TP53 mutant TNBC overexpresses serine biosynthesis genes PSAT1 and PHGDH and may demonstrate vulnerability to dietary glycine supplementation

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SAN ANTONIO BREAST CANCER SYMPOSIUM®

Abstract

Since triple-negative breast cancer (TNBC), by definition, does not respond to antiestrogen or HER2-directed therapy, there is a vital unmet need for identification of novel and specific therapeutic targets. Since most TNBC is characterized by loss-of-function mutations in the tumor suppressor p53, TNBC is likely vulnerable to targeting of pathways upon which p53-mutant breast cancer cells depend. One such pathway is the serine synthesis pathway (SSP) which demonstrates upregulation of biosynthetic enzymes PSAT1 and PHGDH in p53-mutant breast cancer. [18F]fluoroglut**amine** is a PET radiotracer that detects SSP activity through its conversion to [18F]fluoroglut**amate** by intracellular glutaminase to use as a substrate of the PSAT1 enzyme in the conversion of 3-PHP to 3-phosphoserine. We propose a 3-part project in which we would (1) study PET tracer in preclinical experiments (SSP-high vs. low cell lines *in vitro* and *in vivo* in mouse models); (2) develop and validate the clinical-grade PET tracer in the PET radiochemistry core facility at Stony Brook; and (3) perform [18F]fluoroglut**amine** PET scans in human-subject volunteers with p53-mutant vs. p53-wildtype breast cancer. If we can demonstrate that p53-mutant and thus triple-negative breast cancer can be preferentially detected with this PET tracer, we will be able to propose therapeutic studies of pharmacologic inhibitors of PSAT1 and PHGDH in patients with TNBC and/or develop radiotherapeutics targeting the SSP.

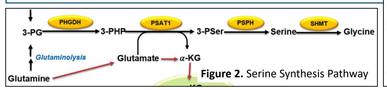


Figure 2. Serine Synthesis Pathway

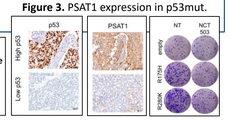


Figure 3. PSAT1 expression in p53mut.

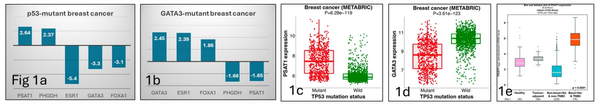
Introduction

Triple negative breast cancer (TNBC), by definition, lacks obvious targets for systemic therapy. While there have been significant developments in the treatment of ER+ and HER2+ breast cancer, treatment of TNBC still relies on traditional cytotoxic chemotherapy. Stage for stage, patients with TNBC have a worse prognosis than other breast cancer (BC) subtypes. Early-stage TNBC patients have an increased risk of recurrence while metastatic TNBC patients have significantly inferior mOS (2 years vs. 5 years in ER+ or HER2+). To date, innovations in TNBC treatment have contributed no more than incremental benefits and only in select subsets of patients. The addition of immune checkpoint inhibitors to chemotherapy adds no more than 7 months to mOS and only in the 40% of patients that overexpress PD-L1. PARP inhibitors are FDA approved for just the 15% of TNBC patients with germline BRCA1/2 mutations. The TROP2-targeted ADC sacituzumab govitecan adds no more than 4 months of mPFS vs. standard chemo. There is a critical unmet need for identification of better therapeutic targets for the 34,000 TNBC patients diagnosed in the US each year.

Research Objectives

One of the hallmarks of TNBC is a loss-of-function mutation in the tumor suppressor gene p53, present in at least 80% of cases. We currently lack safe and effective vectors for gene therapy to restore lost p53 function. Nor can we yet circumvent this limitation with drugs targeted to parallel pathways upon which p53-deficient cells depend. Fortunately, gene-expression profiling of p53-mutant BC reveals novel potential therapeutic targets. p53-mutant BC demonstrates significant upregulation of serine synthesis pathway (SSP) enzymes PSAT1 and PHGDH. Conversely, ER-positive BC cell lines and *ex vivo* breast tumors express low levels of PHGDH (Kim, 2014). We hypothesize that p53-mutant BC depends on increased SSP activity to support its aggressive phenotype and is likely sensitive to inhibition of these biosynthetic enzymes.

We propose a translational research project to study the SSP in BC. Specifically, we propose ultimately to use the PET radiotracer [18F]fluoroglut**amine** ([18F]FGln) to detect SSP activity in human subjects. While a clinical-grade reagent is validated, we will use research-grade reagent to study SSP activity in cell culture and animal models. If we can establish that p53-mutant (and, by implication, triple-negative) BC relies on upregulation of SSP activity, this tracer may help select patients for treatment with SSP inhibitors or novel radiotherapeutics

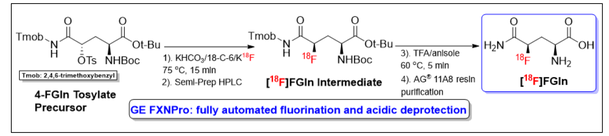


Preliminary Data

We used Breast CancerGene-Expression Miner v5.2, muTarget and GEPIA to interrogate publicly available datasets including METABRIC, GTEx, SCAN-B and TCGA. p53 is the most frequently mutated gene in TNBC. p53 mutations are more common in ER-negative than ER-positive breast cancer (Shah, 2012). In our *in silico* analysis, PSAT1 gene expression was upregulated 2.64 times and PHGDH 2.37 times in p53 mutant versus wildtype breast cancers (Figure 1a). Conversely, ESR1, GATA3 and FOXA1 gene-expression was downregulated (5.4-fold, 3.3-fold and 3.1-fold, respectively). GATA3-mutant breast cancer (largely ER+) shows upregulation of GATA3 (2.45-fold), ESR1 (2.39-fold) and FOXA1 (1.86-fold) gene expression (Figure 1b) and downregulation of PHGDH and PSAT1. PSAT1 expression is upregulated in p53 mutant breast cancer (Figure 1c) while GATA3 is downregulated (Figure 1d). The biggest gene-expression difference between TNBC and non-TNBC is in PSAT1 (Figure 1e). Of note, PHGDH upregulation is one of the TNBC-defining transcripts on the influential PAM50 dataset (Perou, 2011). ER+ cases from PAM50 demonstrate PHGDH downregulation.

Research Design

Specific Aim 1: To demonstrate that [18F]F-Gln selectively identifies p53-mutant TNBC and serves as a biomarker of serine biosynthesis using *in vitro* and *in vivo* models of TNBC. We hypothesize that p53-mutant TNBC, characterized by high PHGDH and PSAT1 expression, will exhibit increased [18F]F-Gln uptake, and that pharmacologic inhibition of the SSP will reduce this uptake, establishing [18F]F-Gln PET as a biomarker of SSP activity and inhibition. We will perform *in vitro* studies in human-derived TNBC cell lines as well as *in vivo* studies using mouse models of those same cell lines to test this hypothesis. **Specific Aim 2:** To synthesize and validate clinical-grade [18F]FGln for use in human subject research. In the past years, we have established a semi-automated synthesis method to prepare (*2S,4R*)-4-[18F]fluoroglutamine To supply for human subject research, we will modify our current synthesis method to established a fully automated synthesis tracer production protocol Once the major parameters being confirmed, the production tests with large scale of fluorine-18 radioactivity (> 250 mCi) will be conducted to fine-tune the operation parameters. With less than 20 times of production test, we expect to obtain an optimized and fully automated tracer production protocol. After that, a detailed batch production document will be compiled and three consecutive full batch productions will be performed and verified, and the corresponding CMC (Chemistry, Manufacturing and Controls) document will be compiled. These documents will form the solid foundation to solicit approval from Regulatory Agency (RDRC from SBU or IND from FDA) and to initiate routine [18F]FGln production for human subject imaging. **Specific Aim 3**: To demonstrate that FGln PET can preferentially detect p53-mutant breast cancer in human subjects. We propose a pilot study to evaluate [^{18}F]fluoroglutamine (FGln) PET/CT as a novel metabolic imaging biomarker for p53-mutant, triple-negative breast cancer (TNBC) compared to p53-wild-type, ER-positive breast cancer. A total of 21 patients will be enrolled: 14 patients with p53-mutant TNBC and 7 patients with p53-wild-type ER-positive disease. Participants will be identified through the Stony Brook Cancer Center and the Phillips Family Cancer Center. Eligible subjects will include both early-stage and metastatic patients, preferably those who are treatment-naïve or distant (>6 months) from prior systemic anticancer therapy. Tumor p53 mutational status will be assessed by DNA sequencing of archived or fresh biopsy material. Expression of PSAT1, PHGDH and p53 will be evaluated by quantitative RT-PCR and immunohistochemistry (IHC). [^{18}F]fluoroglutamine will be synthesized under current Good Manufacturing Practice (cGMP) conditions by the Stony Brook PET Radiochemistry Core Facility. Each participant will receive 185 MBq (5 mCi ± 10%) of [^{18}F]FGln intravenously, followed by a PET/CT scan at 60 ± 10 minutes post-injection. Low-dose CT will be performed for attenuation correction and anatomic localization.



Prior studies of [18F]fluoroglutamine

Figure 4. p53-mutant gliosarcoma in an experimental animal.
Figure 5. High-grade glioma in a human subject participant.
Figure 6. FGln avidity in high-grade vs. low-grade glioma

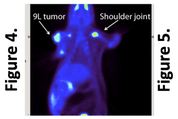


Figure 4.

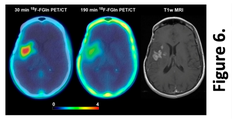
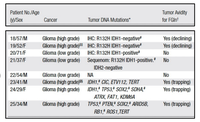


Figure 5.



Patient No./Age /p/Sex	Cancer	Tumor DNA Mutations*	Tumor Acidity for FGln†
18/57.M	Glioma (high grade)	IHC: R132H IDH1-negative#	Yes (declining)
19/62.F	Glioma (high grade)#	IHC: R132H IDH1-negative#	Yes (declining)
20/71.F	Glioma (low grade)	IHC: R132H IDH1-positive#	No
21/37.F	Glioma (low grade)	Sequenom: R132H IDH1-positive,# IDH2-negative	No
22/54.M	Glioma (low grade)	NA	No
23/41.M	Glioma (high grade)#	IDH1.? CIC, ETV1/12, TERT,	Yes (dropping)
24/29.F	Glioma (high grade)	IDH1.? TP53,? SOX2,? SDHA,? ATRX, FAT1, KDM6A	Yes (dropping)
25/34.M	Glioma (high grade)	TP53,? PTEN,? SOX2,? ARID1B, NBN,? RUNX1, TERT	Yes (dropping)

Figure 6.

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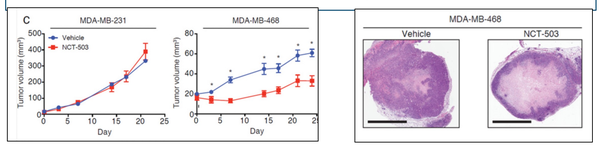


Figure 7. SSP-high cell lines inhibited by PHGDH inhibitor

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Revised Title: Validation of an [18F]Fluoroglutamine PET Radiotracer for Detection of p53-Mutant Breast Cancer through Incorporation into the Serine Synthesis Pathway

*Figures 2-7 adapted from references, above

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