Intratumoral (IT) INT230-6 can cause tumor necrosis in vivo: Preliminary results of a Phase II Randomized Presurgical Window-Of-Opportunity Study in Early Breast Cancers (the INVINCIBLE study) – PART I

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BACKGROUND

INT230-6 is a novel intratumoral (IT) agent consisting of cisplatin (CIS), vinblastine (VIN) and an amphiphilic cell penetration enhancer (SHAO) that enables tumor dispersion and diffusion into cancer cells.

Cisplatin 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)[iii]; Vinblastine, in addition to cytotoxic effects, is capable of inducing dendritic cell maturation. Previously Intensity reported that injection of INT230-6 into the primary and metastatic tumors of stage IV cancer patients has demonstrated the ability to substantially reduce the cancer and induce an adaptive (T-cell) immune response that attacks not only the injected tumor, but also non-injected tumors and unseen micro-metastases (Abscopal effect) (JCO 2020 38(15) S3016).

INT230-6 is designed to improve cancer treatment by more efficiently killing tumors and presenting antigen to activate immune system. In the neoadjuvant breast caner setting INT230-6 may be able to reduce the cancer substantially prior to standard of care (SOC) as well as activate an anti-cancer immune response that may increase the pathological complete response rate for women undergoing the therapy and increase event free survival.

The INVINCIBLE study is a randomized, Phase 2 presurgical Window-Of-Opportunity trial for IT INT230-6 (comprising SHAO, VINblastine (VIN) Cisplatin (VIN)) evaluating clinical and BioLogical Effects in patients with early-stage operable Breast Cancer. INT230-6 also contains a dispersion enhancer molecule (SHAO) designed to facilitate diffusion of the cytotoxic agents into cancer cells and cause tumor necrosis. In this trial, IT injections of INT230-6 are conducted to 1) exploit the potential of regional cytotoxic chemotherapy on breast cancer in vivo and 2) assess the potential for an immune response in the tumor microenvironment and host prior to surgical resection.

CONTACT

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PRECLINICAL DRUG ABSORPTION

- INT230-6 or control (aqueous cisplatin solution) was injected with India ink into the center of a murine pancreatic tumor (BxPc3 ~1.2 cm^3).
- Tumors were immediately imaged and excised for sectioning.
- Results indicated enhanced INT230-6 dispersion throughout the tumor. While the control formulation did not disperse or enter the cancer cells and mostly leaked out of the tumor.

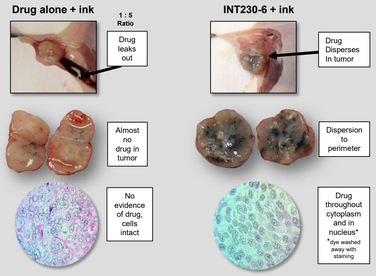

Figure 1: Mouse intratumoral injection. Histology at 100x magnification

RANDOMIZED 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

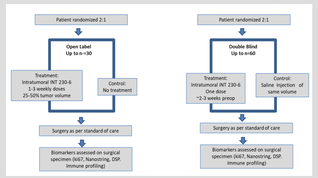

Figure 2:Study Schema

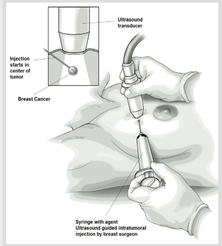

Figure 3: Intratumoral injection

Up to 90 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 IT 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 up to 60 patients where patients will receive one IT dose of INT230-6 vs saline 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.

RESULTS– PART I

Table 1: Patient characteristics

	Total	Treated n=20 (69%)	Control n=9 (31%)
Mean Age (yrs)	29	58.03	58.10
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%)

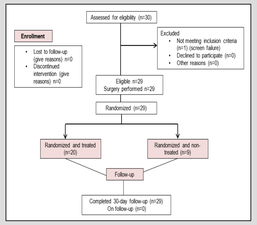

Figure 4: Consort Diagram

- No surgery was delayed or cancelled
- No surgical procedure altered
- Mean wait time to surgery 24 days (range 14-34 days)
- Mean interval between last injected dose to surgery 15 days (range 6-31 days)

SAFETY – PART I

Table 2: Adverse events

	Total	Treated n=20	Adverse Event
Grade 1	25	25(89%)	Pain/burning at injection site– 20/20 patients Skin erythema/induration– 2/20 patients Nausea/Vomiting (grade 1) – 3/20 patients
Grade 2	3	3 (11%)	Skin blister – 1/20 patients Diarrhea (grade 2)- 1/20 patients Skin infection – 1/20 patients
Grade 3	0	0 (0%)	

- 89% of all adverse events were grade 1. All adverse events resolved within 7 days. Pain at the injection site was treated with acetaminophen and/or ibuprofen as needed. No narcotics were required for pain.

BIOMARKERS – PART I

Representative images of H&E and NanoString DSP compartment selection in the cohort

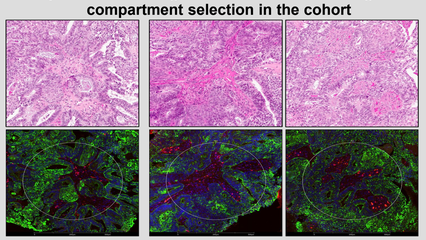

Figure 5. Low resolution immunofluorescence image of the markers that define the selected compartments. Selection of tumour compartment (pan-CK, green) and TME compartment (CD45, red).

Differential gene expression comparing the pre- and post-treatment samples within the tumor compartment

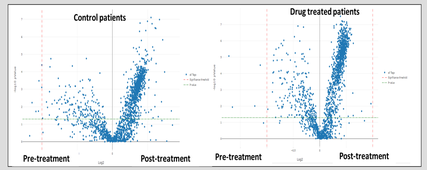

Figure 6. Volcano plots showing differential gene expression comparing pre-and post-treated samples in (A) the control group and (B) the drug treated group.

- In control samples, 918 genes were significantly different pre- vs post- treatment
- In drug treated samples, 1163 genes significantly different pre- vs post-treatment

Relative abundance levels of immune cells present in the tumor compartment

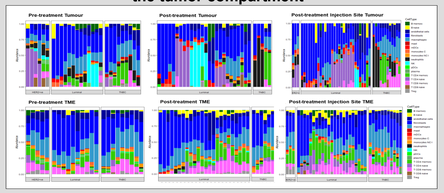

Figure 7. Bar graphs demonstrating the immune cell abundance in cohort separated by subtype.

- 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.
- Further analysis with immune profiling of blood is pending.

EFFICACY – PART I

- Part I demonstrated feasibility, safety and tolerability of presurgical IT injections in breast cancer patients. Preliminary data show histologic evidence of up to 95% tumor necrosis in varying biologic subtypes including lobular carcinoma. Further analysis with Ki67 is pending.

Outline of Tumor Extent
Extent of Necrosis within Tumor (gross pathology assessment

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

Figure 8: 85% necrosis after 2 injections in patient #14

Patient #14:
Histologic analysis shows an increase in tumor infiltrating lymphocytes (TILs) as well as ghost cells consistent with necrosis

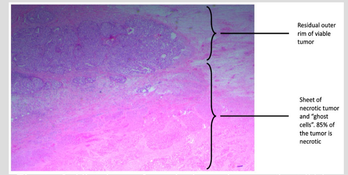

Figure 9: Histology of surgical specimen patient #14

Patient #20:
4.4 cm invasive lobular cancer: Grade 2 ER+PR+Her2- 1 injection (21.3mls)

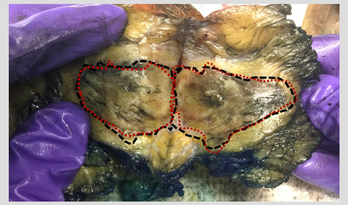

Figure 10: 95% necrosis after 1 injections in patient #20

CONCLUSIONS

Preliminary evidence shows that even a single dose of INT230-6 can cause tumor necrosis and stimulate an immune response in breast cancers prior to surgery with minimal adverse effects and good tolerability. Significant necrosis is demonstrated in invasive lobular cancers which are normally resistant to chemotherapy. PART II of the study, due to be completed in 2022, randomizes patients to either INT230-6 or saline injection and will rule out the potential confounding effect of hydrostatic pressure on tumor necrosis. The results of PART II will further evaluate the potential cytotoxic, immunomodulatory and other biologic effects of INT230-6 and its role as a potential cancer therapy in breast cancer patients awaiting surgery. Window of opportunity clinical trials are a rapid and efficient method to evaluate biologic and clinical effects of novel agents in treatment naïve cancers.