Survival and Immune Response Data from Intratumoral INT230-6 Alone (IT-01) and with Pembrolizumab [KEYNOTE-A10] in Subjects with Locally Advanced, Unresectable and Metastatic Solid Tumors

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BACKGROUND

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

Preclinical 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- 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 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]; 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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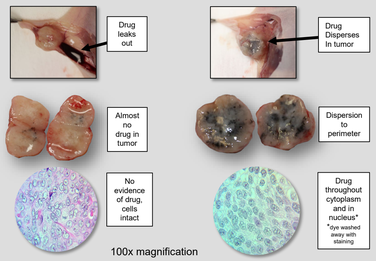
PRECLINICAL STUDY

- 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

- ≥ 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
- Pembro 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
- Ipi q3 weeks x 4 doses

[@]Please see abstract # 536 for additional details.

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

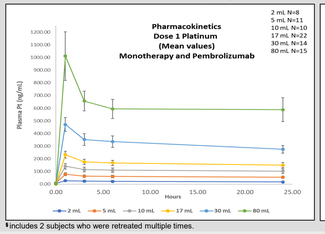
78 subjects (60 mono, 18 combo with Pembrolizumab) have been treated as of July 31 2021. PK data from 80 [§] samples reflects analysis as of August 5 2021.

DEMOGRAPHICS	
Age	Median 62 (34-85)
Gender	42 male, 36 female subjects
ECOG	27% ECOG 0
	67% ECOG 1
	6% ECOG 2
Median number of prior therapies (range)	4 (0-10)
Tumor types	20+ cancer types were accrued; breast cancer and sarcoma were the most frequent.
Sites of injection	Over 550 INT230-6 IT injections were given (280+ to deep tumors, including liver, lung, pancreas, 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.

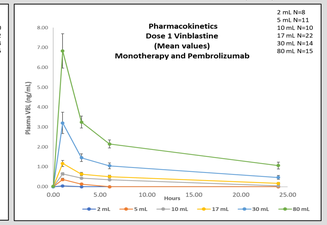
- Demographics were similar, except for tumor types, in subjects enrolled in monotherapy and PD-1 combination arms; and for subjects dosed to ≥ 40% tumor burden vs. < 40% tumor burden.
- Dose response proportionality is observed in the PK profile, with most of the drug cleared by 24 hours. (Figure 2)
- 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 patients previously dosed with platinum agents, Pt metal is detected predose. There is no observed difference on the plasma measurement of the INT230-6 components in the presence of Pembrolizumab.
- Peak plasma concentrations of vinblastine are ~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 vinblastine.

Figure 2

Mean Platinum PK for dose 1 (n=80)



Mean Vinblastine PK for dose 1 (n=80)



[§] includes 2 subjects who were retreated multiple times.

SAFETY

Related Treatment emergent AEs by max severity in >2 subjects (n=60 [§])

INT230-6 Monotherapy	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
	No. of subjects with least 1 AE	21 (35.0%)	24 (40.0%)	7 (11.7%)	0	0	52 (86.7%)
	Localized tumor-related pain	22 (36.7%)	11 (18.3%)	2 (3.3%)	0	0	35 (58.3%)
	Nausea	18 (30.0%)	5 (8.3%)	0	0	0	23 (38.3%)
	Fatigue	5 (8.3%)	11 (18.3%)	1 (1.7%)	0	0	17 (28.3%)
	Vomiting	11 (18.3%)	3 (5.0%)	0	0	0	14 (23.3%)
	Decreased appetite	5 (8.3%)	7 (11.7%)	0	0	0	12 (20.0%)
	Anaemia	1 (1.7%)	4 (6.7%)	2 (3.3%)	0	0	7 (11.7%)
	Abdominal pain	2 (3.3%)	2 (3.3%)	1 (1.7%)	0	0	5 (8.3%)
	Dizziness	4 (6.7%)	1 (1.7%)	0	0	0	5 (8.3%)
	Back pain	3 (5.0%)	1 (1.7%)	0	0	0	4 (6.7%)
	Chills	3 (5.0%)	0	0	0	0	3 (5.0%)
	Dry mouth	2 (3.3%)	1 (1.7%)	0	0	0	3 (5.0%)
	Dysgeusia	2 (3.3%)	1 (1.7%)	0	0	0	3 (5.0%)
	Groin pain	2 (3.3%)	1 (1.7%)	0	0	0	3 (5.0%)
	Myalgia	2 (3.3%)	1 (1.7%)	0	0	0	3 (5.0%)
	Pain in extremity	0	3 (5.0%)	0	0	0	3 (5.0%)

Related Treatment emergent AEs by max severity in >1 subject (n=18)

Pembrolizumab Combo	Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Total
	No. of subjects with least 1 AE	2 (11.1%)	10 (55.6%)	2 (11.1%)	0	0	14 (77.8%)
	Localized tumor-related pain	5 (27.8%)	4 (22.2%)	0	0	0	9 (50.0%)
	Vomiting	4 (22.2%)	0	1 (5.6%)	0	0	5 (27.8%)
	Fatigue	0	3 (16.7%)	1 (5.6%)	0	0	4 (22.2%)
	Nausea	4 (22.2%)	0	0	0	0	4 (22.2%)
	Decreased appetite	1 (5.6%)	2 (11.1%)	0	0	0	3 (16.7%)
	Abdominal pain	0	2 (11.1%)	0	0	0	2 (11.1%)
	Injection site reaction	2 (11.1%)	0	0	0	0	2 (11.1%)
	Pruritus	1 (5.6%)	1 (5.6%)	0	0	0	2 (11.1%)

[§] 2 subjects re-enrolled in subsequent arms of the study, and safety was assessed for each arm.
- Additional related grade 3 AEs reported in 1 subject each of hyponatremia, 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.
- Most adverse events were low grade and transient. There were no related grade 4 or 5 TEAEs. There were no events that were dose limiting.

Monotherapy: 2 related SAEs (localized tumor-related pain and procedural pain).
Pembrolizumab Combination: 4 related SAEs (headache, pneumothorax, sepsis and vomiting).

CANCERS TREATED

IN230-6 Monotherapy Population

Type of Cancer	# of patients	Percent
Sarcoma	9	17%
6 Other* <4% each	8	15%
SCC	6	11%
Melanoma	6	11%
Colorectal	4	8%
H&N	4	8%
Ovarian	4	8%
Breast	3	6%
Cholangiocarcinoma	3	6%
Pancreatic	3	6%
Renal	3	6%

*Other includes: Bladder, Cervical, Eccrine, Thyroid, Chordoma, and Lung

Subjects with total tumor burden or >2 cm^3 and < 700 cm^3

INT230-6 + PEM Population

Type of Cancer	# of patients	Percent
Pancreatic	5	31%
Breast (2 TNBC)	3	19%
Cholangiocarcinoma	3	19%
Colorectal	2	13%
Chordoma	1	6%
Merkel Cell	1	6%
Sarcoma	1	6%

These tables report the various cancers treated in the INT230-6 monotherapy and Pembrolizumab combination groups. There was a higher percentage of pancreatic, breast and cholangiocarcinoma subjects in the Pembrolizumab combination arm.

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 is shown in Figure 3.

Figure 3

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

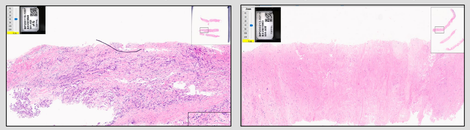
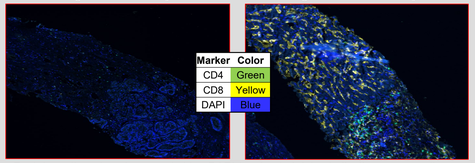


Image A sample pre-dose shows highly proliferative cancer. Image B sample taken on day 28 post two doses (day 0 and day 14) shows significant reduction in cancer cells. (Magnification 400μ)

Multiplex immunohistochemistry (IHC) staining of biopsied tissue from an ampula of vater cancer, typically a "cold" tumor, 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**

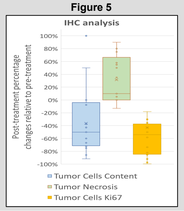


Post dose, there is a decrease in blue DAPI (nuclear) staining indicating cancer cell death and a significant increase in CD4 and CD8 T-cells (green, yellow stains).

IHC analysis of tumor cell content, tumor necrosis and Ki67 from paired (pre- and 28 days post-dose) biopsies from INT230-6 monotherapy subjects (n=17) is shown in Figure 5.

There is a median reduction of 50% in tumor cell content, a median increase of 10% in tumor necrosis and a median reduction of 54% in Ki67.

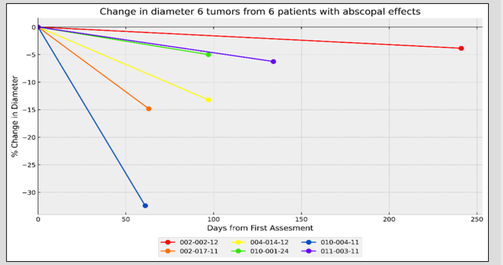
Figure 5



EFFICACY

Abscopal effects were seen in non-injected visceral lesions in 6 INT230-6 monotherapy subjects. (Figure 6)

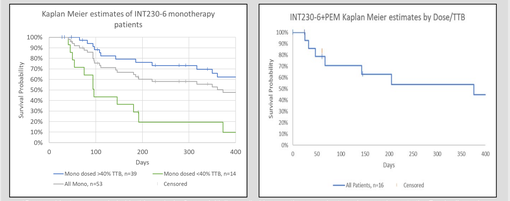
Figure 6



The median overall survival (mOS) of 373 days for monotherapy (n=53)[†] compares favorably to results seen in basket studies of patients having similar prognostic factors (ECOG, LDH, # of metastatic sites). (Figure 7 left panel) In basket studies with similar Phase 1 solid tumor populations, the mOS is 90-160 days.[iv, v]

An exploratory analysis of dose relative to total tumor burden (TTB) showed that subjects receiving a dose of INT230-6 <40% of their reported TTB had a mOS of 96 days. While in subjects receiving a dose of INT230-6 to ≥40% of TTB, mOS was 570 days (HR=0.104, 95% CI(0.038, 0.29)). Abscopal responses in Figure 6 were seen in subjects dosed to ≥ 40% total tumor burden. Patients receiving the Pembrolizumab combination had a mOS 376 days (n=16) [†]. A comparison of <40% vs ≥40% of their TTB was not conducted because data is immature.

Figure 7



[†]Some subjects were excluded in this analysis: 2 were initially enrolled in monotherapy cohorts and crossed over to the Pembrolizumab combination cohorts, and these were only included in the Pembrolizumab combination cohorts. In addition, some subjects were excluded because they had bulky disease (>700cm^3) or a very small tumor (<2cm^3).

CONCLUSIONS

- INT230-6 is well tolerated as monotherapy and in combination with Pembrolizumab.
- INT230-6 demonstrates direct tumor killing in injected lesions. In addition, IHC and abscopal results in non-injected lesions indicate dosing INT230-6 may also activate a T-cell mediated immune response.
- Data suggests that INT230-6 prolongs survival compared to historical data from published basket studies in patients with similar prognostic factors.
- An exploratory analysis suggests survival for monotherapy subjects receiving INT230-6 dose to ≥40% of their total tumor burden compares favorably to the <40% group and to historical survival from Phase 1 solid tumor basket studies with similar prognostic indicators (ECOG, LDH, # of metastatic sites).[iv, v]
- Comparison of the survival data of INT230-6 monotherapy to the Pembrolizumab combo is challenging due to different cancer types in the patient populations.

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