Safety and Efficacy of INT230-6, a Potential First-in-Class Intratumoral Therapy, in Monotherapy and in Combination with Pembrolizumab: Results from the IT-01 Study [KEYNOTE-A10] in Subjects with Locally Advanced, Unresectable and Metastatic Breast Cancer

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NCT:03058289 **Abstract Number: 541**

BACKGROUND

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

Preclinical data indicates in addition to inducing cell death, 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- first INT230-6 dose biopsies 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 (including breast) and induce an adaptive (T-cell) immune response that attacks not only the injected tumor, but also non-injected tumors and unseen micro-metastases. [i,ii] In an ongoing neoadjuvant study IT-02, we have demonstrated that a single INT230-6 dose can kill >95% of a breast tumor.

The role of cytotoxic agents in augmenting the response of checkpoint inhibitors was established based on the KEYNOTE-189 trial in NSCLC.[i] 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. INT230-6 is designed to improve cancer treatment by more efficiently killing tumors, presenting antigen to activate immune system, and reducing the side effects associated with systemic therapies. IT-01 is an open-label phase 1/2 study, currently enrolling adult subjects with solid tumors in phase 2 cohorts. The study seeks to assess the safety and efficacy of intratumoral (IT) INT230-6 alone and in combination with pembrolizumab or ipilimumab.

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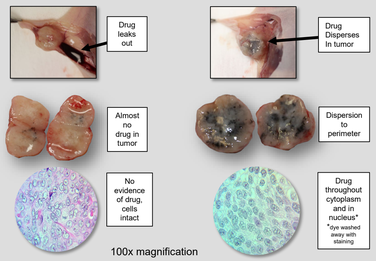
PRECLINICAL DRUG ABSORPTION

- 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). (Figure 1)
- 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.[iii]

Figure 1



CLINICAL STUDY

Phase 1
INT230-6 monotherapy dose escalation by increasing the frequency, dose volume, number of tumors, and concentration of drugs in the tumors.

Phase 2

Note: The data for breast cancer subjects reported in this poster were treated in Phase 1 prior to the ipilimumab cohort was opened.

- ≥ 18 years
- ECOG PS ≤ 1 for Combo and PS ≤ 2 for Mono
- Advanced solid tumors, relapsed/ refractory
- Measurable disease by RECIST 1.1
- At least one target tumor for injection

Dose expansion

Pembrolizumab combination
Pancreatic, MSS colon, bile duct, SCC
- INT230-6 dosing as in Monotherapy
- Pembrolizumab q3 weeks x 2 years

INT230-6 monotherapy
Solid tumors
- INT230-6 1 mL per 3 cc of tumor vol., up to 175 mL; q2 weeks x 5 doses
- Maintenance q9 weeks x 2 years

Ipilimumab combination
Breast cancer, sarcoma, HCC
- INT230-6 dosing as in Monotherapy
- Ipilimumab q3 weeks x 4 doses

IT-01 is an open-label, multi-arm Phase 1/2 clinical study in heavily pre-treated adult subjects with solid tumors.

Primary Outcome Measures for Phase 2:
- Assess the preliminary efficacy of INT230-6 alone or in combination with immunotherapy as measured by disease control rate utilizing iRECIST.

Additional Outcome Measures for Phase 2:
- Characterize the overall safety of INT230-6 alone or in combination with immunotherapy by the rate of grade 3 or higher adverse events (AEs) attributed to the combination.
- Assess the injected tumor response.
- Characterize the pharmacokinetic (PK) profile of multiple doses of the three INT230-6 components (CIS, VBL, and SHAO) after single and then multiple IT tumor site injections.

DEMOGRAPHICS & EXPOSURE

Overall, in Study IT-01 through June 1, 2021, 83 subjects received over 580 INT230-6 IT injections (340 to deep tumors, including liver, lung, and lymph nodes) at doses of 0.3 to 175mL (86 mg CIS, 17.2 mg VIN) in a single session, which are higher amounts than typical IV doses, with repeated intratumoral injections in multiple tumors.

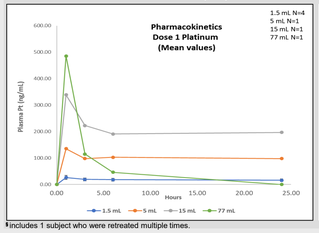
7 BC subjects (4 mono, 3 combo with pembrolizumab) in Phase 1 have been treated as of June 1, 2021.
PK data from 7 [§] BC patients from IT-01 reflects analysis as of August 5, 2021.

DEMOGRAPHICS Breast Cancer Population	
Age	Median 56 (46-82)
Gender	1 male, 6 female subjects
ECOG	43% ECOG 0
	57% ECOG 1
	0% ECOG 2
Median number of prior therapies (range)	6 (2-10)
Breast Cancer subtypes	4 TNBC, 1 ER+ PR- HER2- invasive ductal, 1 ER+ PR+ HER2- adenocarcinoma and 1 angiosarcoma
Injection	83 injections into 2 breast, 3 skin, 21 liver and 57 lymph nodes, including mediastinal lymph nodes

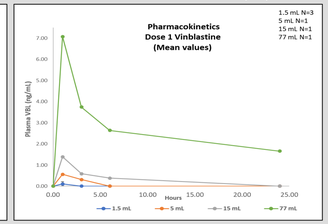
- The monotherapy subjects were more heavily pre-treated with a median of 8 prior therapies vs. 3 for pembrolizumab combination subjects.
- Dose response proportionality is observed in the PK profile, with most of the drug cleared by 24 hours. (Figure 2)
- At 7 days post dose, no accumulation of vinblastine is seen. Cisplatin when dosed is rapidly degraded to platinum (Pt) metal. When dosing cisplatin IV, a slow release of the metal is observed for several months. However, the metal has no known toxicities, and no appreciable accumulation is observed. In subjects previously dosed with platinum agents, Pt metal is detected pre-dose. There is no observed difference on the plasma measurement of the INT230-6 components in the presence of pembrolizumab, or in the breast cancer subject subset.
- Peak plasma concentrations of vinblastine are <~5% of predicted based on historical IV kinetics, indicating most of the drug remains in the tumor. [iii]

Figure 2

Mean Platinum PK for dose 1 (n=7)



Mean Vinblastine PK for dose 1 (n=7)



[§] includes 1 subject who were retreated multiple times.

SAFETY

Related Treatment Emergent AEs by max Severity in Breast Cancer Subjects (n=4 [§])

INT230-6 Monotherapy	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
	No. of subjects with least 1 AE	2 (50.0%)	1 (25.0%)	1 (25.0%)	0	0	4 (100%)
	Nausea*	3 (75.0%)	0	0	0	0	3 (75.0%)
	Localized tumor-related pain	1 (25.0%)	1 (25.0%)	0	0	0	2 (50.0%)
	Anaemia	1 (25.0%)	0	1 (25.0%)	0	0	2 (50.0%)
	Fatigue*	1 (25.0%)	1 (25.0%)	0	0	0	2 (50.0%)
	Vomiting*	1 (25.0%)	1 (25.0%)	0	0	0	2 (50.0%)
	Abdominal discomfort	1 (25.0%)	0	0	0	0	1 (25.0%)
	Abdominal pain	1 (25.0%)	0	0	0	0	1 (25.0%)
	Chills	1 (25.0%)	0	0	0	0	1 (25.0%)
	Dysgeusia	1 (25.0%)	0	0	0	0	1 (25.0%)
	Musculoskeletal chest pain	0	1 (25.0%)	0	0	0	1 (25.0%)
	Neck pain	1 (25.0%)	0	0	0	0	1 (25.0%)
	Pain in extremity	0	1 (25.0%)	0	0	0	1 (25.0%)
	Pyrexia	1 (25.0%)	0	0	0	0	1 (25.0%)
	Stomatitis	1 (25.0%)	0	0	0	0	1 (25.0%)

Related Treatment emergent AEs by max severity in Breast Cancer Subjects (n=3)

Pembrolizumab Combo	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
	No. of subjects with least 1 AE	1 (33.3%)	2 (66.7%)	0	0	0	3 (100%)
	Localized tumor-related pain	1 (33.3%)	2 (66.7%)	0	0	0	3 (100%)
	Axillary pain	1 (33.3%)	0	0	0	0	1 (33.3%)
	Injection site bruising	1 (33.3%)	0	0	0	0	1 (33.3%)
	Muscular weakness	1 (33.3%)	0	0	0	0	1 (33.3%)
	Nausea	1 (33.3%)	0	0	0	0	1 (33.3%)
	Neck pain	0	1 (33.3%)	0	0	0	1 (33.3%)
	Pruritus	1 (33.3%)	0	0	0	0	1 (33.3%)

[§] 1 subject re-enrolled in subsequent arms of the study, and safety was assessed for each arm.
*In the broader monotherapy context, constitutional symptoms do not appear to dose related.
- Most adverse events were low grade and transient.
- There were no related grade 4 or 5 TEAEs.
- There were no related SAEs.
- There were no events that were dose limiting.

BIOMARKERS

Hematoxylin and Eosin (H&E) assessments were conducted to determine the percentage of viable tumor cells and necrotic cells. There were substantial reductions of cancer cells in biopsies from INT230-6 monotherapy subjects after two injections of INT230-6, when compared to baseline. Images from matched pair biopsies from a triple negative breast cancer subject pre and post two INT230-6 injections are shown in Figure 3.

Figure 3

Image A: Pre-dose Day 0 **Image B: Post 2 doses Day 28**

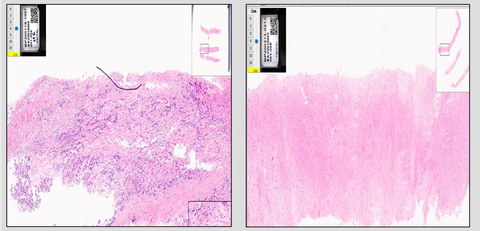
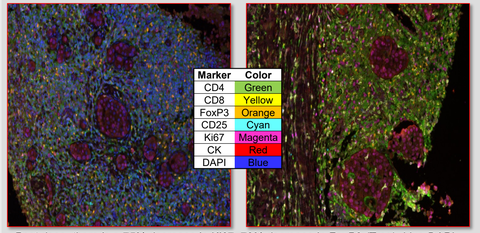


Image A sample pre-dose shows highly proliferative cancer. Image B sample taken on day 28 post two doses (day 0 and day 14) shows significant reduction in cancer cells. (Magnification 400μ)

Multiplex immunohistochemistry (IHC) staining of biopsied tissue from a breast cancer subject pre dose (day 0) and T= 28 days post dose who received 2 doses of INT230-6 (days 0 and 14) is shown in Figure 4.

Figure 4

Image A: Pre-dose Day 0 **Image B: Post 2 doses Day 28**



Post dose, there is a 75% decrease in Ki67, 73% decrease in FoxP3 (Treg), blue DAPI (nuclear) staining indicating cancer cell death and a significant increase in CD4 and CD8 T-cells (green, yellow stains).

Summary of H&E, tissue analysis and injected tumor volumes in matched paired (pre- and 28 days post-dose) biopsies from triple negative metastatic breast cancer subjects (n= 3 naive tumors, evaluable, monotherapy, and combination with pembrolizumab) after two INT230-6 doses.

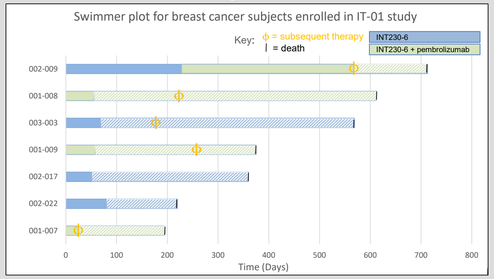
Subject	% Change in H&E assessment of viable tumor cells	Ki67 Values by Image Analysis Pre/Post Treatment	% Change in Ki67 of tumor cells by Image Analysis	% Change in Ki67 of tumor cells by Manual Analysis	Injected tumor volume in cm3 and dose (mL) (C1D0/C1D14)
001-008	-86%	72% / 5%	-93%	-97%	4.91 (1.62/1.62)
001-009	-50%	78% / 33%	-58%	-64%	46.83 (15.45/15.45)
002-022	-72%	98% / 93%	-5%	-5%	412 (60/100)
Average	-69%	83% / 44%	-52%	-55%	

Post dose, there was a substantial reduction in the number of viable tumor cells of the injected tumors. Ki67 was reduced significantly in most subjects. Subject 002-022 received INT230-6 monotherapy, while the other two received INT230-6 + pembrolizumab combination. Subject 002-022's tumor was excessively larger than the others

EFFICACY

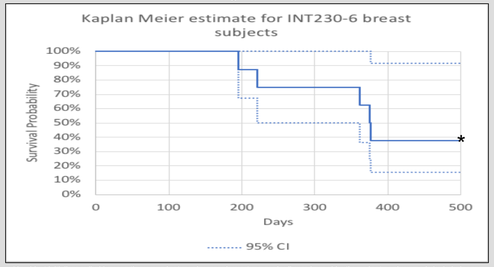
- RECIST measures may not be able to capture treatment-related effects with intratumoral therapies, including INT230-6.
- Disease control rate (DCR), defined as the percent of breast cancer subjects with a complete response, partial response, or stable disease at the first radiologic assessment, was 57%.
- Abscopal effects were seen in a non-injected visceral lesion in 1 out of 4 INT230-6 monotherapy subjects.
- The swimmers plot illustrates the survival of subjects treated with INT230-6 as single agent (blue), and INT230-6 in combination with pembrolizumab (green).
- All subjects were deceased.
- All subjects' treatments were discontinued secondary to clinician's decision, and not for disease progression. One subject (002-009) continued receiving INT230-6 injections despite a new lesion. Figure 5.

Figure 5



The median overall survival (mOS) was 12 months (CI:7.2, NR) (Figure 6),[1] which compares favorably to results seen in Phase 1 studies of subjects with similar prognostic factors (ECOG, LDH, # of metastatic sites), where the mOS is 90-160 days.[iv, v]

Figure 6



[1] 1 subject initially enrolled in monotherapy cohorts and crossed over to a pembrolizumab combination cohort, and was only included once.
* Data cut off at 500 days for illustrative purposes. All subjects deceased after 715 days.

CONCLUSIONS

- INT230-6 is well tolerated as monotherapy and in combination with pembrolizumab in heavily pre-treated adult subjects with breast cancer.
- INT230-6 demonstrates direct tumor killing in injected lesions.
- IHC and abscopal results in non-injected lesions indicate dosing INT230-6 may also activate a T-cell mediated immune response.
- Early data suggests that INT230-6 may prolong survival when compared to historical data from published studies in subjects with similar prognostic factors.
- Enrollment is ongoing with INT230-6 monotherapy and now in combination with ipilimumab in subjects with breast cancer in the Phase 2 expansion portion of the study.

i. Ghandi et al. NEJM 4/2018 ii. Bender et al. Int J Mol Sci 6/2020 iii. historical PK data from IV administration: Owelien J. Cancer Res 8/1977
iv. Laguna-Garrido et al. Cancer 3/2012 v. Chau et al. BMC Cancer 10/2011