Intratumoral INT230-6 causes tumor necrosis and promotes a systemic immune response; results from a multi-center Phase 1/2 study of solid tumors with and without pembrolizumab (PEM) [Intensity IT-01; Merck KEYNOTE-A10]

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BACKGROUND

INT230-6 is a novel product with a unique dual anti-cancer mechanism. 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), that enables drug movement throughout a tumor (including to hypoxic regions) and passive diffusion into cancer cells following intratumoral (IT) delivery. A neoadjuvant study in breast cancer confirms that a single injection can kill 95% of an injected tumor and recruit TILs.[ii]

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 not only the injected tumor, but also non-injected tumors and unseen micro-metastases. [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 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 immune system, and reducing the side effects associated with systemic therapies. IT-01 NCT#03058289 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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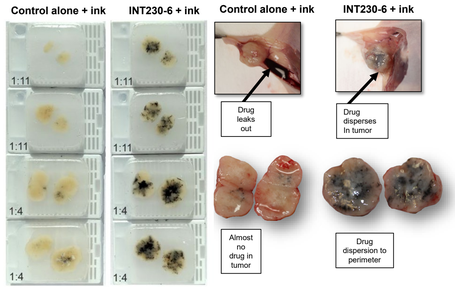
DRUG DISPERSION IN TUMORS

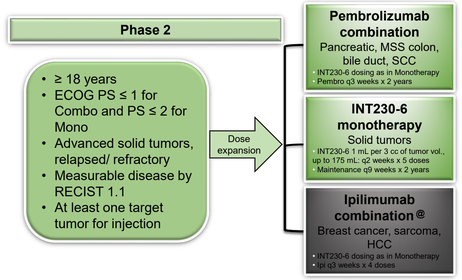
- Control, an aqueous cisplatin solution, (Figures 1A, 1C) or INT230-6 (Figures 1B, ID) were injected with India ink into the center of a murine pancreatic tumors (BxPc3 ~1.2 cm^3). Tumors were immediately imaged and excised for sectioning.
- Results indicated enhanced INT230-6 dispersion throughout 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]

Figure 1A Figure 1B Figure 1C Figure 1D



CLINICAL STUDY

IT-01 is an open-label, multi-arm Phase 1/2 clinical study in heavily pre-treated adult subjects with solid tumors. The clinical program evaluates INT230-6 as a monotherapy and in combination with checkpoint inhibitors (PEM or IPI) given Q3W. Total dose was set by a subject's incoming tumor burden. INT230-6 was escalated in the phase 1 portion from 5 mL every 28 days to up to a 175 mL given every 14 days. In phase 2 INT230-6 dose was limited to a maximum of 175 mL Q2W. Checkpoint drugs were given per label. Several biomarkers are being evaluated using collected blood and tumor tissue.



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

Additional Outcome Measures for Phase 2:

1. 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 study treatments.
2. 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

As of April 1, 2022, there were 93 subjects treated (63 mono, 30 combined with pembrolizumab (two patients were in both groups).

Demographicme	Mono	Pembrolizumab Combo
Age (median, range)	60.7 (35-84)	65.3 (47 – 86)
Gender	50% male	60% male
ECOG	0 (30%), 1 (62%)	0 (23%), 1 (77%)
Median # of prior Therapies (range) / % prior Pt / % prior PD-1	4 (0-10)/48%/48%	3 (1-10)/77%/48%
Overall # of tumors injected (% deep)	469 (53% deep)	164 (65% deep)
# of cancer types treated	19 (>50% sarc, BC, melanoma, ovarian)	7 (>70% PC, CRC, Biliary, TNBC)

Sarc: sarcoma, BC: breast cancer, PC = pancreatic cancer, CRC = colorectal cancer, TNBC = triple negative breast cancer

Tumor types for in subjects enrolled in monotherapy and PD-1 combination arms not similar.
Deep tumors were those that were not palpable and below the surface of the skin or in the body.

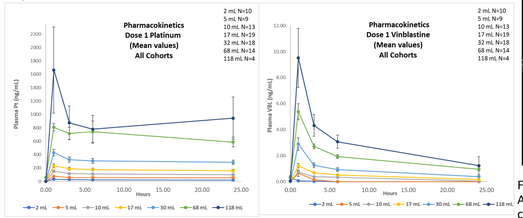
PHARMACOKINECTICS & EXPOSURE

Pharmacokinetic data is from 87[§] samples. Dose response proportionality is observed in the PK profile for doses ranging from 2 to 118 mL of INT230-6. Most of the drugs cleared by 24 hours. (Figure 2). At 7 days post dose, no accumulation of vinblastine is seen. Cisplatin when dosed is rapidly reduced to platinum (Pt) metal. In patients previously dosed with platinum-based agents, Pt metal is detected predose due to a 144-hour half life. SHAO is **G**enerally **R**egarded **A**s **S**afe (**GRAS**) and considered an excipient.

Peak plasma concentrations of vinblastine are ~ 1 to 5% of predicted based on historical IV kinetics, indicating most of the drug remains in the tumor.[iii] Some subjects received 3X the typical systemic IV dose of VIN.

Further analysis showed that there are no differences in INT230-6 PK due to age (< or ≥ 65), ECOG, or gender.

Figure 2



[§] reflects analysis as of April 1, 2022

SAFETY

Treatment related adverse events (TRAEs) by max severity in >4 subjects (n=63) INT230-6 mono (N=63)

INT230-6 Monotherapy	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4/5	Total
	No. of subjects with least 1 AE	21 (33.3%)	26 (41.3%)	7 (11.1%)	0	54 (85.7%)
	Localized tumor-related pain	23 (34.9%)	12 (19.0%)	2 (3.2%)	0	37 (58.7%)
	Nausea	20 (31.7%)	5 (7.93%)	0	0	25 (39.7%)
	Fatigue	5 (7.9%)	12 (19.0%)	1 (1.6%)	0	18 (28.6%)
	Vomiting	12 (19.0%)	3 (4.8%)	0	0	15 (23.8%)
	Decreased appetite	5 (7.9%)	8 (12.7%)	0	0	13 (20.6%)
	Anaemia	1 (1.6%)	5 (7.9%)	2 (3.2%)	0	8 (12.7%)
	Abdominal pain	2 (3.2%)	2 (3.2%)	1 (1.6%)	0	5 (7.9%)
	Dizziness	4 (6.3%)	1 (1.6%)	0	0	5 (7.9%)

i. Ghandiet al. NEJM 4/2018 ii. Bender et al. Int J Mol Sci 6/2020 iii. historical PK data from IV administration: Owellen,J. Cancer Res 8/1977, iv. Laguna-Garrido et al. Cancer 3/2012 v. Chau et al. BMC Cancer 10/2011
See ASCO 2022 Abstract Number: 11515, Poster: 420

SAFETY

Treatment related adverse events (TRAEs) by max severity in >2 subjects Combination (n=30)

Pembrolizumab Combo	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Total
	No. of subjects with least 1 AE	6 (20.0%)	12 (40.0%)	4 (13.3%)	1 (3.3%)	23 (76.7%)
	Localized tumor-related pain	8 (26.7%)	4 (13.3%)	0	0	12 (40.0%)
	Vomiting	5 (16.7%)	0	1 (3.3%)	0	6 (20.0%)
	Fatigue	1 (3.3%)	4 (13.3%)	1 (3.3%)	0	6 (20.0%)
	Nausea	4 (13.3%)	0	0	0	4 (13.3%)
	Decreased appetite	1 (3.3%)	2 (6.7%)	0	0	3 (10.0%)
	Abdominal pain	1(3.3%)	2(6.7%)	0	0	3 (10.0%)

No grade 5 related AEs

* 2 subjects enrolled in both mono & the combo; safety was assessed for each arm.
* In the combo there were 4 drug related immune adverse events (G1 GGT, G1 Rash Maculo papular, G2 Systemic Inflammatory Response Syndrome, G2 Hypothyroidism) Additional related grade 3 AEs reported in 1 subject each of hyponaetremia, neutrophil count decrease, procedural pain, and white blood cell count decrease in INT230-6 monotherapy cohorts; and 1 subject each of anemia, dehydration, dyspnoea, hypoxia, and pneumothorax in the pembrolizumab combination cohort.

Monotherapy: 2 related SAEs (G2 localized tumor-related pain & G3 procedural pain).
Pembrolizumab Combination: 4 related SAEs (G2 headache, G3 pneumothorax, G1 sepsis and G3 vomiting). The related grade 4 AE was a neutrophil decrease that resolved quickly

EFFICACY

RECIST metrics (sum of longest diameters) such as progression free survival PFS or disease control rate (DCR) to gauge a drug's efficacy are validated for use for systemically delivered therapies. The DCR rate >50 days for monotherapy was 47%; however, the INT230-6+PEM DCR was 33.3%. RECIST response principles are not ideal for use with this intratumoral administered therapy. In this study, evaluation of tumors response is complicated by the large amount of INT230-6 repeatedly injected and retained in the tumors prior to the first efficacy scan. In addition, immune infiltration, could increase tumor size. Results in a neoadjuvant setting (ASCO 2022 Abstract Number: 605, Poster: 376) that a single injection causes almost complete necrosis of the entire tumor also indicates that longest diameter is inaccurate in capturing efficacy with this IT technology. A scan of a monotherapy chordoma tumor highlights this issue (Figure 3). Longest diameter declines 15%, whereas 2nd longest diameter declines 42%.

Figure 3

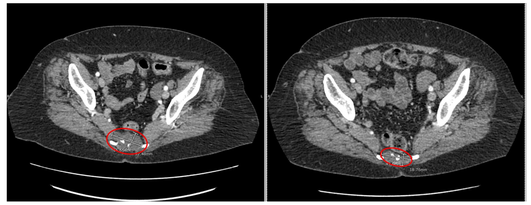
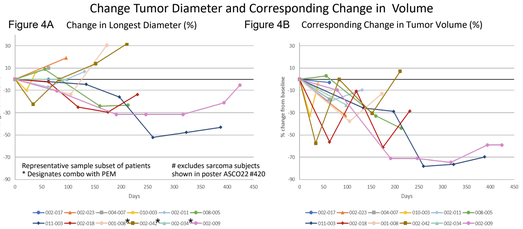


Figure 4 also highlights the observation that evaluating tumors by RECIST is problematic. Panel A reports changes in a tumor longest diameter over time. Panel 4B shows the tumor's corresponding volume change calculated from 3 dimensions using the modified ellipsoid formula. Each line represents an injected tumor from a subject# receiving either INT230-6 with or without PEM. Tumors diameters with a size increase or that were stable often shown a decrease in volume with considerable variability and a lack of correlation.

Change Tumor Diameter and Corresponding Change in Volume

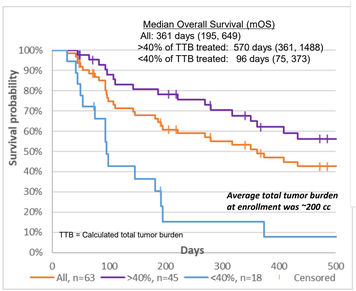
Figure 4A Change in Longest Diameter (%) Figure 4B Corresponding Change in Tumor Volume (%)

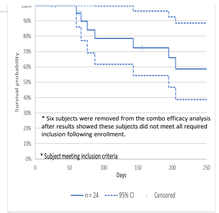


EFFICACY

The Royal Marsden Hospital Index can predict survival based on risk factors (# of metastatic sites, albumin and LDH levels) phase 1/2 studies[v]. In study IT-01 75% of subjects had 1 or more risk factors (predicting a mOS of 90-160 days[v]). An exploratory analysis of cumulative dose relative to a subject's incoming reported total tumor burden (TTB) was performed. Figure 5A reports mOS all monotherapy subjects and those receiving at ≥ or < 40% of their TTB. For the PEM combination with a median follow-up of 143 days the mOS for subjects meeting all inclusion criteria (n=24) has not been met (Figure 5B).

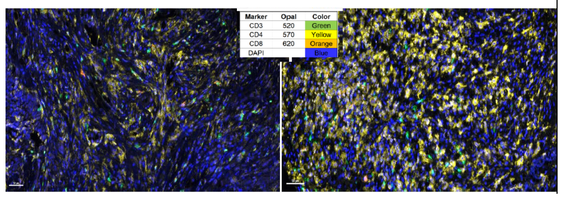
Figure 5A Kaplan Meier estimates of INT230-6 monotherapy Figure 5B Kaplan Meier estimates of INT230-6 + PEM*





BIOMARKERS

Figure 6 C1D0 C2D0



Biopsies from injected tumors for patients are taken pretreatment cycle 1 day 0 (C1D0) and Day 28 (C2D0) for immunohistochemistry (IHC) analysis. Dosing was conducted immediate post the first biopsy and C1D14. IHC analysis included evaluation of CD3+ T-cells, CD4+ T-cells, CD8+, DAPI (cancer). There was a notable reduction in cancer cells in the post dose biopsies and an increase in CD3+ CD4+ and CD8+ T-cells. The above figure 6 shows a significant influx of T-cells from a refractory, monotherapy ovarian cancer patient who prior to enrollment progressed on multiple platinum agents and several cycles of PD-1 antibody therapy. Similar results are seen in PEM combo treatment.

CONCLUSIONS

- INT230-6 is well tolerated as monotherapy and in combination with pembrolizumab.
- INT230-6 components are retained in the tumor and demonstrate direct tumor killing in injected lesions.
- IHC results in injected lesions indicate dosing INT230-6 activates a T-cell mediated immune response. Uninjected tumor regressions is evidence of a potential immune response (see Intensity Therapeutics SITC Abstract 501 and poster)
- Data suggests that INT230-6 prolongs survival compared to historical data from published basket studies in patients.
- An exploratory analysis suggests survival for monotherapy subjects receiving INT230-6 dose to ≥40% of their TTB compares favorably to the <40% group; though sample size is small
- Data is encouraging; though randomized controlled studies in an earlier line patient population in a single cancer type would be needed to assess efficacy for the combination with PEM or INT230-6 alone