# Targeting TRAF3IP2, A Breakthrough Cancer Therapy

## Cancer

Cancer is the second leading cause of death worldwide, and 10 million deaths in 2020 were attributed to cancer. According to the Global Cancer Statistics 2020, an estimated 19.3 million new cancer cases (18.1 million excluding nonmelanoma skin cancer) and almost 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) occurred worldwide in 2020[9]. The most common types of cancer in terms of new cases in 2020 were breast, lung, colon and rectum, prostate, skin (non-melanoma), and stomach cancer. Globally, 18,094,716 million cases of cancer were diagnosed in 2020, and the age-standardized rate for all cancers (excluding non-melanoma skin cancer) for men and women combined was 190 per 100,000 in 2020.

## Cancer Treatment Obstacles:

Despite significant advancements in cancer treatment, mortality benefit remains limited due to therapy inefficacy and treatment failures, attributed to various factors, including the following:

1. Heterogeneity of cancer: Cancer is a complex disease, and different types of cancer can have different genetic mutations, making it difficult to develop a single treatment that works for all types of cancer. This heterogeneity is mainly driven by cancer stem cells, a subset with stem cell properties. The cancer stem cells are able to divide and spread to distant sites to form new tumors.
2. Resistance to treatment: Cancer cells can develop resistance to chemotherapy, radiation, and other treatments, making them less effective over time[10]. Therapeutic resistance is primarily due to cancer stem cells, which are inherently treatment resistant.
3. Side effects: Cancer treatments can cause side effects that can be severe and impact the quality of life of patients. For example, chemotherapy can cause nausea, vomiting, hair loss, fatigue and polyneuropathy.
4. Late diagnosis: Cancer is often diagnosed at a later stage when it has already spread to other parts of the body (metastasis), making it more difficult to treat. In fact, majority of cancer mortality is due to metastasis. Metastasis is driven by cancer stem cells.

5. Cost: Cancer treatments can be expensive, and not all patients can afford them, limiting their access to effective treatments[11].

**Hallmarks of Cancer: Therapeutic Correlations and Limitations**

1. Molecular studies have identified several hallmarks of cancer, which seem to be conserved across most types of cancer (**Figure 1**). Hallmarks of cancer represent mechanisms by which cancer develops, grows, and spreads. "Blocking" or "inhibiting" pathways leading to the hallmarks of cancer has served as a cornerstone of therapies and is the dominant paradigm driving therapeutic development.

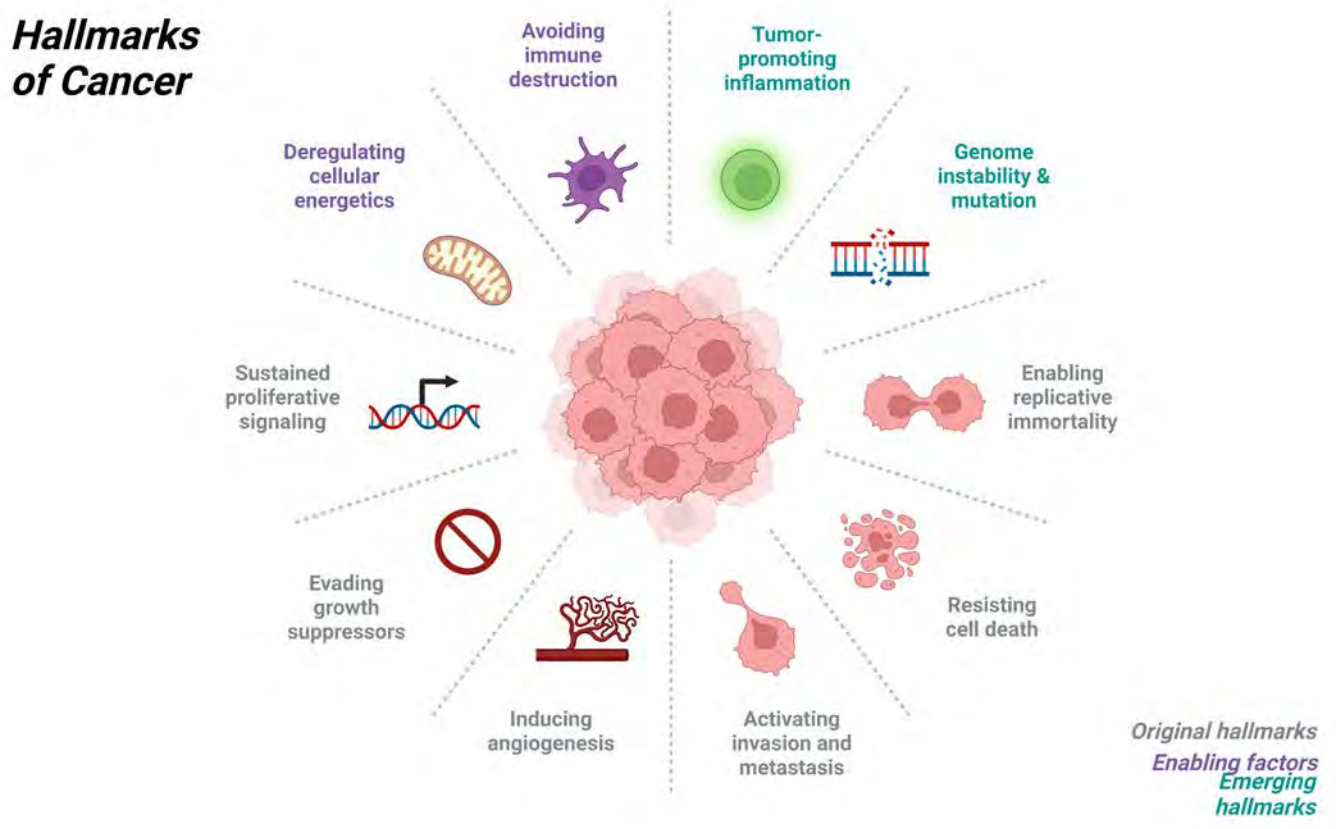


**Figure 1: Hallmarks of Cancer**

2. Most standard therapies (chemotherapy and radiotherapy) and emerging therapies (biologics, specific molecular inhibitors) are focused on targeting a single pathway. Tumors are "flexible" due to cancer stem cells, which survive therapies and subsequently morph into more resistant forms. This is the major factor associated with treatment failure. Cancer stem cells are chiefly responsible for instigating

metastasis, thus designating them a pivotal focal point for therapeutic interventions (Figure 2). However, contemporary treatment methodologies lack precision in addressing these critical cellular entities. Moreover, the intrinsic recalcitrance of cancer stem cells to conventional therapies paradoxically fuels their fortification in the presence of chemotherapy. This confluence of factors ultimately culminates in the resurgence of metastatic afflictions, resulting in significant mortality rates.

a. For example, chemotherapy and radiotherapy aim to reduce sustained proliferative signaling, and slow down tumor growth. However, cancer stem cells are slowly dividing and can become dormant in response to chemo or radiotherapy. Later, when the course of chemo or radiotherapy is over, the cancer stem cells wake from dormancy and result in cancer recurrence. Moreover, the cancer stem cells are inherently chemo and radioresistant, and the new tumors that emerge from them are chemo and radioresistant, often resulting in significant mortality from recurrent disease.

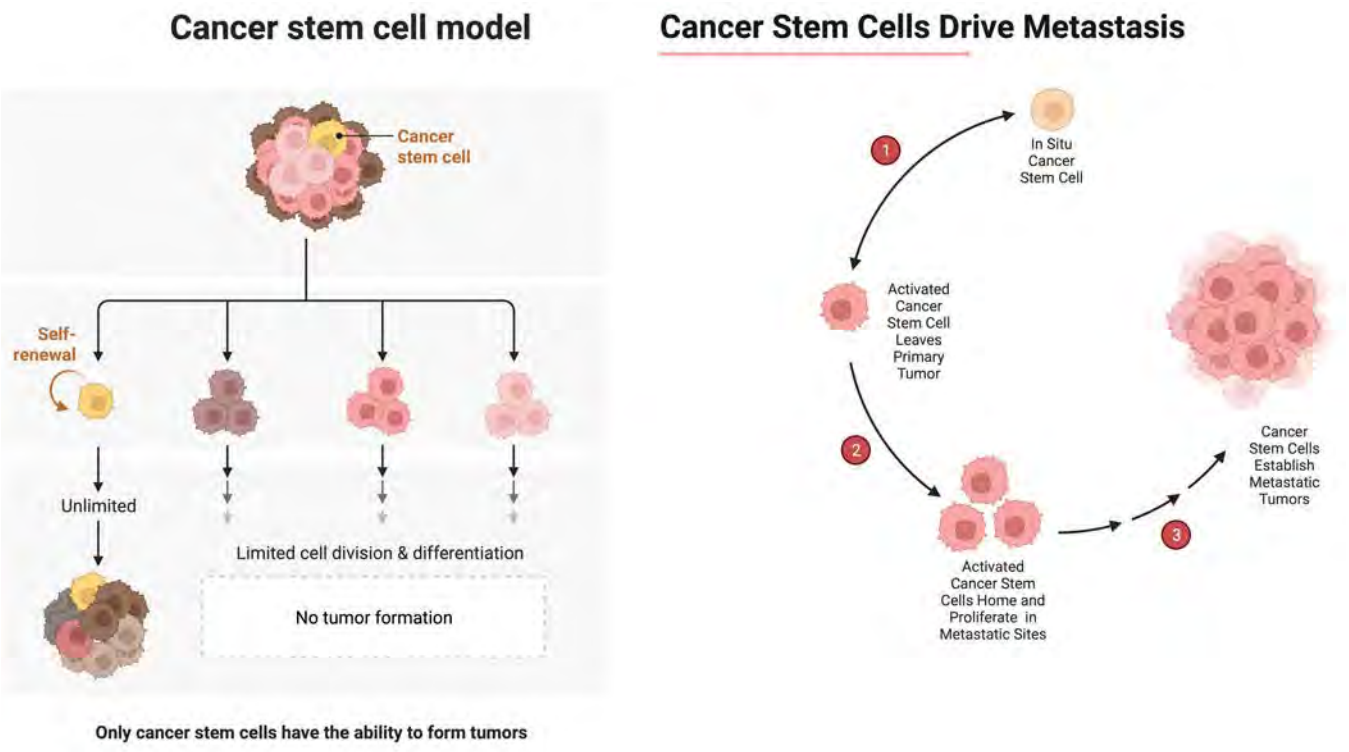


**Figure 2: Cancer Stem Cells Drive Tumorigenesis and Metastasis**

b. Specific therapies such as anti-angiogenics, which block single factors (among many that play roles in angiogenesis) involved in blood vessel growth, are rarely used as monotherapy and are usually

combined with other therapies. Even in combination therapy, these agents are associated with poor clinical responses, as tumors are able to switch phenotypes and use alternative mechanisms of blood vessel growth.

c. Immunotherapies, which aim to activate the body's own immune system to kill cancer cells, have recently gain much traction in the treatment of cancer. However, major therapeutic resistance results from "immune cold" tumors, which are characterized by evasion of cancer cells from the immune system. Like other forms of therapeutic resistance, the "immune cold" phenotype is driven by cancer stem cells.

## TRAF3IP2: A Novel Paradigm in the Treatment of Cancer

We have identified the cytoplasmic adapter molecule **TRAF3IP2** (TRAF3 Interacting Protein 2) as a novel, previously unknown regulator of cancer stem cells and as such of importance for multiple pro-tumorigenic and pro-resistance pathways. Our approach is unique and innovative, as it targets the cancer stem cell, the major factor involved in metastasis, therapeutic resistance, and tumor growth.

Our novel and groundbreaking data indicate the crucial role of TRAF3IP2 in several cancer types. In this document we summarize our research data indicating the important role of TRAF3IP2 in two of the most deadly cancer types, **glioblastoma and triple negative breast cancer**. In both, glioblastoma and triple negative breast cancer, chemotherapies, radiotherapies, and immunotherapies have failed to showmortality benefit, and their prognosis is very poor. The role of cancer stem cells in driving glioblastoma and triple negative breast cancer growth and metastasis, and treatment resistance, is increasingly clear.

### A- TRAF3IP2 in Glioblastoma

**1A)** ***TRAF3IP2 expression is increased in glioblastoma tumor tissues:*** To demonstrate the clinical relevance of TRAF3IP2 in glioblastoma, we analyzed TRAF3IP2 expression in glioblastoma tumor tissue. Immunohistochemistry (IHC) revealed increased TRAF3IP2 expression in ten different primary human glioblastoma tissue samples [1,12-14] (**Figure 3**).

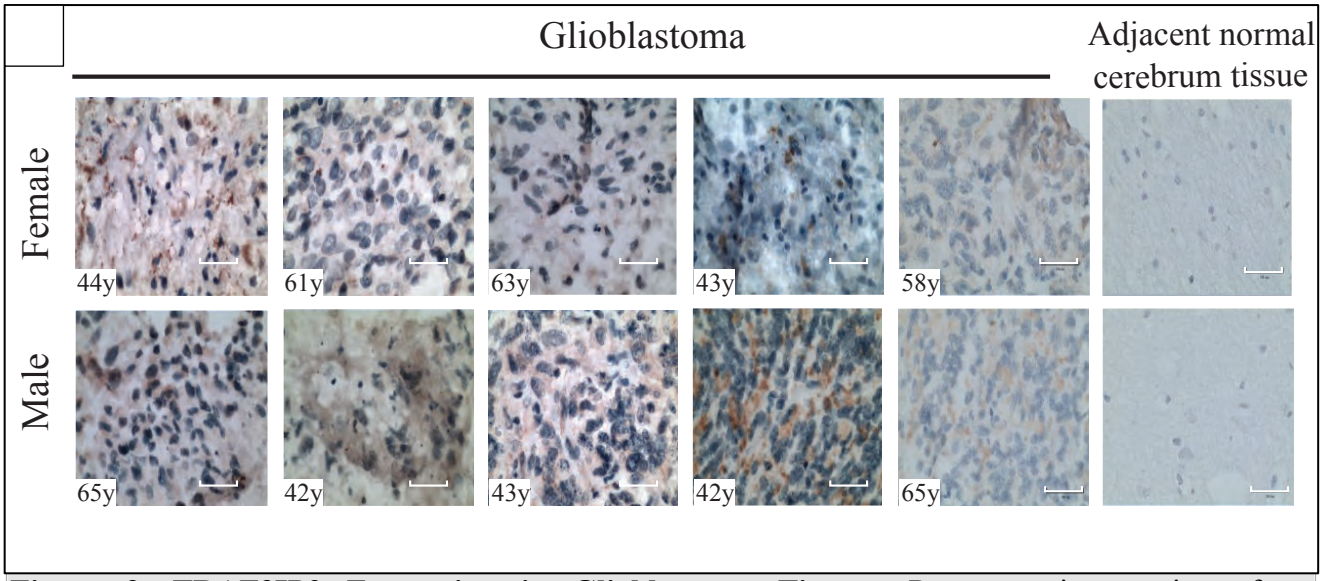


**Figure 3. TRAF3IP2 Expression in Glioblastoma Tissues.** Representative sections from glioblastoma tissues from ten patients show increased TRAF3IP2 expression (brown) by Immune-Histo-Chemistry IHC, counterstained with hematoxylin (blue). Scale bar, 100 mm.

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**2A)** ***Silencing TRAF3IP2 inhibits glioblastoma growth.*** The **glioblastoma cells** (U87) were transduced with lentiviral vector expressing validated TRAF3IP2 shRNA ($U87_{TRAF3IP2KD}$), which silenced TRAF3IP2. Lentiviral vector carrying a non-targeted scrambled shRNA ($U87_{Control\ shRNA}$) served as a

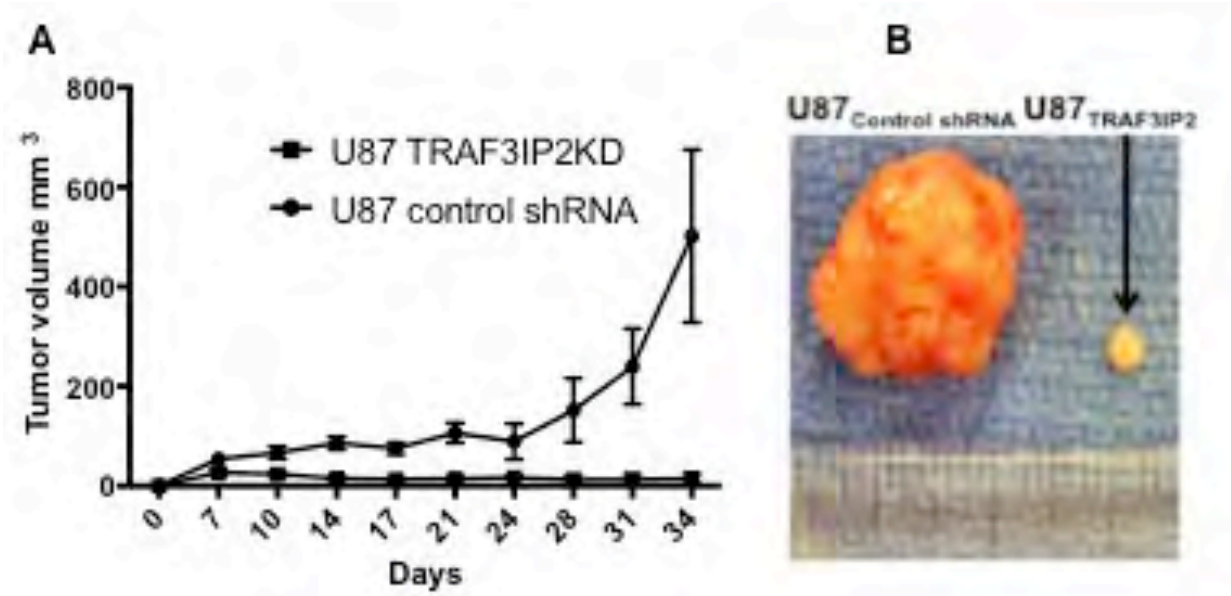


**Figure 4.** Silencing TRAF3IP2 prevents glioblastoma growth. **(A)** Immunodeficient NIH-III mice were injected with $U87_{TRAF3IP2KD}$ cells ($1X10^6$ cells) into the flank region. Control animals were injected with $U87_{control\ shRNA}$ cells ($1X10^6$ cells). Tumor size was measured weekly using calipers. **(B)** $U87_{TRAF3IP2KD}$ cells formed smaller tumors.

control. The $U87_{TRAF3IP2KD}$ cells ($1\times10^6$ cells) were then injected into the flank of immunodeficient mice for tumor induction. The injected animals were euthanized 34 days post-injection due to development of significant tumor size in animals injected with $U87_{Control\ shRNA}$. The data showed that U87 cells transduced with scrambled shRNA ($U87_{control\ shRNA}$) formed markedly larger tumors earlier compared to $U87_{TRAF3IP2KD}$ cells (1790.8 mg *versus* 10.8 mg; $P<0.0001$; **Fig. 4A and B**).

**3A)** ***Therapeutic significance of targeting TRAF3IP2 in the regression of pre-existing glioblastoma tumors.*** Having demonstrated that TRAF3IP2-silenced malignant U87 glioblastoma cells to form significantly smaller tumors, we next determined whether treating existing tumors by lentiviral TRAF3IP2 shRNA regress their size. In this translationally important strategy, tumors were induced at first by injecting luciferase-labeled U87 cells into the flank region of immunodeficient NIH-III mice. Fourteen days later, when tumors were distinctively quantifiable, lentivirus expressing GFP-tagged TRAF3IP2 shRNA (TRAF3IP2 shRNA-LV) was injected subcutaneously onto the tumors. Scrambled shRNA-LV served as a control. Results in **Figure 5A** show a remarkable reduction in tumor size over 50 days post-induction in TRAF3IP2 shRNA-LV-treated animals (*versus* scrambled shRNA-LV; 0.08±.03 g *versus* 1380±48, respectively).

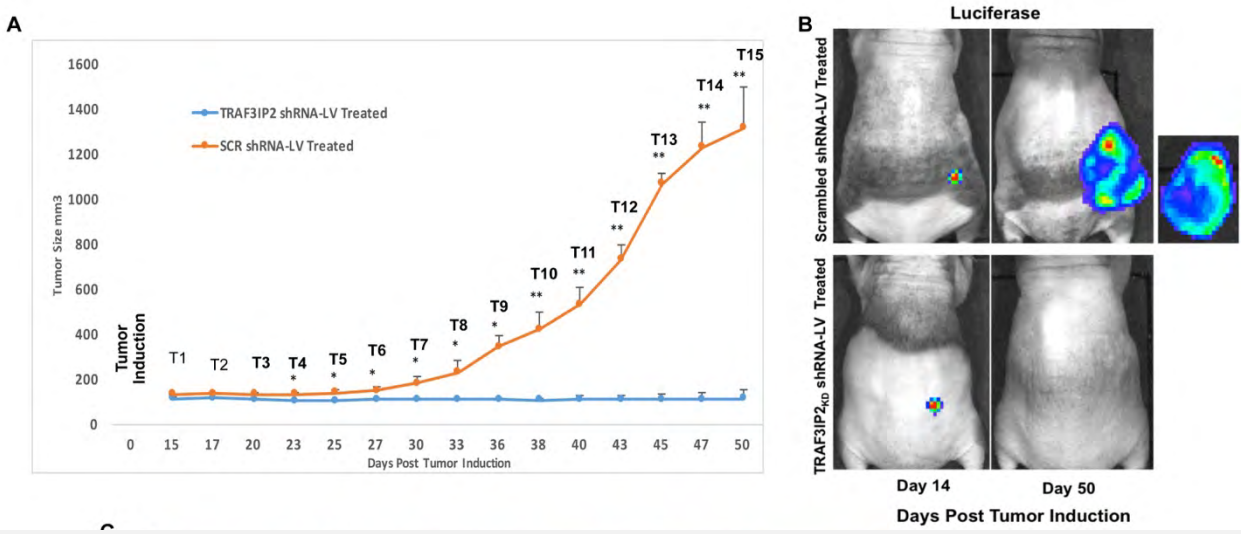


**Figure 5.** Effect of silencing TRAF3IP2 in a flank xenograft model. **(A)** Suppression of glioblastoma tumors by TRAF3IP2 shRNA-LV injected subcutaneously onto tumors compared to scrambled shRNA-LV injected tumors. Frequency of administration is shown in the graph. Tumor size was measured biweekly (*P<0.05; **P<0.001). **(B)** Animals imaged for luciferase weekly.

## B- TRAF3IP2 in Breast Cancer

Triple-negative breast cancer (TNBC) is the most aggressive and deadliest subtype of breast cancer with limited therapeutic options. The **critical barrier** to its successful treatment is the lack of targets that prevent and/or eliminate metastasis, block cancer stem cells function, and increase overall survival (OS) [15-23]. Specifically, in an advanced metastatic stage, TNBC is managed by a combination of chemotherapeutics, which is associated with low response rates and poor OS.

**1B) Analysis of TCGA; TNBC (Triple Negative Breast Cancer Cells) data indicates an inverse correlation between TRAF3IP2 expression and Overall Survival in TNBC patients** (**Figure 6**).

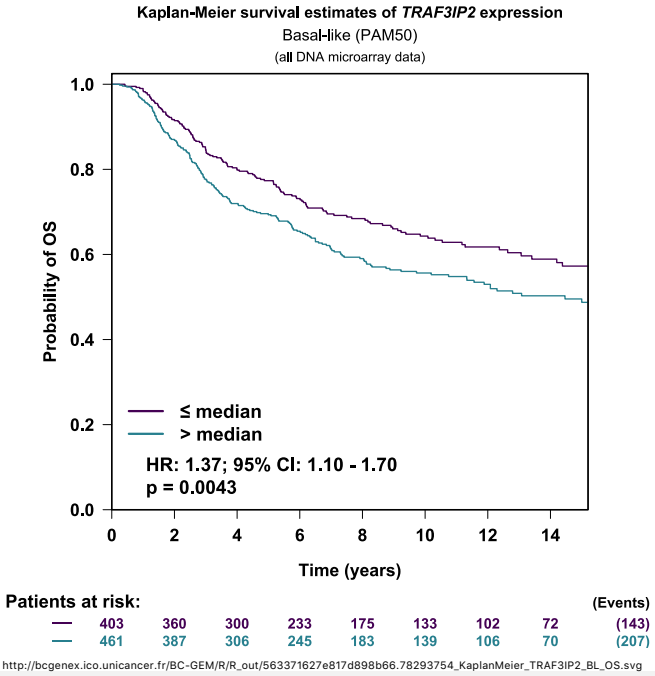


**Figure 6: High levels of TRAF3IP2 are associated with decreased overall survival (OS) in TNBC.** Analysis of pre-existing TCGA data showed a hazard ratio of 1.37 for higher, but not lower, TRAF3IP2 expression (p=0.0043), indicating that higher TRAF3IP2 expression is associated with significantly lower OS in TNBC. OS and hazard ratio were calculated using Cox Proportional-Hazards model across TNBC cases; n=403 low and 461 high TRAF3IP2.

**2B)** ***TRAF3IP2 expression is increased in TNBC cell line and tumor tissues:*** To demonstrate the clinical relevance of TRAF3IP2 in BC, we analyzed TRAF3IP2 expression in MDA-MB 231 cells, a TNBC cell line, compared to normal breast epithelial cells (184A1 cells) and mesenchymal stem cells (MSCs). Data show significantly high expression of TRAF3IP2 in MDA-MB231 cells compared to normal breast

epithelial cells and MSCs (**Figure 7A**). Moreover, IHC revealed increased TRAF3IP2 expression in four different *primary TNBC tissue samples* [12-14] (**Figure 7B**).

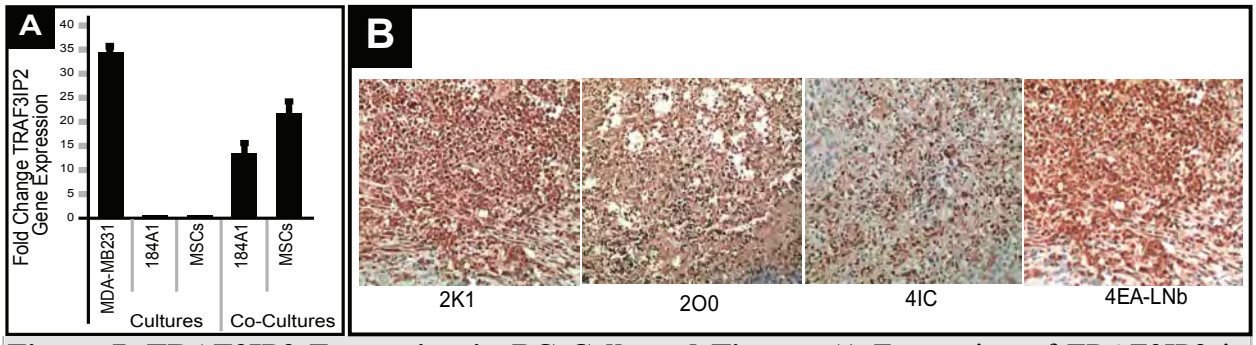


**Figure 7. TRAF3IP2 Expression in BC Cells and Tissues. A)** Expression of TRAF3IP2 in MDA-MB231, 184A1 and MSCs. The expression of TRA3IP2 is significantly higher compared to 184A1 and MSCs. However, co-culture of 184A1 or MSCs with MDA-MB231 cells enhanced TRAF3IP2 expression in both 184A1 and MSCs. **B)** Representative sections from four independent TNBC tissues show increased TRAF3IP2 expression (brown) by IHC, counterstained with hematoxylin (blue).

**3B)** ***Silencing TRAF3IP2 inhibits tumor growth and prevents metastasis in vivo.*** **Primary TNBC cell line** (4IC) was transduced with lentiviral vector expressing validated TRAF3IP2 shRNA ($4IC_{TRAF3IP2\ KD}$). Lentiviral vector carrying a non-targeted scrambled shRNA ($4IC_{Control\ shRNA}$) served as control. The data

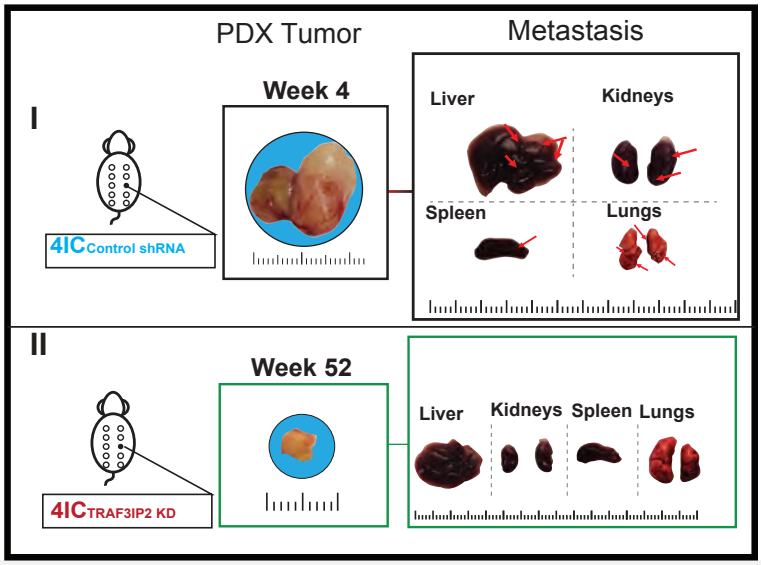


**Figure 8: Silencing TRAF3IP2 suppresses TNBC tumorigenesis.** Female 4- to 6-weeks-old NSG mice were injected intramammary with $4IC_{TRAF3IP2\ KD}$ cells ($1X10^6$ cells in PBS and Matrigel) and compared to the control group ($4IC_{Control\ shRNA}$)(n=10/group). Animals in the control group were euthanatized 4-weeks post-inoculation due to large tumor growth (**I**). Significant gross metastasis was found in these animals in various tissues. The extent of metastasis in the abdomen, liver, kidneys and lungs is shown (arrows) (**I**). In contrast, $4IC_{TRAF3IP2\ KD}$-injected animals showed minimal tumor growth upon euthanasia after week 52 (**II**). Further, no metastasis was detected in major tissues.

from these experiments indicate that silencing TRAF3IP2 reduces tumorigenicity of primary TNBC tissue, resulting in markedly decreased tumor weight, volume, metastatic potential and effecting prolonged survival (**Figure 8**).

### C- Mechanistic Role of TRAF3IP2 in Tumorigenesis and Metastasis: A Novel Paradigm

In this section, we delve into the role of TRAF3IP2 as a master regulator of a multitude of tumorigenic pathways. Additionally, we present our meticulously gathered research findings elucidating how the targeting of TRAF3IP2 manifests as a potent means to impede tumor proliferation and metastatic progression, thereby culminating in a substantial augmentation of overall survival rates.

As shown in **Figure 9**, our data show that targeting TRAF3IP2 several tumorigenic pathways:

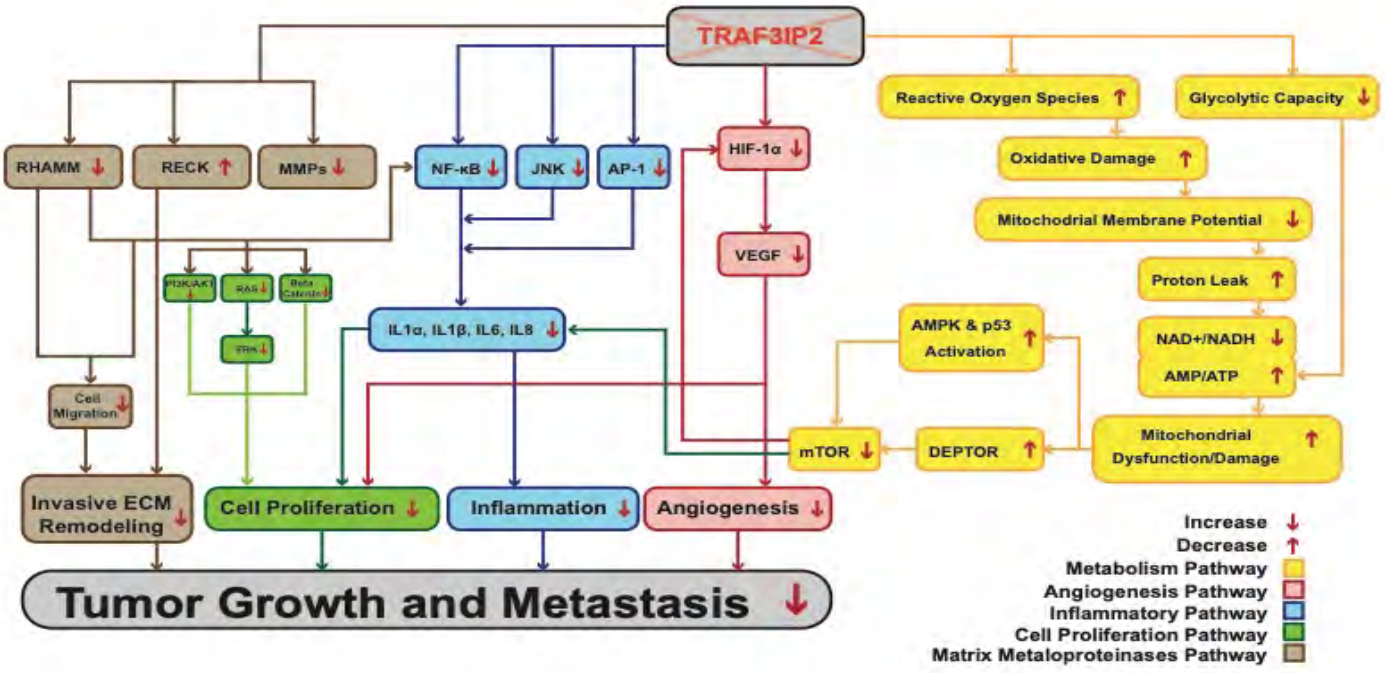


**Figure 9: Targeting TRAF3IP2: Comprehensive Mechanism of Action[1-8]**

Rather than solely targeting single pathways as other therapeutics currently under clinical investigation do, blocking TRAF3IP2 coordinately inhibits several pro-tumorigenic pathways. At this juncture, we

discuss our novel data unveiling how targeting TRAF3IP2 effectively mitigates the existing constraints observed within cancer therapy, as enumerated in the compendium of "Cancer Treatment Obstacles."

**1- Cancer stem cells**:

Heterogeneity of cancer: Cancer constitutes a complex disease, wherein distinct cancer variants can encompass diverse genetic mutations, engendering a formidable challenge in devising a uniform therapeutic approach applicable across all cancer categories. This diversity is primarily instigated by cancer stem cells (CSCs), an inherent subset endowed with traits reminiscent of stem cells. These cancer stem cells possess the unique capacity to undergo division and disseminate to remote locales, instigating the genesis of novel tumor formations. Targeting TRAF3IP2 shows remarkable anti-cancer stem cell effects.

Cancer stem cells are largely responsible for tumorigenesis, metastasis, development of chemoresistance and recurrence in TNBC[24]. Neoadjuvant with or without adjuvant chemotherapy remains as a standard therapeutic regimen in TNBC. In the neoadjuvant setting, chemotherapy serves to debulk the tumor in preparation for surgical resection, while adjuvant chemotherapy aims to eliminate residual cancer post-resection[25]. Neoadjuvant chemotherapy tends to eliminate non-stem bulk tumor cells, while selecting for and enhancing tumor initiating CSCs, which are inherently more resistant to cytotoxic and cytostatic chemotherapy [26,27]. Likewise, adjuvant therapy is not maximally effective in eliminating CSCs post-resection, as evidenced by chemotherapy refractory and recurrent TNBC disease [25,26]. Our data show that

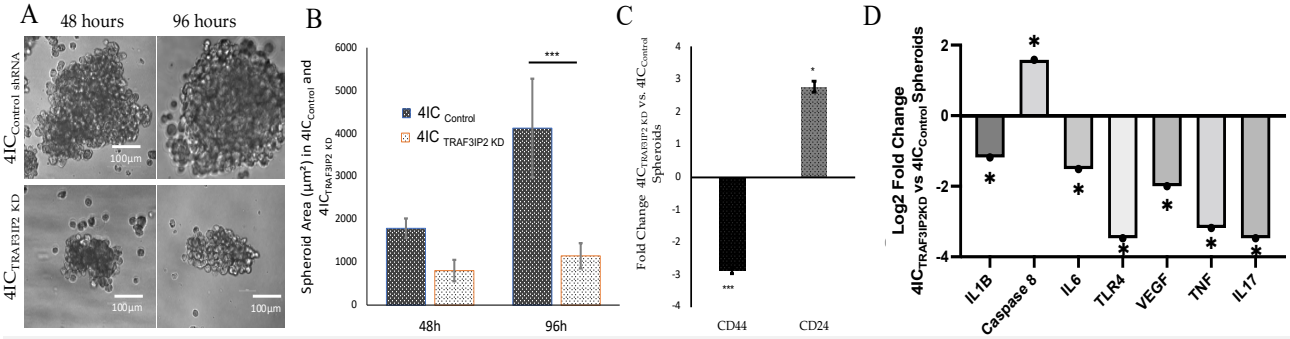


**Figure 10: Effect of targeting TRAF3IP2 on TNBC CSCs.** Once cultured in serum free media to isolate CSCs, the $4IC_{TRAF3IP2KD}$ cells showed a significant impaired ability to form spheroids, while $4IC_{Control}$ cells formed larger spheroids at 48 and 96 hours in culture (A&B). Flow cytometry showed a significant reduction of CD44 cells and increased CD24 cells in $4IC_{TRAF3IP2KD}$ spheroids (C). Transcriptomic analysis showed a downregulation of inflammatory and angiogenic factors and increased in apoptosis marker (D) (n= 5 independent experiments; *p<0.05, **p<0.01, ***p<0.001).

targeting TRAF3IP2 reduces the spheroid formation ability (a hallmark of CSCs) of TNBC cells indicating

reduced CSC numbers. CSCs are identified by the $CD44^{+}/CD24^{-}$ signature. Targeting TRAF3IP2 significantly reduced CD44 and increased CD24, suggesting suppression of the CSC phenotype (**Figure 10**). Also, transcriptomic analysis showed a reduction in inflammatory and angiogenesis markers in CSCs (**Figure 10**).

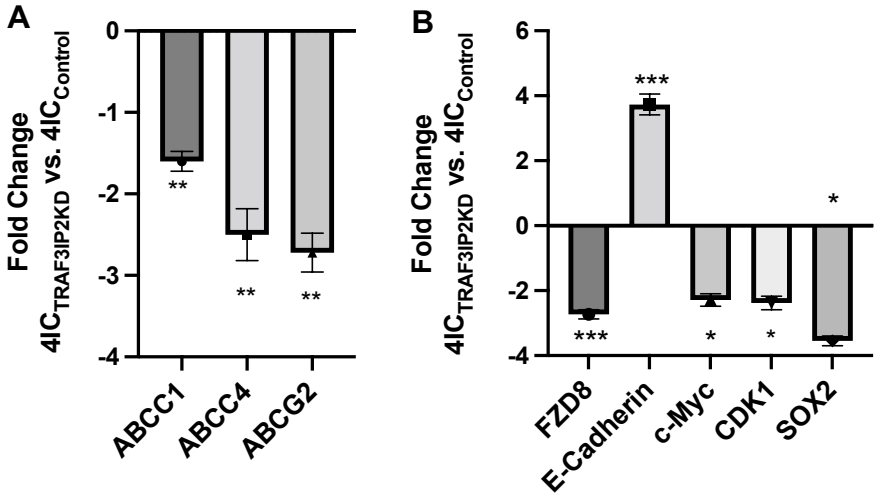


**Figure 11: Silencing TRAF3IP2 perturbs CSCs markers. A)** Targeting TRAF3IP2 significantly reduces levels of ABC Transporters ($p<0.05$). B) Targeting TRAF3IP2 significantly inhibits Wnt/ß-Catenin signaling by downregulating FZD8 and upregulating CDH1, as evidenced by downregulation of c-Myc and CDK1 (n=5; *$p<0.05$, **$p<0.01$, ***$p<0.001$).

In addition, the data in **Figure 11A** demonstrate that targeting TRAF3IP2 decreases the expression levels of ABC transporters, which efflux chemotherapeutics and produce chemoresistance. Further, targeting TRAF3IP2 alters Wnt/ß-Catenin signaling and reduces Sox2, a critical marker of CSCs (**Figure 11B**).

**2- Resistance to treatment:**

Cancer cells have the propensity to evolve resistance against chemotherapy, radiation, and assorted interventions, thereby diminishing their efficacy with the passage of time [10]. The emergence of therapeutic resistance predominantly stems from the presence of cancer stem cells, an intrinsic population inherently impervious to treatment strategies.

Our preliminary results also show that ***targeting TRAF3IP2 enhances the efficacy of chemotherapy agents (i.e. Paclitaxel) in TNBC cells, resulting in suppressed tumor growth.*** In this experiment, tumor was isolated from a metaplastic highly aggressive TNBC patient that showed limited

response to chemotherapy (4IC), silenced for TRAF3IP2 ($4IC_{TRAF3IP2\ KD}$; cells transduced with scrambled shRNA served as a control, $4IC_{Control\ shRNA}$), treated with Paclitaxel and analyzed for cell cycle profile *in vitro*. Paclitaxel has been previously shown to arrest cell cycle progression at the G2/M phase, resulting in apoptosis [28]. Our data show that Paclitaxel treatment alone increased the proportion of cells in G2 by 23%, as compared to a 12% increase from TRAF3IP2 knockdown alone. Importantly, targeting TRAF3IP2 reduced the proportion of cells in S phase by 4%. Notably, combining paclitaxel with TRAF3IP2 knockdown completely prevented entry into S phase, and arrested cells in the G2/M checkpoint (**Figure 12A-D**).

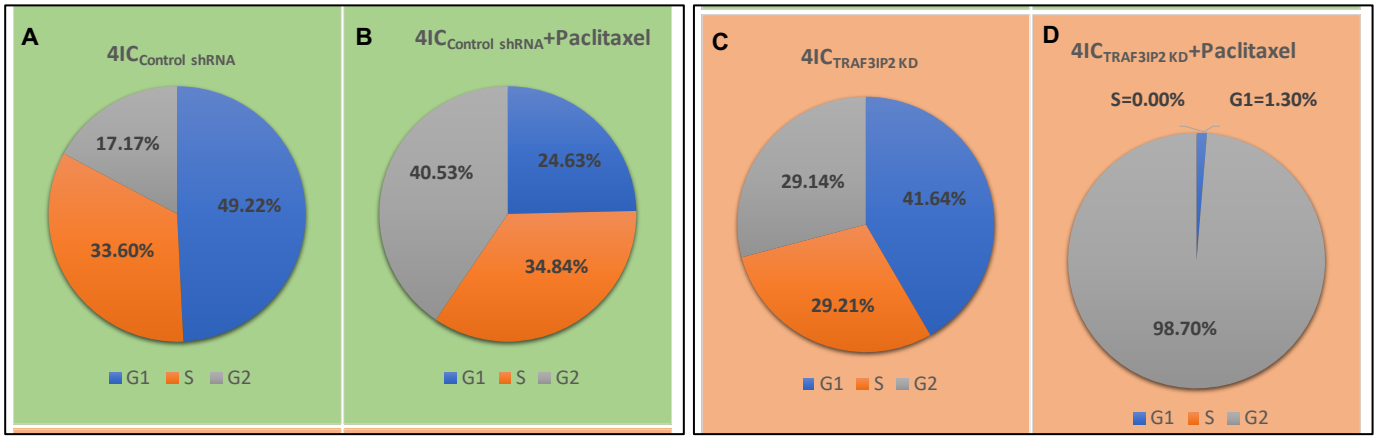


**Figure 12: Targeting TRAF3IP2 enhances the efficacy of Paclitaxel in TNBC, resulting in cell cycle arrest.** Cell cycle profiles of $4IC_{Control\ shRNA}$, $4IC_{Control\ shRNA}$+Paclitaxel, $4IC_{TRAF3IP2\ KD}$ and $4IC_{TRAF3IP2\ KD}$+Paclitaxel (**A-D**). In Paclitaxel treatment alone, there was increase in the proportion of cells in G2 by 23%, as compared to a 12% increase in $4IC_{TRAF3IP2\ KD}$. Combined Paclitaxel administration and TRAF3IP2 silencing completely prevented entry into S phase and arrested cells in the G2/M checkpoint.

Overall our data show that targeting TRAF3IP2 has both the intrinsic and extrinsic effect on both tumor cells and tumor microenvironment, an area where tumor cells and host non-malignant cells communication results in tumor grow and metastasis (**Figure 13**). This dual effect of targeting TRAF3IP2 inhibits chemo/immunotherapeutic resistance.

**TRAF3IP2 inhibits chemo/immunotherapeutic resistance.**

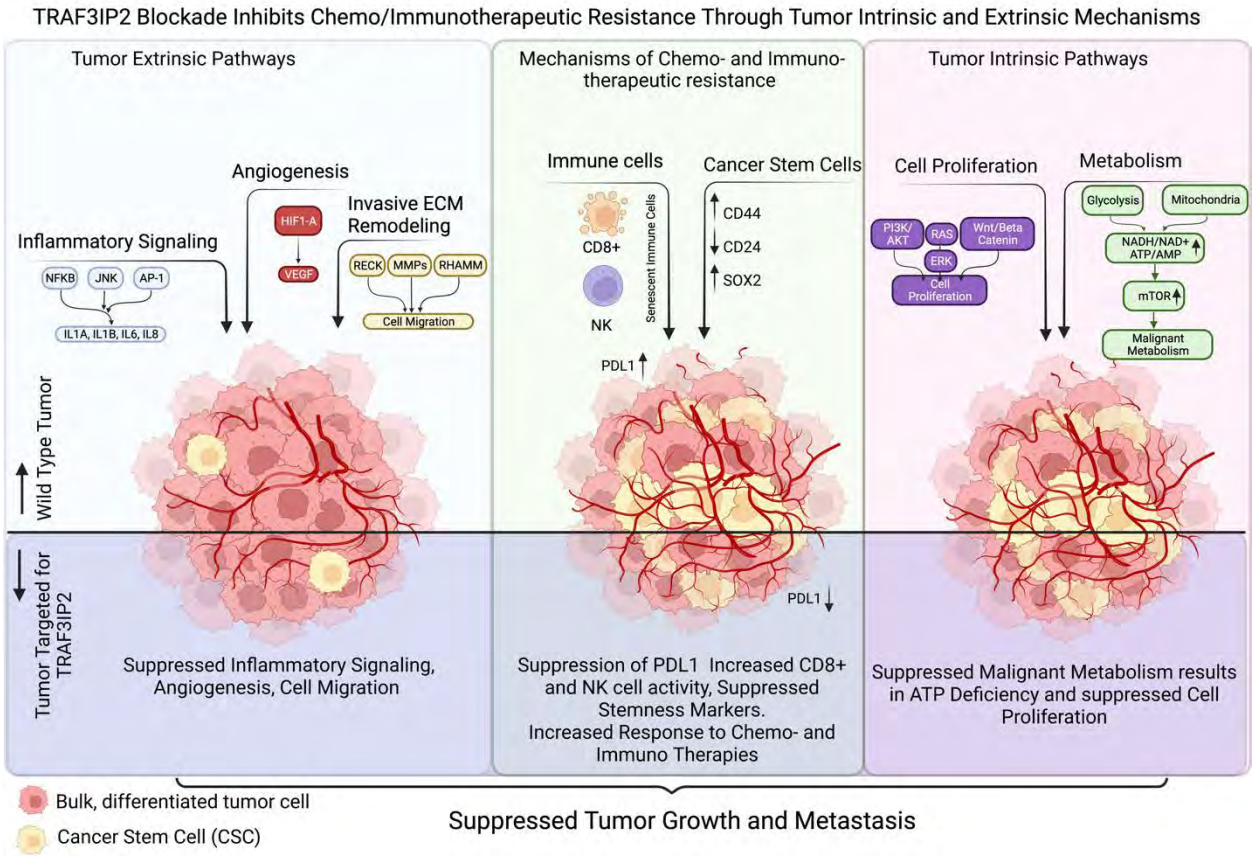


**Figure 13: TRAF3IP2 Blockade Inhibits Chemo/Immunotherapeutic Resistance Through Tumor Intrinsic and Extrinsic Mechanisms**

### 3- Side effects:

Cancer therapies have the potential to induce adverse reactions that range in severity and can significantly compromise the well-being of patients. For instance, chemotherapy is known to elicit side effects including nausea, vomiting, alopecia, and profound fatigue, all of which collectively contribute to a diminished quality of life.

*Genome profiling of messenger RNA in TRAF3IP2-silenced U87 glioblastoma cells*

Total cellular gene expression analysis shows hierarchical clustering of differentially expressed genes in TRAF3IP2-silenced U87 cells compared to control shRNA-transfected cells ($U87_{TRAF3IP2KD}$ *versus* $U87_{control\ shRNA}$ cells). The data show that the expression of 1,297 genes was significantly perturbed in $U87_{TRAF3IP2KD}$ cells, of which 597 were significantly upregulated (>2-fold) and 700 downregulated.

Further, gene ontology analysis revealed that silencing TRAF3IP2 significantly affects the expression of genes involved in cell cycle progression, immune activation, cytokine-cytokine interaction, aging, apoptosis, extracellular matrix organization, DNA replication, repair and metabolism (**Fig. 14**). A significant alteration was also observed in canonical pathways related to angiogenesis in U87$_{\text{TRAF3IP2KD}}$ cells (*versus* U87$_{\text{control shRNA}}$ cells).

Interestingly, our research has unveiled that the directed intervention of TRAF3IP2 exerts a discernible impact on the expression of a substantial pool of 1297 genes within cancer cells. However, intriguingly,

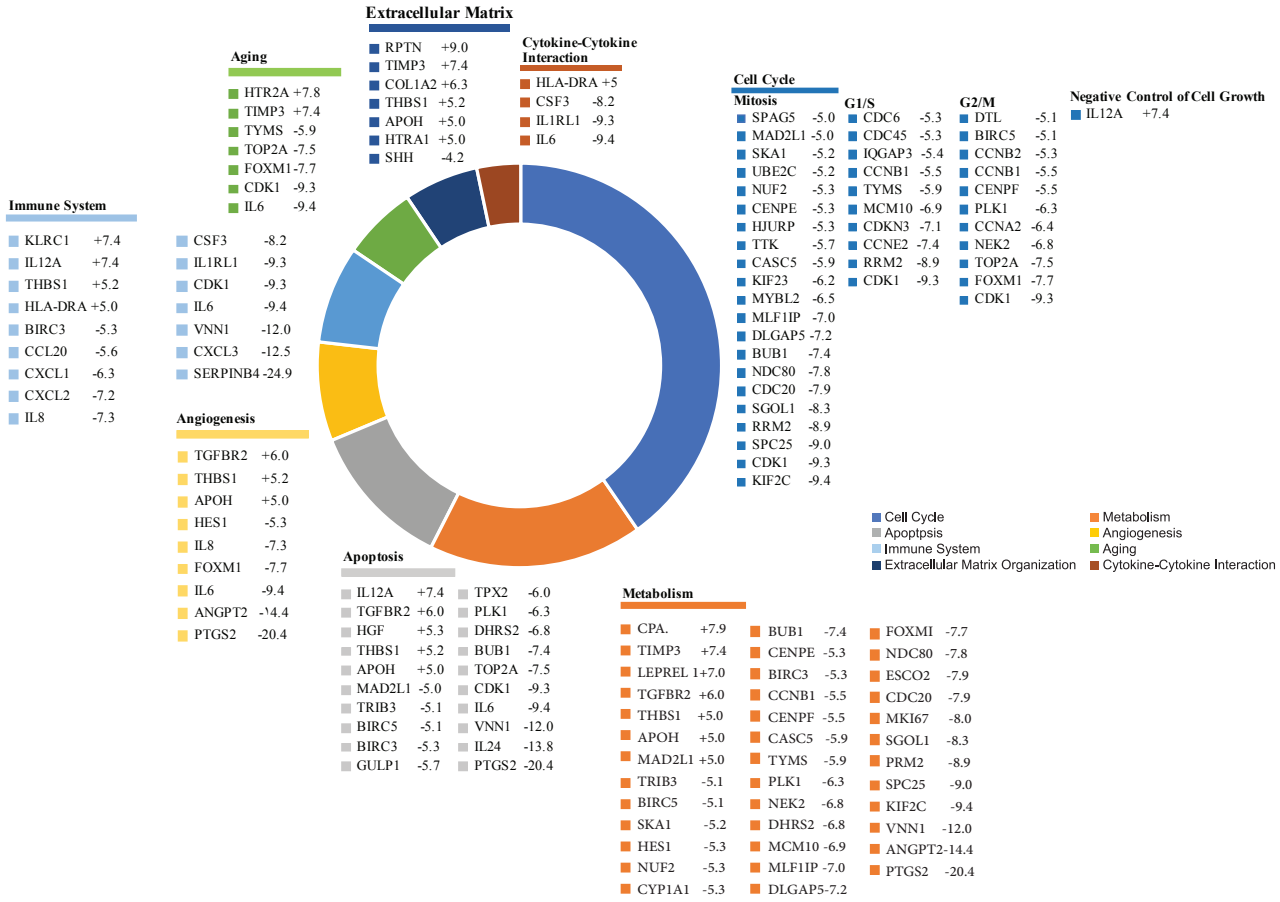


**Figure 14. Differential gene expression in U87$_{\text{TRAF3IP2 KD}}$ and U87$_{\text{control shRNA}}$ cells.** Hierarchical clustering displayed genes differentially expressed in U87$_{\text{TRAF3IP2 KD}}$ and U87$_{\text{control shRNA}}$ cells. Pathway analysis (using Reactome) of a cluster of 1297 perturbed gene expressions in U87$_{\text{TRAF3IP2 KD}}$ cells revealed a statistically significant preponderance of genes involved in cell cycle, metabolism, apoptosis, angiogenesis, immune system, aging, extracellular matrix organization, and cytokine-cytokine interaction. The chart displays genes representative of each pathway displaying greater than 5-fold change in U87$_{\text{TRAF3IP2 KD}}$ versus U87$_{\text{control shRNA}}$ cells ($P$<0.05).

our data also illuminates that when TRAF3IP2 was selectively targeted in non-malignant cells, no discernible alteration in gene expression patterns was observed.

4. **Late diagnosis:**

Frequently, the diagnosis of cancer occurs during advanced stages, characterized by its dissemination to distant body regions—a process known as metastasis—thus compounding the challenges of effective treatment. Notably, a substantial proportion of cancer-related deaths can be attributed to this metastatic progression. The propulsion of metastasis is orchestrated by cancer stem cells, rendering existing therapeutic approaches largely ineffectual in the face of this resilient phenomenon.

We show that targeting TRAF3IP2 prevents glioblastoma growth and intracranial metastasis in a preclinical model. Having demonstrated that silencing TRAF3IP2 significantly inhibits glioblastoma growth and development, this compelling data prompted our further studies on the effect of TRAF3IP2 in tumorigenesis and intracranial dissemination of glioblastoma in a preclinical model. In this experiment the mice were injected into the left hemisphere of the brain (SSCx) with wild type glioblastoma cells ($3X10^5$ cells in PBS and Martigel) to induce tumor for both control and experimental

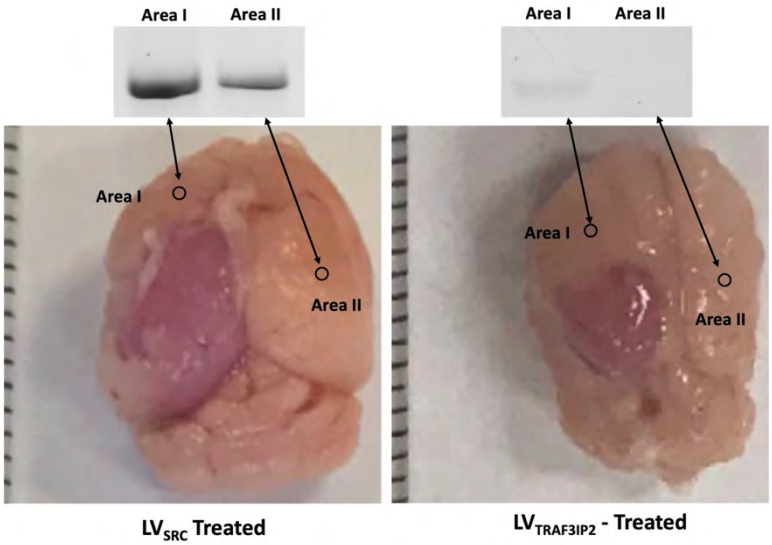


**Figure 15. Detection of intracranial dissemination using PCR.** The intracranial micrometatatsis was analyzed using primers directed towards a human-specific α-satellite DNA sequence of the centromere region of human chromosome 17. Two areas of the brain outside of the tumor region (the left parietal lobe, at a distance of 2mm from the focus of initial tumor inoculation (Area I) and right SSCx equidistant from the interhemispheric fissure relative to the focus of initial tumor inoculation (Area II) were selected. While there was a trace amount of signal detected in Area II, there was no detectable human specific DNA in the area II of $LV_{TRAF3IP2}$ treated brain.

groups (n=10/group). Ten days post-tumor induction, the experimental group was treated with lentiviral vector carrying silencer for TRAF3IP2 (LVTRAF3IP2), while the control group was treated with lentivirual vector carrying scrambled sequence (LVSCR) to serve as control. Four weeks post-tumor induction, adjacent sections of brain were biopsied and evaluated for metastasis and micrometastasis using a human-specific microsatellite PCR-assay using primers that specifically detect human-specific α-satellite DNA sequence of the centromere region of human chromosome 17 [29]. Areas evaluated include the left parietal lobe, at a distance of 2mm from the focus of initial tumor inoculation (Area I) and the right SSCx equidistant from the interhemispheric fissure relative to the focus of initial tumor inoculation (Area II) (**Figure 15**). Results demonstrate markedly reduced metastasis at both sites in the $LV_{TRAF3IP2}$ treated group (3-18 human cells / 100 000 mouse cells) as compared to control $LV_{SCR}$ treated group (400-900 human cells / 100 000 mouse cells) (**Figure 15**); demonstrating the essential and critical role of TRAF3IP2 in enabling and driving metastasis and dissemination. These data indicate that **silencing TRAF3IP2 very effectively inhibits malignant metastasis**, the reason for the low survival of patients with glioblastoma, as at times of diagnosis the cancer has already spread and dissiminated throughout the whole brain.

### 4- Cost and Quality of Life

Cancer therapies often carry a substantial financial burden, rendering them inaccessible to a portion of patients due to financial constraints. This economic barrier hampers the ability of these individuals to avail themselves of efficacious treatment options.

The inadequacy of current treatments necessitates more advanced, but ineffective, therapeutic modalities, incurring escalated expenditures for both the healthcare system and the individual patient. Moreover, the patient's overall quality of life experiences a notable decline. In fact, a recent analysis published in JAMA showed that only 24% of randomized clinical trials of cancer drugs demonstrated quality of life improvement [30]. In stark contrast, targeting TRAF3IP2 eradicates cancer stem cells, eliminates metastatic occurrences, and mitigates resistance to treatment. This multifaceted strategy not only augments the initial success rate of cure but also curtails the likelihood of relapses. Consequently, the necessity for salvage therapies diminishes, concurrently enhancing the well-being of patients and culminating in substantial cost savings for both healthcare systems and individuals.

**References:**


1. Alt, E.U.*, et al.* TRAF3IP2, a novel therapeutic target in glioblastoma multiforme. *Oncotarget* **9**, 29772-29788 (2018).
2. Alt, E.U.*, et al.* Targeting TRAF3IP2, Compared to Rab27, is More Effective in Suppressing the Development and Metastasis of Breast Cancer. *Sci Rep* **10**, 8834 (2020).
3. Bayat, N.*, et al.* The Anti-Angiogenic Effect of Atorvastatin in Glioblastoma Spheroids Tumor Cultured in Fibrin Gel: in 3D in Vitro Model. *Asian Pac J Cancer Prev* **19**, 2553-2560 (2018).
4. Izadpanah, A.*, et al.* Targeting TRAF3IP2 inhibits angiogenesis in glioblastoma. *Front Oncol* **12**, 893820 (2022).
5. Mummidi, S.*, et al.* RECK suppresses interleukin-17/TRAF3IP2-mediated MMP-13 activation and human aortic smooth muscle cell migration and proliferation. *J Cell Physiol* **234**, 22242-22259 (2019).
6. Valente, A.J.*, et al.* Interleukin-18 enhances IL-18R/Nox1 binding, and mediates TRAF3IP2-dependent smooth muscle cell migration. Inhibition by simvastatin. *Cell Signal* **25**, 1447-1456 (2013).
7. Worner, P.M.*, et al.* Breast Tumor Microenvironment Can Transform Naive Mesenchymal Stem Cells into Tumor Forming Cells in Nude Mice. *Stem Cells Dev* (2018).
8. Yoshida, T.*, et al.* Minocycline reverses IL-17A/TRAF3IP2-mediated p38 MAPK/NF-kappaB/iNOS/NO-dependent cardiomyocyte contractile depression and death. *Cell Signal* **73**, 109690 (2020).
9. Sung, H.*, et al.* Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* **71**, 209-249 (2021).
10. Debela, D.T.*, et al.* New approaches and procedures for cancer treatment: Current perspectives. *SAGE Open Med* **9**, 20503121211034366 (2021).
11. Djulbegovic, B.*, et al.* Treatment success in cancer: new cancer treatment successes identified in phase 3 randomized controlled trials conducted by the National Cancer Institute-sponsored cooperative oncology groups, 1955 to 2006. *Arch Intern Med* **168**, 632-642 (2008).
12. Matossian, M.D.*, et al.* Drug resistance profiling of a new triple negative breast cancer patient-derived xenograft model. *BMC Cancer* **19**, 205 (2019).
13. Matossian, M.D.*, et al.* A novel patient-derived xenograft model for claudin-low triple-negative breast cancer. *Breast Cancer Res Treat* **169**, 381-390 (2018).
14. Matossian, M.D.*, et al.* Panobinostat suppresses the mesenchymal phenotype in a novel claudin-low triple negative patient-derived breast cancer model. *Oncoscience* **5**, 99-108 (2018).
15. Blomqvist, C.*, et al.* Influence of treatment schedule on toxicity and efficacy of cyclophosphamide, epirubicin, and fluorouracil in metastatic breast cancer: a randomized trial comparing weekly and every-4-week administration. *J Clin Oncol* **11**, 467-473 (1993).
16. Joensuu, H.*, et al.* Combination chemotherapy versus single-agent therapy as first- and second-line treatment in metastatic breast cancer: a prospective randomized trial. *J Clin Oncol* **16**, 3720-3730 (1998).
17. Mauri, D.*, et al.* Overall survival benefit for weekly vs. three-weekly taxanes regimens in advanced breast cancer: A meta-analysis. *Cancer Treat Rev* **36**, 69-74 (2010).
18. Sparano, J.A.*, et al.* Weekly paclitaxel in the adjuvant treatment of breast cancer. *N Engl J Med* **358**, 1663-1671 (2008).

19. Schmid, P*., et al.* Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. *N Engl J Med* **379**, 2108-2121 (2018).
20. Bardia, A*., et al.* Paclitaxel With Inhibitor of Apoptosis Antagonist, LCL161, for Localized Triple-Negative Breast Cancer, Prospectively Stratified by Gene Signature in a Biomarker-Driven Neoadjuvant Trial. *J Clin Oncol*, JCO2017748392 (2018).
21. Adams, S*., et al.* Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. *Ann Oncol* **30**, 397-404 (2019).
22. Miles, D*., et al.* Primary results from IMpassion131, a double-blind, placebo-controlled, randomised phase III trial of first-line paclitaxel with or without atezolizumab for unresectable locally advanced/metastatic triple-negative breast cancer. *Ann Oncol* **32**, 994-1004 (2021).
23. Emens, L.A*., et al.* First-line atezolizumab plus nab-paclitaxel for unresectable, locally advanced, or metastatic triple-negative breast cancer: IMpassion130 final overall survival analysis. *Ann Oncol* **32**, 983-993 (2021).
24. O'Conor, C.J., Chen, T., Gonzalez, I., Cao, D. & Peng, Y. Cancer stem cells in triple-negative breast cancer: a potential target and prognostic marker. *Biomark Med* **12**, 813-820 (2018).
25. Nedeljkovic, M. & Damjanovic, A. Mechanisms of Chemotherapy Resistance in Triple-Negative Breast Cancer-How We Can Rise to the Challenge. *Cells* **8**(2019).
26. Steward, L., Conant, L., Gao, F. & Margenthaler, J.A. Predictive factors and patterns of recurrence in patients with triple negative breast cancer. *Ann Surg Oncol* **21**, 2165-2171 (2014).
27. He, J*., et al.* Inhibition of USP2 eliminates cancer stem cells and enhances TNBC responsiveness to chemotherapy. *Cell Death Dis* **10**, 285 (2019).
28. Choi, Y.H. & Yoo, Y.H. Taxol-induced growth arrest and apoptosis is associated with the upregulation of the Cdk inhibitor, p21WAF1/CIP1, in human breast cancer cells. *Oncol Rep* **28**, 2163-2169 (2012).
29. Becker, M*., et al.* Sensitive PCR method for the detection and real-time quantification of human cells in xenotransplantation systems. *Br J Cancer* **87**, 1328-1335 (2002).