2015-100
Title: Phase Ib/II Treatment of Advanced Pancreatic Cancer with anti-CD3 x anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs) in Combination with Low Dose IL-2 and GM-CSF

Phase of Study: Phase II
IND Number: 13091
Version: 6/5/15, 8/25/15, 5/27/16

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SUMMARY

Epidermal Growth Factor Receptor (EGFR) is expressed on pancreatic cancer (PC). Our recent phase I study on patients with colorectal cancer and advanced pancreatic showed encouraging results. This protocol will confirm toxicities and estimate the clinical efficacy of combining anti-CD3 x anti-EGFR bispecific antibody (EGFRBi) armed activated T cells (EGFR BATs) given to patients (pt) with locally advanced or metastatic PC who have received at least first line chemotherapy and may have responding, stable or progressive disease. In the phase I study, 5 patients received 3-4 infusions of 2 x 10^{10} BATs per infusion with total 8 x 10^{10} per course of treatment without dose limiting toxicities (DLTs) with a median overall survival (OS) of 14.5 mos.

One treatment of chemotherapy will be given 1-2 weeks before immunotherapy to create immunologic space before the infusions of EGFR BATs. In the single dose phase I portion of this study involving 3-6 pt, we will confirm the safety of twice weekly infusions of up to 10^{10} EGFR BATs infusions in combination with 300,000 IU of interleukin 2 (IL-2)/m^2/day and 250 μg granulocyte-macrophage colony stimulating factor (GM-CSF)/m^2/twice weekly beginning 3 days before the 1st infusion and ending on the day of the last infusion. If there is toxicity in 1 of 3 pt, 3 additional pt will be added to the dose level of 10^{10}. If 2 of 6 patients experience DLTs, then the dose will be reduced to 7.5 x 10^9 per infusion. If only 1 pt has toxicity in the first 6, then the study will proceed to enroll the remaining cohort of pt in the phase II. In the phase II portion of this study, we will estimate the clinical efficacy of 8 infusions of 10^{10} EGFR BATs in combination with IL-2 and GM-CSF in 39 evaluable pt (including the 3-6 pt in the above mentioned single dose phase I). The primary endpoint is an improvement in median OS from historical data of 7 mos to 10.5 mos.

Immune evaluations will be performed to determine if: a) infusions of EGFR BATs significantly increase cellular or humoral anti-PC responses and if those responses persist and b) immune responses correlate with clinical responses. The original tumor paraffin blocks prior to treatment will be evaluated for the presence of CD3, CD4, CD8, PD1/PDL1, monocytes subpopulations, MDSC, and cytoplasmic IFN-γ and IL-10 by Immunohistochemical staining. The type and number of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment will be correlated with clinical responses (secondary objective).

If the pt does not have rapidly progressive disease 2 months after the last infusion, the treatment may be repeated. If the pt has significant progression of disease, the pt will receive oncologist's choice of chemotherapy. Such chemotherapy may be given starting 30 days after the last BATs infusion. The pts will receive 3 months of chemotherapy and continue to be evaluated on protocol.

In summary, the primary objective of the study is to evaluate clinical efficacy after confirming the safety at a dose up to 10^{10} EGFR BATs per infusions for 8 infusions in combination with IL-2 and GM-CSF. The primary endpoint is OS with the design to determine whether infusions of BATs in PC pts could improve the median OS from 7 months to 10.5 mos. Given the poor prognosis for pt with metastatic or locally advanced PC, we will also follow the pt to determine if immunotherapy followed by chemotherapy improve clinical responses over historical responses and improves time to progression free survival (PFS). Patients who are stable, partial responders, or complete responders by Wolchok criteria are eligible for re-treatment with BATs. The secondary objectives are: 1) immune evaluation studies to determine if infusions of BATs significantly increases cellular or humoral anti-PC responses and if those responses persist and correlate with clinical responses and 2) determine whether the type and number of T cells, Th_1/Th_2 secreting T cells, activation markers in the original biopsy paraffin block correlates with clinical outcomes.

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1.0 OBJECTIVES

1.1 Primary Objectives:

In patients who have received at least first line chemotherapy:

a) Confirm in a single dose phase I (3 to 6 pts) that 8 infusions of up to 10^{10} EGFR BATs given twice per week in combination with IL-2 (300,000 IU /m^2/day) and granulocyte-macrophage colony stimulating factor (GM-CSF) (250 µg/m^2/twice weekly) beginning 3 days before the 1st infusion and ending on the day of the last infusion is safe.

b) Perform a phase II clinical trial to estimate the clinical efficacy of 8 infusions of 10^{10} EGFR BATs in combination with IL-2 and GM-CSF in 39 evaluable pts (including the 3-6 pts in the single dose phase I). The primary endpoint is an improvement in median overall survival (OS) from historical data of 7 mos to 10.5 mo. The trial will accrue 39 evaluable pts (including those in the single dose phase I.

1.2 Secondary Objectives:

1.2.1. Determine if infusions of EGFR BATs significantly increase cellular or humoral anti-PC responses by peripheral blood mononuclear cells (PBMC) at different time points after last EGFR BATs infusion and if those responses persist beyond 2 mos

1.2.2 Obtain original tumor paraffin blocks prior to treatment and evaluate blocks for CD3, CD4, CD8, PD1/PDL1, monocytes subpopulations, MDSC, and cytoplasmic IFN-γ and IL-10 by Immunohistochemical staining to quantitate type and number of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment to estimate whether the type and number correlate with clinical responses.

1.2.3 To determine progression free survival (PFS).

2.0 BACKGROUND AND SIGNIFICANCE

2.1 Metastatic and Locally Advanced Pancreatic Cancer:

PC is the fourth most common cause of cancer-related death in the United States with over 48,960 newly diagnosed cases expected in 2015 and an expected mortality rate of approximately 40,560[1]. Most pt diagnosed with PCs have unresectable disease and usually die of metastatic disease even after treatment with chemo and radiation therapy. Despite the recent remarkable advancements in many malignancies, little success has been reflected in the outcome of PC. This malignancy is still considered among the most aggressive and the least curable of all human malignancies. Unfortunately, late diagnosis of PC in most pts with LAPC or MPC is associated with poor prognosis. Without effective treatment, the median survival of less than one year is expected for those with MPC.

2.2 Chemotherapy for the treatment of PC.

Single-agent gem became the first-line treatment of MPC after the pivotal 1997 report, where Burris et al[2] compared gem with 5-FU. The response rate was 5.4% in pt treated with gem alone whereas none of the 5-FU treated pts responded (p = NS). Gem was associated with a statistically significant improvement in pain control, weight loss and functional impairment leading to FDA approval based on improvements in pt performance status. Many other combinations with gem failed to provide any further benefit and.[3] and clinical outcomes remained alarmingly low[2,4-18]. More recently, the combination of nab-paclitaxel plus gem improved OS to 8.5 months compared to 6.7 mos for gem alone. [20] The cocktail of 5-FU, oxaliplatin, irinotecan and leucovorin (FOLFIRINOX) improved OS in the first line setting to 11.1 mos when compared to gem.[19] In 34 second-line chemo clinical trials reviewed by Rahma et al in 2013, pts who received treatments had a OS of 6 mos compared to those who received supportive care of 4 mos. Gem and platinum-based combinations provided a PFS and OS of 4 and 6 mos, respectively' and the combination of 5 FU and

platinum agents provided a PFS and OS of 2.9 and 5.7 mos, respectively.[21] ***With dismal response rates, PFS, and OS in clinical trials, innovative and novel therapeutic approaches are desperately needed to improve OS in pts with LAPC and MPC.***

2.3 EGFR (ERBB-1 Receptor, HER1) as target.

EGFR is over-expressed in 30-50% of PC. In clinical trials, small molecule inhibitors that target the intracellular tyrosine kinase signaling pathways of EGFR, such as gefitinib (Iressa®) or erlotinib (Tarceva®) have been tested without major impact on disease. Though cetuximab was approved by the FDA in 2004 for the treatment of metastatic colon cancer, results from a study in PC with gem and cetuximab were disappointing.[22] Therefore, cetuximab alone is not effective. Alternative strategies that exploit and directly target EGFR over-expression using the targeting ability of cetuximab circumvent the activational or mutation status of EGFR may be more effective at killing tumor cells than antibody alone.

2.4. EGFR BATs:

Arming anti-CD3 activated T cells (ATC) with anti-CD3 x anti-EGFR bispecific antibody (EGFRBi) makes every T cell into a non-MHC restricted EGFR-specific cytotoxic T lymphocyte (CTL).[23,24] Anti-CD3 x anti-HER2 bispecific antibody (BiAb) armed ATC (HER2 BATs) showed repeated killing, proliferation, and release of Th_1 cytokines, Regulated on Activation Normal T Expressed and Secreted (RANTES=CCL5), macrophage inhibitory protein-1 alpha (MIP-1α), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α),and GM-CSF that induce DC maturation. Dying tumor cells release tumor associated antigens (TAA), that can lead to increased cross-presentation of TAA by antigen presenting cells (DC). This mechanism, illustrated in **Fig 1**, may vaccinate pts against their own TAAs. Preclinical studies showed that EGFRBi armed ATC (EGFR BATs) killed pancreatic cancer cell line MiaPaCA, secreted cytokines, and inhibited tumor growth in mice.[24]

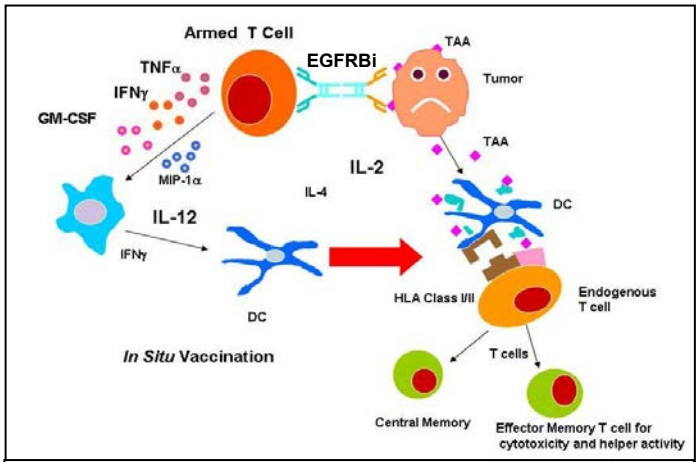


Fig 1: Infusion of targeted T cells induces anti-tumor immune responses. T cells kill tumor cells releasing cytokines and chemokines that induce DC maturation followed by antigen presentation of unknown tumor antigens to "vaccinate" the patient against their own TAAs.

2.5. Unarmed ATC as mobile serial killers and immunokine factories.

Cross-linking the T cell receptor with anti-CD3 triggers activation, proliferation, cytokine synthesis, and non-MHC restricted cytotoxicity directed at tumor targets.[25-28] The tumoricidal cytokines produced include IFN-γ, TNF-α, and GM-CSF.[29-39] In animals, infusions of ATC provide anti-tumor effects[40-43] and improve engraftment when limiting doses of stem cells were given after lethal total body irradiation.[44] In humans, a phase I trial treating pts with cyclophosphamide followed by infusions of activated $CD4^+$ cells resulted in 1 complete, 2 partial and 8 minor responses in 31 advanced cancer pts.[45,46] If nonspecific ATC had enhanced cancer-directed activity after chemoT, BATs may have even greater anti-tumor activity.

2.6. HER2 BATs Target high HER2 SK-BR-3 and low HER2 SUM 1315 expression cell lines.

Our preclinical studies showed that HER2 BATs killed breast cancer cells lines that expressed not only high amounts of HER2 (SK-BR-3, high 3+ expression) but, more importantly, target and lyse cell lines that express little (MCF-7, a negative control for IHC)[47] or essentially no HER2 expression (Sum 1315). Using goat anti-HER2/neu Ab to detect HER2/*neu* receptors on the cells, we showed that Sum 1315 was only 6.7% HER2 positive by flow cytometry (**Fig 2b**) and negative for HER2 expression by Western blotting (**Fig 2a). Fig 2c** shows high levels of specific cytotoxicity of HER2 BATs directed at both Sum 1315 and SK-BR-3 cells using an arming dose of 50 ng/10^6 ATC at the effector:target (E:T) ratios depicted in **Fig 2a**. This is consistent with the observation that as few as 10-30 T cell receptor (TCR)-ligand interactions are sufficient to mediate T cell killing.[48] Arming doses of 50, 1.0, and 0.05 ng of BiAb/10^6 ATC led to 4000, 80, and 4 dimers/cell, respectively (assuming 20% dimer and 20% of BiAb actually binding to the T cells). Therefore, it is clear that only a few molecules of HER2Bi on ATC and a few HER2 on the surface of tumor cells are sufficient to allow binding and triggering of high levels of specific cytotoxicity.

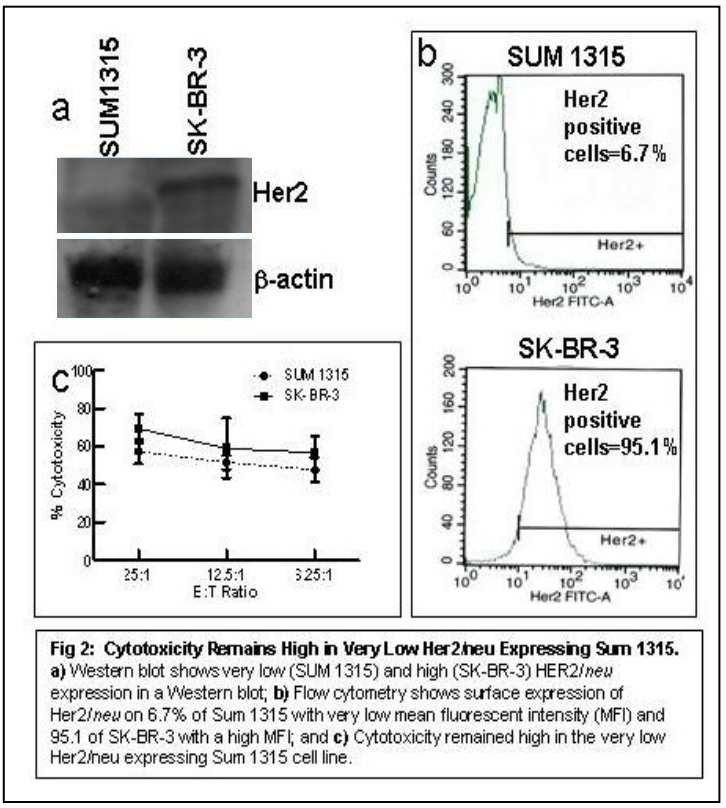


Fig 2: Cytotoxicity Remains High in Very Low Her2/neu Expressing Sum 1315. **a)** Western blot shows very low (SUM 1315) and high (SK-BR-3) HER2/*neu* expression in a Western blot; **b)** Flow cytometry shows surface expression of Her2/*neu* on 6.7% of Sum 1315 with very low mean fluorescent intensity (MFI) and 95.1 of SK-BR-3 with a high MFI; and **c)** Cytotoxicity remained high in the very low Her2/neu expressing Sum 1315 cell line.

2.7. Phase I Safety, Immune and Clinical Responses in Metastatic Breast Cancer (MBC).

HER2 BATs exhibit anti-HER2 cytotoxicity, proliferate, and secrete immunokines upon tumor engagement. In our phase I trial, 23 women with HER2 0-3+ MBC received 8 infusions of HER2 BATs (twice a week for 4 weeks) in combination with IL-2 and GM-CSF to evaluate safety, feasibility, PFS, OS, T cell trafficking and immune responses. The median OS for 8 HER2 3+ pts, 14 HER2 0-2+ pts, and all pts was 57.4, 27.4, and 36.2 mos, respectively (**Fig 3c**). One HER2 1+ pt who was progressing on Femara developed a very good partial response (nearly complete response) that persisted at 7 mos (**Fig 3d**). BATs persisted in the

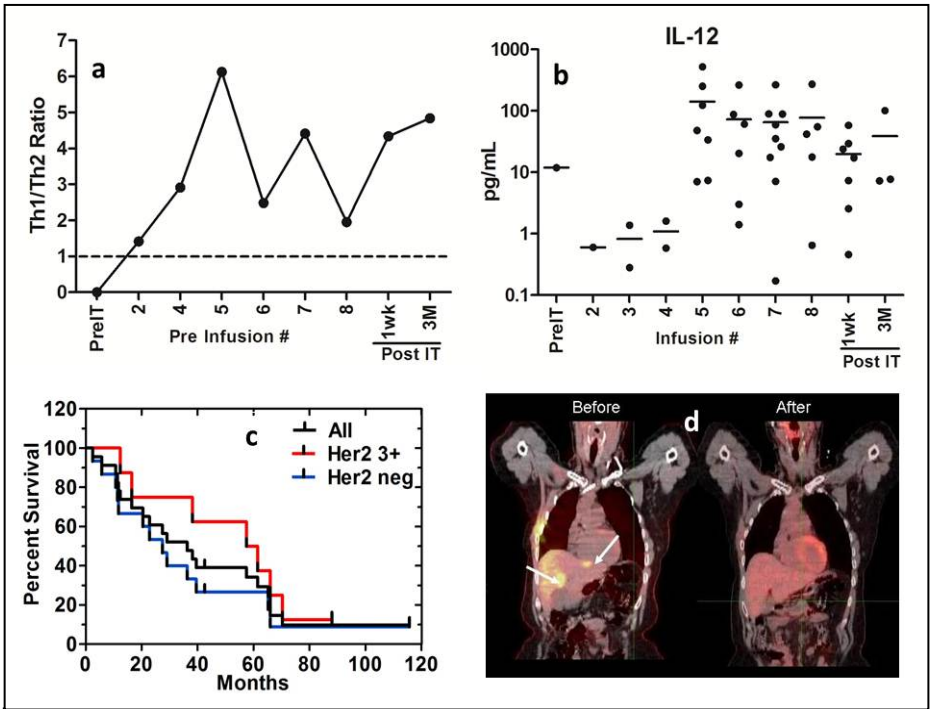


Fig 3. a) Mean Ratio of Th_1/Th_2 cytokine as a function of infusion #, where Th1/Th2 = [IL-2+IFN-γ]/[IL-4+IL-5] ; **b**) IL-12 levels as a function of infusion # with means denoted as horizontal bars; **c**) the median OS is 59.4 months for the HER2 3+ group (**red**), 27.4 months for the HER2 0-2+ (**blue**) group, and is 36.2 months for the entire group (**black**); **d**) HER2-ve partial responder showing 2-liver mets preIT (left) PET/CT which disappeared postIT (right).

blood for at least a week and trafficked to tumors. Targeting HER2 positive and negative tumors induced cytotoxic anti-tumor responses, increases in Th_1 cytokines, and IL-12 serum levels. These robust immune responses suggest HER2 BATs infusions may provide a clinical benefit.[49,50]

2.7.1. Evidence for activation and Immunization of endogenous immune cells in MBC pts. Peripheral blood mononuclear cells (PBMC) exhibited high levels of cytotoxicity directed at SK-BR-3 that persisted up to 4 mos [51]. **Fig 4** shows specific cytotoxicity mediated by endogenous lymphocytes (IgG2a negative population) directed against SK-BR-3 in two representative pts. PBMC from these pts were depleted of IgG2a+ bearing cells (ATC) using Miltenyi columns and the IgG2a negative population was used in the killing assay. The results strongly suggest that endogenous lymphocytes had developed robust immune responses to breast cancer antigens. There were significant increases in serum Th_1 cytokines (**Fig 3a**) and IL-12 (**Fig 3b**)[50] during and after infusions. The marked increases in serum IL-12 (**Fig 3b)** show that monocytes are activated by BATs infusions and secrete high levels of IL-12.

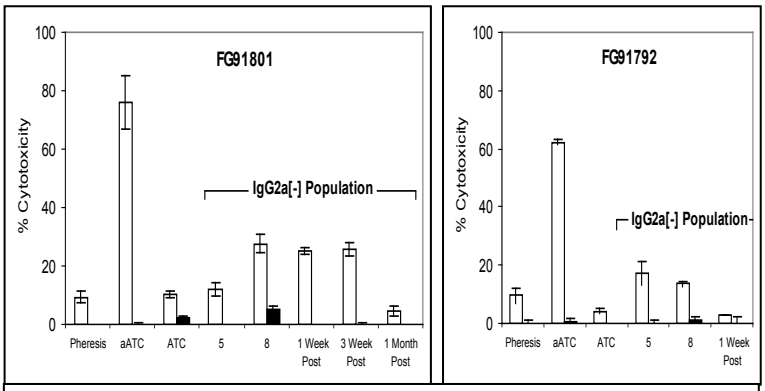


Fig 4: Circulating PBMC depleted of IgG2a+ cells (IgG2a [-]) using Miltenyl beads exhibited cytotoxicity. The IgG2a [-] population was tested for specific cytotoxicity directed at HER2-positive SKBR-3 (25:1 E:T) or HER2-negative Raji as a function of treatment schedule. The IgG2a [-] populations were compared to the pheresis product, unarmed ATC, armed ATC product.

2.7.2. No Human Anti-Mouse Antibody (HAMA) responses. Because anti-CD3 mAb moiety comprising the BiAb is a murine IgG2a monoclonal antibody (mAb), we evaluated pts' sera before and after HER2 BATs infusions for the development of HAMA to IgG2a in an ELISA and did not detect clinically significant levels of IgG anti-mouse. Of the 10 pts evaluated, none developed levels > 10 ng/ml during or up to 8 weeks after immunotherapy.

A.7.3. Vaccination with BATs infusions and Boost after Stem Cell Transplant (SCT) with 'Immune' ATC. In 5 evaluable MBC pts who had undergone the phase I clinical trial consisting of 8 infusions of HER2 BATs in combination with low dose IL-2 and GM-CSF, a second leukapheresis was performed to obtain "anti-breast cancer immune" PBMC. PBMC after 8 infusions of BATs before the second expansion exhibited specific cytotoxicity directed at SK-BR-3 ranged from 2 to 38% with a median of 26%. ATC were produced and cryopreserved for multiple infusions (4-15 infusions) after high dose chemo and SCT. Two pts at Roger Williams Hospital received up to 10^{10} immune ATC 3 times per week for 3 weeks and then once weekly infusions of 2 x 10^{10} ATC for 6 weeks and 4 pts at KCI received ATC once per week for 4 weeks. **Fig 5a** shows immediate non-MHC restricted specific cytotoxicity mediated by uncultured PBMC directed at SK-BR-3 targets at the designated time points for IT and post SCT for 5 pts. There were significant serum titers of anti-SK-BR-3 that increased after the vaccination with 8 HER2 BATs infusions and high levels of anti-SK-BR-3 IgG antibody titers were present at the various time points after HDC and SCT up to 2 years after SCT (data not shown). In order to confirm that transferred memory B cells were responsible for the serum antibodies were transferred in the stem cell product, fresh PBMC from 5 pts were re-stimulated *in vitro* with SK-BR-3 targeted T cells

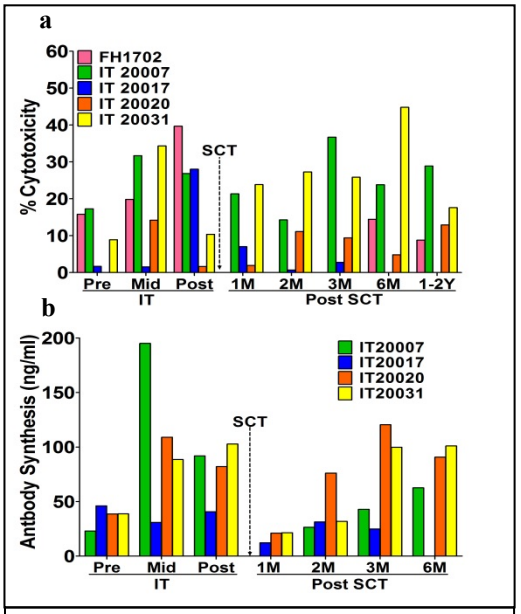


Fig 5: a) Multiple infusions of ATC after SCT promoted the development of cytotoxicity that could be detected during and at 2-4 weeks post IT (MidIT and PostIT), and 3 mos (3M), 6 mos (6M) and 12-24 mos (1-2Y) after SCT. **b)** Shows the anti-SK-BR-3 antibody levels in culture supernatants tested after 14 days of culture. These data suggest that pts had been immunized against her own tumor antigens by multiple infusions of BATs that can be transferred in ATC product after SCT.

and CpG using a new assay for inducing anti-tumor antibodies *in vitro.*[52] The 14 day culture supernatants contained anti-breast cancer antibodies before and after SCT. In other words, the stem cell product contained memory B cells that produce anti-breast cancer antibodies when re-stimulated *in vitro (***Fig 5b***)*. Together, this proof-of-concept pilot study shows that both cellular and humoral anti-tumor immune responses can be detected during and after IT, and these immune responses can be transferred via the "immune" ATC.

2.7.4.Summary of HER2 BATs. The HER2 BATS infused into MBC pts in the phase I were safe and induced immune and clinical responses. BATs infusions induced endogenous immune cells to develop anti-breast cancer CTL and serum antibody activity that could be transferred in pts after autologous SCT in multiple infusions of immune ATC which provided anti-tumor specific helper activity for antibody synthesis. These studies provide PROOF of CONCEPT that BATs can vaccinate pt against their own tumor antigens and the memory effector T cells and specific antibody forming B cells can be transferred and be easily detected after HDC and SCT.

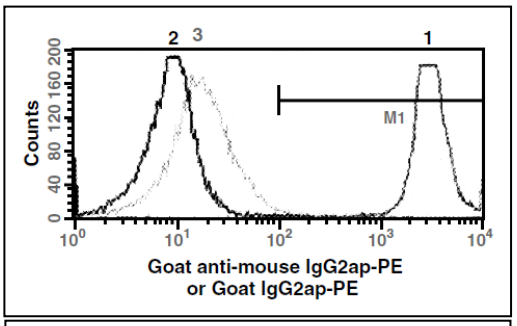


Fig 6 EGFRBi binding to EGFR-positive versus EGFR-negative cells. A431cells (peak1) andMDA-MB-453 cells (peak 3) were incubatedwith EGFRBi (1 Ag/mL) for 30 minutes at room temperature. EGFRBi binding was evaluated by flow cytometry using phycoerythrin-conjugated (PE) goat anti-mouse IgG2a to detect the anti-CD3 moiety of the BiAb. A representative histogram (peak 2) of t he background binding for an isotype control (GIgG-PE) and unarmed ATC binding for each of the cell lines is also shown.

2.8. Preclinical Data that support proposed research:

A.8.1 EGFR BiAb redirects non-MHC restricted cytotoxicity of ATC. Based on the experience generated with HER2 BATs, EGFR directed BATs were generated. EGFR is expressed in several epithelial cancers including non-small cell lung cancer (NSCLC). In NSCLC addition of EGFR directed antibodies (cetuximab and necitumumab) to chemo has shown modest improvement in survival.[53,54] The degree of binding of EGFR BATs to the EGFR-positive NSCLC cell line, A549, compared to an EGFR-negative cell line, MDA-MB-453, was evaluated using phycoerythrin-conjugated (PE) goat anti-mouse IgG2a to detect the OKT3 (anti-CD3) moiety of the BiAb (**Fig 6**). EGFR BATs binding to A549 cells was consistent with their expression of EGFR; in contrast, EGFR BATs bound just above the background of the isotype control to MDA-MB-453 cells. A representative histogram of the background binding for an isotype control to A549 is also shown.

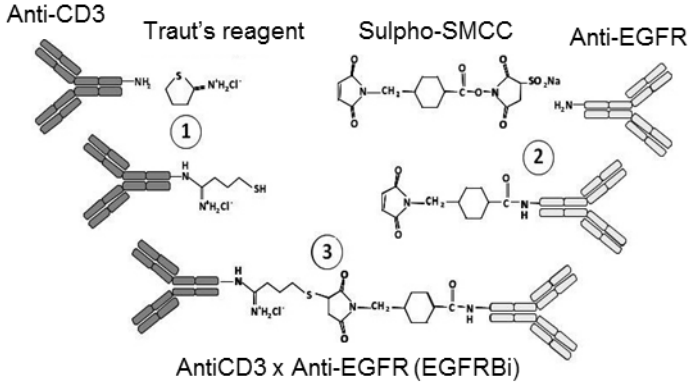


Fig 7: 1) Anti-CD3 (OKT3, Centrocor Ortho-Biotech, Raritan, NJ) was cross-linked with Traut's reagent (2-iminothiolane HCl, Pierce); 2) Anti-EGFR was cross-linked with sulphosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (Sulpho-SMCC); and 3) Cross linked monoclonal antibodies were desalted on PD-10 columns (Pharmacia, Uppsala, Sweden) to remove unbound cross-linker. The cross-linked OKT3 and anti-EGFR will be heteroconjugated overnight.

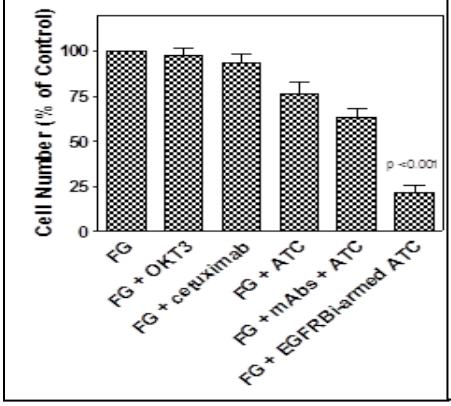


Fig 8 Antiproliferative effects of EGFR BATs compared with mAbs (cetuximab) or unarmed ATC against COLO356/FG cells. COLO 356/FG cells were treated with unconjugated OKT3 (anti-CD3) or cetuximab (500 ng/well), unarmed ATC (107/well), EGFR BATSs (10^7/well armed wi h 50 ng EGFRBi/10^6 ATC), or a combination of the mAbs with unarmed ATC. COLO 356/FG cells were counted after 72 hours. Results from three independent experiments are shown as the number of cells for each treatment as a % of the cells cultured with medium only. After 72 hours, the cells in the control wells had gone through 1.5 doublings. Bars = SD.

2.8.2 Production of EGFRBi. The production of anti-CD3 x anti-EGFR (OKT3 x cetuximab) BiAb by chemical heteroconjugation has been described (**Fig 7**).[47] The heteroconjugated product is analyzed by non-reducing SDS-PAGE and quantified by densitometry using Quantity One software (Bio-Rad, CA). EGFR BATs are prepared and quality controlled and assured per SOP to be pathogen free, and endotoxin levels are quantitated and approved for use by the FDA (IND 13091.[47,55]

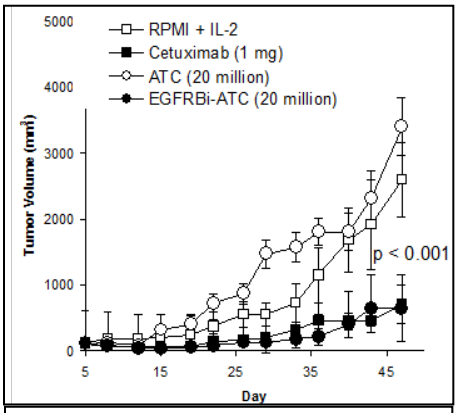
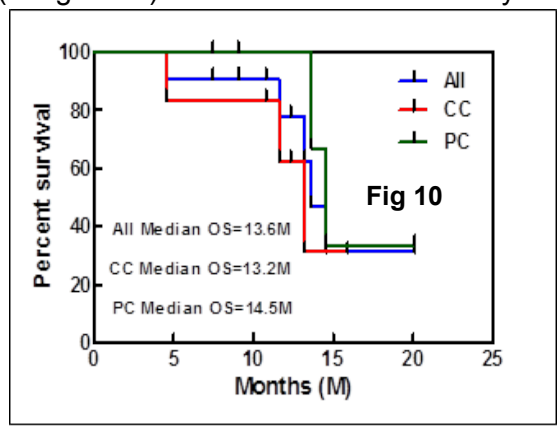


Fig 9 EGFRBi-armed ATC and cetuximab delayed growth of pancreatic tumor xenografts, but only cetuximab delayed growth of colorectal tumor xenografts in SCID-Beige mice. COLO356/FG tumor cells (5 x10^6) were implanted s.c.in SCID-Beige mice. When tumors measured 60 mm^3, treatments were initiated. Mice received EGFR BATs by tail vein injection once per week for 6 weeks, and tumor growth was monitored. Points are median tumor volumes from each treatment group (n = 5mice/treatment group/tumor type); bars, SE.

2.8.3. In vitro and in vivo characterization studies of EGFR BATs performed in a pancreatic cancer cell line. Much of the preliminary characterization of EGFR BATs was initially performed using the pancreatic carcinoma cell line, COLO 356/FG (FG), which demonstrates sensitivity to EGFR BATs-mediated cytotoxicity. We compared the anti-proliferative effect of EGFR BATs to cetuximab in cell proliferation assays. ATC alone, EGFR BATs (E:T of 10) or equivalent concentrations of unconjugated monoclonal antibodies (anti-CD3 or cetuximab) were co-cultured with FG cells. After 72-hours, surviving FG cells were harvested and counted using a Coulter Particle Counter. Results (**Fig 8**), expressed as the number treated cells as a % of FG target cells plated alone (mean ± SD) from 3 independent experiments, show that **although cetuximab alone was ineffective (p > 0.05 compared to control) at inhibiting proliferation of FG, EGFR BATs induced significant killing (p<0.001) of the same target cells.** When FG was grown as subcutaneous xenografts in a SCID-Beige mouse model (T-, B-, and NK cell deficient model), cetuximab (1 mg/week) administered intravenously for 6 weeks significantly delayed tumor growth compared to ATC alone or medium administered on the same schedule, but EGFR BATs administered at a 1000-fold lower dose (20 x 10^6 ATC armed with 50 ng EGFR BiAb/million cells ≈ 0.001 mg cetuximab/week) produced comparable tumor growth inhibition (**Fig 9**). No gross toxicities were observed in any of the treated groups of mice. Body weights of mice over the course of treatments were significantly lower (6-8%) in the unarmed ATC treatment group (p < 0.001), but did not differ significantly between the other 3 treatment groups. Post-mortem necropsy of vital organs showed significant 23-30% and 22-32% increases in liver and kidney weights, respectively, in the mice treated with cetuximab compared to the other 3 treatment groups; liver and kidney are the primary target organs for EGFR-targeted toxicities. These findings are consistent with observations from preclinical and clinical studies exploring other EGFR-targeted therapeutics.[56,57] Importantly, compared to cetuximab, EGFR BATs showed no evidence of organ toxicities in our preclinical studies and produced comparable tumor growth delay suggesting that EGFR BATs may offer a wider therapeutic window with regard to safety. The EGFR BATs total dose used in these mice is equivalent to a 3.2 x 10^{10} total



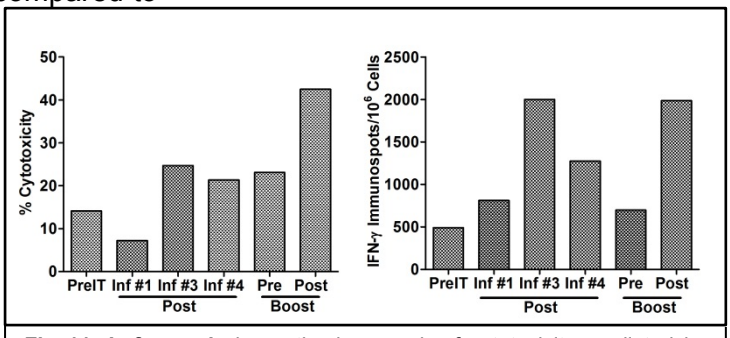


Fig 11: Left panel shows the bar graph of cytotoxicity mediated by fresh PBMC of patient (IT20091) against pancreatic cancer cell line-MiaPaCa-2 preIT, post EGFR BATs infusion #1, #3 and #4, pre and post boost infusion at 3 mos. **Right panel** shows the enhanced IFN-γ EliSpots by PBMC directed at MiaPaCa-2 cells at MiaPaCa-2 post immunotherapy (post) and boost at 3 mos.

dose in humans, but significantly higher doses of ATC armed with other BiAbs, such as HER2Bi, have been attained in humans without encountering DLTs.[58]

2.9. Phase I Clinical Trial targeting colorectal and pancreatic cancer with EGFR BATs.

Five PC and 6 colorectal cancer pts received up to 78.8 x 10^{10} (median of 43, range 20.4 - 78.8 x 10^{9}) EGFR BATs in 3-4 doses given once per week for 3 weeks followed by a boost at 12 weeks. There were no DLTs. The planned doses were 10, 20, and 40 x 10^{9} BATs/infusion. The OS for the entire group, the colorectal, and pancreatic cancer pts was 13.6, 13.2, and 14.5 mos, respectively (**Fig 10)**. Most remarkable are the PC pts. *Three are **alive at 664, 333, and 284 days after pheresis,*** and two died at 375 (IT20087) and 408 (IT20092) days after study enrollment (**Table 1** as of 5/26/2015). **Fig 11** shows the cytotoxicity mediated by fresh PBMC from pt against PC cell line-MiaPaCa-2 after each infusion as well as the pre and post booster (4th infusion) results for cytotoxicity (left panel) and IFN-γ EliSpots (right panel). **Fig 12** shows cytotoxicity of PBMC from 9 of the pts before (preIT) and 2 weeks post infusions #3 directed at a colorectal cell line. These data suggest that IT may sensitize or make tumor cells more susceptible to chemo leading to remarkable complete responses. Pt IT20102 had an abdominal lymph node decrease in size at 3 mos, received his booster infusion, and decreased by 27% at 6 mos on CT scan. This pt was interviewed (with consent) by the local Fox News affiliate. His story is available at the link below.

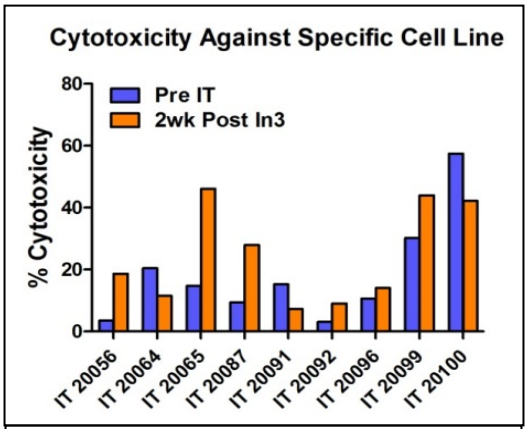


Fig 12: Specific cytotoxicity mediated by PBMC from colorectal and pancreatic cancer pts before and after 3 infusions of EGFR BATs.

Table 1

Pt	Age	Disease	Prior Tx	BATs x 10^{9}	TTP (days)	OS (days)	Comments
IT20087	58	Mets to liver	Folfirinox	47	186	Dead (409)	Progressed
IT20091	63	T3 N1Mets to liver. Post Whipple	5FU,Leuk/5FU Folfirinox	9.3 78.8	**CR,** 138	**Alive (664)**	Chemo Induced **CR** after Treated Twice IT with Folfirinox, responding to Folfirinox again.
IT20092	64	T2b Abd Nodes, post Whipple	gemcitaine, 5FU, radiation,	36	211	Dead (436)	Had chronic diarrhea; Appendicitis With PC tumor
IT20102	56	T4, Mets to liver, lungs	Folfirinox	74	**Stable (near PR)**	**Alive (333)**	Lesion decreased by 27% at 6 mos, stable at 8 mos
IT20104	51	T4, Abd Nodes	FOLFOX stable 1 yr then Xeloda	72	71, **CR**	**Alive (284)**	Chemo Induced CR after IT

Table 2: Side Effects Incidence and Grade at Dose Levels EGFR BATs						
			Total # of Episodes by Grade			
Dose Level	Reaction	# Patients Affected (% at Dose Level)	1	2	3	4
Non Evaluable*	Palpitations	1 (12.5%)	1			
(n=8)	Nausea	3 (37.5%)	3			
	Vomiting	3 (37.5%)	3			
	Chills	6 (75%)	7	6		
	Fatigue	5 (62.5%)	14			
	Fever	2 (25%)	3			
	Headache	5 (62.5%)	10	1		
	Hypertension	2 (25%)	1	1		
	Hypotension	1 (12.5%)		2		
Level 1	Nausea	2 (67%)	3			
(n=3)	Vomiting	1 (33%)	2			
	Chills	3 (100%)	6			
	Fatigue	3 (100%)	10			
	Fever	1 (33%)	1			
	Headache	3 (100%)	6			
	Hypertension	1 (33%)	2			
	Hypotension	0				
Level 2	Nausea	1 (33%)	1			
(n=3)	Vomiting	0				
	Chills	3 (100%)	4	1		
	Fatigue	2 (67%)	4			
	Fever	0				
	Headache	2 (67%)	4			
	Hypertension	0				
	Hypotension	0				

http://www.clickondetroit.com/lifestyle/health/a-new-cuttingedge-treatment-for-a-deadly-disease/32488192

2.9.1. Toxicity of EGFR BATs. There have been no DLTs and only eleven episodes of Grade 2 toxicities (**Table 2**). There were no grade 3 or 4 toxicities. Toxicities related to the cellular infusion typically resolved with medication within 24-48 hours after an infusion.[59]

2.10. Summary of Preliminary Data on In vitro and Clinical Use of EGFR BATs.

Our preclinical studies clearly show that EGFR BATs lyse EGFR positive colorectal and pancreatic cell lines *in vitro* and significantly inhibited the growth of EGFR positive tumors in immunodeficient mice. The phase I clinical trial in colorectal and pancreatic cancer pts was particularly encouraging and remarkable in the PC pts who, as summarized in **Table 1**, had substantial amounts of metastatic disease. A median survival of 14.5 mos with a near PR in IT20102 without any other treatment, and complete responses (CR), and a persistent CR response to chemotherapy in IT20104 are striking. It is not clear what the mechanism is for responses to chemotherapy in IT20091 and IT20104. Our working hypothesis is that immunotherapy sensitized the tumor or modulated the tumor so that subsequent chemotherapy was more effective. Studies to dissect and understand these results are underway.

3. ELIGIBILITY

3.1 Eligibility (Inclusion and Exclusion Criteria):

Inclusion Criteria:

- Histological or cytological proof of pancreatic adenocarcinoma. Must have locally advanced or metastatic pancreatic cancer who have received ***at least first line chemotherapy*** and may have responding, stable or progressive disease
- Expected survival ≥ 3 months
- KPS ≥ 70% or SWOG Performance Status 0 or 1
- Absolute Neutrophil Count (ANC) ≥ 1,200/mm^3
- Lymphocyte count ≥ 400/mm^3
- Platelet Count ≥ 75,000/mm^3
- Hemoglobin ≥ 8 g/dL
- Serum Creatinine < 2.0 mg/dl, Creatinine Clearance ≥50 ml/mm (can be calculated or measured)
- Total Bilirubin ≤ 2 mg/dl (biliary stent is allowed)
- SGPT and SGOT < 5.0 times normal

- LVEF ≥ 45% at rest (MUGA or Echo)
- Age ≥ 18 years at the time of consent (Written informed consent and HIPAA authorization for release of personal health information)
- Females of childbearing potential, and males, must be willing to use an effective method of contraception
- Females of childbearing potential must have a negative pregnancy test within 7 days of being registered for protocol therapy

Exclusion Criteria:

- Any chemotherapy related toxicities from prior treatment.(>grade 2 per CTCAE v4.0)
- Known hypersensitivity to cetuximab or other EGFR antibody
- Treatment with any investigational agent within 14 days prior to being registered for protocol therapy
- Symptomatic brain metastasis
- Chronic treatment with systemic steroids or another immuno-suppressive agent
- Serious non-healing wound, ulcer, bone fracture, major surgical procedure, open biopsy or significant traumatic injury within 28 days prior to being registered for protocol therapy
- Active liver disease such as cirrhosis, chronic active hepatitis or chronic persistent hepatitis
- Known HIV infection
- Active bleeding or a pathological condition that is associated with a high risk of bleeding (therapeutic anticoagulation is allowed)
- Uncontrolled systemic disease like active infections
- Nonmalignant medical illnesses that are uncontrolled or a controlled illness that may be jeopardized by the treatment with protocol therapy
- Females must not be breastfeeding
- Pt may be excluded if, in the opinion of the PI and investigator team, the pt is not capable of being compliant

Minor changes from these guidelines will be allowed at the discretion of the attending team under special circumstances. The reasons for exceptions will be documented.

3.2 Screening Assessment

Medical history, physical examination, Karnofsky or SWOG performance status, EKG, tumor markers, MUGA or ECHO, laboratory tests (hematology, chemistry) and staging CT, MRI or PET/CT will be performed ≤ 4 weeks prior to registration.

3.3 Informed Consent and Registration

Each pt must be aware of the nature of his/her disease process and must willingly consent to treatment after being informed of alternatives, potential benefits, side effects, and risks. Patients will be evaluated, consented, and registered after meeting all eligibility criteria, and prior to leukopheresis. Patient's disease will be staged. Baseline studies will be completed within 4 weeks prior to registration.

4. TREATMENT PLAN

4.1 Treatment Summary

One treatment of chemotherapy (see section 4.2) will be given 1-2 weeks before immunotherapy to create immunologic space before the infusions of EGFR BATs. In the single dose phase I portion of this study involving 3-6 pt, we will confirm the safety of twice weekly infusions of up to 10^{10} EGFR BATs infusions in combination with 300,000 IU of interleukin 2 (IL-2)/m^2/day and 250 µg granulocyte-macrophage colony stimulating factor (GM-CSF)/m^2/twice weekly beginning 3 days before the 1st infusion and ending on the day of the last infusion. The pt will receive a single treatment with chemotherapy up to

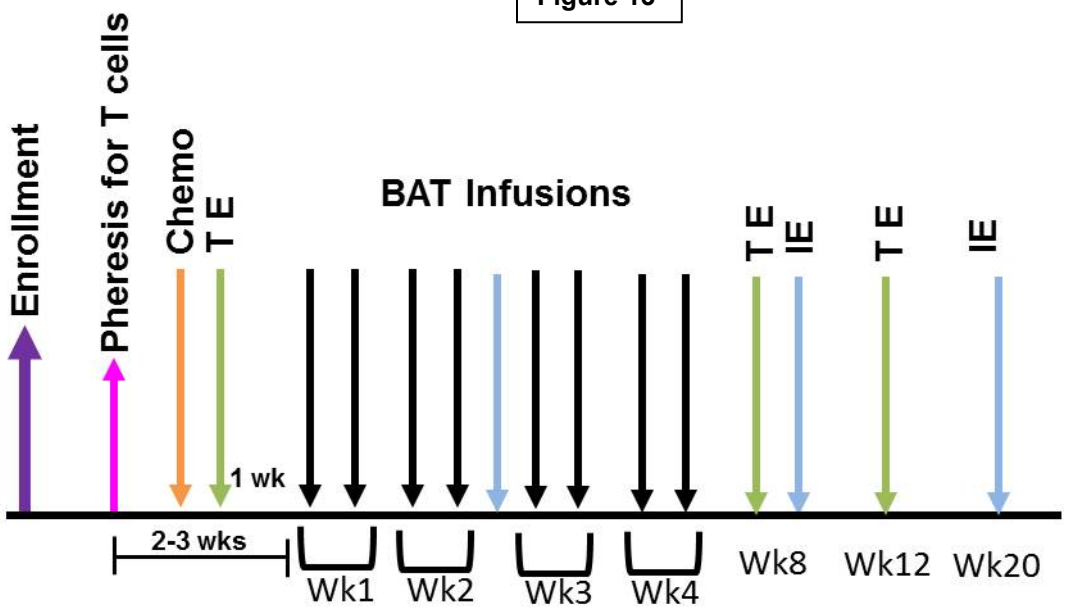
1-2 weeks prior to starting the BATs infusions. If there is toxicity in 1 of 3 pt, 3 additional pt will be added to the dose level of up to 10^{10}. If 2 of 6 pt experience DLTs, then the dose will be reduced to 7.5 x 10^{9} per infusion. If only 1 pt has toxicity in the first 6, then the study will proceed to enroll the remaining cohort of pt in the phase II.

In the phase II portion of this study, we will estimate the clinical efficacy of 8 infusions of 10^{10} EGFR BATs in combination with IL-2 and GM-CSF in 39 evaluable pt (including the 3-6 pt in the abovementioned single dose phase I). The primary endpoint is an improvement in median OS from historical data of 7 mos to 10.5 mo.

Immune evaluations will be performed to determine if: a) infusions of EGFR BATs significantly increase cellular or humoral anti-PC responses at 8 weeks after last EGFR BATs infusion and if those responses persist at 26 weeks or 52 weeks and b) immune responses correlate with clinical responses. ~~The original tumor paraffin blocks prior to treatment will be evaluated for the presence of CD3, CD4, CD8, PD1/PDL1, monocytes subpopulations, MDSC, and cytoplasmic IFN-γ and IL-10 by Immunohistochemical staining. The type and number of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment will be correlated with clinical responses.~~

If the pt does not have rapidly progressive disease 2 mos after the last infusion, the treatment may be repeated. If the pt has significant progression of disease, the pt will receive oncologist choice of chemotherapy. Oncologist choice chemotherapy will be given starting 30 days after the last BATs infusion if the repeat tumor evaluation shows progression of disease. **(The MDT team will specially discuss the management of each pt at the time of progression).** The pt will continue to be followed for disease and overall survival status and blood studies may be done optionally to continue evaluate immune responses to tumor every 3 months.

Figure 13



The primary objective of the study is to evaluate clinical efficacy and to confirm the safety at a dose up to 10^{10} EGFR BATs per infusions for 8 infusions. The primary endpoint is OS with the design to determine

whether infusions of BATs in PC pts could impro ve the median OS from 7 mos to 10.5 mos. Given the poor prognosis for patients with metastatic or locally advanced PC, we will also follow the patients to determine if immunotherapy followed by chemotherapy improve clinical responses over historical responses and improves progression free survival. Patients who are stable, partial responders, or complete responders by RECIST criteria are eligible for re-treatment with BATs.

The secondary objectives are: 1) immune evaluation studies to determine if infusions of BATs significantly increase cellular or humoral anti-PC responses at 8 weeks after last BATs infusion and if those responses persist at 26 weeks or 52 weeks; and 2) progression free survival.

Each pt will be given two infusions per week with doses ranging up to 10 x ~~10^9~~10^{10} per infusions per week for 4 weeks in a row. Restaging CT and immune evaluations will be done 8 weeks after the last BATs infusions and then every 2 months as per standard of care until disease progression.

Baseline serum and cell will be aliquoted from the pheresis. Blood draws (4 green top tubes containing approximately 40 mls of anti-coagulated blood and 1 red top tube containing 8 mls of serum) will be **pre-BAT** infusion, **after the 4th** infusion, and then approximately **2 weeks** (14±4 days), **2 months** (60 days±7 days, optional), **4 months** (120 days±7 days) and **optionally every 6 months** (180 days±7 days) **after the last infusion.** Optional IEs will be done if there continue to be immune responses at 6 month (± 2 weeks) intervals until the responses have disappeared.

Given the poor prognosis for patients with advanced pancreatic cancer, we will also follow the patients to determine if immunotherapy has any impact on survival and response rate (secondary endpoint). Patients who are stable, partial responders, or complete responders by RECIST criteria are eligible for re-treatment with EGFR BATs. Patients who show evidence of progression at 8 weeks after the last BATs infusion will be given oncologist's choice chemotherapy.

4.2 Chemotherapy

The time from leukopheresis to harvest of ATC is about 3 weeks. One treatment of standard chemotherapy: 1) gemcitabine; 2) gemcitabine and nab-paclitaxel; 3) modified FOLFOX6 (oxaliplatin, 5-fluorouracil and leucovorin); or 4) FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxalipalatin) to create immune space. In patients with advanced pancreatic cancer with no other standard chemotherapy available or allergy, immunotherapy may be given within 1 week after a single dose of cyclophosphamide (1 gram/m^2).

4.3 Immunotherapy

4.3.1 EGFR BATs infusions: EGFR BATs infusions will be done in an outpatient setting at KCI. All appropriate assurances for identification of product, patient, sterility, etc. will be performed prior to infusion. The identity of the frozen BATs will be verified with patient's identifiers and thawed at the bedside of the pt just prior to infusion. BATs will be infused intravenously (IV) with the rate of infusion based on the endotoxin content of the product. All patients will be observed for at least 4 hours after an infusion. If stable, patients will be discharged home. **BATs** infusions will begin approximately1- 2 weeks after chemotherapy and the subsequent doses will be administered twice weekly for 4 weeks.

4.3.2 Subcutaneous IL-2 Injections: All patients will receive SQ IL-2 (300,000 IU/m^2/day), beginning 3 days before the first ATC infusion and ending at the last BATs infusion.

4.3.3 GM-CSF Injections: All patients will receive SQ GM-CSF (150 µg/m^2/daily), to start 3 days before the first ATC infusion and ending at the last BATs infusion.

4.4 Recommended Concomitant Medications:

Anti-emetics such as ondansetron, dolasetron, or granisetron should be used prior to chemotherapy. 30 minutes prior to each EGFR BATs infusions, all patients will be pre-medicated with Benadryl (50 mg IV or PO) and Tylenol 650 mg PO. Demerol (25-50 mg IV) will also be administered post infusion if the pt develops grade 3 chills and then may be administered 30 minutes prior to subsequent BATs infusions dependent upon the severity of the initial reaction. The pt will alternate ibuprofen 600 mg every 8 hours with acetominophen 650 mg every 8 hours for 24 hours after the BATs infusion.

- **Ancillary Therapy:**
  Patients will receive full supportive care including transfusion of blood and blood products, antibiotics, and anti-emetics, when appropriate. The reason(s) for treatment, dosage and the dates of treatment will be recorded.
- **Steroids/Other Therapy:**
  With the exception of steroids for anti-emetic regimen, adrenal failure, septic shock, pulmonary toxicity or hormones administered for non-disease-related conditions (e.g. insulin for diabetes), steroids will not be administered. The patients will stop hormonal therapy 1 week prior to immunotherapy. Hydrocortisone (50-100 mg IV every 6 hrs) for severe adverse reactions related to BATs infusions is allowed. Life-threatening reactions may be treated with Situximab (anti-IL-6 mab to control cytokine storm).

4.5 Quality Control and Assurance of BATs

Lists and lots of suppliers of mAbs, heteroconjugation reagents, and culture reagents are maintained. Release criteria for armed products for clinical use include: negative results for sterility check with ID (7 days, negative for mycoplasma, endotoxin less than 2.5 EU/mL (otherwise a calculated infusion rate will be used to ensure the pt receives no more than 5 EU/kg/hr), ≥10% cytotoxicity directed at EGFR+ cancer cell line (25:1 E/T) compared to the respective unarmed ATC, >50% CD3+ cells and <10%: CD19, CD20, CD16; and CD56+ cells will be measured in ATC product.

4.6 Tumor Measurements

All tumors will be measured in millimeters or centimeters prior to initiation of therapy. These measurements will consist of the longest diameter and the perpendicular diameter at the widest portion of the tumor and will be made and recorded by the physician or his designee. An estimate of overall objective and subjective response will be made and recorded at the end of treatment. Disease status will be evaluated in the patients and their responses will be confirmed a minimum of four (4) weeks after the first response has been recorded using standard **RECIST 1.1** criteria. Responses will be noted as Complete Response (CR), Partial Response (PR), Minor Response (MR), Stable Disease (SD) or Progressive Disease (PD). Duration of response will be measured from first observation of the response. All patients will be staged before IT and at designated follow up times after IT (see section 8).

4.7 Accrual and Duration of Study

Our anticipated accrual rate is 11 patients per year. Thus, it should take approximately 4 years to accrue the 43 patients needed for the trial. Allowing for 18 months of follow-up to obtain the primary endpoint on the last pt enrolled and 6 months to assemble, analyze and interpret the data the total study duration is projected to be at most 6 years.

4.8 Re-start Criteria

Patients removed from the study due to unrelated toxicity may be re-entered upon complete resolution of toxicity. Re-entry criteria are the same criteria required for initial treatment.

5.0 TOXICITIES, DOSE MODIFICATIONS AND MANAGEMENT

5.1 Leukapheresis Procedure

Reported toxicity from leukopheresis is minimal. Patients have a small potential for infection from the placement of central catheters used for pheresis. Use of the anticoagulant citrate dextrose may cause symptoms of mild hypocalcemia, which are controlled with calcium replacement.

5.2 Granulocyte-Macrophage-Colony Stimulating Factor (GM-CSF).

Therapeutic Classification: Colony stimulating factor.

Known Side Effects and Toxicities: Patients receiving GM-CSF (Leukine-Sagramostim) have experienced fever 60-90 min after administration (duration 1-4 hrs); chills; nausea; vomiting; diarrhea; fatigue; weakness; headache; decreased appetite; thrombosis; rapid or irregular heartbeat or other heart problems; feeling of faintness; facial flushing; pain in the bones, muscles, chest, abdomen, or joints; local reaction at the site of injection; rashes; and kidney and liver dysfunction. Eosinophilia or other blood component abnormalities may occur. There have been infrequent reports of fluid accumulation or worsening of preexisting fluid accumulation in the extremities, in the lungs, and around the heart which may result in breathing problems or heart failure. Rarely, patients have developed acute allergic reactions. There have also been reports of low blood pressure, hypoxia, transient loss of consciousness, and difficulty in breathing after the first injection of Sargramostim. These signs may or may not recur with additional injections of Sargramostim. Patients with prior heart, lung, kidney, or liver problems may have worsening of their symptoms following administration of Sargramostim. There may be other side effects that could occur.

5.3 Toxicity of Low-Dose Subcutanous (SQ) Interleukin 2 (IL-2). Therapeutic classification: Lymphokine Known Side Effects and Toxicities: Administration of low dose SQ IL-2 (2×10^5 IU/m^2/day) for 90 days resulted in no grade III toxicities. None of the patients experienced pulmonary capillary leak syndrome, severe hypotension, oliguria, azotemia, or hyperbilirubinemia. The most frequent toxicities included fatigue, fever, and nausea. None of the patients had to stop their SQ IL-2 therapy due to side effects. Therefore, it is unlikely that major toxicities associated with the low dose SQ IL-2 will occur although death due to high dose IL-2 toxicity has been reported.[60].

5.4 EGFR BATs

Toxicity associated with BATs has been minimal. The reported symptoms have been reversible with cessation of therapy. Most patients treated with BATs some experienced chills, headaches, fatigue, and hypotension after infusions. Fever, chills, fatigue, and headache were the most common reactions. Other rarely reported effects included mild gastrointestinal and neurologic symptoms.

5.4.1 Dose Modification for EGFRBi-armed ATC-related Toxicities

All patients with treatment related, grade 4 non-hematologic toxicity will be removed. If the left ventricular ejection fraction (LVEF by MUGA) falls by more than 10% from the previous value after EGFRBi BATs, therapy will be withheld and the pt removed from the protocol (no one has been removed for cardiac-related toxicities in our phase I study). If there is persistent grade-3 toxicity at any time, treatment will be held until toxicity improves to grade 0 or 1. If grade 3 toxicities recur, the BATs infusions will be stopped (this would be considered a DLT). Toxicity will be assessed daily for 7 days after each reinfusion and then weekly for unresolved toxicities.

It is anticipated that in some cases, maximal T-cell expansion will not achieve the desired dose per infusion. In this case, the available T-cells will still be infused in 8 divided doses. Patients who receive less than this dose will be evaluable for clinical efficacy and immune responses.

5.5 Monitoring of patients getting chemotherapy

The major toxicities expected with chemotherapy are myelosuppression, nausea, vomiting, diarrhea, neuropathy and loss of appetite. No dose modification is planned since patients are receiving only one course of treatment. No prophylactic antibiotics are planned. The patients will receive prophylactic anti-emetics and will be monitored for signs of acute toxicity.

5.5.1. Cetuximab toxicity

Acne-like Rash: The most common adverse event associated with cetuximab administration is acne-like rash. Acne-like rash usually occurs on the face, upper chest, and back, but occasionally extends to the extremities and is characterized by multiple follicular or pustular appearing lesions characterized histologically as a lymphocytic perifolliculitis or suppurative superficial folliculitis. The onset of rash is generally within the first 2 weeks of therapy.

A number of therapeutic interventions have been attempted, including oral and topical antibiotics, topical steroids, and rarely, oral steroids. The value of these measures is unknown since definitive clinical trials have not bee performed. The etiology of the acne-like skin rash is believed to be the result of cetuximab binding to EGFR in the epidermis. When cetuximab was chemically heteroconjugated to OKT3 in the bispecific antibody format and infused on activated T cells in the phase I clinical trials, there were no nail disorders, allergic reactions, acute infusional reactions, cardiac toxicities, or skin rashes (Choi 2015, GI ASCO abstract).

Nail Disorder: Nail disorders were reported in 6.4% of patients receiving cetuximab as a single agent. The nail disorder is characterized as paronychial inflammation with associated swelling of the lateral nail folds of the toes and fingers. The most commonly affected digits are the great toes and thumbs. According to investigators, the nail disorder persists for up to 3 months after discontinuation of cetuximab. Soaks in aluminum acetate (Burow's) solution BID-QID will prevent secondary infection. Symptom relief may be achieved with standard bandages or with the application of liquid bandages (cyanoacrylate preparations). Preliminary analysis in patients treated at usual doses (400 mg/m^2 initial dose, followed by 250 mg/m^2 weekly), revealed that the incidence of nail disorders is greater in patients who received more than 6 infusions (approximately10%) compared to patients treated with 6 or less infusion of cetuximab (approximately 3%).

Allergic Reactions: The majority of the allergic/hypersensitivity reactions described have been grade 1 to 2 toxicities with less occurrences of grade 3-4. All reactions responded promptly to appropriate medical intervention.

Infusion Reaction: Infusion reactions are distinct from allergic or hypersensitivity reactions, although some of the manifestation are overlapping. Infusion reactions generally develop during or shortly after the infusion. Mild infusion reactions (chills, fever, dyspnea) have been reported in 23% of patients receiving cetuximab as a single agent. Severe infusion reactions (airway obstruction [bronchospasm, stridor, hoarseness], urticaria, hypotension) were reported in 2% of patients receiving single agent cetuximab. Infusion reactions occur most often with the first dose.

Pulmonary Toxicity: Interstitial lung disease has been reported in less than 1% of patients who have received cetuximab for advanced colorectal cancer, and in one pt with head and neck cancer.

Cardiac Toxicity: Recent reports described the occurrence of cardiac dysfunction, chest pain, and/or cardiac ischemia/infarction. These events occurred in patients who were receiving cetuximab in addition to 5-FU-containing therapy. Cardiac ischemia/infarction and acute cariomyopathy are known side effects

of 5-fluorouracil-based chemotherapy. It is presently unclear if the addition of cetuximab may increase the risk of 5-FU-related cardiac events.

Other Toxicities: Other reported or potential toxicities associated with cetuximab include:

Skin—Pruritis (10%), alopecia
Gastrointestinal—Diarrhea (28%, 2% grade 3/4), nausea/vomiting (29%),
stomatitis/mucositis (11%), anorexia, constipation
Metabolites—Hypomagnesemia (rarely grade 4 requiring aggressive IV repletion)
Pulmonary—Interstitial pneumonitis was reported in 3 of 633 (<0.5%) patients;
one pt died as a consequence.
EENT—Conjunctivitis (7%)
Constitutional—Fatigue/malaise, asthenia, infection
Musculoskeletal—Back pain
Hematologic—Anemia, leukopenia
CNS—Headache (25%, 3% grade 3/4)

5.6 Unexpected or Life-Threatening Toxicity

Reporting requirements and procedures depend upon: (1) whether agents are suspected of causing toxicity, (2) whether possibility of such atoxicity was reported in the consent form, or manufacturer's literature, (3) the severity or grade of the toxicity.

- **Expected toxicities:**
  Grade 4 myelosuppression: Report only as part of regular data submission. All other toxicities, Grades 4 and 5: Written report to principal and co-investigators within 10 working days. All other toxicities, Grades 1 to 3: Report only as part of regular data submission.
- **Unexpected Toxicities:**
  Unexpected toxicities are toxicities that are not listed in the toxicity management section of the protocol, the consent form, or the manufacturer's package insert.
- **REPORTING ADVERSE REACTIONS:**
  All serious and unexpected adverse drug reactions will be reported to the IRB and FDA within 5 days of the event.

5.7 Toxicity Grading

The NCI CTEP CTCAE v4.0 Toxicity Tables and 0-4 Grading Scale will be used.

5.8 Study Stopping Criteria:

- Death due to toxicity (not from disease progression)
- Occurrence of 2 patients with grade 4 adverse events attributable to study treatment

6.0 DRUG FORMULATION

6.1 Murmonab OKT3 : This is a murine IgG2a monoclonal antibody directed at human CD3 and is commercially available from Miltenyl, Auburn, CA). It is purchased in vials containing 5 mg/5 ml of reconstituted bacteriostatic water. OKT3 is used to activate T cells for growth and for heteroconjugation with Cetuximab to produce anti-CD3 x anti-EGFR bispecific antibody.

6.2 Anti-EGFR monoclonal antibody Cetuximab (Bristol-Myers Squibb, New York, NY): Cetuximab is a humanized murine monoclonal antibody directed at EGFR, and is commercially available as single-use, 50 ml vials containing 100 mg of drug as a sterile, preservative-free, injectable liquid. The binding characteristics and its ability to mediate cytotoxicity towards non-small cell lung cancer cells (A-549) and other lung cancer cell types (Calu-6) have been well

documented in our preclinical studies.

6.3 Anti-CD3 (OKT3) x anti-EGFR (cetuximab, Erbitux®) heteroconjugated bispecific antibody (EGFRBi): See section 7.3 on the production of anti-CD3 x anti-EGFR per GMP conditions. Investigational New Drug (IND) application BB-IND #13091: Product Name: Autologous T Cells Activated with OKT3 and Expanded with Interleukin-2 (Prometheus) armed with OKT3 (anti-CD3) x Cetuximab (anti-EGFR) given with Granulocyte Macrophage Colony-Stimulating Factor and Interleukin 2. Sponsor: L.G. Lum. Approved 8/06 to date. BB-IND #13091 has SOPS that specifies the production of bispecific antibody, sterility testing, and the standard operation procedures for arming of activated T cells.

6.4 Leucovorin: Leucovorin is commercially available in parenteral formulations (3- and 5-mg ampules; 50-,100- and 350-mg vials). In situations where leucovorin (folinic acid) is not available because of shortage, then the pure l-stereioisomer, levoleucovorin (levo-folinic acid) may be administered as a substitute, at one-half of the protocol-stipulated leucovorin dose (200 mg/m2 of levoleucovorin in most situations) in order to facilitate continuity of pt care.

6.4.1 Leucovorin Storage and Preparation: All dosage forms are stored at room temperature. The reconstituted parenteral solution,10 mg/mL, is stable for at least 7 days at room temperature. At concentrations of 0.5 to 0.9 mg/mL the drug is chemically stable for at least 24 hours at room temperature under normal laboratory light. The 50- and 100-mg vials for injection are reconstituted with 5 and 10 mL of sterile water or bacteriostatic water, respectively, resulting in a 10-mg/mL solution. The 350-mg vial is reconstituted with 17 mL of sterile water resulting in a 20-mg/mL solution. Leucovorin (0.5–0.9 mg/mL) is chemically stable for at least 24 hours in normal saline, 5% dextrose, 10% dextrose, Ringer's injection, or lactated Ringer's injection. Leucovorin (0.03, 0.24, and 0.96 mg/mL) is stable for 48 hours at room and refrigeration temperatures when admixed with floxuridine (FUDR, 1, 2, and 4 mg/mL) in normal saline. Leucovorin is also compatible with fluorouracil.

6.5 Fluorouracil: Fluorouracil is a fluorinated Pyrimidine Antimetabolite that inhibits thymidylate synthetase, blocking the methylation of deoxyuridylic acid to thymidylic acid, interfering with DNA, and to a lesser degree, RNA synthesis. Fluorouracil appears to be phase specific for the G1 and S phases of the cell cycle. Pharmacies or clinics shall obtain supplies from normal commercial supply chain or wholesaler for injection 50 mg/mL (10 mL, 20 mL, 50 mL, and 100 mL). Fluorouracil may be given IV push or IV infusion.

6.5.1 Fluorouracil Drug Product Storage and Preparation

Store intact vials at room temperature and protect from light. A slight discoloration may occur with storage but usually does not denote decomposition. Dilute in 50 to 1000 mL of 0.9% NaCl or D5W. If exposed to cold, a precipitate may form; gentle heating to 60ºC will dissolve the precipitate without impairing the potency. Solutions in 50 to 1000 mL 0.9% NaCl or D5W or undiluted solutions in syringes are stable for 72 hours at room temperature. Fluorouracil and leucovorin are compatible for 14 days at room temperature.

6.6 Oxaliplatin

Oxaliplatin is used in combination with infusional 5-fluorouracil/leucovorin for the treatment of cancer. Oxaliplatin is commercially available as a 50-mg or 100-mg vial. Refer to the label for additional information regarding treatment with oxaliplatin.

6.6.1 Oxaliplatin Storage and Preparation

Oxaliplatin is provided as a sterile, preservative-free, lyophilized powder for reconstitution in vials containing 50 mg and 100 mg of oxaliplatin. The lyophilized powder is reconstituted by adding 10 mL (for the 50-mg vials) or 20 mL (for the 100-mg vials) of Water for Injection or 5% dextrose solution and then by diluting in an infusion solution of 250 mL of 5% dextrose solution. Oxaliplatin should be stored at 25ºC (77ºF) in the light-proof packaging provided. Temperature excursions to 15ºC to 30ºC (59ºF to 86ºF) are permitted. **DO NOT mix with solutions containing normal saline or other chloride-containing fluids/diluents. DO NOT combine with alkaline medications or media (such as basic solutions of 5-FU, trometamol) which cause OXAL to degrade.** Do not use needles or intravenous infusion sets containing aluminum items for the preparation or administration (risk of degradation of OXAL on contact with aluminum).

6.7 Proleukin

Proleukin (IL-2; Prometheus, San Diego, CA) is purchased as a preservative-free lyophilized powder in vials containing 22 million International Units (IU) for expanding T cells. Patients will not receive IL-2 infusions.

6.8 Granulocyte-macrophage colony stimulating factor (GM-CSF, Leukine)

GM-CSF (rhu -GM-CSF, sargramostim) is a 127 amino acid glycoprotein produced in a yeast (S. cerevisiae) expression system by Immunex, which differs from the native GM-CSF by substitution of leucine for arginine at position 23. GM-CSF is a hematopoietic growth factor that stimulates the proliferation and differentiation of hematopoietic progenitor cells. GM-CSF is provided as a lyophilized powder in glass vials, and is suitable for parenteral administration following reconstitution with 1 ml sterile water for injection, USP without preservative. Active Ingredient: rhu GM-CSF 250 µg or 500 µg with a specific activity of approximately 5×10^7 U/mg in a normal human bone marrow colony assay. Inactive Ingredients: Mannitol, USP 40 mg, Sucrose, USP 10 mg, and tromethamine, USP 1.2 mg. Syringe: 14 day stability refrigerated. Minibag: (50cc NS/D5W) 48 hour stability refrigerated.

7.0 PREPARATION OF EGFRBi-ARMED ACTIVATED T-CELLS

7.1 Facility:

Qualified personnel who are familiar with procedures which minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of immunotherapeutic agents in a self-contained protective environment. Cells will be generated in the Cancer Immunotherapy Laboratory (GMP facility in Karmanos Cancer Center) under a FDA approved IND #BB-13091 with standard operating procedures (SOPs) for growing, splitting, harvesting, arming of ATC, cryopreservation, and infusion of BATs.

7.2 Activation, Culture, and Freezing of Armed ATC:

Lymphocytes are obtained by pheresis and cultured at a density of 1-3 x 10^6 cells/ml in RPMI 1640 media (Lonza), containing 100 IU/ml of IL-2, 10-20 ng/ml of OKT3 (Janssen Biotech, Inc or Miltenyi Biotech), and 2% human serum (Valley Biomedical). Cells will be cultured for a maximum of 14 days in media.

7.3 Preparation of anti-CD3 x anti-EGFR Bispecific Antibody:

BB-IND #13091 entitled “Autologous T Cells Activated with OKT3 and Expanded with Interleukin-2 armed with OKT3 (anti-CD3) x Cetuximab (anti-EGFR) given with Granulocyte Macrophage Colony-Stimulating Factor and Interleukin 2” specifies details for the production, purification, and quality control testing are part of the new IND that was submitted to the FDA on 3/29/2011. Lawrence G. Lum, MD, DSc is the sponsor. (Phone number 313-576-8326 and e-mail: luml@karmanos.org).

7.4 Arming of ATC with EGFRBi:

The harvested ATC will be counted and a dose of 50 ng of EGFRBi per million ATC will be added to the solution and incubated for 1 hr at 4^{o}C. The BATs will be washed, counted, and re-suspended in the final solution that will be cryopreserved in aliquots specific for each infusion.

7.5 Cytotoxicity Assay:

Cytotoxicity is measured in a 20 hr ^{51}Cr -release assay. The EGFR+ cell line, will be used as a positive control and an EGFR- cell line will be used as a negative control. Tumor cells are plated in a flat-bottomed microtiter plate and incubated at 37^{o}C. The targets are washed and labeled the next day with ^{51}Cr at 37^{o}C. The wells containing tumor cells will be washed and armed or unarmed ATC will be plated at different E:T ratios and incubated for 20 hrs at 37^{o}C. The next day, the supernatants harvested from the microtiter wells will be counted and the percent specific lysis will be calculated[47].

7.6 Quality Assurance of ATC Cell Product:

Lists of suppliers of monoclonal antibodies, heteroconjugation reagents, and culture reagents will be maintained as well as lot numbers used and supplier provided documentation of sterility and documentation that all reagents are free of endotoxin and mycoplasma. The final bag of cells to be administered will be tested for sterility, mycoplasma, and endotoxin level. Records of all quality control measures will be maintained by the laboratory.

8.0 REGISTRATION AND REQUIRED DATA

8.1 Pre-Study.

All patients enrolled will be evaluated and staged prior to initiation of therapy. Baseline studies will be completed within ≤4 weeks prior to study registration.

8.2 Protocol Registration

The attending physicians in the gastrointestinal oncology service will consider the pt for the study. All pt information will be forwarded to Data Manager at (313-576-8506 or Stephanie Bower at 313-576-9276) at the Karmanos Cancer Institute Clinical Trial Office for determination of eligibility. Upon informed consent and eligibility confirmation, the pt will be registered to the study.

8.3 Tests and Observations

Immune evaluations will be conducted as delineated in **Table 1**. Baseline serum and cell will be aliquoted from the pheresis. Blood draws (4 green top tubes containing approximately 40 mls of anti-coagulated blood and 1 red top tube containing 8 mls of serum) will be **pre-BAT** infusion, **after the 4th** infusion, and then approximately **2 weeks** (14±4 days), **2 months** (60 days±7 days, optional), **4 months** (120 days±7 days) and **optionally every 6 months** (180 days±7 days) **after the last infusion.** Optional IEs will be done if there continue to be immune responses at **6 month** (± 2 weeks) **intervals** until the responses have disappeared.

8.3 Table 1: Tests and Observations	Pre –Registration[1]	Pheresis	Chemotherapy	Pre - BAT	Week 1		Week 2		Post BAT #4	Week 3		Week 4		1-2 wks Post #8	2 mos after last infusion	4 mos after last infusion	6 mos after last infusion
					BAT #1[2]	BAT #2	BAT #3	BAT #4		BAT #5	BAT #6	BAT #7	BAT #8				
History and Physical[3]	X			X					X					X	X	X	X
Performance Status[3]	X			X			X		X					X	X	X	X
CBC	X			X					X					X	X	X	X
Metabolic Panel w/ Mg + Phosphorus	X			X					X					X	X	X	
CEA or CA 19-9	X			X					X					X	X	X	X
Serum Pregnancy Test	X																
HBsAg B & C and HIV	X																
Urinalysis & CrCl[4]	X																
Immune Studies[5]		X		X[9]					X					X	(X)	X	(X)
EKG	X																
MUGA or ECHO	X													X			
CTor PET/CT[6]	X			X											X		
Tumor Measurements[7]	X			X										X	X		
Pheresis[8]		X															
Bridging chemo			X														
BAT Infusion					X	X	X	X		X	X	X	X				
Safety Assessment					X	X	X	X		X	X	X	X				

Table Legend

1. All tests performed ≤4 Weeks prior to study registration.
2. Pt should receive BAT #1-8 infusions twice per week for 4 weeks (+/- 2 days).
3. H&P and Performance: To be performed by one of the protocol investigators or their designee.
4. Creatinine clearance may be calculated.
5. Optional (X) immune evaluation assessment (± 1 week). Immune function testing samples will be delivered to Dr. Lum's laboratory (HWCRC RM 723). Please call Dana Schalk at 313-576-8320 for specimen pickup. Do not leave the blood overnight. A total of ~~60~~ 48 mls of blood will be drawn at each of the designated points. One 10 ml red top tube and five 10 ml green or lavender top tubes containing anti-coagulant.
6. CT or PET/CT: To be repeated approximately every 8-12 weeks to until progression of disease
7. Tumor evaluation will be done with the discretion of the PI according to the schema figure above

8. T-cells will be collected using pheresis and transported by Dr. Lum;s staff at Karmanos Cancer Institute to the cGMP facility. Collected cells will be cultured and armed in Dr. Lum's laboratory according to established protocols.
9. Prior to BAT infusion. (-3 days to day of infusion)

9.0 CRITERIA FOR RESPONSE, PROGRESSION, AND RELAPSE

9.1 Tumor Measurements

All tumor measurements will be measured in centimeters prior to initiation of therapy. These measurements should be done according to Wolchok criteria. An estimate of overall objective and subjective response will be made and recorded at the end of treatment, 2 months after the first infusion. Since it is expected that immune activity would lead to tumor flare, the restaging CT's may be repeated 2 months later based on immune-related response criteria (irRC).[61,62] and the designation of CR, PR, SD, or PD will be made at that time.

9.2 Response Definitions: Response will be defined by using the irRC criteria with the allowance of an initial mild PD due to the nature of immunologic therapies as above.

"Only measurable lesions are taken into consideration. Measures are taken bidimensionally for each lesion. Measurability is defined as 5 × 5 mm or more on helical computer tomography scans. The **sum of the perpendicular diameters** (SPD) of index lesions at baseline is added to that of new lesions to calculate total tumor burden according to the following formula:

$$\textbf{Tumor Burden} = \textbf{SPD}_{\textbf{index lesions}} + \textbf{SPD}_{\textbf{new, measurable lesions}}$$

Response categories are defined as immune-related complete response (irCR), immune-related partial response (irPR), immune-related stable disease (irSD), and immune-related progressive disease (irPD) using the same thresholds defined by standard WHO criteria". Decreases in total measurable tumor burden compared to the baseline tumor burden (SPD of all index lesions at baseline). irPD should be confirmed at 2 consecutive times mandated for irPR or irCR. New lesions are allowed when the overall tumor burden decreases qualifying for partial response (≥50% decrease) or stable disease (<50% decrease to >25% increase) as described inTable 2. These new patterns are considered clinically meaningful because they appear to be associated with favorable

Table 2. Derivation of irRC overall responses

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25?	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).
†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.

survival.[61,63] Importantly, early increases in the size of lesions may be due to infiltration of lymphocytes and does not preclude an irCR, irPR, or irSD at the next consecutive time point. If a pt is classified as having irPD, confirmation by a second scan in the absence of rapid clinical deterioration is required. ***Thus, the definition of confirmation of progression represents an increase in tumor burden of at least 25% compared with baseline at two consecutive time points at least 4 weeks apart. It is recommended that this confirmation be done at the discretion of the investigator in the context of the patient's tumor type, disease stage, and***

clinical status because awaiting a response after tumor burden increase may not be appropriate for patients with rapid symptomatic progression accompanied by a decline in performance status."

9.2.1 Complete Response (irCR): Disappearance of all clinical evidence of active tumor for a minimum of eight (8) weeks. The pt must be free of all symptoms. All measurable, evaluable and non-evaluable lesions and sites must be assessed.

9.2.2 Partial Response (irPR): a decrease ≥ 50% decrease in SPD compared to the baseline SPD. This category includes changes in non-index lesions and new, nonmeasurable lesions under "non measurable response" in Table 2. Unequivocal progression of non-index lesions if there is a decrease ≥ 50% in SPD is an irPR.

9.2.3 Stable Disease (irSD): a decrease <50% to <25% of index and new, and measurable lesions with absent/stable non-index lesions or any new, non measurable lesions.

9.2.4 Progressive Disease (irPD): ≥ 25% increase with any non-index lesions and any new, nonmeasurable lesions.

9.3 Evaluation of best overall response. The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time will be classified as having "symptomatic deterioration". Every effort will be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, the residual lesion may be investigated (fine needle aspirate/biopsy), if possible, to confirm the complete response status.

9.4 Duration of overall response. The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

9.5 Duration of stable disease. SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

9.6 Reporting of results. All patients included in the study will be assessed for response to treatment, even if there are major protocol treatment deviations. Each pt will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 8) unknown (not assessable, insufficient data). Patients with no measurable disease at the start of treatment will be evaluated based on recurrence-free survival.

9.7 Additional Assessments. All patients will have a baseline tumor marker assessment and testing of tumor markers at the designated follow-up time points. Changes in serial tumor marker measurements, in

those patients who have baseline elevations, will be utilized as a surrogate marker of clinical response, stable disease or disease progression and reported as such.

9.8 Patients without residual disease: Patients whose primary tumor or metastatic disease has been removed or successfully treated, respectively, prior to entry into the phase I trial will be evaluated based on duration of relapse-free survival.

9.9. Progression free survival: length of time from enrollment in the study until disease progression or death.

10.0 CRITERIA FOR REMOVAL OF PATIENTS FROM STUDY

10.1 Disease Progression: Patients with progressive disease following BATs infusions will be removed from treatment, their disease pathologically documented, and the pt will be followed for survival and the immunologic endpoints.

10.2 Extraordinary Medical Circumstances: If at any time the constraints of this protocol are detrimental to the patient's health, the pt shall be withdrawn from treatment. In this event:

10.2.1 Document reasons for withdrawal and record whether this action was pt or physician directed.

10.2.2 The pt will remain technically on-study although treatment has been discontinued. The patients will be followed for relapse and survival.

10.3 Unexpected or Life-Threatening Toxicity (CTCAE v4.0 Table):

10.3.1 Direct questions regarding drug therapy to the Principal Investigator.

10.3.2 Reporting requirements and procedures depend upon: (1) whether agents are suspected of causing toxicity, (2) whether possibility of such a toxicity was reported in the consent form, or manufacturer's literature (Published Toxicity), (3) the severity or grade of the toxicity.

10.3.3 Expected toxicities include:

10.3.3.1 Grade 4 myelosuppression: Report only as part of regular data submission.

10.3.3.2 All other toxicities, Grades 4: Written Report to Principal and Co-Investigators, within 10 working days.

10.3.3.3 All other toxicities, Grades 1 to 3: Report only as part of regular data submission.

10.3.4 Unexpected Toxicities: Unexpected toxicities are toxicities that are not listed in the toxicity management section of the protocol, the consent form, or the manufacturer's package insert. All unexpected toxicities will be reported to Principal and Co-Investigators, within 10 working days.

10.4 Patient noncompliance with the protocol requirements

10.5 Patient request to withdraw

10.6 Development of serious intercurrent illness during the course of treatment

10.7 Patient removed at the discretion of the PI

10.8 Patient cells do not grow during activation and arming process or cannot be used for other reasons (e.g., viability, contamination or bag breakage in the freezer).

11.0 ADVERSE EVENT (AE) REPORTING

WSU IRB Adverse Event reporting

Adverse Event reporting to begin after initiation of any immunotherapy drugs (IL-2 and GM-CSF).

Adverse event reporting will be done in compliance with the Wayne State University Institutional Review Board (IRB) as follows:

A. Death or Life Threatening Adverse Reaction or Unexpected Event:

If a death or immediately life-threatening Adverse Reaction/Unexpected Event occurs, it must be reported to the IRB office within five (5) business days of the PI becoming aware, even if the information in the report is incomplete. As more information is forthcoming, one or more additional reports should be filed with the WSU IRB Administration Office. The initial report of the Adverse Reaction or Unexpected Event and follow-up reports should be filed using the IRB Unexpected Problem Report Form.

B. Serious Adverse Reaction or Unexpected Event:

If a Serious Adverse Reaction/Unexpected Event occurs (one that necessitates or prolongs hospitalization, results in a permanent or significant disability or congenital anomaly, or is judged to be serious by the principal investigator), and is **unexpected**, (not listed in the consent form) it must be reported to the IRB within five (5) business days of the PI becoming aware. If the Serious Adverse Event is listed in the consent form but a relationship to the study intervention/activity cannot be ruled out by the PI, it must be reported to the IRB within five (5) business days. If the information is incomplete on the initial filing, follow-up reporting is required. The IRB Unexpected Problem Report Form should be used in all reports pertaining to these reactions/events.

C. Non-Serious (Moderate or Minor) Adverse Reaction or Unexpected Event:

If a non-serious Adverse Reaction occurs that is **unexpected** (not listed in the informed consent), it must be reported to the IRB within five (5) business days of awareness, **unless the Principal Investigator can rule out the relationship to the study agent or intervention.** If a non-serious Adverse Reaction occurs that is **expected** (listed in the informed consent), but is more frequent, more intense or longer lasting that expected, or requires medical treatment, it must be reported to the IRB within five (5) business days. If the reaction that is listed in the consent form occurs as described in the informed consent, **it does not need to be reported to the IRB.**

If an Unexpected Event occurs, it must be reported to the IRB within five (5) business days of the awareness by the Principal Investigator. The Adverse Reaction/ Unexpected Event Form should be used for these submissions.

D. Possibly-Related Adverse Reactions/ Unexpected Events

In the rare case where a sponsor requires that a non-reportable (i.e., possibly related) Adverse Reaction/ Unexpected Event be reported to the IRB, an amendment should be submitted by the PI requesting that the non-reportable safety report be appended to the IRB file. It remains the primary responsibility of the WSU PI, the study sponsor, and any associated DSMC to identify trends that might require the event to be elevated to definitely or probably related status.

If a trend is identified that elevates the Adverse Reaction to definitely or probably related status, an Unexpected Problem Report Form should be completed, along with an amendment for consent form changes, if needed, and submitted to the IRB.

Safety Reporting Requirements for IND Holders
In accordance with 21 CFR 212.32, sponsor-investigators of studies conducted under an IND must comply with following safety reporting requirements:

a. Expedited IND Safety Reports:

7 Calendar-Day Telephone or Fax Report:
The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of investigational agent. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND. All telephone conversations will be log as note to file in the IND BB-#13091.

15 Calendar-Day Written Report:
The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the use of the investigational agent. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, Genentech Drug Safety, and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500a Form but alternative formats are acceptable (e.g. summary letter).

FDA fax number for IND Safety Reports:

1 – The reviewers for the FDA are at:

Dr. TBD
U.S. Food and Drug Administration
Center for Biologic Evaluation and Research
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

All written IND Safety Reports submitted to the FDA by the Sponsor-Investigator must also be faxed to CBER at:

Jean F. Gildner, MSHS, MT (ASCP), CQA (ASQ)
Regulatory Project Manager
FDA/CBER/OCTGT
10903 New Hampshire Avenue
Bldg. 71, RM. 5222
Silver Spring, MD 20993-0002
Phone: (240) 402-8296

Fax: (301) 595-1303
jean.gildner@fda.hhs.gov

AND:

BB-IND #13091 Sponsor Contact Information
Lawrence G. Lum MD, DSc
Rm 740.1, 7HWCRC, 4100 John R
Detroit, MI 48201
Tel# 313-576-8326 and fax # 313-576-8939

12.0 STATISTICAL CONSIDERATIONS

12.1 Study Design/Endpoints

The objectives of this single arm phase II clinical trial are to evaluate the clinical efficacy and to confirm the safety of anti-CD3 x anti-EGFR-Bispecific Antibody Armed Activated T-Cells (BATs) therapy for locally advanced or metastatic pancreatic cancer who have received at least first line chemotherapy. The primary endpoint will test whether the treatment improves overall survival (OS) as compared to an historical standard. The secondary objectives include evaluating PFS after the last BATs infusion. Events that are considered progression events are detailed in section 9.

12.2 Sample Size

Previous studies indicated that the median survival of 2nd line pancreatic cancer patients receiving chemotherapy is 7 months[21,64]. The hypothesis in this trial is that the BAT therapy as described in this protocol will increase the median overall survival to 10.5 months (a 50% increase). A sample size of 39 yields 0.80 power to detect an increase in median survival of 3.5 months assuming exponentially distributed death times, 48 months of accrual and 18 months minimum follow up with a one-sided Type I error of 0.05. We plan on enrolling 10% more patients, 43 total to ensure that we obtain 39 evaluable patients (patients who complete all BAT infusions) at the conclusion of the trial. The 3-6 six patients in the phase I portion, assuming no dose limiting toxicities, will be analyzed as part of the phase II. No additional patients would be needed (section 4.1 Treatment Plan).

12.3 Analysis

Descriptive statistics (point and exact 90% confidence interval estimates from the resultant Kaplan-Meier curve) will be generated for the primary objective as well for the PFS endpoint in the secondary objectives. The median OS and PFS will be estimated on an intention-to-treat basis (using all registered patients), and on a response-evaluable basis (using all patients who completed all BAT infusions) using the Kaplan-Meier method. Toxicity rates will be estimated using all treated patients. Point and 90% Wilson's confidence intervals will be estimated to describe binary endpoints including toxicity rate and response rates. Patients who did not have an event 18 months after the last pt was enrolled will be censored for the survival endpoints (date of last contact for OS and date of last evaluation for progression for PFS). Exploratory Cox regression analyses will be undertaken to determine if any of the clinical covariates measured at the beginning of the trial (e.g., age, race, group assignment) are associated with OS and/or PFS.

12.4 Evaluation of toxicity

All registered patients will be evaluated for toxicity probably related to the treatment. Toxicity data will be

collected at least weekly during the course of treatment. Specific toxic outcomes are detailed in section 5 of this protocol.

12.5 Other toxic outcomes

Data on other toxic side effects (excluding those detailed in section 5.0) that patients may experience will also be collected. We have chosen a safety threshold of 0.25 to monitor other toxic side effects. We would recommend reconsidering the phase II portion of the study for safety reasons if there were X many occurrences of grade 3 or higher toxicity (using the CTCAE guidelines) among the first N (or fewer) patients treated, as it would result in an upper confidence limit greater than 0.25:

N	X	p	UCL
8	1	0.125	0.255
13	2	0.154	0.256
18	3	0.167	0.253
22	4	0.181	0.261
27	5	0.185	0.256
32	6	0.188	0.252
36	7	0.194	0.256
41	8	0.195	0.252

In the above table, N = the number of patients treated; X = the cumulative number of patients with a grade 3 or higher toxicity currently observed; p = the observed toxicity rate; and UCL = the exact 1-sided upper 80% confidence limit for p, using Wilson's method without a continuity correction. After treating 41 patients, the potential toxicity risk of this regimen should be well defined, and thereafter the possible need for termination of the study based on toxicity should be minimal.

12.6 Expected accrual rate, accrual duration, and study duration

Our anticipated accrual rate is 11 patients per year. Thus, it should take approximately 4 years to accrue the 43 patients needed for the trial. Allowing for 18 months of follow-up to obtain the primary endpoint on the last pt enrolled and 6 months to assemble, analyze and interpret the data the total study duration is projected to be at most 6 years.

12.7 Secondary Objectives

12.7.1: Immune Evaluations: For the quantitative immune response variables measured in this objective, we will produce summary statistics (including means, medians, and standard deviations) pre- and post-BATs treatment. Subsequent analyses will compare the immune response variables (after a suitable transformation, if necessary) pre- and post-treatment using a paired t-test (or Wilcoxon sign ranked test if the data are not approximately normally distributed). To explore whether immune responses associate with clinical responses, the association between the baseline of each biomarker and clinical endpoints (such as response, or OS) will be analyzed using logistic regression for binary endpoints and Cox regression for time to event endpoints. Analyses will adjust for other covariates such as age and tumor stage. No multiplicity adjustment is planned due to the nature of being exploratory. A two-sided p-value of < 0.05 will be considered significant. **Power calculation**: Given the anticipated 39 pts enrolled on this trial. We have 80% power to detect a difference in paired mean of an immune response variable pre- and post- treatment of 0.46 standard deviation units using a two-sided Type I error rate of 0.05. The minimum detectable effect size for the logistic and Cox regression analyses will be a function of the response and event rates. For example, assuming an event rate of 20%, we have 80% power to detect a hazard ratio of 2.7 with a two-sided Type error rate of 0.05.

12.7.2 Paraffin Block Staining: Each biomarker and computed biomarker (e.g., Th_1/Th_2) will be evaluated for normality and transformed (including non-parametric) if necessary to achieve normality. We will produce summary statistics (including means, medians, and standard deviations) for each variable and subsequently associate each variable with OS using Cox regression. In addition, a threshold for each variable associating with OS will be defined using classification and regression trees.[65] **Power calculation:** Assuming an event rate of 20%, we have 80% power to detect a hazard ratio of 2.7 with a two-sided Type error rate of 0.05 with 39 patients.

13. HUMAN SUBJECTS

13.1 Recruitment

In address concerns related to decreased minority recruitment in local and national protocols, we will take specific steps to enhance the awareness in the Detroit Metro Region and the State of Michigan to recruit minority groups for these studies. The location of the KCI provides a unique opportunity to recruit African Americans to the protocol. Our previous accrual record also indicated that we have been successful in recruitment to this pt population and with enrollment of African Americans.

13.2 Informed Consent

Patients will be enrolled only after informed consent is obtained after a full explanation and discussion of all alternatives with the investigator/s in a consultation visit of approximately 1 hr with a follow up and reinforcement of the risks and benefits with the nurse coordinator. The Consent forms are signed in the presence of one of the investigators and/or the Immunotherapy Nurse Coordinator. No links to pt names will be presented. The patients are given consent forms after the consult and asked to write down any questions after reading the consent forms and to bring the questions to the signing meeting.

13.3 Data and Safety ~~and~~ Monitoring Plan

Pt safety will be monitored by the PI and protocol data manager(s) on a monthly basis. The safety of protocol participants will be reviewed and adherence to stopping rules will be monitored. Adherence to the protocol, i.e., protocol violations, and data completeness and integrity will also be reviewed. Overall assessment of accrual, toxicities and responses will be done at this time to determine whether significant benefits or risks are occurring that would warrant study closure. A monthly summary will be provided to the Karmanos Cancer Institute Data and Safety Monitoring Committee and will be reviewed quarterly. One month prior to anniversary date of the IRB original approval, a yearly summary report of trial activities will be made to all participating co-investigators and the Karmanos Cancer Institute Protocol Review and Monitoring Committee (PRMC). This report will include the number of patients, the number of patients treated, a summary of all adverse events reported to date, a specific list of serious adverse events requiring immediate reporting and any significant developments that may affect the safety of the participants or ethics of the study.

13.3.1 Meetings

Scheduled meetings will be held monthly on the activity of the protocol. These meetings will include the protocol investigators, data managers, and key personnel involved with the conduct of the protocol.

13.3.2 Evaluation

During these meetings the investigators will discuss matters related to:

- Safety of protocol participants (Adverse Event reporting)
- Validity and integrity of the data

- Enrollment rate relative to expectation of target accrual, characteristics of the participants
- Retention of participants, adherence to the protocol (potential or real protocol violations)
- Data completeness on case report forms and complete source documentation

13.3.3 Completed Data and Safety Monitoring

Reports of these regular investigator meetings will be kept on file in the office of the Clinical Trials Core. The data manager assigned to the clinical trial will be responsible for completing the report form. The completed reports will be reviewed and signed off by the Principal Investigator (PI) or by one of the Co-PI's in the absence of the PI. The signed off forms will then be forwarded to the Quality Manager, Clinical Trials Office for review of completeness and processing with the Karmanos Cancer Institute Data and Safety Monitoring Committee.

13.3.4 Data Safety Monitoring Committee

The Barbara Ann KARMANOS Cancer Institute, Data and Safety Monitoring Committee will meet on a monthly basis to review the prior months Serious Adverse Event forms and Data and Safety Monitoring study specific reports that have been filed.

13.3.5 Retention Of Records

All documentation of adverse events, records of study drug receipt and dispensation, and all IRB correspondence will be retained for a minimum of 2 years after the investigation is completed.

13.3.6 Protocol Activation

This protocol will not be activated until the Karmanos Cancer Institute Protocol Review Committee, the Wayne State University IRB, and the US Food and Drug Administration have approved the initiation of this protocol.

14.0 References

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