A Phase II Randomized Window of Opportunity Trial Evaluating Cytotoxic and Immunomodulatory effects of Intratumoral INT230-6 (Cisplatin, Vinblastine) in Early-Stage Breast Cancer: the INVINCIBLE Trial

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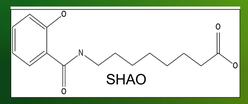
BACKGROUND

INT230-6 is a novel product with a unique dual anti-cancer mechanism that achieves both direct cancer cell necrosis and immune cell activation. The drug is comprised of cisplatin (CIS) and vinblastine (VIN) co-formulated with an, cell penetration and unique tissue dispersing enhancing molecule, 8-((2-hydroxybenzoyl)amino) octanoate (SHAO) shown in the figure below.



Following direct intratumoral (IT) injection, SHAO enables rapid drug dispersion throughout a tumor (including to hypoxic regions) and passive diffusion into cancer cells of the active cytotoxic agents. Post-resection pathology from this neoadjuvant study in breast cancer confirms that a single injection can cause necrosis of up to 95% of a 4.4 cm breast cancer tumor.[1]

Data indicates INT230-6 increases influx of antigen presenting cells (APCs) to the tumor microenvironment and increases the amount and quality of expressed antigens. On pre- and post-biopsies following two INT230-6 treatments in humans, we have previously reported a reduction in Ki67+ cells, FoxP3 and viable tumor cells, with increases in CD4 and CD8 T-cells in the tumor micro-environment using whole section image analysis of paired samples for assessment. Previously we reported that INT230-6 has demonstrated the ability to substantially reduce the cancer in large tumors and induce an adaptive (T-cell mediated) immune response that attacks the injected tumor and also non-injected tumors. [i, ii]

The role of cytotoxic agents in augmenting the response of checkpoint inhibitors was established based on the KEYNOTE-189 trial in NSCLC.[i] Cisplatin's immune effects include modulation of STAT signaling; induction of an immunogenic type of cancer cell death through exposure of calreticulin and release of ATP and high-mobility group protein box-1 (HMGB-1)ii resulting in binding to T-cells via TLR. The vinca compound vinblastine, in addition to cytotoxic effects, is capable of inducing dendritic cell maturation.

INT230-6 is designed to improve cancer treatment by more efficiently killing tumors, presenting antigen to activate the immune system, and reducing the side effects associated with systemic therapies. Study IT-01 NCT#03058289 is an open-label phase 1/2 study, that enrolled 110 adult subjects with solid tumors in phase 2 cohorts. The study seeks to assess the safety and efficacy of intratumoral (IT) injections of INT230-6 in early stage presurgical breast cancer.

1. ASCO 2022 abstract 605 poster 376

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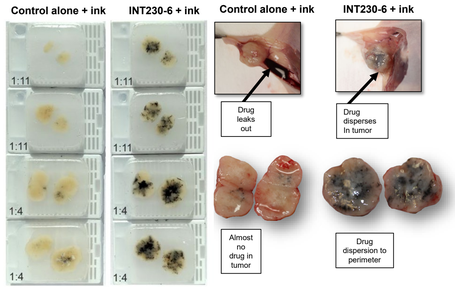
Further information can also be found on www.Intensitytherapeutics.com

DRUG DISPERSION IN TUMORS

- Control, an aqueous cisplatin solution, (Figures 1A, 1C) or INT230-6 (Figures 1B, ID) were injected with India ink into the center of a murine pancreatic tumors (BxPc3 ~1.2 cm^3). Tumors were immediately imaged and excised for sectioning.
- Results indicated enhanced INT230-6 dispersion throughout the tumor (1B and 1D) compared to controls. The control formulation did not disperse, was not retained by the cancer cells and mostly leaked out of the tumors (1A and 1C).[ii]



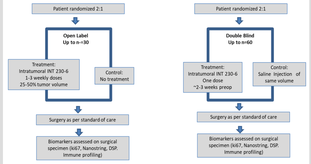
WINDOW OF OPPORTUNITY CLINICAL TRIAL

Part I: INT230-6 monotherapy safety and dose finding vs. no treatment
Part II: INT230-6 monotherapy safety and efficacy

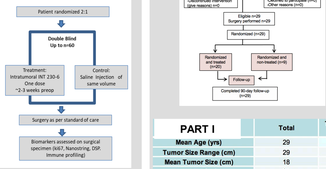
Eligibility Criteria

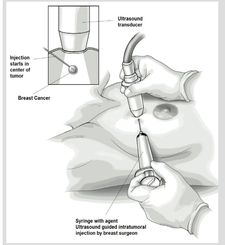
- Female breast cancer patients awaiting surgery
- Clinical stage I or II
- Histologic grade ≥ 2
- ≥ 1.5cm palpable
- Invasive ductal/lobular/NOS
- ECOG 0-2

PART I: n= 29



PART n= 58





87 women with newly diagnosed operable early-stage intermediate or high-grade T1-T2 invasive breast cancers are randomly allocated (2:1) prior to resection to Intratumoral injections of INT230-6, no treatment or saline sham. This study has two parts. **Part I** (N=29) was a randomized trial comparing 1-3 doses of INT230-6 injected weekly vs no treatment prior to surgery to evaluate safety, feasibility, and optimal drug dosing. **PART II** is a double-blinded randomized trial of 58 patients where patients received one intratumoral dose of INT230-6 vs saline sham injection (2:1).

STUDY OBJECTIVES

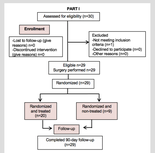
Hypothesis and Primary Outcome
Intratumoral INT230-6 is superior to no treatment or saline injection, as measured by an increase the proportion of patients achieving complete cell cycle arrest (CCCA), defined as a reduction in the proportion of cells staining positive for Ki67 as assessed by immunohistochemistry to less than a natural logarithm, or ≤2.7%, at the post-treatment specimen.

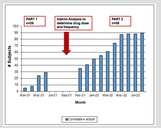
Secondary Outcome Measures
1) To assess for the proportion of patients that achieved a complete pathologic response (PCR) on surgical pathology, using the US FDA Guidance for Industry titled *Pathological Complete Response in Neoadjuvant Treatment of High-Risk Early-Stage Breast Cancer: Use as an Endpoint to Support Accelerated Approval*, as assessed by the local pathologist at the time of definitive surgery
2) To assess the Residual Cancer Burden after treatment with INT230-6
3) To characterize the overall safety of INT230-6 injected prior to surgery.
4) To assess the effect of INT230-6 on cell death pathways such as necrosis and apoptosis

Exploratory Outcomes
1. To assess the effect on infiltrating immune cells such as macrophages, NK, DC, CD4 T-cells, CD8 T-cells, regulatory T-cells.
2. To perform broad immune profiling of the blood and assess changes in CD4/CD8 T cells and identification of CD8 tetramers.

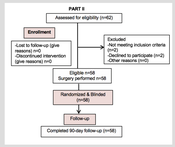
PATIENT CHARACTERISTICS AND SAFETY

PART I
Up to 3 Intratumoral Injections Preoperatively
(Control did not have injection)





PART II
One Intratumoral Injection Preoperatively
2:1 Drug vs Saline Injection
(**Not yet unblinded)



PART I	Total	Treated n=20 (69%)	Control n=9 (31%)
Mean Age (yrs)	29	58.1	55.2
Tumor Size Range (cm)	29	1.5-4.4	1.5-2.5
Mean Tumor Size (cm)	18	2.4	1.6
Invasive Ductal	24	17 (85%)	7 (77.78%)
Invasive Lobular	3	2 (10%)	1 (11.11%)
Invasive Carcinoma NOS	2	1 (5%)	1 (11.11%)
ER/PR+ Her2-	19	13(44.8%)	6 (20.68%)
ER/PR+ Her2+	4	3 (15%)	1 (11.11%)
ER-PR-Her2-	6	4 (20%)	2 (22.22%)
1 injection	4	4(13.7%)	N/A
2 injections	13	31 (44.8%)	N/A
3 injections	3	3 (10.34%)	N/A
Dose Range	29	0.3-21.3ml	N/A
Lumpectomy	24	18 (90%)	6 (66.67%)
Mastectomy	5	2 (10%)	3 (33.33%)

PART II	Total	Injection n= 58
Mean Age (yrs)	58	58.6
Tumor Size Range (cm)	58	1.5-4.0
Mean Tumor Size (cm)	58	2.4
Invasive Ductal	40	68.90%
Invasive Lobular	15	25.86%
Invasive Carcinoma NOS	3	5.17%
ER/PR+ Her2-	40	68.90%
ER/PR+ Her2+	2	3.44%
ER-PR-Her2-	0	0%
1 injection	58	100%
2 injections	0	0%
3 injections	0	0%
Dose Range	58	2-24.5ml
Lumpectomy	48	83%
Mastectomy	10	17%

Adverse events were mostly grade 1 with expected pain at the breast injection site; very little systemic adverse effects. Most patients had 2 injections preoperatively.

Event	Grade 1	Grade 2	Grade 3	Total
All	20(100%)	1(15%)	0(0%)	20(100%)
Pain injection site	20(100%)	2(10%)	0(0%)	20(100%)
Injection site reaction	2(10%)	0(%)	0(0%)	2(10%)
Injection site infection	0(%)	1(5%)	0(0%)	1(5%)
Nausea	1(5%)	0(%)	0(0%)	1(5%)
vomiting	2(10%)	0(%)	0(0%)	0(0%)
Pneumothorax	0(%)	0(%)	0(0%)	0(0%)

Injection Doses:
Average dose injected = 3cc (2.4cm tumor).
Dose range 0.8-21 cc.

Injection Timing:
2-14 days from surgery

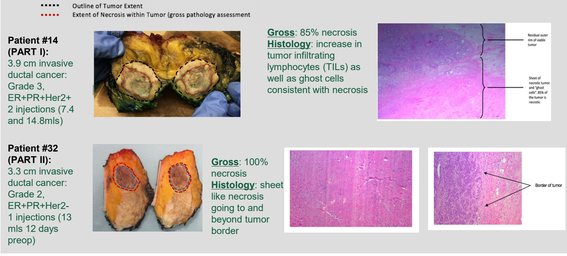
Overall Perioperative Sequelae:
No surgeries delayed.

Most common adverse events were grade I pain at the breast injection site. One patient had pneumothorax as the tumor was deep on the chest wall, treated promptly with chest tube and no surgical delay. Of the 58 subjects 18 received sham.

Event	Grade 1	Grade 2	Grade 3	Total
All	47(74.1%)	7(12.1%)	3(3.4%)	57(87.9%)
Pain injection site	40 (69%)	9(5.2%)	1(1.7%)	44(75.86%)
Injection site reaction	4(6.9%)	0(0%)	0(0%)	4(6.89%)
Injection site infection	2(3.4%)	4(6.9%)	0(0%)	6(10.34%)
Nausea	6(10.3%)	0(0%)	0(0%)	6(10.34%)
vomiting	2(3.4%)	0(0%)	0(0%)	2(3.44%)
Pneumothorax	0(0%)	0(0%)	1(1.7%)	1(1.72%)

5/90 (5%) infections total, 2 postoperative and 3 preoperative. All were treated with a short course antibiotics with complete resolution.

EFFICACY - GROSS PATHOLOGY AND HISTOLOGY



Patient #14 (PART I): 3.9 cm invasive ductal cancer: Grade 3, ER+PR+Her2+ 2 injections (7.4 and 14.8mls)

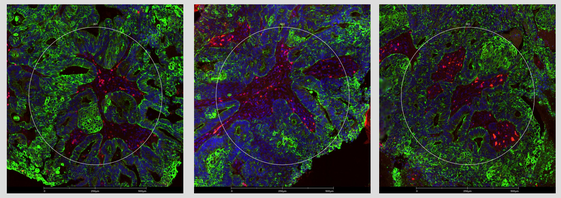
Gross: 85% necrosis
Histology: increase in tumor infiltrating lymphocytes (TILs) as well as ghost cells consistent with necrosis

Patient #32 (PART II): 3.3 cm invasive ductal cancer: Grade 2, ER+PR+Her2- 1 injections (13 mls 12 days preop)

Gross: 100% necrosis
Histology: sheet like necrosis going to and beyond tumor border

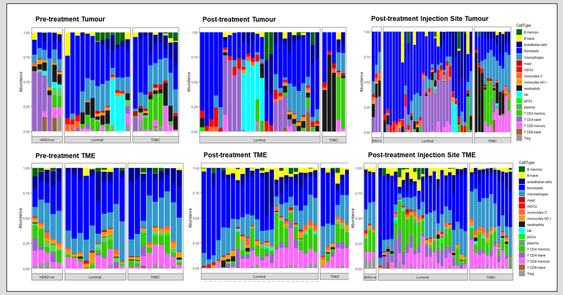
TRANSLATIONAL STUDIES: PART I

Representative NanoString DSP compartment selection in the cohort



In situ transcriptomic profiling, using the NanoString Digital Spatial Profiler (DSP) cancer transcriptomic atlas (CTA) assay was performed on the diagnostic biopsy and the surgical resection (at the injection site and resected tumour). At least three regions of interest were selected per sample. The immunofluorescence image of the markers that define the selected compartments. Selection of tumour compartment (pan-CK, green) and TME compartment (CD45, red). In situ spatial quantification of 1800 tumour and immune genes across 60 patients revealed heterogeneity of tumour and immune genes in most patients.

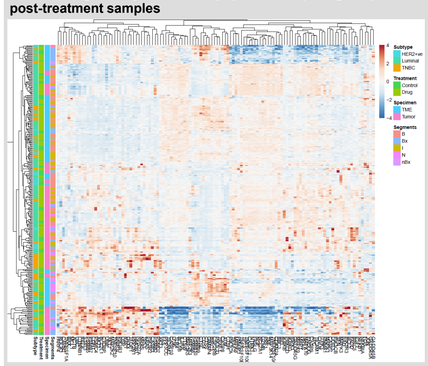
Relative abundance levels of immune cells present in the tumor compartment



A spatial deconvolution algorithm was performed to examine the relative levels of immune cells present. Within the tumour compartment there was a relative increase in abundance of CD4 T naïve and B and NK cells post treatment. Within the TME compartment there was a relative increase in abundance of CD8 memory T, CD4 naïve and B cells post treatment.

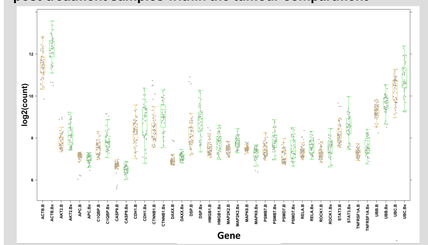
TRANSLATIONAL STUDIES: PART I

Differential gene expression of apoptosis comparing the pre- and post-treatment samples



Cluster analysis of differential gene expression. Heatmap of the apoptosis genes showing differential expression between the pre- and post-treatment samples within the tumor and tumor microenvironment. An increase in apoptosis was observed in the tumour regions in patients treated with the drug.

Differential gene expression of apoptosis comparing the pre- and post-treatment samples within the tumour compartment



Boxplot of the top 20 apoptosis genes showing differential expression between the pre- and post-treatment samples within the tumor compartment. An increase in apoptosis was observed in sampled post-treatment.

CONCLUSION

This window of opportunity pre-surgical clinical trial demonstrates that INT230-6 injection is a novel and simple method to convert traditionally immune quiescent breast cancers into immunogenic tumors with minimal adverse effects and good tolerability. An increase in immune cells present in the tumor compartment was demonstrated after treatment. In addition, within the tumor compartment an increase in apoptotic genes expression was observed.
Patient acceptability was high and accrual was fast. These results indicate a future potential for INT230-6 as an immunotherapeutic option in early-stage breast cancer.