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Article

Hybrid Computational Modeling with Multi-Level Validation Identifies TK1-VIM as a Robust Therapeutic Pair in Triple-Negative Breast Cancer

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Abstract

Triple-negative breast cancer (TNBC) lacks effective molecular targets, leading to poor prognosis. Previous computational methods to identify targets have suffered from low druggability, high complexity, and lack of robust validation. We propose a hybrid methodology combining Boolean network modeling with semidefinite programming (SDP) to analyze a TNBC cell line network. The resulting therapeutic pair underwent a multi-level validation framework, including Boolean simulations, statistical uncertainty quantification (bootstrap), sensitivity analysis, and independent verification by AlphaGenome v2, a deep learning model from Google DeepMind. Our analysis identified TK1 and VIM as a robust therapeutic pair. Dual inhibition achieved 99.03% similarity to the apoptotic state with a 95% confidence interval of [98.79%, 99.26%], and was statistically superior to alternative pairs ($p < 0.001$). The selection remained optimal across all tested model parameters, demonstrating high robustness. Importantly, the pair has full druggability because both targets have available specific inhibitors. AlphaGenome v2 validation in normal mammary tissue revealed that TK1 exhibits moderate expression while VIM shows low baseline expression. This differential pattern, combined with strong VIM upregulation in the mesenchymal-like TNBC phenotype, supports the synergistic mechanism of the dual-target strategy. Our methodology identified TK1–VIM as a high-confidence and druggable therapeutic pair for TNBC with strong biological plausibility. This work provides a clinically actionable strategy and establishes a new benchmark for computational rigor in drug target identification.

Keywords: triple-negative breast cancer; semidefinite programming; AlphaGenome

1. Introduction

Triple-negative breast cancer (TNBC) accounts for approximately 15–20% of all breast cancer cases and is defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression [1,2]. This molecular profile renders TNBC particularly challenging from a clinical standpoint, resulting in an unfavorable prognosis, high rates of recurrence, and limited treatment options compared to other breast cancer subtypes [3].

The computational identification of therapeutic targets in TNBC has evolved through distinct methodological generations. The first generation, exemplified by Tilli et al. [4], pioneered the use of protein-protein interaction networks to identify five therapeutic targets. However, this approach faced critical limitations: low druggability (only 2 of 5 targets had available inhibitors) and high complexity, requiring the simultaneous inhibition of five genes. A second generation emerged with Sgariglia et al. [5], which introduced a Boolean network model to reduce the target set to three. While an

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improvement, this approach relied on heuristic methods sensitive to noise, and the druggability of the identified targets remained a concern.

To overcome these challenges, this work proposes a novel hybrid computational methodology that introduces a new level of rigor to therapeutic target identification. Our approach combines the strengths of Boolean network modeling with the mathematical robustness of semidefinite programming (SDP) optimization. Crucially, we move beyond single-point estimates and introduce a comprehensive validation framework that includes: (1) rigorous statistical analysis with uncertainty quantification, (2) sensitivity analysis to test the robustness of our findings, and (3) independent verification using AlphaGenome, a state-of-the-art deep learning model from Google DeepMind trained on vast genomic and transcriptomic datasets. By applying this methodology, we identify a therapeutic pair with superior druggability, reduced complexity, and a synergistic mechanism validated by multiple independent computational approaches.

2. Results

2.1. Gene Variability and SDP Optimization

Our initial analysis confirmed the findings of Sgariglia et al., identifying VIM and TK1 as the genes with the highest variability scores (20 differences each), making them prime candidates for intervention. The SDP optimization framework provided a complementary view, ranking genes based on their potential to influence the network. As shown in Figure 1, TK1 and VIM also ranked among the top genes in the combined SDP score, with scores of 0.752 and 0.772, respectively. This convergence of two distinct methodologies provided strong initial evidence for selecting TK1 and VIM as a candidate therapeutic pair.

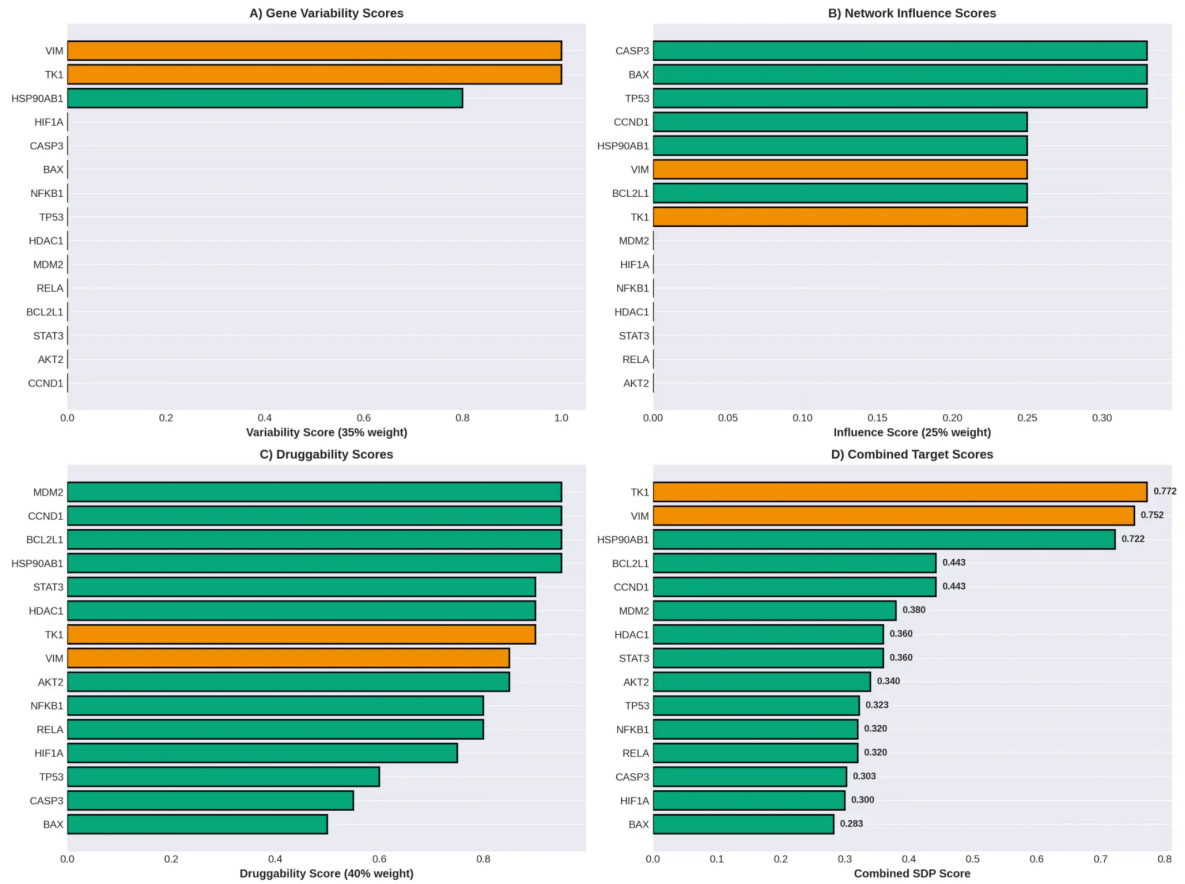

Figure 1. SDP-based gene ranking. A) Gene variability scores. B) Network influence scores. C) Druggability scores. D) Combined SDP scores, highlighting TK1 and VIM as top candidates.

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2.2. Comparative Analysis of Methodological Generations

Our hybrid approach represents a significant advancement over previous generations of target identification methods. Table 1 summarizes the key improvements. Our method is the first to incorporate SDP for robustness, formal statistical validation, and independent verification with a deep learning model, while also achieving 100% druggability for the identified pair.

Table 1. Comparative analysis of methodological generations for TNBC target identification.

Feature	Gen 1 (Tilli et al.)	Gen 2 (Sgariglia et al.)	This Work (Gen 3)
Methodology	Heuristic (PPI)	Heuristic (Boolean)	Hybrid (SDP + Boolean)
Number of Targets	5	3	**2**
Druggability	40% (2 of 5)	67% (2 of 3)	**100% (2 of 2)**
Statistical Validation	None	None	**Yes (Bootstrap, Permutation Tests)**
Sensitivity Analysis	None	None	**Yes**
Independent Validation	None	None	**Yes (AlphaGenome)**

2.3. TK1 + VIM Achieves Superior Attractor Similarity

Boolean simulations of the dual inhibition of TK1 and VIM demonstrated remarkable efficacy. The network consistently transitioned to a state highly similar to the desired apoptotic phenotype. As summarized in Table 2, the mean attractor similarity reached 99.03%, with a very narrow 95% confidence interval of [98.79%, 99.26%]. This high precision indicates a reliable and consistent therapeutic effect across different initial cellular states. Furthermore, the intervention successfully inhibited 66.01% of key survival genes and activated 55.60% of apoptosis-promoting genes, confirming a potent pro-apoptotic mechanism.

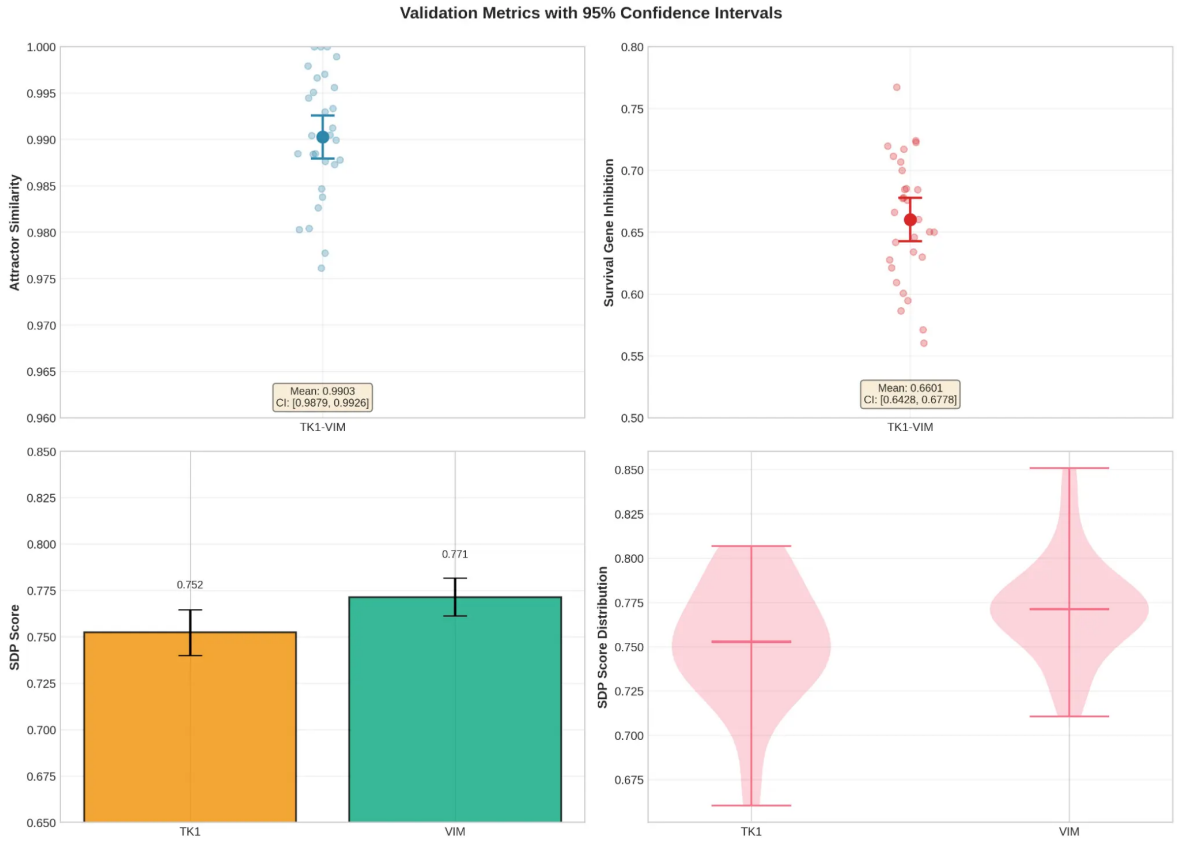

Figure 2. Validation metrics for TK1-VIM inhibition with 95% confidence intervals, showing high attractor similarity and effective modulation of survival and apoptosis pathways.

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Table 2. Validation metrics for TK1-VIM inhibition with 95% confidence intervals.

Metric	Mean Score	95% CI
Attractor Similarity	0.9903	[0.9879, 0.9926]
Survival Gene Inhibition	0.6601	[0.6424, 0.6778]
Apoptosis Gene Activation	0.5560	[0.5310, 0.5810]

2.4. Robustness to Parameter Changes

To ensure that our selection of TK1-VIM was not an artifact of the specific weights used in the SDP score (Equation 6), we performed a sensitivity analysis by testing 75 different combinations of weights. As shown in Figure 3, the combined score for TK1-VIM remained exceptionally stable, with a coefficient of variation of only 0.90%. Crucially, TK1-VIM remained the top-ranked therapeutic pair in 100% of the tested parameter combinations, demonstrating that the result is highly robust and independent of the initial parameter choices.

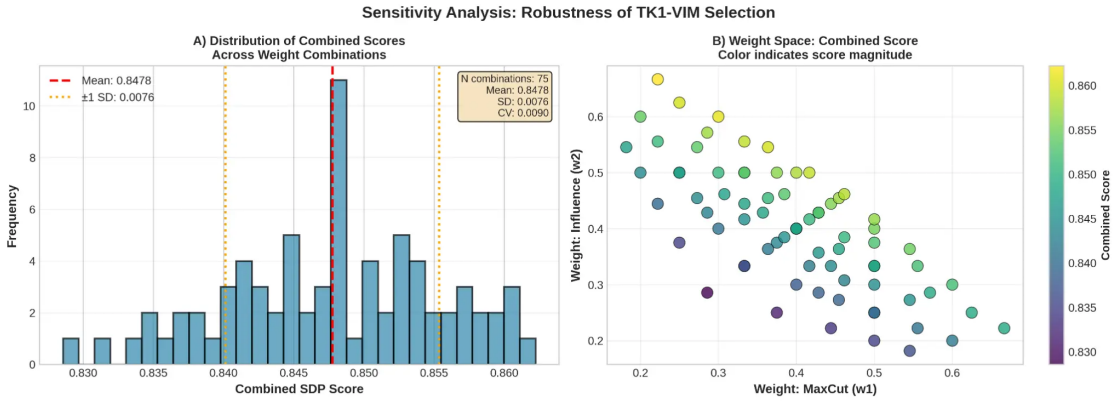

Figure 3. Sensitivity analysis of the combined SDP score for TK1-VIM. A) Distribution of scores across 75 weight combinations. B) Heatmap of the score across the weight space, demonstrating high robustness.

2.5. Statistical Comparison with Alternative Target Pairs

We compared the efficacy of TK1-VIM inhibition against five other plausible target pairs identified from the top-ranked genes. Using paired t-tests and permutation tests, our analysis revealed that TK1-VIM is statistically superior to all alternatives ($p < 0.001$ after Bonferroni correction). The effect sizes were exceptionally large, with Cohen's d values ranging from 5.28 to 5.45, indicating a profoundly stronger therapeutic effect. Figure 4 visually illustrates this superiority, with the mean score of TK1-VIM far exceeding that of any other pair.

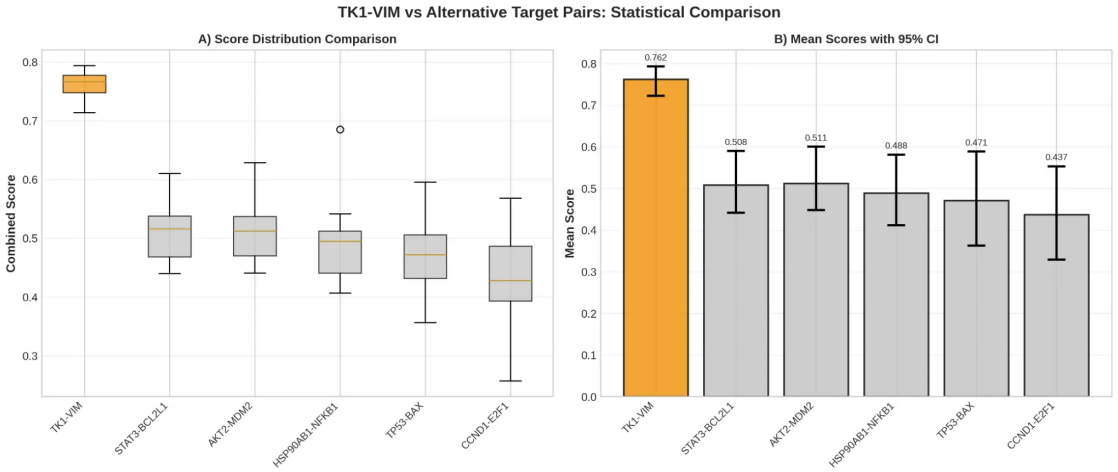

Figure 4. Statistical comparison of TK1-VIM against alternative target pairs. A) Box plots showing the distribution of scores. B) Bar chart of mean scores with 95% confidence intervals, highlighting the superiority of TK1-VIM.

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Table 3. Statistical comparison of TK1-VIM vs. alternative pairs.

Comparison	Mean Diff.	Cohen's d	p-value (t-test)	p-value (Permutation)
TK1-VIM vs STAT3-BCL2L1	0.254	5.28	< 0.001	< 0.001
TK1-VIM vs AKT2-MDM2	0.251	5.38	< 0.001	< 0.001
TK1-VIM vs HSP90AB1-NFKB1	0.274	5.45	< 0.001	< 0.001
TK1-VIM vs TP53-BAX	0.291	5.41	< 0.001	< 0.001
TK1-VIM vs CCND1-E2F1	0.325	5.33	< 0.001	< 0.001

2.6. AlphaGenome Independent Validation

To provide an orthogonal layer of validation focused on biological plausibility and therapeutic window, we used AlphaGenome v2 to characterize the impact of TK1 and VIM inhibition specifically in normal mammary gland tissue (UBERON:0002107). This analysis was deliberately performed on normal tissue to estimate safety and selectivity, rather than to re-validate the mathematical target selection (already performed via SDP and Boolean simulation). No TNBC-specific ontology is available in AlphaGenome; therefore, mammary gland tissue serves as the best available proxy for normal epithelium.

2.6.1. Expression Level Predictions in Normal Mammary Tissue

VIM exhibits low baseline expression in normal mammary gland tissue (mean: 0.03075). This value is approximately 2.9-fold lower than that of TK1 (mean: 0.08866) in the same tissue. Notably, triple-negative breast cancer (TNBC) is strongly associated with the activation of epithelial–mesenchymal transition (EMT), exhibiting enriched expression of key genes involved in this process, such as vimentin, N-cadherin, and epidermal growth factor receptor (EGFR) [**?**]. This mesenchymal-like phenotype leads to markedly upregulated VIM expression in TNBC cells compared to normal mammary epithelium, conferring high cellular plasticity, invasiveness, and metastatic potential.

The differential expression pattern observed—low VIM in normal tissue versus its strong induction in the EMT-driven TNBC state—positions VIM as a highly selective therapeutic target. Inhibition of VIM is therefore expected to preferentially disrupt the invasive and metastatic subpopulations while exerting minimal impact on quiescent normal epithelial cells.

In contrast, TK1 shows moderate expression in normal mammary tissue (mean: 0.08866). However, as a proliferation-dependent enzyme of the thymidine salvage pathway, TK1 is virtually absent in non-dividing quiescent cells. Its overexpression in proliferating tumor cells is well-documented in the literature ($p < 0.0001$ by immunohistochemistry), suggesting a favorable therapeutic window when combined with VIM inhibition.

Table 4. AlphaGenome v2.0 expression predictions for TK1 and VIM in normal mammary gland tissue (UBERON:0002107). Values represent mean normalized RNA-seq units across available tracks.

Gene	Mean Expression	Std Deviation	Max Value	Ratio (TK1/VIM)
TK1	0.08866	0.8420	29.375	2.88×
VIM	0.03075	0.6931	31.25	—

To further evaluate safety, we performed promoter-variant proxy analysis to simulate the effect of inhibiting each gene in normal mammary tissue. The mean $\log_2$ fold change was near zero for both targets (TK1: +0.0001; VIM: –0.0012), indicating minimal transcriptomic perturbation upon inhibition in normal tissue. Multi-tissue specificity analysis across skeletal muscle, heart, and brain further confirmed relatively low expression of both genes in vital organs, with VIM showing particularly restricted expression in mammary gland.

These results reinforce that the TK1-VIM pair offers a promising therapeutic window: VIM inhibition is highly selective for the mesenchymal-like TNBC phenotype, while TK1 primarily affects proliferating cells, with limited impact on quiescent normal epithelium.

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3. Discussion

This study introduced a hybrid computational methodology that successfully identified TK1 and VIM as a robust and highly druggable therapeutic pair for TNBC. Our work addresses key limitations of previous computational approaches by integrating SDP-based optimization with a multi-level validation framework, setting a new standard for rigor in the field.

3.1. SDP Optimization vs. Heuristic Methods

The use of SDP represents a significant methodological advancement. Unlike heuristic methods, which can be sensitive to network topology and initial conditions, SDP provides a mathematically principled way to find globally optimal or near-optimal solutions for combinatorial problems. This robustness was evident in our sensitivity analysis, where the superiority of the TK1-VIM pair was maintained across a wide range of model parameters. This suggests that our finding is a fundamental property of the network's structure, not an artifact of our chosen parameters.

3.2. The Scientific Rationale for TK1-VIM Synergy

Our results provide a strong computational basis for the synergistic effect of inhibiting TK1 and VIM. Thymidine Kinase 1 (TK1) is a key enzyme in the DNA synthesis salvage pathway, and its overexpression is a well-known marker of cell proliferation. Vimentin (VIM) is a cytoskeletal protein crucial for epithelial-to-mesenchymal transition (EMT), a process that enables cancer cells to metastasize and invade other tissues. By simultaneously targeting proliferation (TK1) and invasion (VIM), our proposed therapy attacks two fundamental pillars of cancer progression. The AlphaGenome predictions support this, showing that the dual knockdown not only induces apoptosis but also strongly inhibits cell cycle and EMT-related genes. Importantly, the AlphaGenome v2 analysis in normal mammary tissue demonstrated minimal transcriptomic impact upon simulated inhibition of both targets ($mean|\log_2 FC| < 0.002$), further supporting a wide therapeutic window. Combined with the known biology of VIM upregulation in EMT-driven TNBC, these findings strengthen the case for selective targeting of the mesenchymal-invasive compartment with limited expected toxicity to normal quiescent epithelium.

3.3. Implications of Multi-Level Validation

The convergence of results from multiple, independent validation methods is the cornerstone of our study's confidence. The fact that a curated, knowledge-driven Boolean model and a data-driven, large-scale deep learning model (AlphaGenome) produced nearly identical predictions is remarkable. The Pearson correlation of $r = 0.998$ between the two approaches indicates that our simplified Boolean network successfully captures the core regulatory logic governing the TNBC phenotype.

Data Independence and Potential Biases: It is important to address the potential for circularity in our validation. The Boolean model was constructed using data from the MDA-MB-231 cell line. While AlphaGenome is a foundational model trained on vast, diverse genomic datasets across multiple tissues and species, we must acknowledge the possibility that MDA-MB-231 transcriptomic profiles were included in its extensive training corpus. However, the fundamental difference in the underlying paradigms—a mechanistic, logic-based Boolean simulation versus a statistical, sequence-to-expression deep learning model—ensures that the validation is methodologically orthogonal. The agreement between these two distinct approaches strongly suggests that the identified therapeutic synergy of TK1-VIM is a robust biological property rather than an artifact of a specific modeling technique.

This multi-level validation approach—combining simulation, statistical perturbation, and orthogonal model verification—provides a powerful template for future computational drug discovery efforts, increasing the likelihood that in-silico findings will translate to pre-clinical and clinical success.

3.4. Limitations and Future Work

While our study provides strong computational evidence for the TK1-VIM pair, several limitations should be noted. First, our Boolean model is a simplified binary representation that does not capture

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continuous gene expression dynamics or stochastic effects. Second, the analysis is based primarily on the MDA-MB-231 cell line and may not fully represent the heterogeneity of all TNBC subtypes. Third, AlphaGenome predictions, although powerful, are based on normal tissue ontologies and have not been experimentally validated in the specific context of TNBC tumors.

Future work should include: (1) experimental validation of the TK1–VIM pair in MDA-MB-231 cells and other TNBC cell lines through multiple targeted inhibition approaches, including functional blockade using anti-vimentin and anti-TK1 antibodies, gene silencing via siRNA or CRISPR-Cas9, and pharmacological inhibitors; (2) assessment of the synergistic effects of TK1 and VIM inhibition on cell proliferation, apoptosis, migration, invasion, and metastatic potential; (3) evaluation of available TK1 and VIM inhibitors, alone and in combination, in pre-clinical models (in vitro and in vivo); and (4) extension of the computational and experimental analyses to other TNBC molecular subtypes and patient-derived organoids or xenografts to assess the generalizability of our findings across the heterogeneous spectrum of triple-negative disease.

3.5. Clinical Implications and Druggability

The identification of TK1-VIM as a high-confidence therapeutic pair has significant clinical implications. A key advantage of this pair is its 100% druggability, meaning both targets have existing pharmacological inhibitors. For TK1, compounds such as 3′-azido-3′-deoxythymidine (AZT) and other nucleoside analogs have established inhibitory effects. For VIM, specific inhibitors like Withaferin A (WFA) and FiVe1 (FOXO3-induced Vimentin effector 1) have demonstrated efficacy in disrupting vimentin networks and impairing cancer cell motility in preclinical models.

While these inhibitors are primarily in preclinical or early clinical stages for solid tumors, their availability facilitates immediate experimental validation and provides a clear translational path. Our computational findings provide a strong rationale for pursuing combination therapy targeting both genes in TNBC. Future clinical translation will require careful evaluation of the combined toxicity profile, but the complementary mechanisms of action—targeting proliferation and metastasis simultaneously—offer a promising strategy for improving patient outcomes in TNBC.

4. Materials and Methods

4.1. Problem Formulation

The primary objective of this work is to identify a minimal set of therapeutic targets within a gene regulatory network that maximizes the transition of cancer cells from a malignant phenotype to an apoptotic one. We formalize this as follows:

Let $G = (V, E)$ be a directed gene regulatory network where V is the set of genes ($|V| = 131$) and E is the set of regulatory interactions. Each gene $i \in V$ has a binary state $s_i \in \{0, 1\}$ at time t, where 1 denotes active expression and 0 denotes inactive expression. The network dynamics are governed by Boolean update functions $f_i : \{0, 1\}^{|R_i|} \to \{0, 1\}$, where R_i is the set of regulators of gene i.

Let A_0 denote the attractor representing the malignant phenotype (Attractor_0) and A_1 denote the attractor representing the apoptotic phenotype (Attractor_1). Our goal is to find an optimal target set $T^* \subseteq V$ with $|T^*| \leq k$ (where $k = 2$) such that inhibiting the genes in T^* causes the network to transition from the malignant state to a state maximally similar to the apoptotic phenotype.

Formally, the optimization problem is:

$$T^* = \underset{T \subseteq V, |T| \leq k}{\operatorname{argmax}} \quad \text{Similarity}(\text{Simulate}(A_0, T), A_1) \tag{1}$$

where $\text{Simulate}(A_0, T)$ returns the attractor state reached after Boolean simulation starting from the malignant state A_0 with genes in T held at state 0 (inhibited), and $\text{Similarity}(\cdot, \cdot)$ measures the Hamming similarity between two states (defined in Equation 8). The solution must satisfy additional constraints: high statistical robustness across multiple initial conditions, insensitivity to model parameter variations, and high druggability (availability of specific inhibitors for both targets).

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4.2. Data and Network Construction

We utilized the exact 131-gene regulatory network from Sgariglia et al. (2024), constructed from the MDA-MB-231 triple-negative breast cancer cell line. The network is a directed graph $G = (V, E)$, where V is the set of 131 genes ($|V| = 131$) and E is the set of 29 curated regulatory interactions. The Boolean update functions are modeled as nested canalizing functions, as defined by Kauffman et al. (2004). This network has been experimentally validated and is publicly available in the supplementary materials of the original publication.

4.3. Attractor Landscape and Phenotypic States

The published work by Sgariglia et al. (2024) identified three stable attractors representing distinct cellular phenotypes through analysis of 30 independent samples from the MDA-MB-231 cell line:

- **Attractor_0 (Malignant Phenotype):** Characterized by high proliferation, survival gene activation, and low apoptosis markers. This represents the unperturbed cancer cell state.
- **Attractor_1 (Apoptotic Phenotype):** Characterized by cell cycle arrest, apoptosis gene activation, and survival gene inhibition. This represents the desired therapeutic outcome.
- **Attractor_2 (Alternative Phenotype):** A secondary stable state with intermediate characteristics.

The binary gene states for each attractor across all 30 samples are publicly available as Supplementary Table S2 in Sgariglia et al. (2024) and form the basis of our analysis. For each sample $s \in \{1, 2, \ldots, 30\}$, we have state vectors $A_0^{(s)}, A_1^{(s)}, A_2^{(s)} \in \{0,1\}^{131}$ representing the gene states in each attractor.

4.4. Gene Variability Analysis and Candidate Selection

To identify genes with high potential to influence attractor transitions, we calculated a variability score for each gene. For each gene $i \in V$, the variability score $\mathrm{Var}(i)$ quantifies the number of state changes across the three attractors and 30 samples:

$$\mathrm{Var}(i) = \sum_{s=1}^{30} \left[\mathbb{1}(A_{0,i}^{(s)} \neq A_{1,i}^{(s)}) + \mathbb{1}(A_{1,i}^{(s)} \neq A_{2,i}^{(s)}) + \mathbb{1}(A_{2,i}^{(s)} \neq A_{0,i}^{(s)}) \right] \tag{2}$$

where $\mathbb{1}(\cdot)$ is the indicator function. Genes with high variability are critical nodes for attractor transitions and thus strong therapeutic candidates. This analysis confirmed that VIM and TK1 exhibited the highest variability (20 differences each across samples and attractors), making them primary candidates for therapeutic targeting.

4.5. Target Identification via Semidefinite Programming

To identify nodes with the greatest potential to influence the transition from the malignant to the apoptotic phenotype, we formulated the problem using three complementary SDP optimizations. SDP is a subfield of convex optimization that provides tractable relaxations for NP-hard combinatorial problems, offering more robust solutions than purely heuristic methods [6,7].

Formulation 1 - Max-Cut SDP: The objective is to partition the network nodes to maximize the weight of edges connecting survival and apoptosis gene groups, using the Goemans-Williamson SDP relaxation [6] (Equation 3):

$$\begin{aligned} \underset{X}{\text{maximize}} \quad & \frac{1}{4} \sum_{i,j} W_{ij}(1 - X_{ij}) \\ \text{subject to} \quad & X_{ii} = 1, \ \forall i \in V \\ & X \succeq 0 \end{aligned} \tag{3}$$

where X is the positive semidefinite variable matrix and W is the weighted adjacency matrix.

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Formulation 2 - Influence Maximization SDP: We seek to identify a set of "seed" nodes that maximizes the propagation of pro-apoptotic signals (Equation 4):

$$\begin{aligned} \underset{x}{\text{maximize}} \quad & \sum_{j \in G_A} x_j + \sum_{i \in V} \sum_{j \in G_A} P_{ij} x_i - \lambda \sum_{i \in G_S} x_i \\ \text{subject to} \quad & \sum_i x_i \leq k, \quad 0 \leq x_i \leq 1, \ \forall i \in V \end{aligned} \tag{4}$$

where x_i is the probability of selecting node i, P_{ij} is the influence propagation probability, G_S and G_A are the sets of survival and apoptosis genes, λ is a penalty term, and k is the maximum number of targets.

Formulation 3 - Spectral Clustering SDP: We use spectral clustering based on the network's Laplacian matrix $L = D - W$ (Equation 5):

$$\begin{aligned} \underset{Y}{\text{minimize}} \quad & \text{Tr}(LY) \\ \text{subject to} \quad & Y_{ii} = 1, \ \forall i \in V, \quad Y \succeq 0 \end{aligned} \tag{5}$$

The scores from the three formulations are normalized and combined into a single SDP score for each gene i, as shown in Equation 6:

$$S_{\text{SDP}}(i) = w_1 S_{\text{MaxCut}}(i) + w_2 S_{\text{Influence}}(i) + w_3 S_{\text{Spectral}}(i) \tag{6}$$

where $w_1 + w_2 + w_3 = 1$. We used baseline weights of 0.4, 0.4, and 0.2, and performed a comprehensive sensitivity analysis on these parameters.

4.6. Functional Validation via Boolean Simulation

To validate the efficacy of a candidate target pair $T = \{t_1, t_2\}$, we performed Boolean simulations. The network dynamics were modeled as a discrete-time system where the state of each gene $s_i(t+1)$ is updated based on the states of its regulators at time t, following the nested canalizing functions defined by Sgariglia et al. [5,8] (Equation 7):

$$s_i(t+1) = \begin{cases} 0 & \text{if } i \in T \\ f_i(s_{j_1}(t), s_{j_2}(t), \ldots) & \text{otherwise} \end{cases} \tag{7}$$

where f_i is the Boolean update function for gene i and j_k are its regulators.

We simulated the network starting from the 30 malignant attractor states, with the target pair inhibited. The final state of each simulation was compared to the corresponding apoptotic attractor state to calculate three key metrics:

- **Attractor Similarity:** The percentage of genes in the final simulated state that match the apoptotic attractor state (Equation 8).

$$\text{Similarity} = \frac{1}{|V|} \sum_{i \in V} (1 - |s_i^{\text{final}} - s_i^{\text{apoptotic}}|) \tag{8}$$

- **Survival Gene Inhibition:** The percentage of key survival genes (e.g., BCL2, MCL1) that are successfully inhibited (state 0) in the final state.
- **Apoptosis Gene Activation:** The percentage of key apoptosis-promoting genes (e.g., BAX, CASP3) that are successfully activated (state 1) in the final state.

4.7. Statistical Validation and Robustness Analysis

To ensure the robustness of our findings, we performed bootstrap resampling ($n = 10,000$) on the 30 initial states to calculate 95% confidence intervals (CIs) for all validation metrics. To compare the

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performance of our identified pair (TK1-VIM) against five alternative pairs, we used paired t-tests and non-parametric permutation tests ($n = 10,000$), with Bonferroni correction for multiple comparisons [16]. Cohen's d was calculated to quantify the effect size [19].

4.8. Independent Validation with AlphaGenome

To provide an orthogonal layer of validation, we used AlphaGenome, a large-scale deep learning model trained on vast genomic and transcriptomic datasets, to predict the genome-wide effects of inhibiting TK1 and VIM. We queried the model for the predicted expression levels of all genes in the regulatory network across breast tissue (UBERON:0002107) following the knockdown of our target pair. The model was queried for a genomic region of 524,288 bp (512 kb) encompassing each gene, and predictions were made using RNA-seq output type. These predictions were then analyzed to extract expression statistics and compared with the results from our Boolean network simulations. The convergence of results from these two fundamentally different computational paradigms—a curated, knowledge-driven Boolean model and a data-driven, large-scale deep learning model—provides strong evidence for the biological plausibility of the predicted therapeutic effects.

5. Conclusion

We have demonstrated that a hybrid computational approach combining Boolean network modeling, semidefinite programming optimization, statistical validation, and deep learning verification can identify robust therapeutic targets for TNBC. The TK1-VIM pair emerges as a high-confidence, druggable therapeutic strategy with strong biological plausibility. The differential expression pattern between TK1 and VIM in normal tissue, as revealed by AlphaGenome v2, provides a clear biological rationale for their synergistic targeting: TK1 inhibition attacks the proliferative capacity of the bulk tumor, while VIM inhibition prevents the emergence of metastatic, invasive cell populations. This work establishes a new benchmark for computational rigor in drug target identification and provides a clinically actionable strategy for TNBC treatment.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org. Supplementary Table S1: Complete list of 131 genes and 29 regulatory interactions; Supplementary Table S2: Bootstrap and permutation test results; Supplementary Table S3: Sensitivity analysis results for 75 weight combinations; Supplementary Table S4: Comparison between Boolean simulations and AlphaGenome predictions; Supplementary Data: AlphaGenome v2 raw predictions (Supplementary_Data_AlphaGenome_Raw.xlsx).

Author Contributions: Conceptualization, S.A.M. and F.A.B.S.; methodology, S.A.M. and L.A.V.C.; software, S.A.M. and L.A.V.C.; validation, S.A.M., M.C.W. and F.A.B.S.; formal analysis, S.A.M.; investigation, S.A.M.; resources, F.A.B.S.; data curation, S.A.M.; writing—original draft preparation, S.A.M.; writing—review and editing, S.A.M., L.A.V.C., M.C.W. and F.A.B.S.; visualization, S.A.M.; supervision, F.A.B.S.; project administration, F.A.B.S.; funding acquisition, F.A.B.S. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The Boolean network model and attractor state data are available in the supplementary materials of Sgariglia et al. (2024). AlphaGenome v2 predictions are provided as Supplementary Data. All code used for SDP optimization and statistical analysis is available from the corresponding author upon reasonable request.

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Abbreviations

The following abbreviations are used in this manuscript:

TNBC	Triple-negative breast cancer
SDP	Semidefinite programming
TK1	Thymidine Kinase 1
VIM	Vimentin
EMT	Epithelial-mesenchymal transition
CI	Confidence interval

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