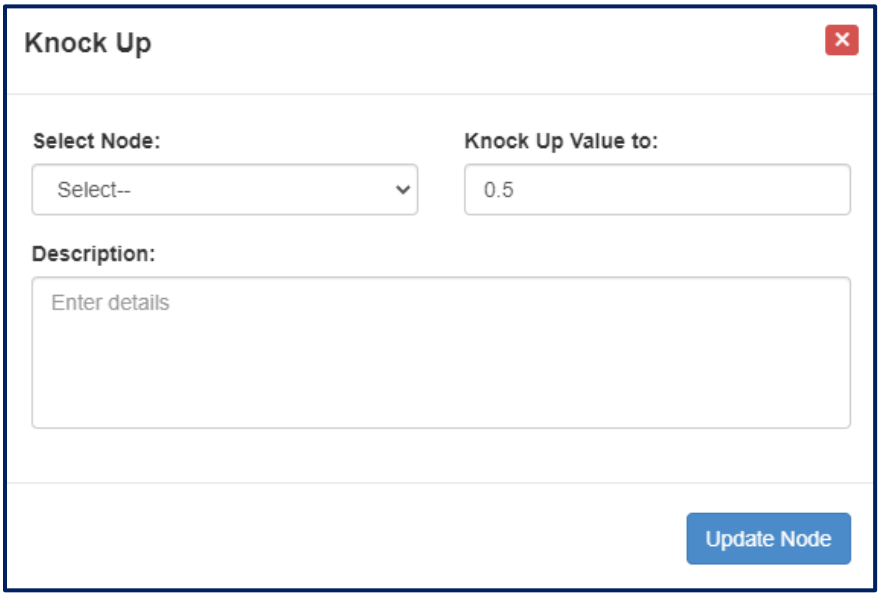


Figure 2.4.1.18 - 'Knock Up' window for a weight-based network.

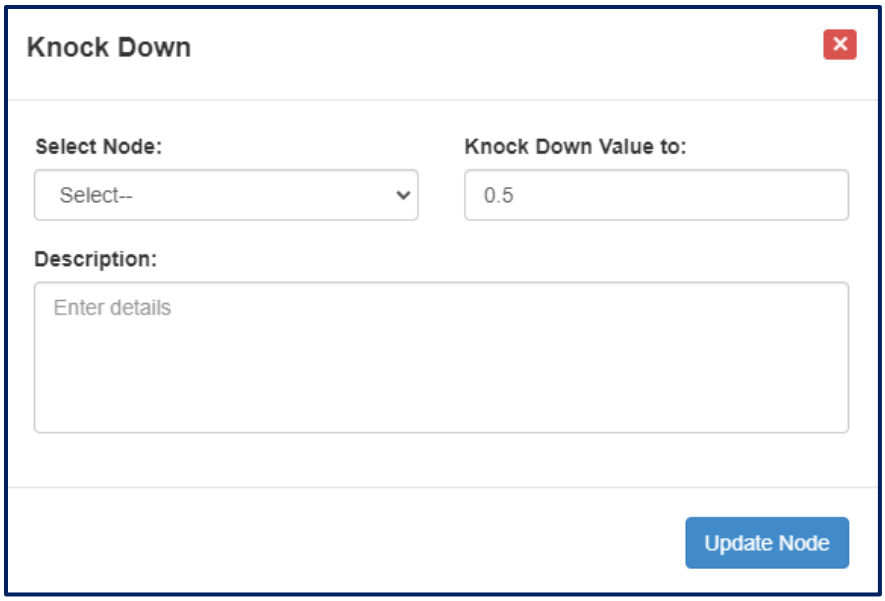


Figure 2.4.1.19 – 'Knock Down' window for a weight-based network.

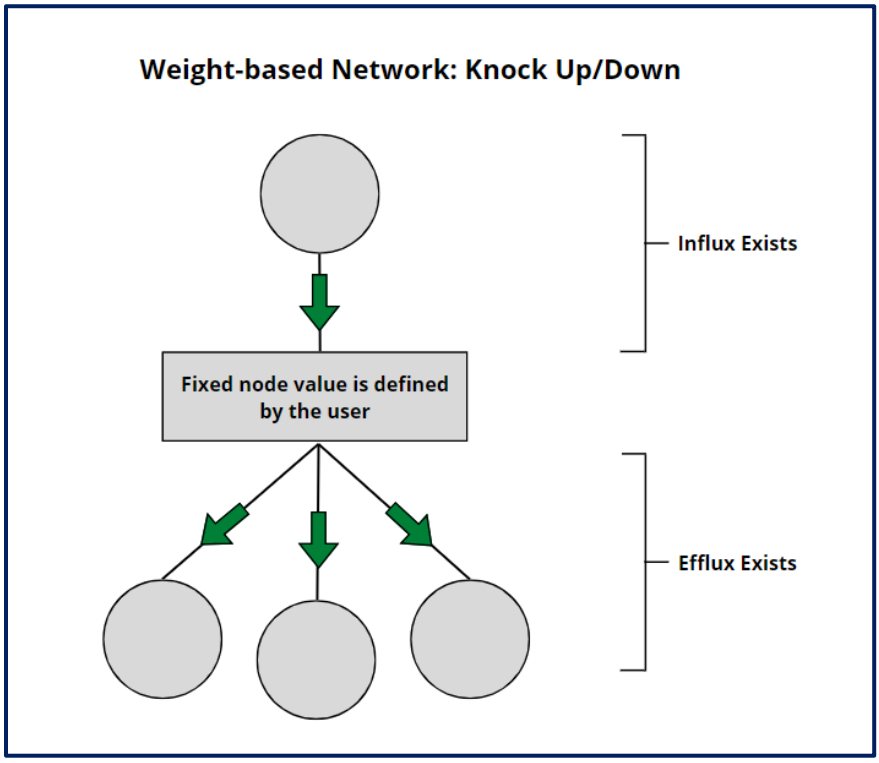


Figure 2.4.1.20 - Implementation of 'Knock Up/Down' in a weight-based network.

ii. Knock Out

On selecting the 'Knock Out' option, the modal shown in **Figure 2.4.1.21** appears. The user can choose a node from the drop-down list that appears when clicking 'Select Node'. The selected node will be assigned '0' as a basal value and its incoming and outgoing edges (regulations controlling other nodes) are removed. Once 'Update Node' is clicked, this drug/mutation will be shown in yellow underneath the therapeutic step (**Figure 2.4.1.27**). The implementation of this feature has been summarized below in **Figure 2.4.1.22.**

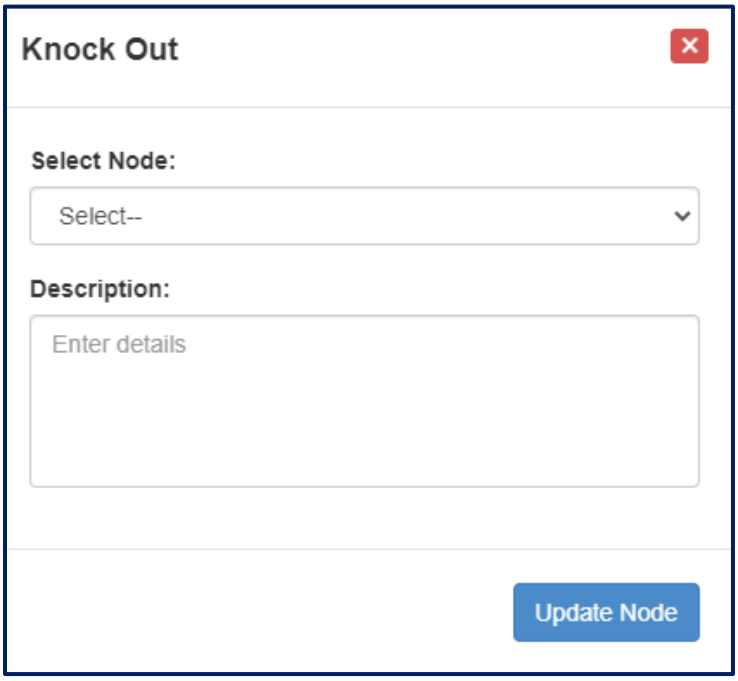


Figure 2.4.1.21 - 'Knock Out' window for a weight-based network.

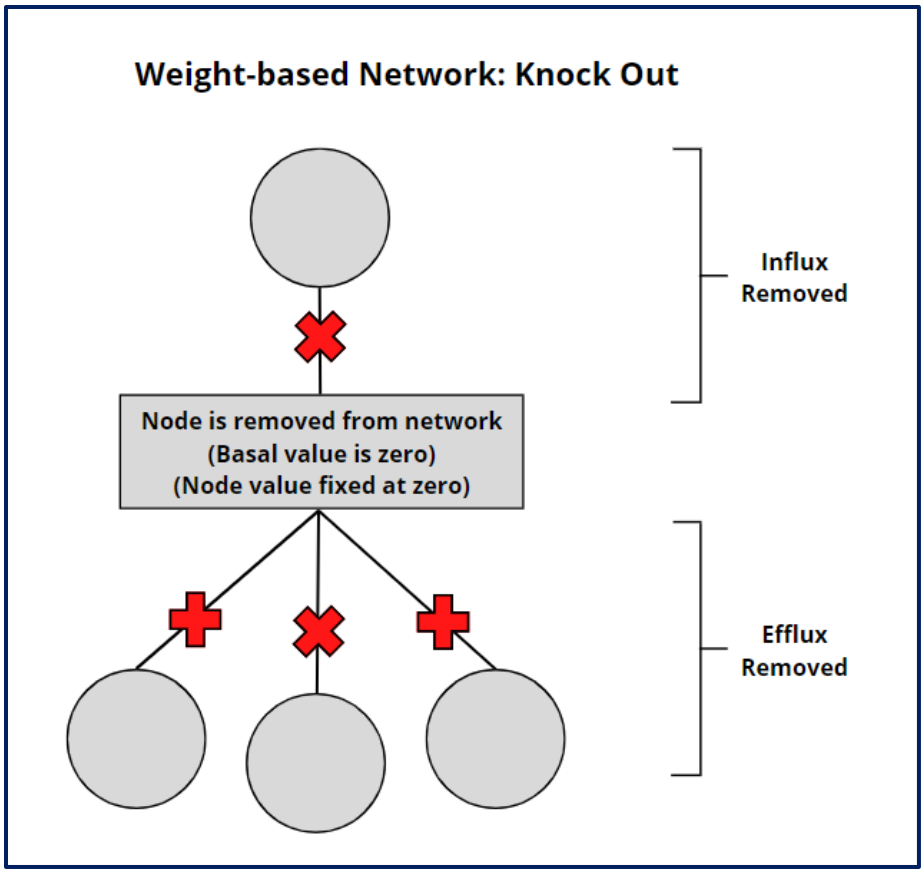


Figure 2.4.1.22 - Implementation of 'Knock Out' in a weight-based network.

iii. Knock In

Selecting the 'Knock In' option allows the user to add another node into the network. The user must add the node name along with its expression value or fixed node value and optional description in the window shown in **Figure 2.4.1.23**. Next, the interaction between this new node and the already present network nodes must be specified by providing the link type, interaction type, node name, and the corresponding edge weight with other nodes in the network. Clicking 'Add New' will add these characteristics to the interaction table. The user can add multiple interactions for each new node. Once 'Update Node' is clicked, the name of the new node is shown in blue underneath the therapeutic step (**Figure 2.4.1.27**). The implementation of this feature is summarized below in **Figure 2.4.1.24**.

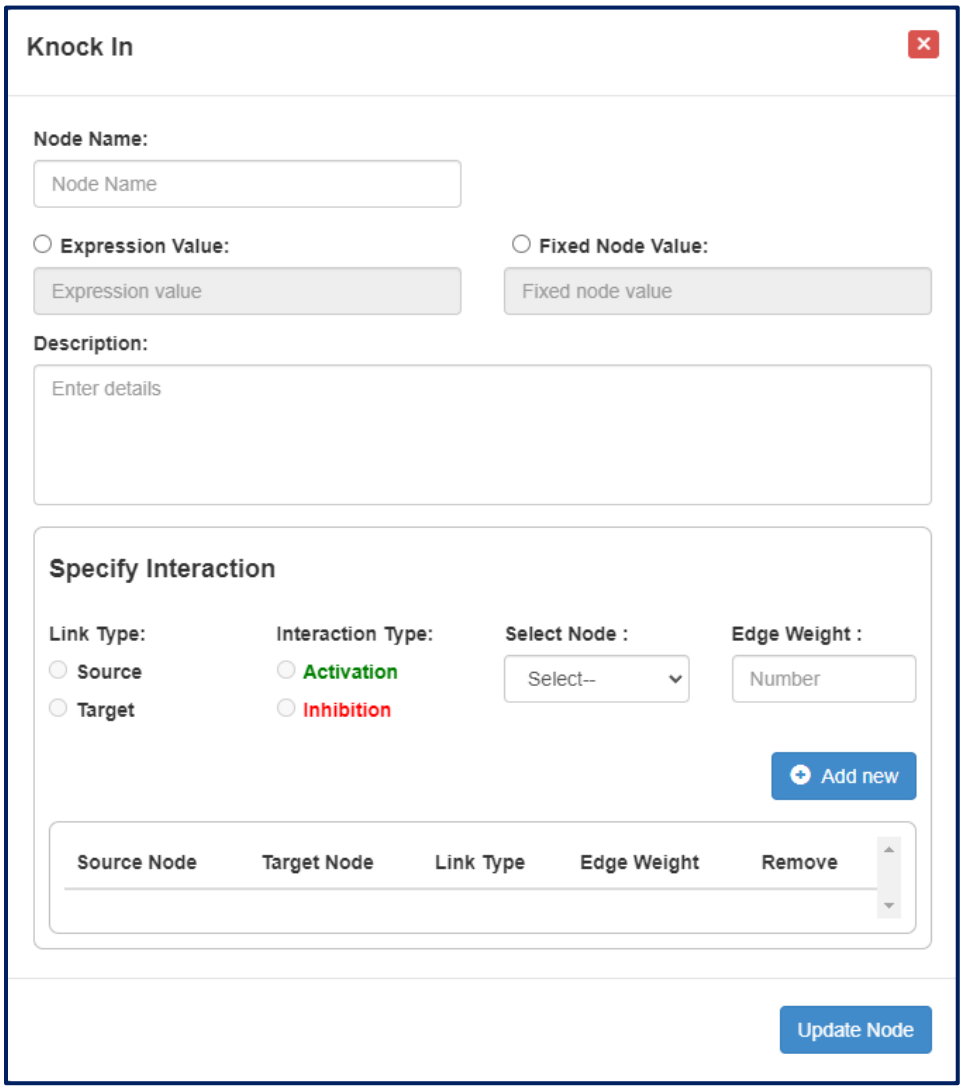


Figure 2.4.1.23 - 'Knock In' window for a weight-based network.

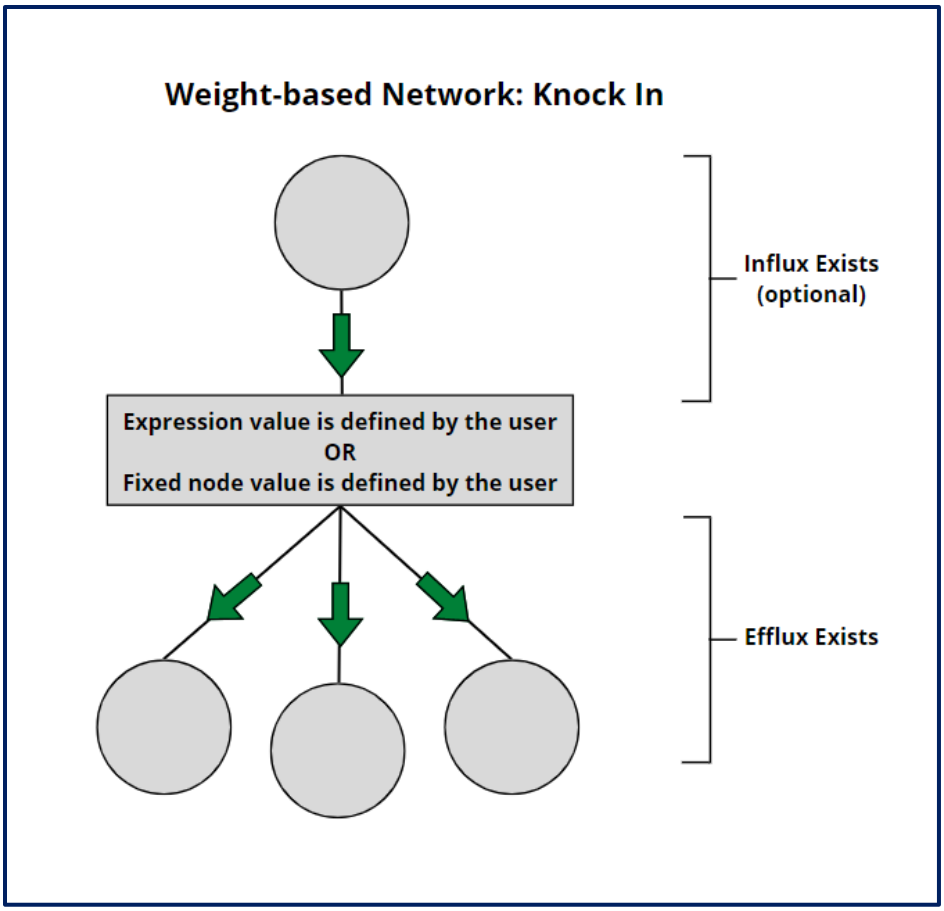


Figure 2.4.1.24 - Implementation of 'Knock In' in a weight-based network.

iv. Target Link

If the user selects 'Target Link', the modal shown in **Figure 2.4.1.25** appears. For updating a link/interaction between two nodes, the user must select the 'Source' (the node which regulates another node) and 'Target' (the node which gets regulated by the source node). The weight assigned to the link between the two nodes will be updated according to the user-specified value. Once 'Update Edge Value' button is clicked, this drug/mutation will be shown in blue underneath the therapeutic step (**Figure 2.4.1.27.**

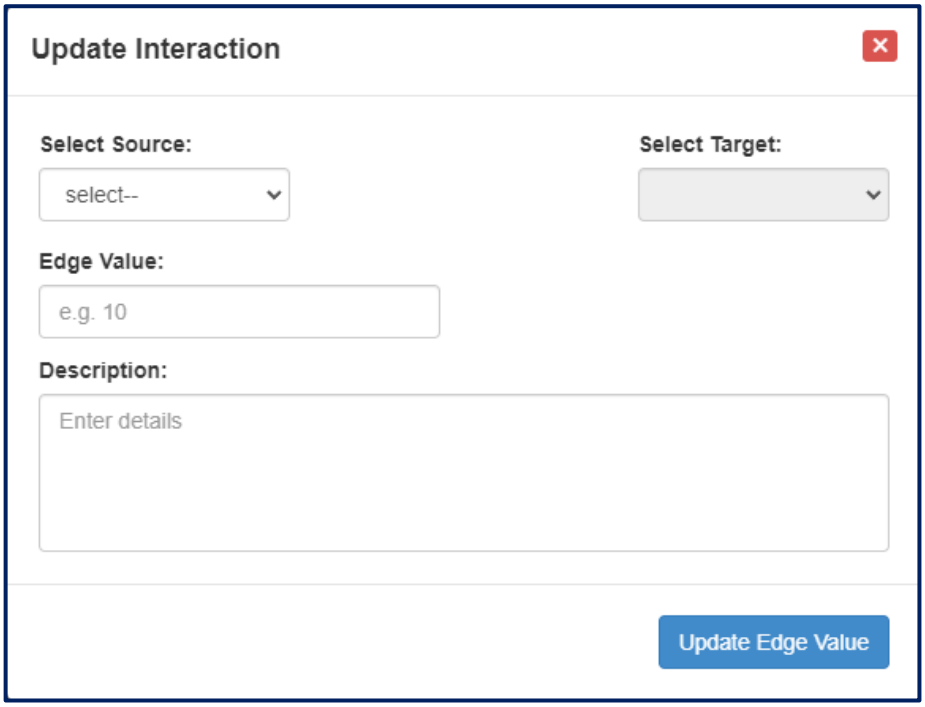


Figure 2.4.1.25 - 'Target Link' window for a weight-based network.

v. Edge Knock In

The user can also add a new edge interaction in the network by selecting the 'Edge Knock In' option as shown in **Figure 2.4.1.26.** The user must select the 'Source' and 'Target' node and assign an 'Edge Value' to the new interaction. Once 'Update Node' button is clicked, this 'Edge Knock In' will be shown in purple underneath the therapeutic step (**Figure 2.4.1.27**)

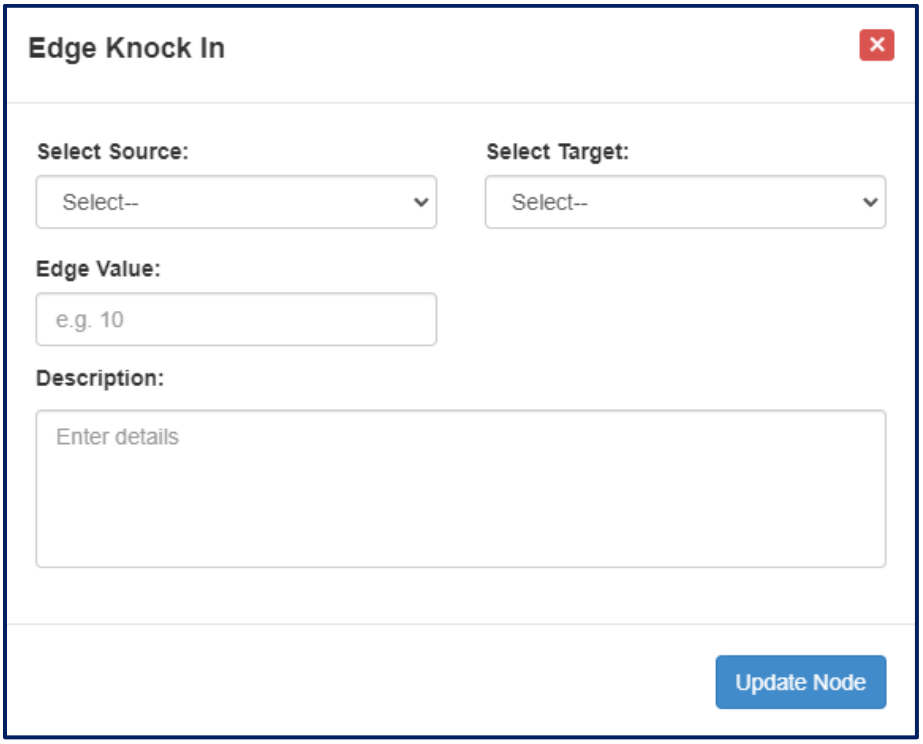


Figure 2.4.1.26 - 'Edge Knock In' window for a weight-based network.

All the modified nodes (‘Knock Up’, ‘Knock Down’, ‘Knock Out’,’ Knock In’, ‘Target Link’, ‘Edge Knock In’) along with their assigned values appear under the ‘New Node’ (**Figure 2.4.1.27**).

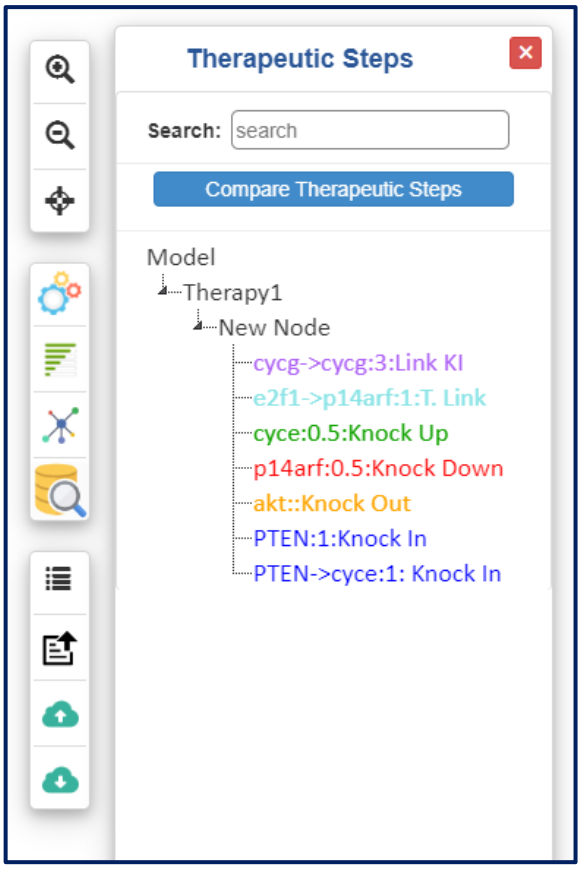
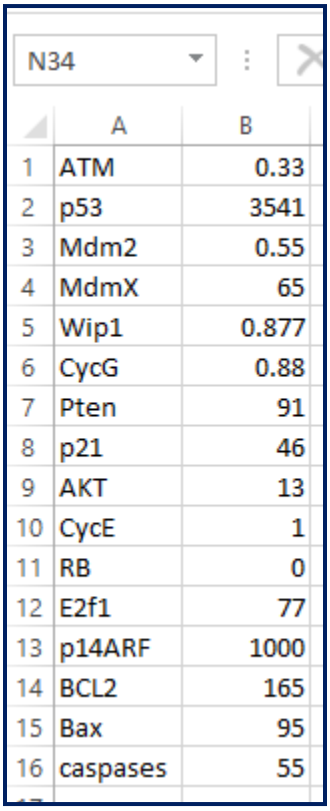


Figure 2.4.1.27 - Target nodes along with their assigned custom values appear color-coded under the therapeutic step in a weight-based therapy.

iv. Add/Remove Expression File

A weight-based network can also be personalized using expression files in TE. The option allows the user to customize the therapy by employing patient-specific expression data such as RNA-seq or microarray expression for calculating and updating the network's basal values. For that, the user must upload an expression file (format.csv) that contains raw or normalized RNA-seq and raw or normalized microarray expression values extracted from a database such as The Cancer Genome Atlas (TCGA) or Gene Expression Omnibus (GEO) (**Figure 2.4.1.28**). The gene expression file, either raw or normalized expression values, can be uploaded by selecting the 'Choose File' option, displayed in **Figure 2.4.1.29.**



N34

	A	B
1	ATM	0.33
2	p53	3541
3	Mdm2	0.55
4	MdmX	65
5	Wip1	0.877
6	CycG	0.88
7	Pten	91
8	p21	46
9	AKT	13
10	CycE	1
11	RB	0
12	E2f1	77
13	p14ARF	1000
14	BCL2	165
15	Bax	95
16	caspases	55

Figure 2.4.1.28 - Example expression file for basal value calculation.

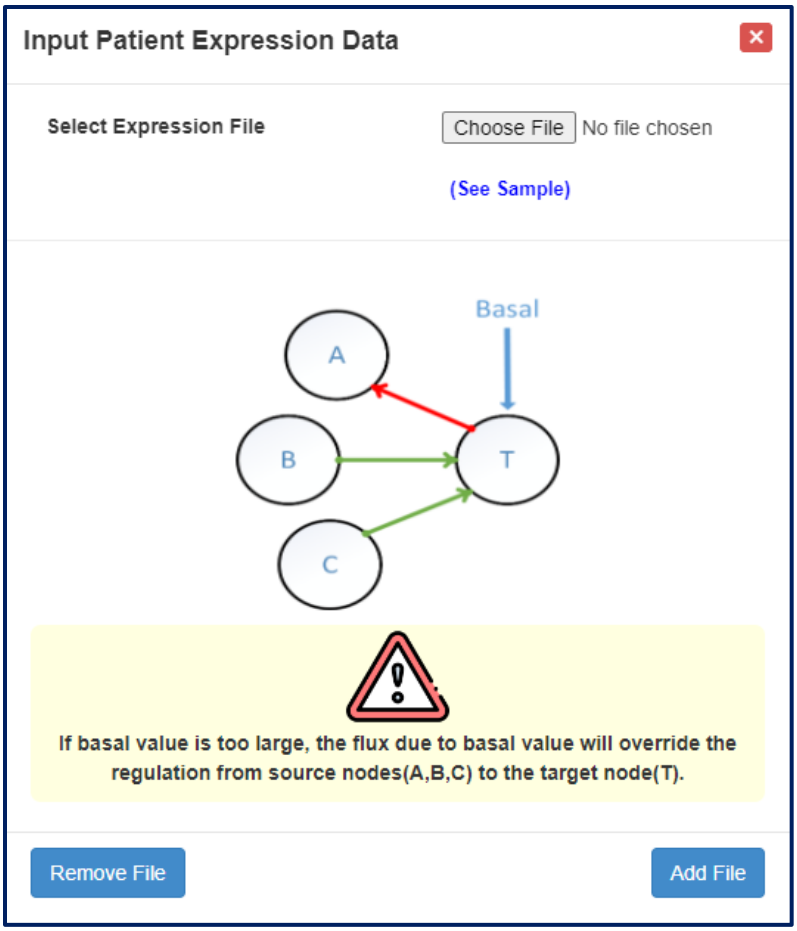


Figure 2.4.1.29 - Calculation Network personalization window for basal value estimation.

i. Basal Value Calculation: The b_i is the basal value of the node, x_j is the experimental value, and w_{ij} represents the network's adjacency matrix.

$$x_i(t+1) = \begin{cases} 1 & if \sum_j (w_{ij}x_j(t)) + b_i > 0 \\ 0 & else\ if \sum_j (w_{ij}x_j(t)) + b_i < 0 \\ x_i & else\ if \sum_j (w_{ij}x_j(t)) + b_i = 0 \end{cases}$$

$$b_i = x_i(t+1) - \sum_j \left(w_{ij}x_j(t)\right)$$

This feature allows users to employ expression data to calculate the basal expression of each node towards personalizing the resultant network with real patient data. This is exemplified in **Figure 2.4.1.30.**

$$b_i = \left((2.22 \times 0.65) + (0.56 \times 0.91) + (7.43 \times -0.81) + (4.61 \times 4.61)\right) - 3.65 = 13.5364$$

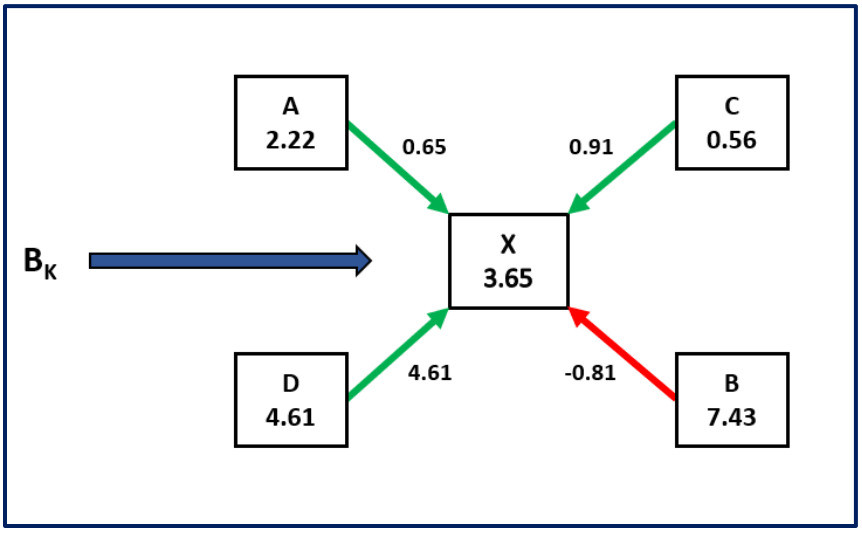


Figure 2.4.1.30 - Example of basal value estimation.

On selecting 'Update Network', the basal values of the network will be updated on the canvas as well.

ii. Expression Value Normalization (*recommended*): Please note that in the case of personalizing a network with expression data, it is recommended that users should not use the raw expression data. Instead, users must normalize the expression values for all genes between 0 and 1, e.g., for a particular gene, divide the expression values across all patients by the maximum expression value for that gene. Next, the basal values will be computed with respect to normalized gene expressions**.** The raw RNA-seq gene expression data and its normalized values are displayed in **Figure 2.4.1.31** and **Figure 2.4.1.32 .** respectively.

Network_Genes	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Max
ATM	1.510623	2.518765	0.908262	2.058787	1.316602	1.522188	1.622792	1.86019032	2.518765
Tp53	5.022743	7.702509	42.84711	32.09936	18.91071	59.49285	34.63199	7.22958871	59.49285
MDM2	4.552624	5.701428	5.859361	8.787163	4.90684	5.118764	3.646182	4.24858655	8.787163
CDKN1A	29.53484	60.16466	47.50169	60.80388	89.00106	44.90698	116.884	28.0266711	116.884

Figure 2.4.1.31 - Raw expression (.csv) file.

Network_Genes	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
ATM	0.599747	1	0.360598	0.817379	0.522717	0.604339	0.644281	0.73853272
Tp53	0.084426	0.129469	0.720206	0.53955	0.317865	1	0.58212	0.12152029
MDM2	0.518099	0.648836	0.666809	1	0.55841	0.582528	0.414944	0.48349926
CDKN1A	0.252685	0.514738	0.4064	0.520207	0.761448	0.384201	1	0.23978203

Figure 2.4.1.32 - Normalized expression (.csv) file.

Drug therapy construction includes two modes of targeted implementation.

1) **Horizontal Therapy**
2) **Vertical Therapy**

2.4.1.1 Horizontal Therapy:

In 'Horizontal Therapy', each drug can be programmed to target multiple nodes or node interactions concurrently, as shown in **Figure 2.4.1.1.1.** In this case, multiple node values or interactions can be altered within a single drug (therapeutic step). Each of these changes will be independently implemented upon a network for analysis, with no sequential overlay. After therapeutic analysis, two files will be generated: one for 'Model' and the other for the drug's result.

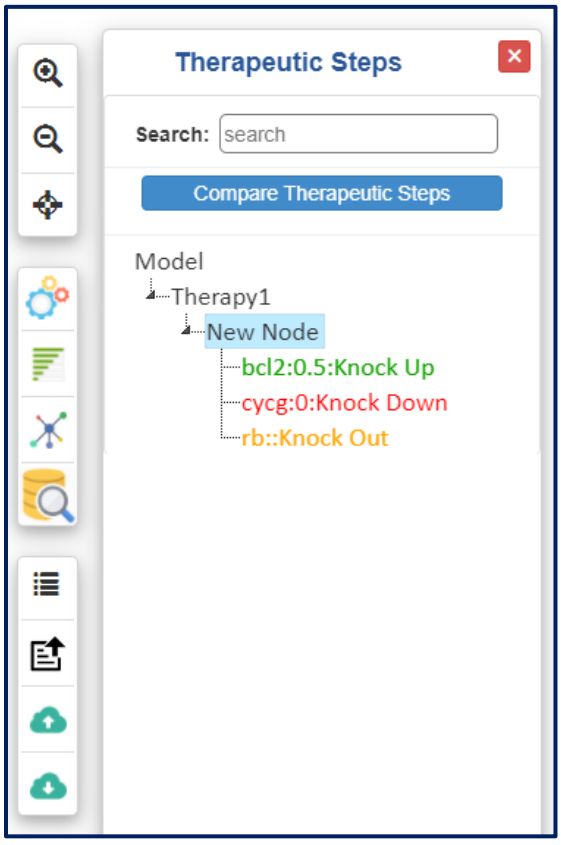


Figure 2.4.1.1.1 - Horizontal therapy construction.

2.4.1.2 Vertical Therapy:

In 'Vertical Therapy', multiple drugs with distinct node targets can be collated to act in tandem, as shown in **Figure 2.4.1.2.1.** The results for this mode of therapeutic analysis will contain the 'Model' and the cumulative effects of each drug upon the network. TE further offers a comparative analysis feature, which allows the user to visually compare the results of therapeutic evaluation between drugs and control cases within a therapy.

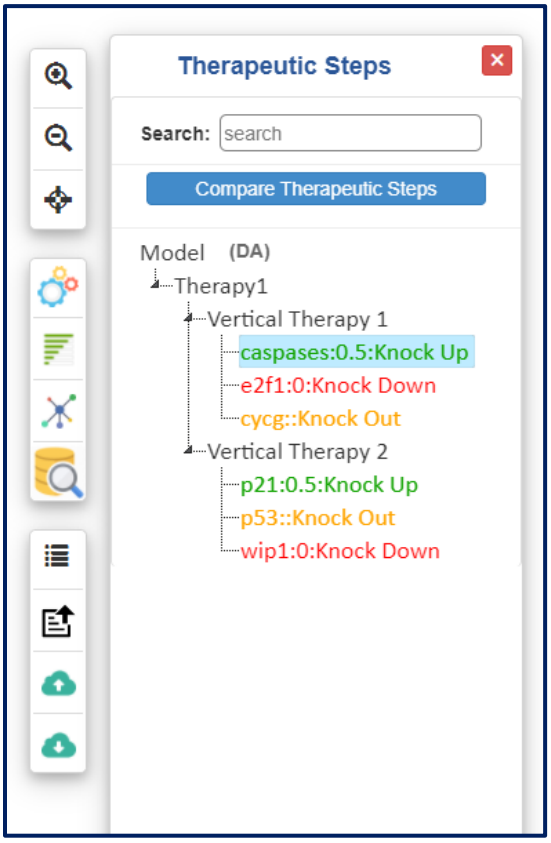


Figure 2.4.1.2.1 - Vertical therapy construction.

This mode of therapy involves the sequential implementation of the effect of each drug. In a multi-drug therapy, the network analysis results of the first drug are used as default node values (for a rules-based or a weight-based network) for the implementation of the effects of the second drug. The average node values from the results of the first drug are multiplied with the basin size ratio or probability of its attractor to generate its node value within the attractor. Summation of the node values from each attractor generate average node propensities, which are used as default node values for the proceeding network analysis to implement the effects of the second drug. This process is repeated for the sequential implementation of each drug within a therapy.

Right-clicking on the newly added target node will allow the user to *Delete* it. (**Figure 2.4.1.2.2**).

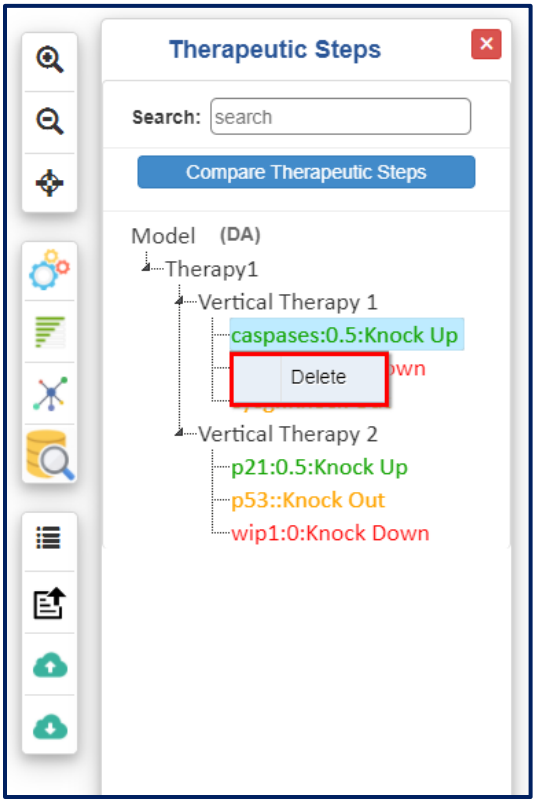


Figure 2.4.1.2.2 - 'Delete' a therapy option.

2.4.2 Viewing Results

'View Results' option (**Figure 2.4.2.1**) will allow the user to view the results in the form of an 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape' and 'Probability Landscape' after performing the network analysis for therapy.

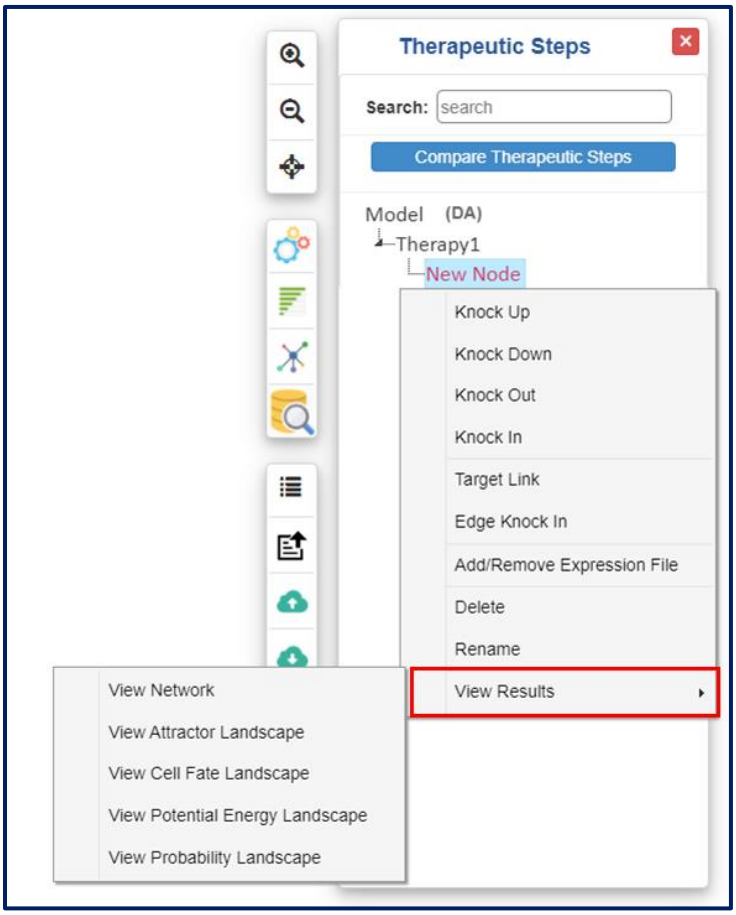


Figure 2.4.2.1 - 'View Results' options.

i. Attractor Landscape

An 'Attractor Landscape' can be viewed in **Figure 2.4.2.2.** The height of attractor peaks indicates the propensity of that particular network state.

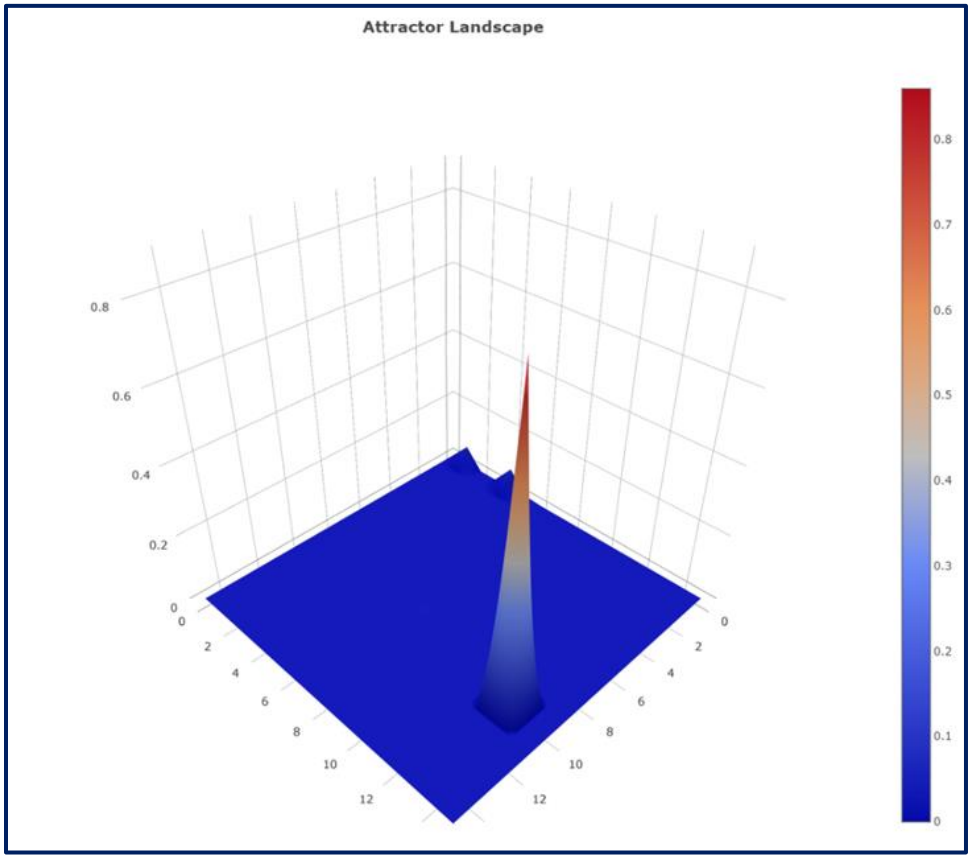


Figure 2.4.2.2 - Attractor Landscape.

ii. Cell Fate Landscape

If cell fate classification has been performed by uploading the relevant file, the user can also view their results in the form of a 'Cell Fate Landscape' as shown in **Figure 2.4.2.3.**

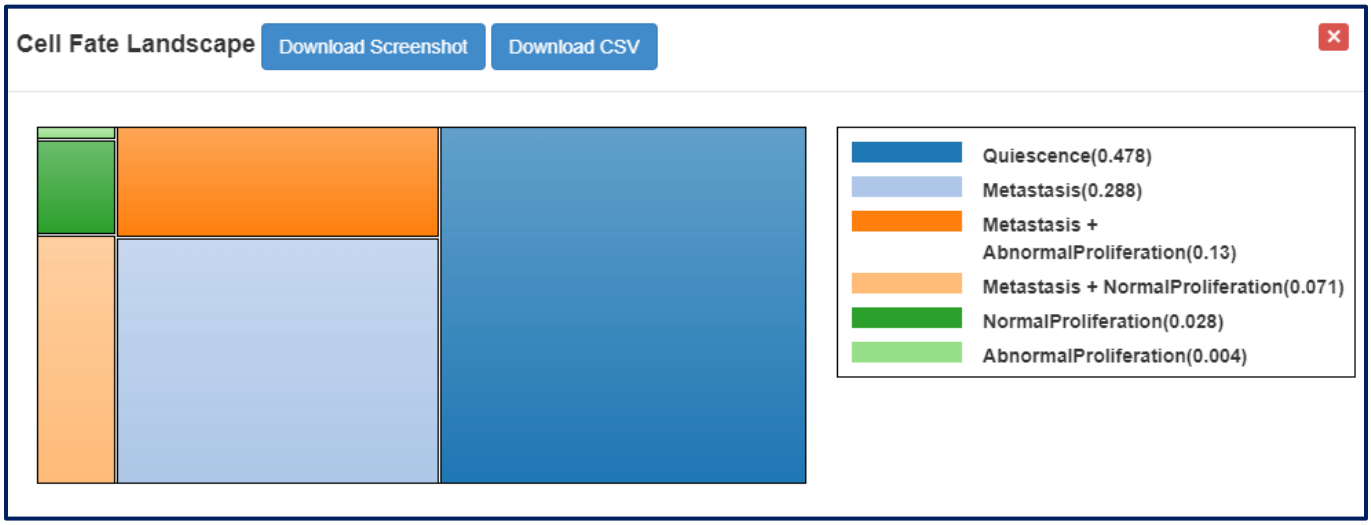


Figure 2.4.2.3 - Cell Fate Landscape.

iii. Potential Energy Landscape

After probabilistic analysis has been performed, the user can view their results in the form of a 'Potential Energy Landscape' where the peaks represent low energy/stable states. A potential energy landscape is shown in **Figure 2.4.2.4.**

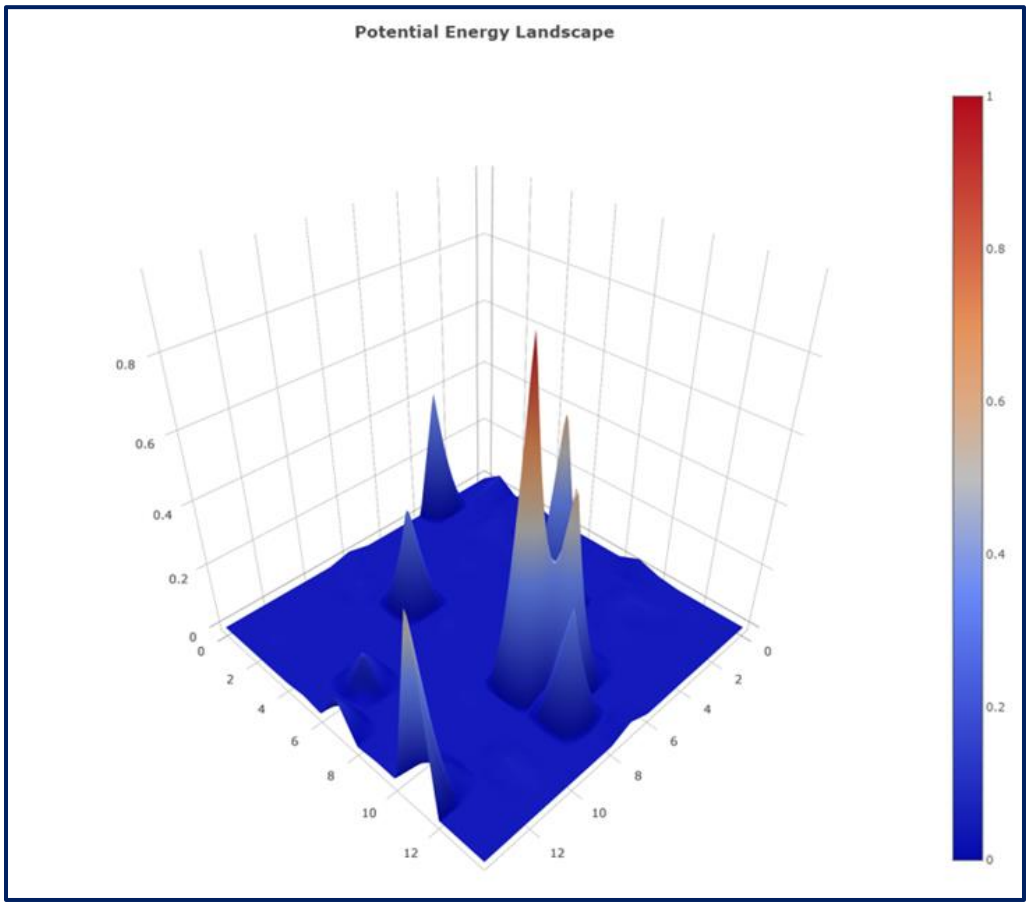


Figure 2.4.2.4 - Potential Energy Landscape.

iv. Probability Landscape

After probabilistic analysis has been performed, the user can view their results in the form of a 'Probability Landscape' where the peaks represent high probability states. A 'Probability Landscape' is shown in **Figure 2.4.2.5.**

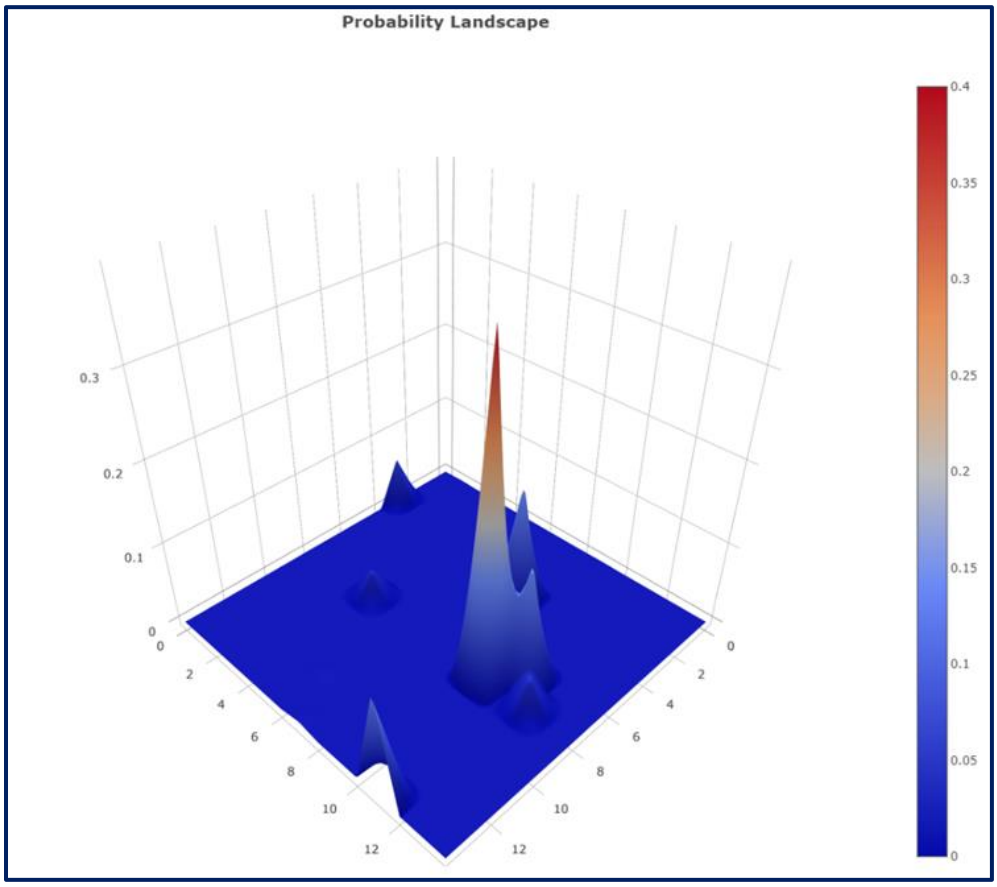


Figure 2.4.2.5 - Probability Landscape.

2.5 TISON's Therapeutics Editor Video Tutorials

Table 2.5.1 - Video tutorials for TE.

Video No.	Video Tutorial Name
1.	Upload Therapeutics Case Study from Editor and Perform Deterministic Analysis
2.	Uploading Network from File, Construct Therapy and Compare Pre and Post Results

2.6 Bibliography

1. Kim, Y., Choi, S., Shin, D. & Cho, K. H. Quantitative evaluation and reversion analysis of the attractor landscapes of an intracellular regulatory network for colorectal cancer. *BMC Syst. Biol.* **11**, 1–22 (2017).

2. Kauffman, S. A. Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.* **22**, 437–467 (1969).

3. Kauffman, S. A. Metabolic stability and epigenesis in randomly constructed genetic nets. *J. Theor. Biol.* **22**, 437–467 (1969).

4. Shmulevich, I., Dougherty, E. R., Kim, S. & Zhang, W. Probabilistic Boolean networks: a rule-based uncertainty model for gene regulatory networks. *Bioinformatics* **18**, 261–274 (2002).

5. Guo, J., Lin, F., Zhang, X., Tanavde, V. & Zheng, J. NetLand: Quantitative modeling and visualization of Waddington's epigenetic landscape using probabilistic potential. *Bioinformatics* **33**, 1583–1585 (2017).