Preliminary results of an in progress, first-in-human Phase 1 study of Decoy20, an intravenous, killed, multiple immune receptor agonist bacterial product in patients with advanced solid tumors

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Background

Systemic activation of multiple immune receptors, such as Toll-like (TLR), Nucleotide oligomerization domain (NOD)-like, and Stimulator of interferon genes (STING) may be required for efficient anti-tumor immune responses. Decoy10 and Decoy20 are attenuated and killed, non-pathogenic, bacterial products with ~90% reduction of lipopolysaccharide (LPS)-endotoxin (TLR4 agonist) activity to enhance intravenous (IV) safety, and with retention of endogenous TLR2,8,9, NOD2 and STING agonist activity. Decoy products produced pre-clinical single-agent and/or combination-mediated anti-tumor activity with breast, colorectal, hepatocellular, and pancreatic carcinomas and non-Hodgkin's lymphoma, including innate/adaptive immune-mediated eradication of established tumors with induction of immunological memory. Tumor-eradicating synergy was observed with Decoy products in combination with anti-PD-1, indomethacin, cyclophosphamide and/or rituximab [1]. We hypothesized that, due to clearance of systemic bacteria by the liver and spleen within minutes to a few hours, systemically-administered Decoy products might produce passively targeted, transient immune activation, suitable as monotherapy or combination with approved agents (passively-targeted, pulse-prime hypothesis). Decoy20 was produced from a non-pathogenic K-12 strain of *Escherichia coli* (*E. coli*).

Study Design and Methods

INDP-D101 (NCT05651022) is a first-in-human, open label, single dose escalation and multi-dose expansion, multicenter Phase 1 trial of Decoy20 in patients with advanced/metastatic solid tumors with an initial DLT period of 28 days. Primary objectives: safety/tolerability. Secondary objectives: anti-drug immunogenicity, pharmacokinetics (PK) and preliminary anti-tumor activity. Exploratory objective: systemic immune activation via immune biomarkers. Eligible patients must have measurable tumors relapsed or refractory to standard therapies. Single-Ascending Dose (SAD) cohort evaluations precede Multiple Dose (MD) cohorts and use a standard statistical 3+3 design. The starting dose is a 1-hour i.v. infusion of 7 x 10^7 killed Decoy20 bacteria and is based on the No Observed Adverse Effect Level in rabbits, the relevant non-clinical toxicology species for LPS. Plasma biomarkers were determined by Meso Scale Discovery Electroluminescence (Figure 1) or Luminex Platform (Figure 2). Decoy20 pharmacokinetics (PK) was determined by a digital drop (dd) PCR method.

Ethics Approval: This study was approved by the following institutions' Ethics Boards: WIRB/Copernicus covering Atlantic and Karmanos with approval number 20223025; USC approval number HS-22-00497.

Trial Registration: NCT05651022

Patient and Disease Characteristics

Subject #	Age/Sex	Primary Disease Site	Dominant Sites of Disease	Prior Systemic Therapy
1	45, F	Tongue Adenoid Cystic CA	Floor of mouth and neck recurrences, measurable lung metastases	Lenvatinib
2	71, M	Piriform sinus squamous cell CA	Lung metastases, mediastinal lymphadenopathy	5-FU / Carboplatin/ Docetaxel; Cetuximab; Pembrolizumab; Pembrolizumab/ Trastuzumab; DF1001/Nab-Paclitaxel
3	48, M	Appendiceal adeno CA	Peritoneal carcinomatosis with intra-abdominal masses	FOLFOX; FOLFIRI + Bevacizumab; Q702-ONC-P1-US001 Clinical Trial; VMO-01C Clinical Trial
4	47, M	Colon CA (MSS)	Lung metastases	FOLFOX; FOLFIRI + Bevacizumab

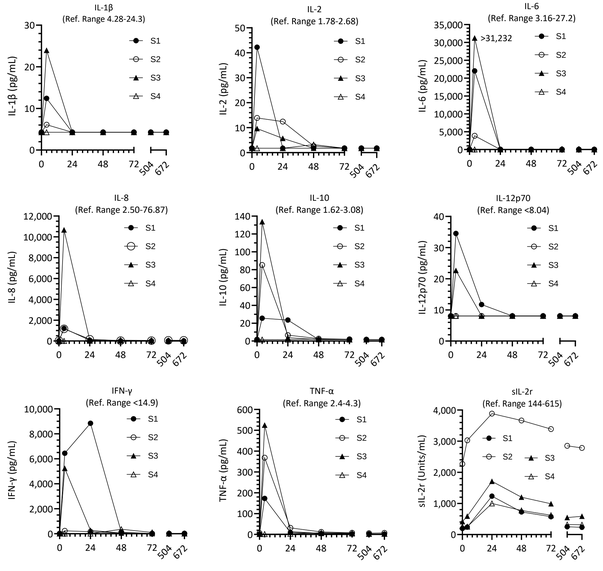
Results (Safety and Plasma Biomarkers)

Table 1 (right). Treatment-Related Adverse Events. One dose limiting toxicity of Grade (G) 3 bradycardia occurred and resolved in <30 minutes following bolus normal saline, acetaminophen, meperidine, and oxygen; G3 malaise in the same patient resolved within 2 days. Two patients experienced G3 AST increase, improving to G1 within 1-2 days. Overall, G1 chills, fatigue, fever, G2 vomiting, hypotension, G1-2 ALT increase resolved within 1-2 days and G4 lymphopenia resolved within 2-3 days, all potentially expected following exposure to LPS (TLR4 agonist), an active ingredient of Decoy20 [2,3].

Post-Dose Adverse Event	INDP-D101 Cohort # / Subject # 1/1	1/2	1/3	1/4
	Grade			
Alkaline Phosphatase Increased		G1		
ALT Increased		G1	G2	
Anorexia	G1	G1		
AST Increased		G3	G3	
Bloating			G1	
Bradycardia				G3
Chills	G1		G1	
Constipation	G2			
Creatinine Increased		G1		
Edema				
Fatigue	G1	G1		
Fever			G1	G1
Headache			G1	G2
Hemoptysis				G1
Herpes simplex reactivation			G2	
Hot Flashes	G1			
Hypercalcemia		G2		
Hypomagnesemia	G1			
Hypophosphatemia	G2			G2
Hypotension	G2			G2
Infusion-Related Reaction (IRR)		G2		G3[1]
Lymphopenia	G4	G4	G4	G4
Malaise				G3
Myalgia				
Nausea	G1			G1
Paresthesia, Intermittent	G1			
Rigors				G2
Tachycardia			G1	
Vomiting	G2		G2	

[1]SAE due to requirement for hospitalization
Note: Data are from an open database and are subject to change

Figure 1 (below). Decoy20 induces transient plasma cytokine, chemokine and biomarker expression. Analysis was carried out at pre-dose, 4, 24, 48, 72 hr, 3 weeks (504 hr) and 4 weeks (672 hr) after end of infusion. Peak cytokine and chemokine induction occurred within ~4 to 24 hours and returned to baseline by 24-48 hours. Soluble IL-2 receptor (sIL-2r), a marker of T cell activation, peaked at ~24 hours and remained elevated up to at least 72 hours. Reference ranges (healthy volunteers, pg/mL or Units/mL) are provided. Most baseline measurements reflect lower limits of assay quantitation.



Results (Plasma Biomarkers)

Table 2. Expanded single time-point plasma cytokine, chemokine and biomarker analysis. Luminex analysis was carried out at pre-dose, 0.5, 1, 2, 4, 6, 24, 48, 72 hr, and 4 weeks after end of infusion. Most induced analytes peaked within 2-4 hr and resolved within 24-48 hours. Data in Table represent the maximum fold induction or reduction.

Biomarker	Subject 1	2	3	4
	Maximum Fold Induction ≥4-Fold or Fold Reduction ≥4-Fold			
April	5			5
BAFF	10	4		
BLC (CXCL13)	6	4 reduced		8
CD30				4
CD40L (CD154)	6			
CRP				
ENA-78 (CXCL5)			5	
Eotaxin (CCL11)	9		7	6
Eotaxin-2 (CCL24)		5 reduced		
Eotaxin-3 (CCL26)				
Ferritin	6			
FGF-2				
Fractalkine (CX3CL1)		6		
G-CSF (CSF-3)	18		67	9
GM-CSF				5
Gro-alpha (CXCL1)	4	15	43	6
HGF	8		63	21
IFN-alpha				
IFN-beta			4	
IFN-gamma	27	(16)	(352)	18
IL-1-alpha	7	17	5	5
IL-1-beta	23	5	6	10
IL-1ra (IL-1F3)	1,216	833	AP	AP
IL-2	(24)	(8)	(5)	
sIL-2r	133		5	120
IL-3				
IL-4	(12)		4	5
IL-5				
IL-6	83	5	71	39
IL-7				
IL-8 (CXCL8)	31	(33)	31	24
IL-9	6	13		
IL-10	(15)	6	13	6
IL-12p40			7	
IL-12p70	6			
IL-13				
IL-15				
IL-16		5 reduced		5
IL-17a (CTLA-8)				
IL-18	8	4		
IL-20				
IL-21			7	
IL-22				
IL-23				
IL-27	32		15	
IL-31	14			9
IL-33				
LIF	6		44	
IP-10 (CXCL10)	28		28	28
I-TAC (CXCL11)	24	5	14	17
MCP-1 (CCL2)	40	19	22	51
MCP-2 (CCL8)	15		10	25
MCP-3 (CCL7)	4			
MCP-4 (CCL13)	6	7	11	
M-CSF		9		
MDC (CCL22)				
MIF		8 reduced		18
MIG (CXCL9)	42		6	24
MIP-1a (CCL3)	22		34	27
MIP-1b (CCL4)	36	25	253	47
MIP-3a (CCL20)	25	25	92	78
MMP-1				
NGF-beta	6			
SCF			6	4
SDF-1a (CXCL12)	5			9
TARC (CCL17)	7		25	
TNF-alpha	17	18	95	(5)
TNF-beta				6
TNF-RII	5			11
TRAIL (CD253)		6	8	7
TWEAK				
TSLP	4		25	4
VEGF-A	38		6	23

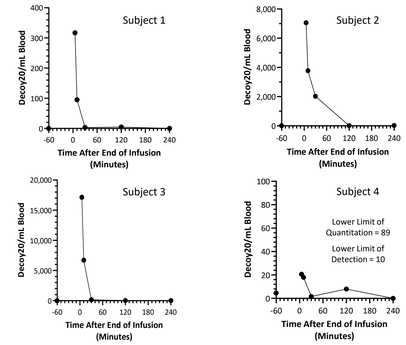
No data entry indicates no induction ≥4-fold
AP = Analysis Pending
Values in parenthesis from Meso Scale Discovery Electrochemiluminescence panel

Table 3. Cytokines and chemokines associated with innate and adaptive anti-tumor immune responses (not exhaustive). Most cytokines and chemokines have been shown to play a positive role in immune responses, but can also produce toxicity if present at abnormally high levels for extended periods. A single dose of i.v. Decoy20 produced transient ≥4-fold induction of the cytokines and chemokines highlighted in yellow in the Table.

Cytokines and Chemokines Inducing Migration, Activation, Maturation and/or Proliferation of Immune Cells	Responsive Immune Cell Type: All Participate in Anti-Tumor Immune Responses
GM-CSF, IL-1β, IL-4, IL-12, IL-15, IFN-αβ, IFN-γ	Dendritic Cells
IL-2, IL-12, IL-18, TNF-α	Gamma-Delta (γδ) T-Cells
IL-1β, IL-8, IFN-αβ, IFN-γ, MIP-1α, TNF-α	M1 Macrophage
IL-2, IL-10, IL-12, IL-15, IL-18, IL-21, IFN-αβ, IFN-γ	NK Cells
IL-12, IL-18, IL-21, IFN-αβ, IFN-γ	NKT Cells
GM-CSF, IFN-αβ, IL-4, IL-8, MIP-1α, TNF-α	Neutrophils
GM-CSF, IL-1β, IL-2, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-15, IL-18, IL-21, IFN-αβ, IFN-γ, MIP-1α, TNF-α, TNF-β	T-Cells (Th1, Th17 or Th2 CD4+ or CD8+) Including CIK, CTL, LAK

Results (Pharmacokinetics)

Figure 2. Pharmacokinetic analysis confirms rapid clearance of systemically-administered Decoy20. A ddPCR method with lower limit of detection/quantitation of 10/89 Decoy20 bacteria per mL blood was developed and used to determine Decoy20 levels in Subject blood pre-dose and 5, 10, 30, 120, 240 minutes, 24 hr, and 4 weeks after the end of the infusion. Decoy20 was cleared from blood within 30 to 120 minutes after the end of the infusion. Due to peak concentration within 5 minutes of end of infusion and the steep elimination slope, differences in peak height between subjects may reflect slight differences in clearance time during infusion. Additional time-points will be required to determine if there is also significant biological variability with respect to exposure.



Results (Preliminary Activity)

Post-dose tumor re-staging at 4 weeks demonstrated stable disease by RECIST 1.1 in all 4 subjects. Three of the subjects had progressive disease prior to Decoy20 administration.

Discussion and Conclusions

A single i.v. dose of Decoy20 was cleared from blood within 30-120 minutes and produced transient induction in plasma of over 50 biomarkers, many of which have been associated with stimulation of innate and/or adaptive immune responses.

Blood immune cell profiling demonstrated a rapid increase in neutrophils, and rapid decrease in essentially all other leukocytes, with recovery of all cell types within approximately 72 hours, suggesting that Decoy20 induces a transient, but significant leukocyte trafficking or re-distribution event (data not shown).

Despite the presence of agonists for TLR2,4,8,9, NOD2 and STING and other molecules associated with intact bacteria, the safety profile of Decoy20 was largely as expected for i.v. administration of purified LPS-endotoxin, based on published clinical experience [2,3]. Adverse effects were generally tolerable and resolved with or without treatment within 30 minutes to 3 days.

Administration of a single dose of Decoy20 produced initial stable disease in all 4 subjects, including 3 progressing prior to Decoy20 administration.

The results are supportive of our passively-targeted, pulse-prime hypothesis.

A second part of the study will enroll subjects to receive multi-dosing of Decoy20.

References

1. Newman M. A systemically administered killed bacteria-based multiple immune receptor agonist for pulsed anti-tumor immunotherapy. [abstract]. In: Proceedings of the American Association for Cancer Research Annual Meeting 2023; Part 1 (Regular and Invited Abstracts); 2023 Apr 14-19; Orlando, FL. Philadelphia (PA): AACR; Cancer Res 2023;83(7_Suppl):Abstract nr 4165.
2. Engelhardt R, Mackensen A, Galanos C. Phase 1 trial of intravenously administered endotoxin (*Salmonella abortus equi*) in cancer patients. Cancer Res. 1991; 51:2524-2530.
3. Bahador M, Cross A. From therapy to experimental model: a hundred years of endotoxin administration to human subjects. J Endotoxin Res. 2007; 13:251-279.