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Sensitivity of Breast Cancer Stem Cells to TRA-8 Anti-DR5 Monoclonal Antibody

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14. ABSTRACT Basal-like breast cancers (BLBCs) generally become resistant to cytotoxic agents and resistance has been attributed to the presence of tumor initiating cancer stem cells (CSCs). Furthermore, LRP6/Wnt appears to play a crucial role in BLBC and CSC progression, and may represent an excellent therapeutic target. We have previously described that TRA-8, a monoclonal antibody specific to death receptor 5, kills both the CSCs and non-CSC population of BLBCs. This study examined two questions: whether niclosamide (an FDA approved anthelmintic, that inhibits Wnt/ β -catenin signaling) is cytotoxic to BLBCs and its CSC population; and whether niclosamide in combination with TRA-8 produces synergistic cytotoxicity. We characterized non-adherent tumorsphere enriched (NATE) cells as a CSC enriched population from BLBC cell lines. Both Adherent and NATE cells from 2LMP, SUM159, HCC1187, HCC1143 cell lines and patient pleural effusion samples showed that niclosamide inhibited Wnt/ β -catenin pathway activation, down regulated LRP6, and decreased downstream β -catenin signaling. The combination of TRA-8 and niclosamide showed additive to synergistic cytotoxicity and further reduced Wnt/ β -catenin activity. <i>In vivo</i> studies also showed that intraperitoneal administration of niclosamide in combination with TRA-8 suppressed growth of established 2LMP orthotopic tumor xenografts. Treatment with niclosamide in combination with TRA-8 may be an effective therapy against BLBC.					
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Introduction

Basal-Like Breast Cancer (BLBC) accounts for 13% of all breast carcinomas [1]. It is characterized by a unique mRNA profile with CK5/6 expression, inactivation of *BRCA1* and lack of estrogen receptor and HER-2 amplification [1-3]. BLBC is considered one of the most aggressive, metastatic, and chemoresistant breast cancer subtypes [4]. Its poor prognosis is linked to enrichment for tumor initiating cancer stem cells (CSC) [5]. The *Cancer Stem Cell Model* suggests that tumors, similar to normal tissue, are organized in a cellular hierarchy, with (CSC) at the top, as the cells with potentially limitless proliferation abilities [6]. The more 'differentiated' descendants, which account for the majority of the tumor population, may also be able to proliferate, but regenerative ability is limited [6]. Traditional chemotherapy agents target these differentiated cells, but unfortunately fail to kill the stem cell progenitor population [7]. The chemoresistant stem cells are thought to be responsible for relapse and metastasis of many tumor types [7]. Several investigators have shown that BLBC cell lines and patient samples contain a subpopulation of breast cancer stem cells (BrCSC) [8, 9]. These BrCSC are identified based on their enhanced tumorigenicity, tumorsphere forming ability, expression of CD44⁺/CD24⁻, elevated enzymatic activity of aldehyde dehydrogenase (ALDH), and dysregulation of self-renewing pathways, including Wnt, Hedgehog, and Notch signaling [10, 11]. BrCSC also overexpress ABC efflux transporters, detoxification enzymes, and have slower turnover rate that make this sub-population likely to become resistant to chemotherapy [12, 13]. Thus an effective BLBC therapeutic strategy must kill these propagating and chemoresistant BrCSC in addition to the proliferating non-stem cell cancer population [14].

One promising approach to prevent BLBC recurrence and metastasis is to target pathways that regulate tumor initiation such as the Wnt/ β -catenin pathway [4, 15]. A cell surface receptor called LRP6, essential for Wnt/ β -catenin signaling, is a potential target because its expression is frequently up-regulated in 20-36% of human breast cancers. Furthermore, suppression of LRP6 is sufficient to inhibit Wnt signaling in breast cancer [16]. Specifically in BLBC, LRP6 is expressed at elevated levels not seen in any other breast cancer subtype, thus providing an excellent potential target for treatment [15-18]. The Wnt/ β -catenin pathway can be inhibited by Niclosamide, which degrades LRP6 and interacts with Frizzled thus preventing proliferation and causing apoptosis [19-21]. Niclosamide (trade name Niclocide) is a teniacide in the antihelminth family that has been FDA approved for the treatment of tapeworms and has been used in humans for nearly 50 years [22-24]. Niclosamide has also been shown to be cytotoxic against prostate and colorectal cancer, myelogenous leukemia, and in ovarian cancer it suppresses CSC and metastasis [21, 25-27]. TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) also inhibits Wnt/ β -catenin signaling by promoting caspase 3 and 8 mediated cleavage of β -catenin [28, 29]. TRAIL also induces apoptosis in BLBC, however, these promising aspects of TRAIL have been mitigated by the hepatic cytotoxicity seen with some TRAIL preparations [30, 31]. TRA-8 is a non-hepatotoxic agonistic monoclonal antibody (mAb) to TRAIL death receptor 5 (DR5) [32, 33]. BLBC, are preferentially sensitive to TRA-8 induced apoptosis; however, some BLBC cells lines are resistant to treatment [34]. My hypothesis is that suppression of Wnt activity by Niclosamide will further sensitize BLBC and its CSC population to treatment with TRA-8.

Specific Aims

Aim 1. To isolate CD44⁺/CD24⁻/ALDEFLUOR⁺ CSC from established human breast cancer cell lines and patient plural effusion samples and evaluate their functional characteristics such as tumorigenicity, tumorsphere formation and chemoresistance.

Aim 2. To evaluate *in vitro* and *in vivo* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines.

Aim 3. To evaluate *in vitro* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines and patient samples in combination with the Wnt inhibitor, Niclosamide.

Aim 4. To evaluate the *in vivo* therapeutic efficacy of anti-DR5 in combination with Niclosamide in xenograft models.

Body

Aim #1:

Task 1. To isolate CD44⁺/CD24⁻/ALDEFLUOR⁺ CSC from established human breast cancer cell lines and patient plural effusion samples and evaluate their functional characteristics such as tumorigenicity, tumorsphere formation and chemoresistance.

Task 1a. Analyze sorted BrCSC population for retention of BrCSC markers (months 1-5)

Task 1b. Functional BrCSC assays (months 6-10)

Task 1a and 1b have been completed. Detailed results were reported in the 2011 annual report. The summary of previous results pertinent for this report are the identification and characterization of non-adherent tumorsphere enriched (NATE) cells from BLBC cell lines. NATE cells were identified from freshly formed tumorspheres and were compared to adherent cells that were sorted for the CSC marker, ALDH⁺, at 6, 12 and 24 h. From this, NATE cells were identified within the newly formed tumorspheres. NATE cells showed 70-80% enrichment for ALDH activity at the 12 h time point compared to sorted ALDH⁺ cells. In the 2LMP, SUM159, and HCC1143 cell lines, ALDH activity was lost over time for both sorted and NATE cells (Fig. 1A). NATE cells (obtained from the tumorspheres at the 12 h time point) showed an enhanced ability to form tumors after mammary fat pad injection (MFP) injection of 20,000 cells in NOD/SCID mice compared to non-adherent cells (obtained from tumorspheres after they had been in non-adherent culture for 4 days). The NATE cells formed aggressive large tumors faster than the non-adherent cells (Fig. 1B) (p=0.01). NATE cells were generated as a tool to analyze the effect of drugs on CSC enriched populations. Because CSCs represent a small fraction of the total population, it is difficult to run mechanistic studies with these cells especially by techniques such as Western blot and TOPflash reporter assay that require a large number of cells. The isolation of NATE cells allowed us to test these novel drug combinations on a larger scale. Further studies need to be conducted to better understand what epigenetic alterations such as EMT/MET are occurring to enrich for this transient CSC population [35]. Non-adherent cells alone do not represent an aggressive population. Also, patient pleural effusion samples were characterized by immunohistochemistry for positive Moc31 staining (adenocarcinoma) and negative Calretinin staining (mesothelial cells) (Figure 2).

Aim #2:

To evaluate *in vitro* and *in vivo* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines.

Aim 2 has been completed. Detailed results were reported in the 2011 annual report and in a published manuscript [36].

Aim #3:

To evaluate *in vitro* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines and patient samples, alone and in combination with the Wnt inhibitor, Niclosamide.

Task 3a. Evaluate Niclosamide cytotoxicity in BLBC

NATE adherent cell lines and patient pleural effusion samples were treated with Niclosamide for 48 h. NATE cells responded to a lower dose range (0.11-0.35 μ M) of Niclosamide treatment compared to adherent cells (0.31-0.54 μ M). However, both NATE and adherent cells had IC₅₀ values of 1 μ M or less. Three of four NATE cell lines (SUM159, HCC1187 and HCC1143 but not 2LMP) had significantly lower IC₅₀ values compared to the adherent parental cell lines (p-values = 0.015, 0.0004 and 0.0002) (Table 1). MCF10A nonmalignant mammary epithelial cells did not show significant sensitivity to 48 h treatment with Niclosamide (Fig. 3). All four patient pleural effusion samples showed greater than 50% reduction in viability. Patient sample UAB05 did not show a dose response past 50% and patient sample UAB04 reached 70% reduction at the lowest dose but also did not show a dose response. UAB03 and UAB01 both reached a 70% reduction in viability with the lowest doses of 1 μ M and showed a dose response with two-fold increasing concentrations of Niclosamide (Fig. 4C).

Task 3b. Niclosamide inhibition of Wnt/ β -catenin in BLBC and NATE cells

Active Wnt/ β -catenin signaling causes increased activation of TCF/LEF transcription factors. To confirm the inhibitory effect of Niclosamide on the Wnt/ β -catenin pathway, we performed the signaling TOPflash luciferase reporter assay to test for inhibition of Wnt/ β -catenin activity. 2LMP, SUM159, HCC1187 and HCC1143 adherent cells were transiently transfected with TOPflash and treated with Niclosamide as shown in Figure 4A. Niclosamide significantly blocked Wnt/ β -catenin activity at levels greater than 60% in 2LMP, SUM159 and HCC1143 cell lines (p values = 0.0005, 0.002, 0.006). HCC1187 Wnt/ β -catenin TOPflash activity was only significantly inhibited in the presence of Wnt3A ligand (data not shown). Inhibition of Wnt/ β -catenin was confirmed by Western blot analysis by examining the total levels of Wnt co-receptor LRP6, phosphorylated LRP6, free β -catenin, total β -catenin and Axin2 after 24 h treatment with Niclosamide (0.5, 0.25, 0.125 μ M) on 2LMP adherent and NATE cells (Fig. 4B). As shown in Figure 2B, increasing doses of Niclosamide reduced total LRP6 and p-LRP6 expression in both adherent and NATE cells for both 2LMP and HCC1187 cell lines. There was a dose-dependent reduction in the expression of total and free β -catenin with increasing levels of Niclosamide. Total and free β -catenin expression was inhibited in NATE cells with a 0.25 μ M concentration of Niclosamide. Axin2 is a specific transcriptional target of the Wnt/ β -catenin signaling pathway [37]. Increasing doses of Niclosamide in both adherent and NATE cells caused a reduction in Axin2. Niclosamide also showed inhibition of the Wnt/ β -catenin pathway in patient sample UAB03 (Fig. 4D).

Task 3c. *In vitro* combination treatment with TRA-8 and Niclosamide

All 4 BLBC cell lines showed inhibition of secondary tumorsphere formation, with Niclosamide concentrations resulting in half the maximal dose required for tumorsphere inhibition (IT₅₀) of 0.25 μ M for 2LMP, 0.09 μ M for HCC1143, and 0.175 μ M for both HCC1187 and SUM159 (Fig. 5A). Pre-treatment with Niclosamide followed by treatment with TRA-8 resulted in increased inhibition of secondary tumorsphere formation. Niclosamide and TRA-8 combined inhibited 80-90% of secondary tumorsphere formation relative to an average of 60% by Niclosamide alone and 40% by TRA-8 as single agent (p-value < 0.00007) (Fig. 5B). Treatment with Niclosamide alone disrupted tumorsphere formation, with the most significant amount of disruption seen in the HCC1143 cell line (Fig. 5C). Adherent 2LMP, SUM159, HCC1143 and HCC1187 cells were treated with Niclosamide and TRA-8 for 24 h and this resulted in decreased Wnt/ β -catenin activity compared to untreated cells. For the 2LMP, SUM159 and HCC1143 cell lines, either agent alone produced greater than 50% reduction in TOPflash activity and the combination resulted in significantly greater reduction compared to Niclosamide treatment alone. The HCC1187 cell line treated with 0.25 ng/mL TRA-8 and 0.25 μ M Niclosamide, only showed significant inhibition of Wnt activity compared to untreated controls (Fig. 6).

Task 3d. Confirm and further elucidate the mechanism of anti-Wnt and anti-DR5 pathway interaction. Develop shRNA LRP6 knockdown of 2LMP and HCC1187. Observe if shRNA knockdown of LRP6 in cell lines produces enhanced sensitivity to TRA-8 treatment. Investigate expression of Survivin and death receptor 5 after treatment with Niclosamide. Detect by Western-Blot analysis β -catenin degradation after treatment with TRA-8.

To be completed

Aim #4:

To evaluate the *in vivo* therapeutic efficacy of anti-DR5 in combination with Niclosamide in xenograft models.

Task 4a. *Ex vivo*

Effect of *ex vivo* treatment of BrCSC enriched cells on tumorigenicity in NOD/SCID mice. 2LMP and SUM159 tumorspheres were treated with TRA-8, Niclosamide, or the combination and IgG control antibody for 3 h and implanted into the MFP of groups of five NOD/SCID mice.

Table 2 represents the frequency of tumor engraftment and the average tumor size of the tumors that developed. In the 2LMP cell line 4 of 5 small, slow growing tumors were observed to develop within 44 days with TRA-8 and Niclosamide treated cells while 5 of 5 large tumors developed in the IgG control group. With the SUM159 cell line, 3 of 5 tumors developed in the control group, 1 of 5 in the TRA-8 group, 2 of 5 in the Niclosamide treated group, and 0 of 5 tumors developed in the combination group at 46 days after implantation.

Task 4b. *In vivo* treatment with TRA-8 and Niclosamide

Niclosamide in combination with TRA-8 inhibited 2LMP tumor growth *in vivo*. Synergistic cytotoxicity was observed *in vitro* suggesting that the combination would suppress tumor growth *in vivo*. To test this hypothesis, 4-week-old female athymic nude mice were injected in the MFP with 2×10^6 2LMP cells. After a 7-day period, tumors reached 16 mm² average size and mice were treated intraperitoneal with TRA-8 (200 ng) and or Niclosamide (12.5 mg/kg). As shown

in Figure 7, Niclosamide did not inhibit tumor growth at this dose but when administered in combination with TRA-8 there was a significantly reduced tumor size (p-value = 0.001).

Task 4c. *In vivo* treatment using various doses of Niclosamide in both 2LMP and HCC1187 BLBC orthotopic tumor animal models.

To be completed

Key research accomplishments:

- BrCSC marker expression is maintained for ~24 h after sorting and cells grown in mammosphere media/low attachment plates acquire elevated levels of ALDH activity by 12 h. This transient population was named non-adherent tumorsphere enriched (NATE).
- TRA-8 is not cytotoxic to normal breast cells or breast stem cells.
- BrCSC enriched populations have DR5 expression similar to parental cells.
- TRA-8 treatment of basal-like BrCSC populations activates caspase 8 and 3.
- TRA-8 treatment of BrCSC populations dramatically inhibits tumorsphere formation.
- BrCSC from 5 out of 8 cell lines were significantly more sensitive to TRA-8 than parental cells.
- Tumorigenicity of BrCSC was inhibited by treatment with TRA-8.
- Adherent and NATE cells from 2LMP, SUM159, HCC1187 and HCC1143 cell lines and patient pleural effusions showed that Niclosamide inhibited Wnt/ β -catenin pathway activation, down regulated LRP6, and decreased downstream β -catenin signaling.
- MCF10 non-malignant cell line did not respond to treatment with Niclosamide.
- The combination of TRA-8 and Niclosamide showed additive to synergistic secondary tumorsphere inhibition and further reduced Wnt/ β -catenin activity.
- *Ex vivo* studies using 2LMP and SUM159 cell lines showed the combination treatment with TRA-8 and Niclosamide produced greater inhibition of tumorigenicity.
- *In vivo* studies showed that intraperitoneal administration of Niclosamide in combination with TRA-8 produced increased growth suppression of established 2LMP orthotopic tumor xenografts.

Reportable outcomes:

Publications related to aims:

1. Lin C, Lu W, Zhang W, **Londoño-Joshi AI**, Buchsbaum DJ, Bu G, Li Y: The C-terminal region Mesd peptide mimics full-length Mesd and acts as an inhibitor of Wnt/ β -catenin signaling in cancer cells. *PLoS ONE* In press 2013.
2. **Londoño-Joshi AI**, Oliver PG, Li Y, Lee CH, Forero-Torres A, LoBuglio AF, Buchsbaum DJ: Basal-like breast cancer stem cells are sensitive to anti-DR5 mediated cytotoxicity. *Breast Cancer Res Treat* 133:437-445, 2012.

Publications not related to aims:

1. Swindall AF, **Londoño-Joshi AI**, Schultz MJ, Fineberg N, Buchsbaum DJ, Bellis SL: ST6Gal-I protein expression is upregulated in human epithelial tumors and correlates with stem cell markers in normal tissues and colon cancer cell lines. *Cancer Res* In press 2013.

2. Whitworth JM, **Londoño-Joshi AI**, Sellers JC, Oliver PG, Muccio DD, Atigadda VR, Straughn JM Jr, Buchsbaum DJ: The impact of novel retinoids in combination with platinum chemotherapy on ovarian cancer stem cells. *Gynecol Oncol* 125:226-230, 2012.
3. Bevis KS, McNally LR, Sellers JC, Della Manna D, **Londoño-Joshi AI**, Amm H, Straughn JM Jr, Buchsbaum DJ: Anti-tumor activity of an anti-DR5 monoclonal antibody, TRA-8, in combination with taxane/platinum-based chemotherapy in an ovarian cancer model. *Gynecol Oncol*, 121:193-199, 2011.

Awards:

1. AACR Minority Scholar in Cancer Research Award- 2013
2. Susan G. Komen for the Cure Travel Scholarship - AACR Annual Conference, Chicago, 2012.

Other:

1. Selected to serve on the Metastasis Research Society Council, International, 2013.
2. Attended the 3rd International Conference on Stem Cells and Cancer, New Delhi, India 2012.
3. AACR representative at One Voice Against Cancer Hill Day, Washington DC, 2012.
4. Attended annual meeting of the AACR, Chicago, IL 2012.
5. Please see additional reportable outcomes in *Curriculum Vitae* attached as Appendix A.

Conclusion:

Results described in this report indicate that targeting β -catenin in BLBC might be an effective means of increasing TRA-8 sensitivity. Combining TRA-8 with Niclosamide is an approach to ameliorate TRA-8 resistance in cancer cells by antagonizing the β -catenin pathway. Niclosamide is a well-studied drug in humans and is relatively inexpensive compared to other chemotherapeutic agents. The combination of these two well-tolerated drugs can be quickly translated into clinical trials, potentially helping BLBC patients who have very few treatment options available to them. This study provides valuable pre-clinical research results that gives important insight into using these novel agents in clinical trials in patients with BLBC.

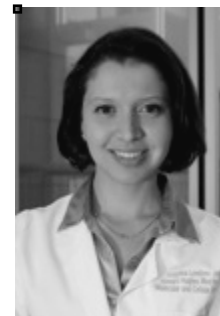
References:

1. Liedtke, C., A.-M. Gonzalez-Angulo, and L. Pusztai, *Definition of Triple-Negative Breast Cancer and Relationship to Basal-Like Molecular Subtype*. PPO Updates Prin. Prac. Onc, 2010. **24**(2): p. 1-6.
2. Nielsen, T.O., et al., *Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma*. Clin Cancer Res, 2004. **10**(16): p. 5367-74.
3. Lakhani, S.R., et al., *Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype*. Clin Cancer Res, 2005. **11**(14): p. 5175-80.
4. Rakha, E.A., J.S. Reis-Filho, and I.O. Ellis, *Basal-like breast cancer: a critical review*. J Clin Oncol, 2008. **26**(15): p. 2568-81.
5. Honeth, G., et al., *The CD44+/CD24- phenotype is enriched in basal-like breast tumors*. Breast Cancer Res, 2008. **10**(3): p. R53.
6. Dontu, G., *Breast cancer stem cell markers - the rocky road to clinical applications*. Breast Cancer Res, 2008. **10**(5): p. 110.
7. Fillmore, C.M. and C. Kuperwasser, *Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy*. Breast Cancer Res, 2008. **10**(2): p. R25.
8. Giatromanolaki, A., et al., *The CD44+/CD24- phenotype relates to 'triple-negative' state and unfavorable prognosis in breast cancer patients*. Med Oncol.
9. Charafe-Jauffret, E., et al., *Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature*. Cancer Research, 2009. **69**(4): p. 1302-13.
10. Al-Hajj, M., et al., *Prospective identification of tumorigenic breast cancer cells*. Proc Natl Acad Sci U S A, 2003. **100**(7): p. 3983-8.
11. Ginestier, C., et al., *ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome*. Cell Stem Cell, 2007. **1**(5): p. 555-567.
12. Dean, M., *ABC transporters, drug resistance, and cancer stem cells*. J Mammary Gland Biol Neoplasia, 2009. **14**(1): p. 3-9.
13. Dave, B. and J. Chang, *Treatment resistance in stem cells and breast cancer*. J Mammary Gland Biol Neoplasia, 2009. **14**(1): p. 79-82.
14. Chuthapisith, S., et al., *Breast cancer chemoresistance: emerging importance of cancer stem cells*. Surg Oncol. **19**(1): p. 27-32.
15. King, T.D., M.J. Suto, and Y. Li, *The Wnt/beta-catenin signaling pathway: a potential therapeutic target in the treatment of triple negative breast cancer*. J Cell Biochem, 2012. **113**(1): p. 13-8.
16. Liu, C.C., et al., *LRP6 overexpression defines a class of breast cancer subtype and is a target for therapy*. Proc Natl Acad Sci U S A, 2010. **107**(11): p. 5136-41.
17. DiMeo, T.A., et al., *A novel lung metastasis signature links Wnt signaling with cancer cell self-renewal and epithelial-mesenchymal transition in basal-like breast cancer*. Cancer Res, 2009. **69**(13): p. 5364-73.
18. Lindvall, C., et al., *The Wnt co-receptor Lrp6 is required for normal mouse mammary gland development*. PLoS One, 2009. **4**(6): p. e5813.
19. Lu, W., et al., *Niclosamide suppresses cancer cell growth by inducing Wnt co-receptor LRP6 degradation and inhibiting the Wnt/beta-catenin pathway*. PLoS One, 2011. **6**(12): p. e29290.

20. Chen, M., et al., *The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling*. Biochemistry, 2009. **48**(43): p. 10267-74.
21. Osada, T., et al., *Antihelminth compound niclosamide downregulates Wnt signaling and elicits antitumor responses in tumors with activating APC mutations*. Cancer Res, 2011. **71**(12): p. 4172-82.
22. Tanowitz, H.B., L.M. Weiss, and M. Wittner, *Diagnosis and treatment of intestinal helminths. I. Common intestinal cestodes*. Gastroenterologist, 1993. **1**(4): p. 265-73.
23. Craig, P. and A. Ito, *Intestinal cestodes*. Curr Opin Infect Dis, 2007. **20**(5): p. 524-32.
24. Merschjohann, K. and D. Steverding, *In vitro trypanocidal activity of the anti-helminthic drug niclosamide*. Exp Parasitol, 2008. **118**(4): p. 637-40.
25. Sack, U., et al., *Novel effect of antihelminthic Niclosamide on S100A4-mediated metastatic progression in colon cancer*. J Natl Cancer Inst, 2011. **103**(13): p. 1018-36.
26. Yo, Y.T., et al., *Growth inhibition of ovarian tumor-initiating cells by niclosamide*. Mol Cancer Ther, 2012. **11**(8): p. 1703-12.
27. Jin, Y., et al., *Antineoplastic mechanisms of niclosamide in acute myelogenous leukemia stem cells: inactivation of the NF-kappaB pathway and generation of reactive oxygen species*. Cancer Res, 2010. **70**(6): p. 2516-27.
28. Senthivinayagam, S., et al., *Caspase-mediated cleavage of beta-catenin precedes drug-induced apoptosis in resistant cancer cells*. J Biol Chem, 2009. **284**(20): p. 13577-88.
29. Cantarella, G., et al., *TRAIL-related neurotoxicity implies interaction with the Wnt pathway in human neuronal cells in vitro*. J Neurochem, 2008. **105**(5): p. 1915-23.
30. Rahman, M., et al., *TRAIL induces apoptosis in triple-negative breast cancer cells with a mesenchymal phenotype*. Breast Cancer Res Treat, 2009. **113**(2): p. 217-30.
31. Higuchi, H., et al., *Cholestasis increases tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-R2/DR5 expression and sensitizes the liver to TRAIL-mediated cytotoxicity*. J Pharmacol Exp Ther, 2002. **303**(2): p. 461-7.
32. Ichikawa, K., et al., *Tumoricidal activity of a novel anti-human DR5 monoclonal antibody without hepatocyte cytotoxicity*. Nat. Med., 2001. **7**: p. 954-960.
33. Buchsbaum, D.J., et al., *Antitumor efficacy of TRA-8 anti-DR5 monoclonal antibody alone or in combination with chemotherapy and/or radiation therapy in a human breast cancer model*. Clin. Cancer Res., 2003. **9**: p. 3731-3741.
34. Oliver, P.G., et al., *Effect of anti-DR5 and chemotherapy on basal-like breast cancer*. Breast Cancer Res Treat, 2012. **133**(2): p. 417-26.
35. Morrison, B.J., J.C. Steel, and J.C. Morris, *Sphere culture of murine lung cancer cell lines are enriched with cancer initiating cells*. PLoS One, 2012. **7**(11): p. e49752.
36. Londono-Joshi, A.I., et al., *Basal-like breast cancer stem cells are sensitive to anti-DR5 mediated cytotoxicity*. Breast Cancer Res Treat, 2012. **133**(2): p. 437-45.
37. Leung, J.Y., et al., *Activation of AXIN2 expression by beta-catenin-T cell factor. A feedback repressor pathway regulating Wnt signaling*. J Biol Chem, 2002. **277**(24): p. 21657-65.

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EDUCATION

University of Alabama at Birmingham (UAB) Ph.D. Candidate Department of Pathology/ Division of Molecular and Cellular Pathology Howard Hughes Medical Institute (HHMI) Med-Grad Predoctoral Fellow Expected date of graduation, May 2013 GPA: 4.0/4.0	2007-present
University of Wisconsin-Madison, College of Agricultural Life Science B.S. in Natural Sciences Major: Genetics Graduated May 2006 GPA: 3.5/4.0 Dean's List 2003, 2004, 2005, 2006	2002-2006
Study Abroad-Universidad Complutense, Madrid, Spain Trans-Atlantic Student Science Exchange Program	2005
Study Abroad-University of California, Santa Barbara Extended Learning Wildland Studies in Melbourne, Australia	2003

AWARDS

• AACR Minority Scholar in Cancer Research Award	2013
• Department of Defense Breast Cancer Research Program Predoctoral Fellowship	2011-present
• UAB Comprehensive Cancer Center - John R. Durant Award for Excellence in Cancer Research-Graduate Student Category	2011
• UAB Department of Pathology - Betty Spencer Pritchett Award for Outstanding Cancer Research	2011
- AACR-Pancreatic Cancer Special Conference, Lake Tahoe, NV, travel award	2012
• Susan G. Komen for the Cure Travel Scholarship - American Association for Cancer Research (AACR) annual conference Chicago, IL	2012
- AACR annual conference Washington, DC	2010
• Howard Hughes Medical Institute (HHMI) - HHMI Med-Grad Predoctoral Fellowship	2007-present
- Science Education Alliance Teaching Assistant Award	2011
- Genomic Analysis Training Janelia Farm, VA, travel award	2010
- Translational Medicine Symposium, HHMI Peer Cluster Meeting, travel award	2010
- AACR Annual Conference- Denver, CO, travel award	2009
• McNair Scholars Graduate School Program	2004-2006
• Pathway Scholars Program Summer Research Internship	2004
• Undergraduate Research Scholar	2002-2004
• College of Agricultural Life Science Honors Program	2002-2005
• Exxon Mobil Mathematics Youth Award-Hispanic Heritage Awards Foundation	2002
• POSSE Foundation Four-Year Full Tuition Undergraduate Scholarship	2001-2006

RESEARCH EXPERIENCE

UAB School of Medicine Division of Radiation Biology Predoctoral work in the lab of Dr. Donald J. Buchsbaum <i>Treatment of Breast Cancer Stem Cells with TRA-8 anti-DR5 Monoclonal Antibody</i>	2009-present
UAB Department of Cellular Molecular Biology Spring Rotation with Dr. Christopher Klug <i>Development of a syngeneic luciferase-pancreatic cancer model system for in vivo visualization of tumor progression</i> <i>Gene Expression Profiling to Identify Novel Cell-Surface Antigens on Malignant Pancreatic Ductal Epithelium</i>	2008
UAB Department of Pathology Division of Molecular and Cellular Pathology Winter Rotation with Dr. Selvarangan Ponnazhagan <i>Chromatin Insulators and their Potential Use in rAAV Transgene Expression</i>	2008
UAB Department Immunology and Molecular and Cellular Biology Fall Rotation with Dr. Zedenk Hel <i>Induction of Long-Term Immunity via Transplantation of Antigen- Expressing Hematopoietic Stem Cells</i>	2007
UAB- Gene Therapy Center Summer Rotation with Dr. David Curiel and Dr. Larisa Pereboeva <i>Overexpression of HOXB4 in Mesenchymal Stem Cells</i>	2007
University of Wisconsin-Madison, Department of Radiology and Human Oncology Undergraduate research mentor, Dr. Jamey Weichert <i>Validation of Non-Invasive Tissue Distribution by MicroPET Scanning In Mice</i> <i>PLD Expression in Breast Cancer Tumor Cells Versus Normal Cells</i> <i>In vivo Cell Trafficking of Metastasis of Colon Cancer</i>	2003-2006
University of Wisconsin-Madison, Department of Biochemistry HHMI investigator Dr. Judith Kimble <i>The Role of F17A9.3 in Germ Line Development of C. Elegans</i>	2002-2003

PUBLICATIONS

- * Contributed equally
1. **Londoño-Joshi AI**, Buchsbaum DJ: Inhibition of Wnt co-receptor LRP6 sensitizes basal-like breast cancer stem cells to anti-DR5 monoclonal antibody alone or in combination with chemotherapy. *In preparation*
 2. Fauci JM, **Londoño-Joshi AI**, Sellers J, Oliver P, Della Manna D, Aristizabal L, Straughn JM Jr, Wang YY, Sabbatino F, Ferrone S, Buchsbaum DJ: Monoclonal antibody-based immunotherapy of ovarian cancer: targeting ovarian cancer cells with the B7-H3-specific mAb 376.96. *In preparation*
 3. Swindall AF, **Londoño-Joshi AI**, Schultz MJ, Fineberg N, Buchsbaum DJ, Bellis SL: ST6Gal-I protein expression is highly upregulated in human epithelial tumors and correlates with stem cell markers in normal tissues and colon cancer cell lines *accepted in Cancer Res January 2013*
 4. Lin C, Lu W, Zhang W, **Londoño-Joshi AI**, Buchsbaum DJ, Bu G, Li Y: The C-terminal region Mesd peptide mimics full-length Mesd and acts as an inhibitor of Wnt/ β -catenin signaling in cancer cells. *PLoS ONE* In press 2013.
 5. Whitworth JM, **Londoño-Joshi AI**, Sellers JC, Oliver PG, Muccio DD, Atigadda VR, Straughn JM Jr, Buchsbaum DJ: The impact of novel retinoids in combination with platinum chemotherapy on ovarian cancer stem cells. *Gynecol Oncol* 125:226-230, 2012

6. **Londoño-Joshi AI**, Oliver PG, Li Y, Lee CH, Forero-Torres A, LoBuglio AF, Buchsbaum DJ: Basal-like breast cancer stem cells are sensitive to anti-DR5 mediated cytotoxicity. *Breast Cancer Res Treat* 133:437-445, 2012
7. Bevis KS, McNally LR, Sellers JC, Della Manna D, **Londoño-Joshi AI**, Amm H, Straughn JM, Jr, Buchsbaum DJ: Anti-tumor activity of an anti-DR5 monoclonal antibody, TRA-8, in combination with taxane/platinum-based chemotherapy in an ovarian cancer model. *Gynecol Oncol*, 121:193-199, 2011

ORAL PRESENTATIONS

**Invited Speaker*

1. *Investigation of death receptor-5 mediated apoptosis breast cancer stem cells. Hematology and Oncology Seminar Series, Birmingham, AL, 2010
2. *Investigation of death receptor-5 mediated apoptosis in basal-like breast cancer stem cells. Science Hour, Department of Radiation Oncology, Birmingham, AL, 2010
3. *Sensitivity of basal-like breast cancer stem cells to death receptor-5 mediated apoptosis. Komen Breast Cancer Research Roundtable, Birmingham, AL, 2010
4. Treatment of triple negative metastatic breast cancer stem cells with TRA-8 anti-death receptor 5 monoclonal antibody. Graduate Student Research Day, Birmingham, AL, 2009
5. Treatment of triple negative metastatic breast cancer with TRA-8 anti death receptor 5 (DR5) monoclonal antibody. Pathology Seminar, Birmingham, AL 2009
6. Development of a luciferase-expressing pancreatic cancer cell line. Pathology Seminar, Birmingham AL, 2008
7. Chromatin insulators and their potential use in rAAV transgene expression. Pathology Seminar, Birmingham, AL, 2008
8. Induction of long-term immunity via transplantation of antigen- expressing hematopoietic stem cell. Pathology Seminar, Birmingham, AL, 2007
9. Validation of non invasive tissue distribution by MicroPET scanning in mice, Undergraduate Symposium, Madison, WI, 2006
10. Phospholipase-D expression in breast cancer tumor cells versus normal cells. Pathways Scholars Symposium, Madison, WI, The National McNair Scholars Symposium, Berkeley, CA, 2004
11. Evaluating metastasis characteristics of CT-26 Cell with florescence labeled NM404. The Undergraduate Symposium, Madison, WI, 2004
12. F17A9.3 role in C. Elegans germ line development. The Undergraduate Research Scholar presentations, Madison, WI, 2002

POSTER PRESENTATIONS

1. **Londoño-Joshi AI**, Forero-Torres A, Fineberg NS, Oliver PG, Zhou T, LoBuglio AF, Buchsbaum DJ: Basal-like breast cancer stem cells are sensitive to combination treatment with anti-DR5 monoclonal antibody and chemotherapy
 - *Center for Clinical and Translational Science*, Birmingham, AL, 2011
 - *Proc Comprehensive Cancer Center 2011 Annual Research Retreat*, Birmingham, AL, 2011
 - *Pathology Research Day*, Birmingham, AL, 2011

2. **Londoño-Joshi AI**, Monti DL: Reading, writing, and arithmetic in the UAB phage explorations lab. *Science Education Alliance*, Janelia Farm, VA, 2001
3. **Londoño-Joshi AI**, Forero-Torres A, Fineberg NS, Oliver PG, Zhou T, LoBuglio AF, Buchsbaum DJ: Sensitivity of breast cancer stem cells to TRA-8 anti-DR5 monoclonal antibody
 - *AACR National Conference*, Washington, DC, 2010
 - *HHMI Southern Regional Conference*, Chapel Hill, NC, 2010
4. **Londoño-Joshi AI**, Forero-Torres A, Fineberg NS, Oliver PG, Zhou T, LoBuglio AF, Buchsbaum DJ: Treatment of triple negative breast cancer stem cells with TRA-8 anti-DR5 monoclonal antibody and 4HPR. *Molecular and Cellular Pathology Research Day*, Birmingham, AL, 2009
5. **Londoño-Joshi AI**, Forero-Torres A, Fineberg NS, Oliver PG, Zhou T, LoBuglio AF, Buchsbaum DJ: Treatment of triple negative breast cancer stem cells with TRA-8 anti-DR5 monoclonal antibody. *Stem Cell Symposium and Cancer Center Retreat*, Birmingham, AL, 2009
6. **Orozco AI**, Klug K: Gene expression profiling to identify novel cell-surface antigens on malignant pancreatic ductal epithelium. *Howard Hughes Med-to Grad Regional Conference*, Birmingham, AL, 2008

ABSTRACTS: non-presenting author

1. **Londoño-Joshi AI**, Della Manna DL, Ravi S, Oliver PG, Gillespie Y, Buchsbaum DJ: Treatment of glioblastoma cells with TRA-8 and AT-406. *The 2012 Brain SPORE Roundtable Meeting*, AZ, 2012
2. Ravi S, **Londoño-Joshi AI**, Oliver PG, Gillespie Y, Buchsbaum DJ: Characterization and treatment of glioma stem-like cells with temozolomide and TRA-8 combination therapy. *Patho-biology and Molecular Medicine Symposium*, Birmingham, AL, 2011
3. Whitworth JM, **Londoño-Joshi AI**, Sellers JC, Oliver PG, Muccio DD, Atigadda VR, Straughn JM, Jr, Buchsbaum DJ: The impact of novel retinoids in combination with platinum chemotherapy on ovarian cancer stem cells. *Proc Comprehensive Cancer Center 2011 Annual Research Retreat*, Birmingham, AL, 2011
4. Aristizabal A, **Londoño-Joshi AI**, Forero-Torres A, LoBuglio AF, Buchsbaum DJ: Characterization of CD44⁺/CD24⁻/ALDH⁺ Marker expression as a model for drug development against basal-like breast cancer tumor initiating cells. *Undergraduate Research Expo*, Birmingham, AL, 2011
5. Ahmed A, Cain G, Clark D, Duckworth M, Dunkerley C, Johnston C, Mentreddy A, Nance C, **Londoño-Joshi AI**, Monti DL: Characterization of novel mycobacteriophages isolated from Alabama environmental samples. *Undergraduate Research Expo*, Birmingham, AL, 2011
6. Alakija O, Blair P, Chang M, Layfield S, Lee P, Liu M, Patel M, Thomas S, **Londono-Joshi AI**, Monti DL: Genomic analysis of the novel B1 mycobacteriophage, OSmaximus. *Undergraduate Research Expo*, Birmingham, AL, 2011
7. Forero-Torres A, Oliver PG, **Londoño-Joshi AI**, Zhou T, LoBuglio AF, Buchsbaum DJ: Death receptor 5, a therapeutic target for triple negative breast cancer. *The 2010 Breast Cancer Symposium*, National Harbor, MD, 2010
8. Buchsbaum DJ, Oliver PG, **Londoño-Joshi AI**, Zhou T, LoBuglio AF, Forero-Torres A: Death receptor 5 (DR5), a therapeutic target for triple negative breast cancer (TNBC). *The 2010 Breast SPORE Roundtable Meeting*, National Harbor, MD, 2010
9. **Londoño-Joshi AI**, Oliver PG, LoBuglio AF, Buchsbaum DJ: Treatment of triple negative breast cancer stem cells with TRA-8 anti-DR5 monoclonal antibody. *Proc Comprehensive Cancer Center 2009 Annual Research Retreat*, Birmingham, AL, 2009

PATENT

- Targeting Cancer Stem Cells March 2010. US Provisional Patent Application (# 61/315,413)

PUBLISHED GENOME SEQUENCES

- Mycobacterium phage OSmaximus, complete genome. GenBank: JN006064.1
- Mycobacterium phage Wee, complete genome. GenBank: HQ728524.1

TEACHING AND MENTORING EXPERIENCE

HHMI Science Education Alliance Phage Exploration, National Genomics Research Initiative Professor Dr. Denise Monti

- University of Alabama at Birmingham BY213 Phage Genomics I
Teaching Assistant
Fall 2010, Fall 2011, 4 credits lectured and taught lab for 2 months while professor was on leave
16 students (primarily honors biology majors)
- University of Alabama at Birmingham BY214 Phage Genomics II
Teaching Assistant
Spring 2011, 4 credits lecture and lab
16 students (primarily honors biology majors)

University of Alabama at Birmingham McNair Scholars Program

- Summer Research Peer Mentor
Summer 2009, 2010 and 2011
5 students (McNair Scholars undergraduate students)
- Tutor Undergraduate Students
Fall 2009 Molecular Genetics, Spring 2010 Cancer Biology, and Fall 2010 Genetics

Research Mentoring at University of Alabama at Birmingham

- Supervised Undergraduate Student Research
Summer 2009, 2010, 2011
Andres Aristizabal; 2011 third place winner at state-wide poster competition
Laura Aristizabal; 2012 second place winner at Summer Expo poster competition
- Supervised Graduate Student Rotation
Saranya Ravi, Fall 2011; *Amber Guidry*, Spring 2010
- Supervised Gyn-Oncology Medical Fellow Research
Keri Bevis, 2010; *Jenny Whitworth*, 2011; *Janelle Fauci*, 2012; *Rebecca Arend*, 2013

LEADERSHIP EXPERIENCE

Associate Member Council for the American Association for Cancer Research (AACR)

Scientific Presentations Professional Advancement Series	2012-2013
Programming, Advocacy Professional Advancement Series	2011-2012
Sub-committee, Associate Membership and Recruitment	2011-2012

Toastmasters International Club

President	2011-2012
Vice-President of Education	2010-2011

Founding Officer	2010
Graduate Student Association	
Treasurer	2010
Budget Committee Chair	2010
Cultural Activities Committee Co-Chair	2008-2010
Senator- Pathology Department	2008-2011
Howard Hughes Med to Grad	
Peer Cluster Regional Southern Conference Coordinator	2008
Volunteering Co-Chair	2008
La Colectiva (Hispanic Student Organization), President	2003-2006
Coalition of South American Students, Co-Chair	2003-2006
International Student Services Committee- Board member	2002-2006

EXTRACURRICULAR ACTIVITIES

- University of Alabama at Birmingham Graduate Biomedical Sciences
 - Cancer Biology Admission Committee 2010-2012
- Pancreatic Action Network Birmingham Affiliate
 - Helped organized first annual Purple Stride 5K, 1K Race, Birmingham, AL 2011-2013
 - Organized lab tour for pancreatic cancer patients and advocates, Birmingham, AL 2011
 - Represented Alabama, Jefferson County at Advocacy Days, Washington, DC 2011
- Hispanic Interest Coalition of Alabama: Medical translator, Birmingham AL 2008-present
- McNair Oral and Poster Presentation for Undergraduate Students: Judge, Birmingham, AL 2008-2011
- POSSE Foundation Undergraduate Student Scholarship: Selection committee, Chicago, IL 2006
- Bi-monthly weekend outings with handicapped and mentally disabled children, Madrid, Spain 2005
- Assisted terminally ill patients at Clinico Moncloa, Madrid Spain 2005
- American Multicultural Student Leadership Conference, Madison, WI 2004
- University of Wisconsin Hospital Burn Unit: Medical translator, Madison, WI 2004
- Boys and Girls Club: Holiday workshops and mentoring, Madison, WI 2003-2006
- World Health Day: Medical translator, Madison, WI 2003-2006
- Extended Learning Services, Little River Earth Sanctuary Environmental Project, Melbourne, Australia 2003

WORK EXPERIENCE

Hyde Park Dermatology- Medical Assistant/Insurance Coder	2007
Latin Solutions Marketing- Brand Ambassador	2006-2007
University of Wisconsin Health Service- Student Health Advocate	2003-2004
Southport Health Center- Assistant Manager	2000-2002
Instituto Cervantes of Chicago- Library Assistant	2001-2002
Illinois Public Interest Research Group- Canvasser	2001

CERTIFICATES

Toastmasters International Speaking and Leadership Certificates: Competent Communicator and Competent Leadership
 Small Rodent Procedures: Pancreatic cancer orthotopic surgery. Mammary-fat-pad orthotopic injections. Intravenous, Intracardiac and Intraperitoneal injections
 Health Insurance Probability and Accountability Act (HIPAA)
 PADI Open Underwater Scuba Diver

PROFESSIONAL MEMBERSHIPS

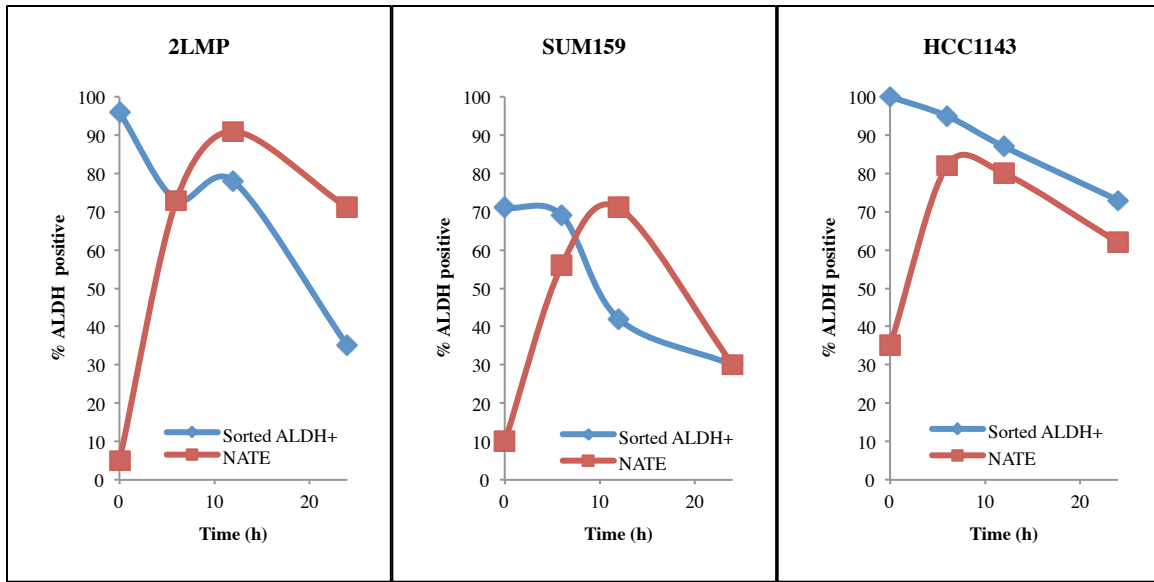
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)

Center for Clinical and Translational Science (CCTS)
American Association for Cancer Research (AACR)
Birmingham International Center (BIC)
Toastmasters International (TMI)

LANGUAGES

Native proficiency in English and Spanish

A.



B.

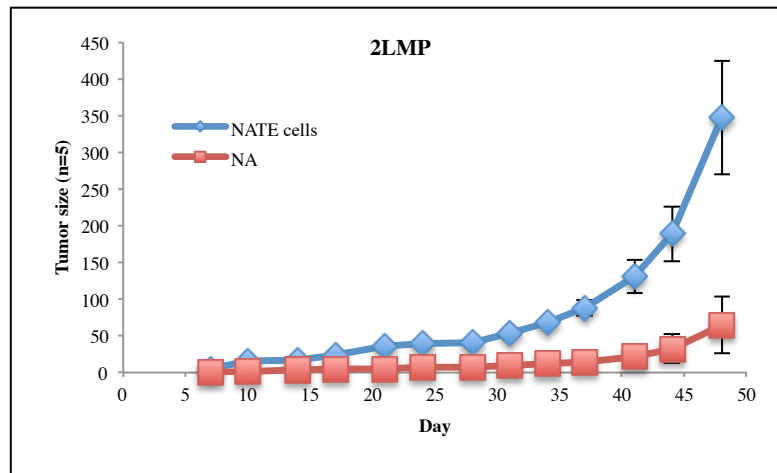


Figure 1. Characterization of non-adherent tumorsphere enriched (NATE) cells. **(A)** 2LMP, SUM159 and HCC1187 cells were FACS sorted for ALDH⁺ cells and analyzed for ALDH expression at 6, 12 and 24 h after sorting (blue line) and compared to cells allowed to form tumorspheres (red line). NATE cells rapidly gained ALDH enzymatic expression reaching ~80% enrichment between 12-16 h. Cells harvested at this 12-16 h time point are called NATE cells. **(B)** 20,000 2LMP NATE cells were injected into the MFP of NOD/SCID mice and showed enhanced tumorigenicity compared to cells cultured in non-adherent conditions for 4 days ($p=0.01$).

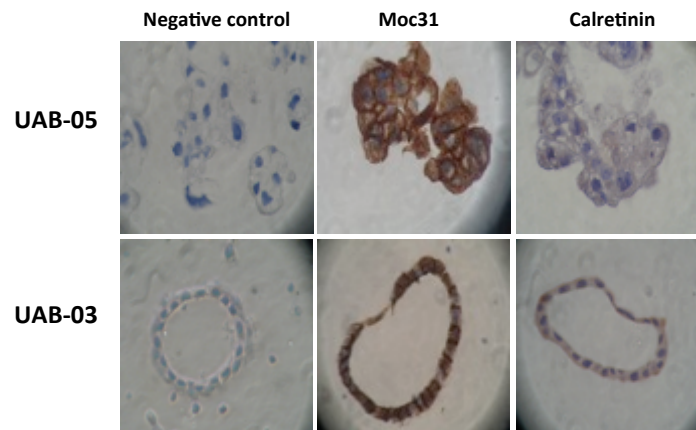


Figure 2. Immunohistochemistry characterization of patient pleural effusion samples. Patient sample name: UAB-05 and UAB-03 were stained for markers that indicate sample represents breast cancer cells and not mesothelial cells. 1st column negative control, 2nd column positive for Moc31 (adenocarcinoma), 3rd column negative for Calretinin (not mesothelial cells).

Cell Line	NATE IC ₅₀ uM	Adherent IC ₅₀ uM	<i>P</i> value NATE vs. Adherent
SUM159	0.11 (± 0.06)	0.31 (± 0.02)	0.015
HCC1187	0.18 (± 0.06)	1.0 (± 0.02)	0.0004
HCC1143	0.18 (± 0.08)	1.0 (± 0.02)	0.0002
2LMP	0.35 (± 0.13)	0.54 (± 0.34)	0.42

Table 1. Sensitivity of BLBC cell lines to niclosamide mediated cytotoxicity. SUM159, HCC1187, HCC1143 and 2LMP BLBC cell lines were treated for 48 h with niclosamide and analyzed for cell viability using ATPlite assay. Three of the four BLBC NATE cell lines showed significantly enhanced sensitivity compared to adherent cells. All individual experiments were assayed in quadruplicate and values represent the mean and SD from a minimum of three independent experiments.

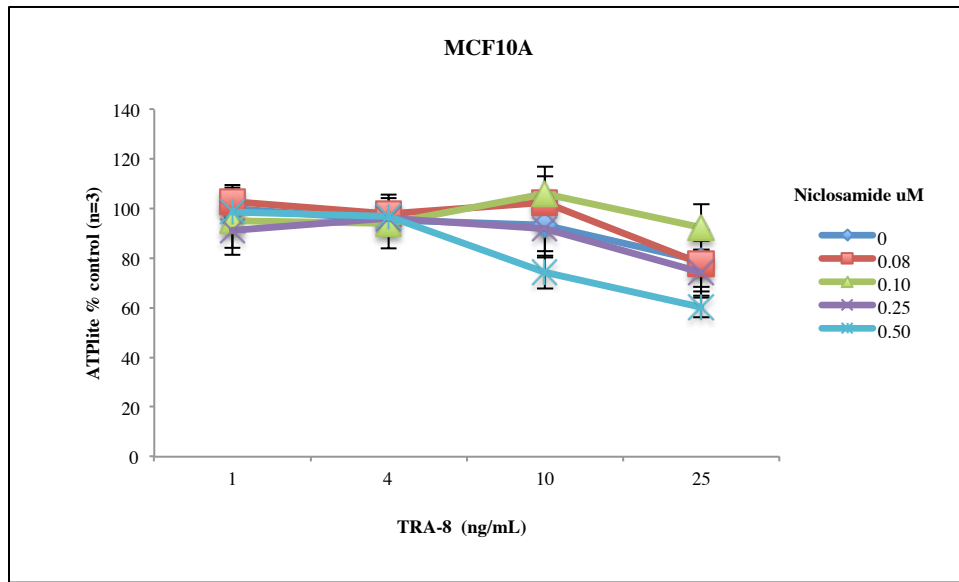
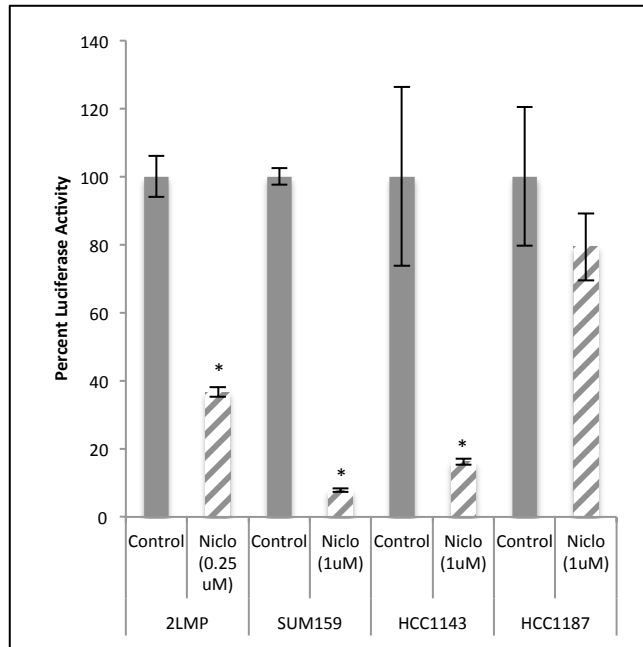
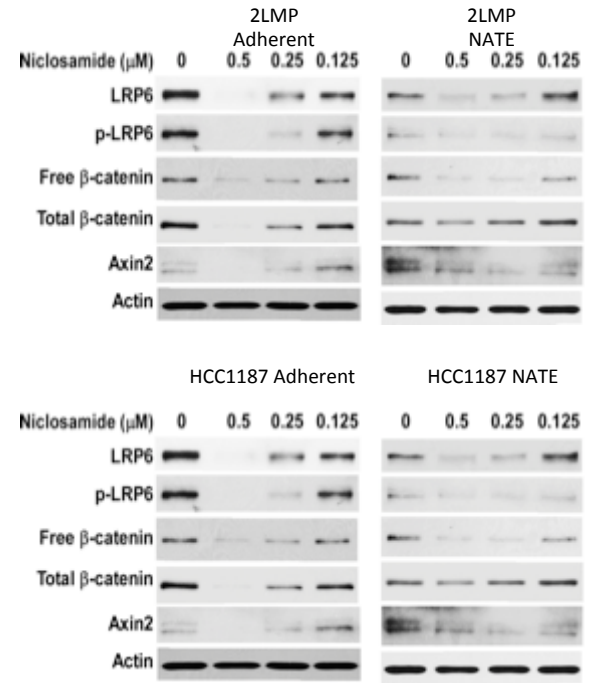


Figure 3. Sensitivity of MF10A cell line to niclosamide mediated cytotoxicity. Attached MCF10A cell line was treated for 48 h with Niclosamide and TRA-8 and analyzed for cell viability using ATPlite assay. All individual experiments were assayed in quadruplicate and values represent the mean and SE from three independent experiments.

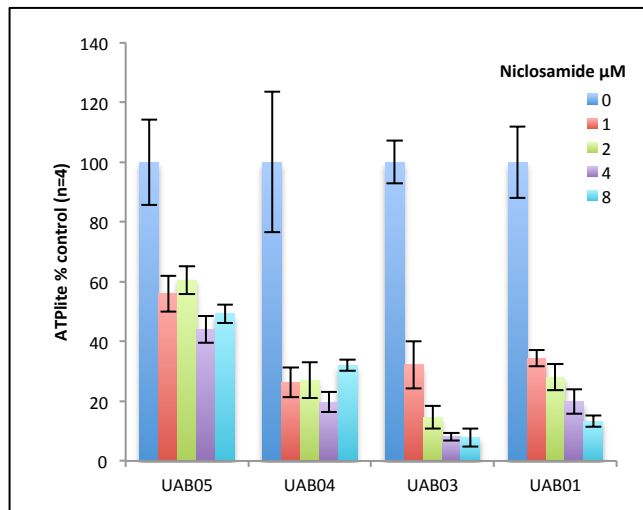
A.



B.



C.



D.

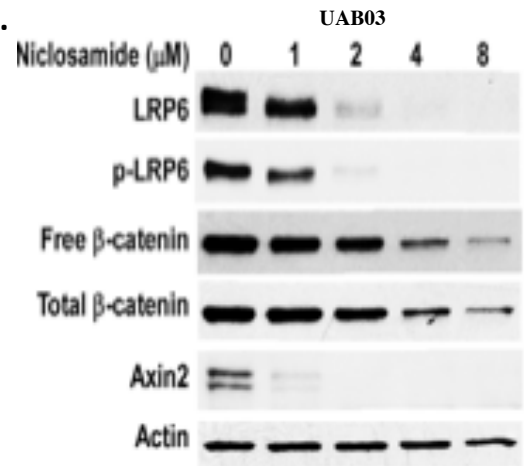


Figure 4. Niclosamide inhibits Wnt/ β -catenin signaling in BLBC cell lines and patient samples. **(A)** 2LMP, SUM159, HCC143 and HCC1187 cells were treated Niclosamide for 24 h (SUM159, HCC1143 and HCC1187 were treated with 1 μ M Niclosamide, and 2LMP with 0.25 μ M Niclosamide). Niclosamide significantly inhibited activity of TCF/LEF luciferase TOPflash reporter in 2LMP, SUM159 and HCC1143 cell lines (* p = 0.0005, 0.002, 0.006). The experiment was performed in triplicate. The bars represent means $\pm$ SE. **(B)** Western blot analysis for 24 h treatment of 2LMP and HCC1187 cell line with niclosamide (0, 0.5, 0.25, 0.125 μ M) on both adherent and NATE cells. LRP6 (top blot) p-LRP6 (second blot), free β -catenin (third blot), total β -catenin (fourth blot), Axin2 (fifth blot), Actin (sixth blot). Increasing doses of niclosamide reduced expression of Wnt/ β -catenin signaling proteins **(C)** All four patient pleural effusion samples showed niclosamide mediated cytotoxicity with two samples showing a dose response (UAB03, UAB01 (1, 2, 4 and 8 μ M). Samples were run in quadruplicates, bars represent means $\pm$ SE. **(D)** Western blot analysis for 24 h treatment of patient pleural effusion effusion sample UAB 03.

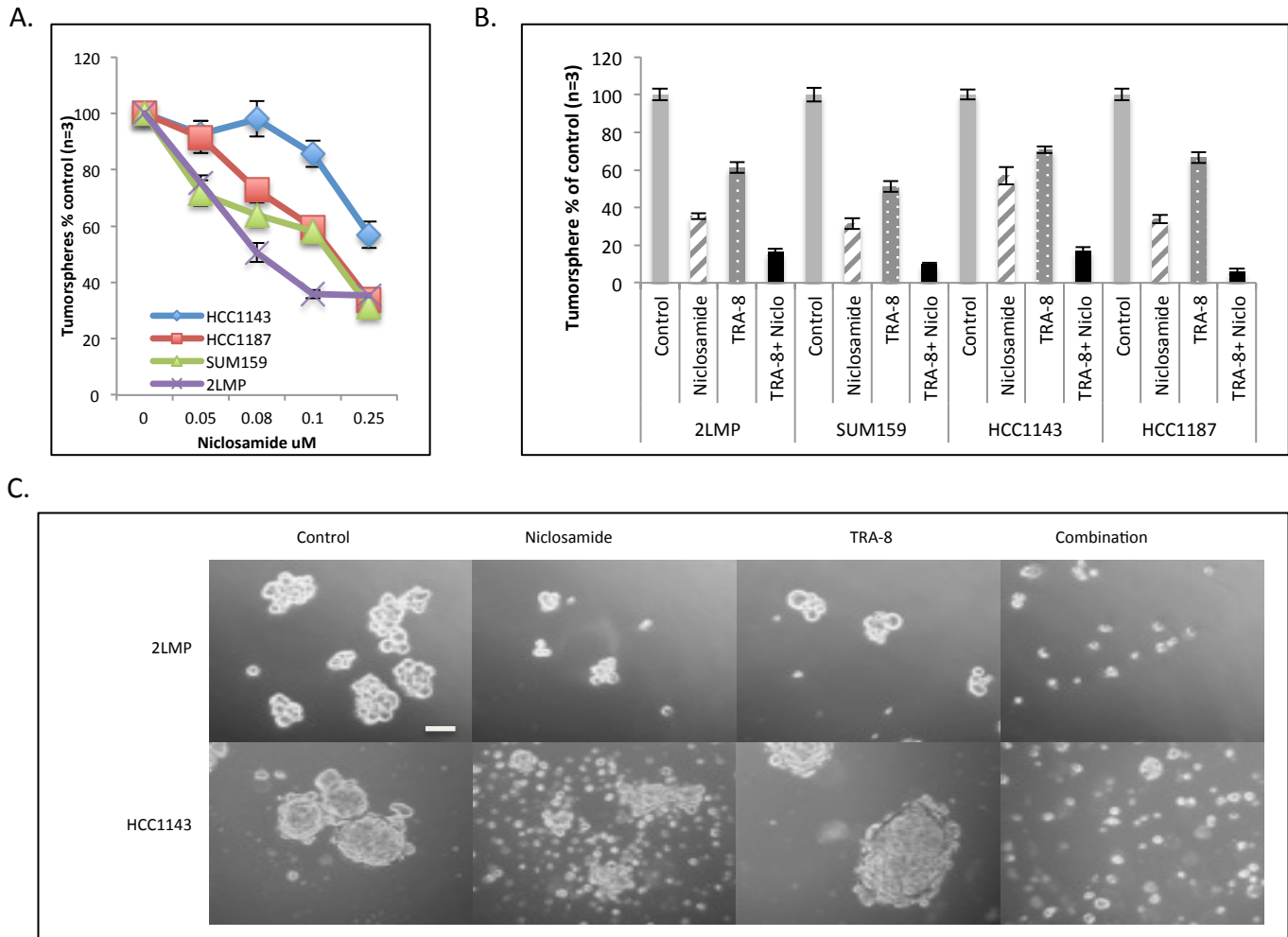


Figure 5. TRA-8 in combination with Niclosamide inhibits Wnt signaling in BLBC NATE cells. **(A)** Secondary tumorsphere inhibition by Niclosamide. HCC1143, HCC1187, SUM159 and 2LMP NATE cell lines were treated for 48 h with Niclosamide (0.05, 0.08, 0.1 and 0.25 μM). **(B)** Inhibition of secondary tumorsphere formation in combination with niclosamide and TRA-8. NATE cells were pre-treated with niclosamide for 24 (0.25 μM), followed with treatment with TRA-8 for an additional 24 h (1 ng/mL 2LMP, 0.5 ng/mL SUM159, 25 ng/mL HCC1143 and 5 ng/mL HCC1187). Secondary tumorsphere inhibition was visually counted using a reticle eye piece (50-150 μm). **(C)** Phase contrast pictures of 2LMP and HCC1143 cell lines pre-treated with niclosamide (0.25 μM) for 24 h, followed by TRA-8 (1 ng/mL 2LMP, 25 ng/mL HCC1143) and the combination, scale bar (100 μm). All individual experiments were assayed in quadruplicates, values represent the mean relative to control and error bars represent SE from three independent experiments. * $p < 0.007$

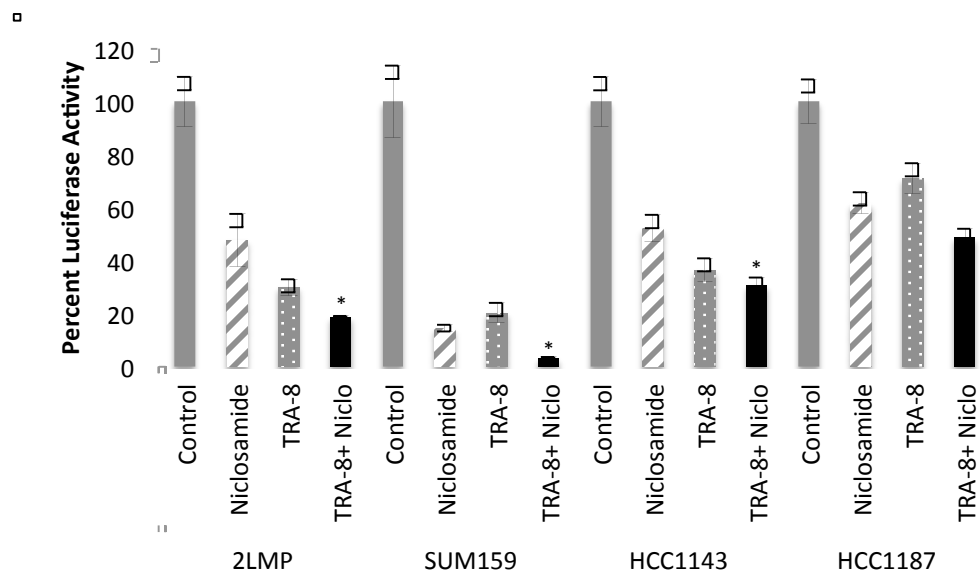


Figure 6. Niclosamide in combination with TRA-8 inhibits Wnt/ β -catenin signaling. Activity of TCF/LEF reporter, TOPFlash was evaluated in 2LMP, SUM159, HCC1143 and HCC1187 adherent cells. All cell lines were treated with 0.25 μ M niclosamide and 0.25 ng/mL TRA-8 for 24 h. The experiment was performed in triplicate. The error bars represent means $\pm$ SE.

	2LMP		SUM159	
	frequency	Mean (SE)	frequency	Mean (SE)
Control	5/5	189 mm ²	3/5	58 mm ²
TRA-8 (10nM)	3/5	158 mm ²	1/5	9 mm ²
Niclosamide (1uM)	4/5	127 mm ²	2/5	13 mm ²
TRA-8 + Niclosamide	4/5	71 mm ²	0/5	

Table 2. Effect of *ex vivo* treatment of BrCSC enriched cells on tumorigenicity in NOD/SCID mice. 2LMP and SUM159 tumorspheres were treated with TRA- 8, Niclosamide, combination and IgG control for 3 h and implanted into the MFP of groups of five NOD/SCID mice. The table represents the frequency of tumor engraftment and the average tumor size of the tumors that developed. In the 2LMP cell line 4/5 small, slow growing tumors were observed to develop within 44 days with TRA-8 and niclosamide treated cells while 5/5 tumors developed in the IgG, TRA-8 3/5, and Niclosamide treatment 4/5 groups. In the SUM159 cell line 3/5 tumors developed in the control group, 1/5 in TRA-8, 2/5 in the niclosamide treated group and 0/5 after 46 days after implantation.

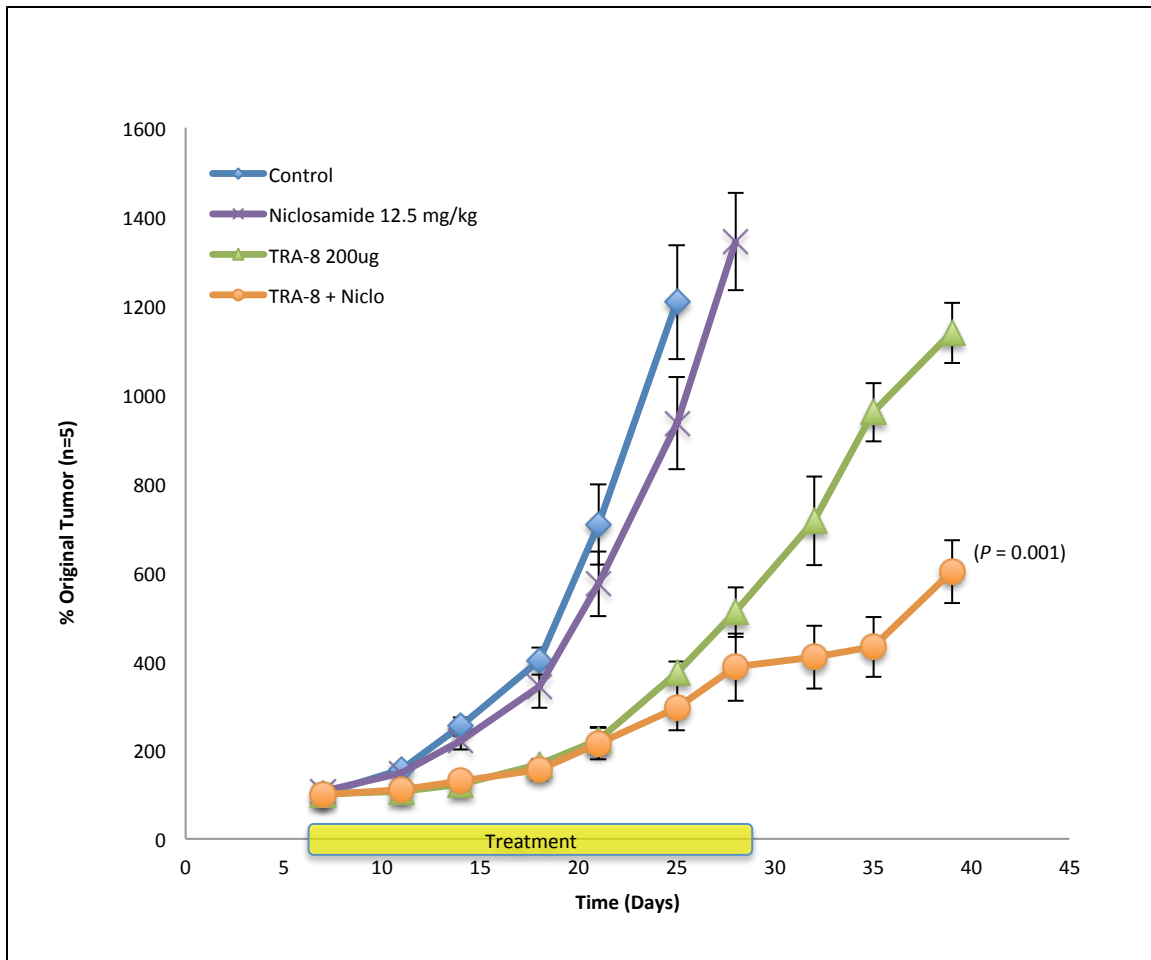


Figure 7. Antitumor effect of niclosamide in combination with TRA-8 *in vivo* on BLBC 2LMP cell line. Niclosamide in combination with TRA-8 reduced the tumor volume in athymic nude mice. Each point in the curve represents the mean + SE (n=5). The therapy started when tumors reached a volume of 16 mm². Niclosamide was given daily for 21 days, TRA-8 was given 2x weekly for 3 weeks. Niclosamide in combination with TRA-8 inhibited tumor growth. Tumor size was measured twice a week until mice were euthanized (combination vs. TRA-8 p=0.05, combination vs. control p=0.001).

Statement of Work 2012-2014
Sensitivity of Breast Cancer Stem Cells to TRA-8 anti-DR5 Monoclonal Antibody

(Task 1) Aim 1: To isolate CD44⁺/CD24⁻/ALDEFLUOR⁺ cancer stem cells from established human breast cancer cell lines and patient plural effusion samples and evaluate their functional characteristics such as tumorigenicity, tumorsphere formation and chemoresistance. (Months 1-10) - *completed*

- a. Analyze sorted BrCSC population for retention of BrCSC markers**
- b. Functional BrCSC assays**

(Task 2) Aim 2: To evaluate *in vitro* and *in vivo* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines. (Months 11-15) – *completed and published findings in 2012*

- a. Evaluate DR5 expression on CSC**
- b. Assess for cytotoxicity and caspase activation of CSC after treatment with TRA-8**
- c. Demonstrate secondary tumorsphere and inhibition using TRA-8**
- d. *Examine* inhibition of CSC tumorigenicity with *ex vivo* treatment using TRA-8**

(Task 3) Aim 3: To evaluate *in vitro* induction of apoptosis by anti-DR5 monoclonal antibody in BrCSC isolated from cell lines and patient samples, alone and in combination with Wnt inhibitor, Niclosamide. (Months 16-25)

- a. Evaluate Niclosamide inhibition in BLBC *completed***
- b. Niclosamide inhibition of Wnt/ β -catenin in BLBC and NATE cells *completed***
- c. *In vitro* combination with TRA-8 and Niclosamide *completed***
- d. Confirm and further elucidate mechanism of anti-Wnt and anti-DR5 pathway interaction**
To be completed
 - Develop shRNA LRP6 knockdown of 2LMP and HCC1187
 - Observe if shRNA knockdown cell lines have enhanced sensitivity to TRA-8
 - Discern expression of survivin and death receptor 5 after treatment with niclosamide
 - Detect by Western-Blot analysis and β -catenin degradation after treatment with TRA-8

(Task 4) Aim 4: To evaluate the *in vivo* therapeutic efficacy of anti-DR5 in combination with Niclosamide in xenograft models. (Months 20-25)

- a. *Ex vivo* anti-DR5 in combination with Niclosamide *completed***
- b. *In vivo* combination with TRA-8 and Niclosamide *To be completed***
 - Test preliminary *in vivo* combination using 2LMP
 - *In vivo* combination using 2LMP and HCC1187 with various doses