Abstract #1497

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

Diana L. Hanna[1], Angela T. Alistar[2], Roger J. Waltzman[3], Jeffery A. Nieves[3], Michael J. Newman[3], Manali Bhave[4], Carlos Becerra[5], Simon Khagi[5], Anthony El-Khoueiry[1], Jacob S. Thomas[1], Mohammed N. Al Hallak[6]

[1]University of Southern California Norris Comprehensive Cancer Center, Los Angeles, CA; [2]Atlantic Health System, Morristown, NJ; [3]Indaptus Therapeutics, Inc., New York, NY; [4]Emory Winship Cancer Institute, Atlanta, GA; [5]Hoag Memorial Hospital Presbyterian, Newport Beach, CA; [6]Karmanos Cancer Institute, Detroit, MI

Pulse-Prime Hypothesis Confirmed

Background

- Systemic activation of toll-like receptor (TLR), nucleotide oligomerization domain (NOD)-like, and Stimulator of interferon genes (STING) are important for anti-tumor immune responses
- **Decoy20** is an attenuated and 100% killed intact bacterial product with ~90% reduction of lipopolysaccharide (LPS)-endotoxin (TLR4 agonist) activity
- Produced from a non-pathogenic K-12 strain of Escherichia coli
- In addition to reduced TLR4 agonist, **Decoy** bacteria retain endogenous TLR2, 8, 9, NOD2, and STING agonist activity
- Produced pre-clinical *in vivo* single-agent and/or combination-mediated anti-tumor activity (colon, hepatocellular, pancreatic carcinomas, and non-Hodgkin's lymphoma)
- Immune-mediated eradication of established murine and human tumors with induction of immunological memory
- Tumor-eradicating synergy observed in combination with anti-PD-1, indomethacin, cyclophosphamide, and/or rituximab
- Tumor eradications dependent on NK, CD4+ T, and CD8+ T cells [1]
- Induces maturation or activation of multiple innate and adaptive human immune cell types *in vitro*, including NK, NKT, DC, CD4+ T and CD8+ T cells [2]
- Hypothesis: due to rapid clearance of systemic bacteria by the liver and spleen, systemically-administered **Decoy20** might produce passively targeted, broad but transient immune activation
- Suitable as monotherapy or in combination with approved agents (passively-targeted, pulse-prime hypothesis, **Figure 1**)

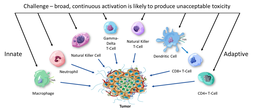


Figure 1. **Decoy20** is designed to activate both innate and adaptive pathways using a Pulse-Prime approach

Study Design and Methods

Tumor specific cohorts selected based on pre-clinical evidence of efficacy and mechanism of action of Decoy20.

- INDP-D101 (NCT05651022): first-in-human, open-label, single-dose escalation and weekly dose expansion, multicenter Phase 1 trial of **Decoy20** in patients with advanced/metastatic solid tumors refractory to standard therapies
- Primary objectives: safety/tolerability
- Secondary objectives: anti-drug immunogenicity, pharmacokinetics, and anti-tumor activity
- Exploratory objective: systemic immune activation via immune biomarkers
- Eligibility: measurable disease per RECIST 1.1; adequate organ function, ECOG 0-1
- Single-Ascending Dose (SAD) cohort evaluations precede weekly dose cohorts
- 3+3 design
- Starting dose: 1-hour IV infusion of 7x10^7 **Decoy20** bacteria
  - Based on No Observed Adverse Effect Level in rabbits, the relevant non-clinical toxicology species for LPS
- **Decoy20** pharmacokinetics (**Figure 2**) determined by a digital drop (dd)PCR method
- Plasma biomarkers: Luminex platform (**Table 5** and **6**)
- Serum biomarkers: Electroluminescence (MSD) platform (**Table 7**)

Enrollment (3 cohorts):

- In Part 1, each received a single dose of **Decoy20** (3 or 7x10^7) to assess for immediate and late-onset toxicities; both doses had a single DLT among 6 or 7 participants
- In Part 2a, each received weekly doses of **Decoy20** 3x10^7 until intolerance or disease progression. This and the higher dose cohort continue to recruit participants.
- Imaging obtained every 8-12 weeks

Table 1. Participant Characteristics

	Part 1 (single dose)		Part 2a (weekly dosing)
	7x10^7	3x10^7	3x10^7
n	6	7	6
Median age (y)	47.5	61	61
Age range (y)	32-81	42-71	44-65

Table 2. Disease Characteristics and Treatment History in Part 2a

Participant	Age/Sex	Tumor Type	Prior Systemic Therapy
1	63/F	Pancreatic CA	FOLFIRINOX; gemcitabine + nab-paclitaxel
2	44/M	MSS Rectal CA	FOLFOXIRI
3	59/M	NSCLC	Platinum + etoposide + XRT + durvalumab; carboplatin + pembrolizumab + bevacizumab
4	48/F	MSS Colon CA	Adjuvant CAPOX; FOLFIRI + bevacizumab
5	65/M	SCCHN	Carboplatin + paclitaxel + XRT; durvalumab; SBRT to liver; PEF ablation
6	64/F	Pancreatic CA	FOLFOXIRI

CA, carcinoma; MSS, microsatellite stable; NSCLC, non-small cell lung cancer; SBRT, stereotactic body radiation therapy; SCCHN, squamous cell carcinoma of head and neck; XRT, radiotherapy; PEF, pulsed electrical field ablation

Safety Results

Table 3. Safety Summary: With weekly Decoy20 administration, AEs were predominantly Grade 1-2 and transient in duration.

Weekly administration of **Decoy20** 3x10^7 (n = 6) revealed mostly grade (G) 1-2 adverse events (AEs). The most commonly observed AE (**Table 3**) was fatigue (n=3); AEs that occurred in 2 participants included increased ALT, increased AST, chills, diarrhea, hypokalemia, hypophosphatemia, IRRs, and lymphopenia. G≥3 AEs were limited in number (**Table 4**). Three were deemed treatment-related (increased AST in 1 participant and lymphopenia in 2 participants). Transaminase elevations peaked at G3 and are expected for **Decoy20** due to the presence of LPS and given the presumed rapid passive hepatic clearance.

Among all 3 cohorts, G3 treatment-related AEs included increased AST, increased ALT, infusion-related reactions (IRR), bradycardia, pain, and malaise; the only related G4 AE was lymphopenia. Bradycardia of <1 day duration (at 7x10^7) and increased AST for >72 hours (at 3x10^7) were dose limiting. Lymphopenia resolved in 2-3 days, an expected pharmacodynamic outcome suggesting trafficking of lymphocytes.

Table 3. All Treatment-Related Adverse Events in Part 2A

Preferred Term	Weekly Dose, 3x10^7 (N = 6)	
	Grades 1 and 2	Grades 3 and 4
Alanine aminotransferase increased	2	0
Arthralgia	1	0
Aspartate aminotransferase increased	1	1
Back pain	1	0
Blood alkaline phosphatase increased	1	0
Blood bilirubin increased	1	0
Chills	2	0
Diarrhea	2	0
Early satiety	1	0
Fatigue	3	0
Headache	1	0
Hyperhidrosis	1	0
Hypokalemia	2	0
Hypomagnesemia	1	0
Hypophosphatemia	2	0
Hypotension	1	0
Infusion-related reaction	2	0
Leukopenia	1	0
Localized edema	1	0
Lymphopenia	0	2
Malaise	1	0
Nausea	1	0
Platelet count decreased	1	0
Pyrexia	1	0
Tachycardia	1	0
Vomiting	1	0

Table 4. All AEs Grade 3 or Higher Irrespective of Relatedness in Part 2A

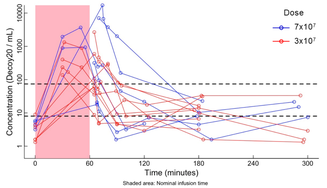
Preferred Term	Weekly Dose, 3x10^7 (N = 6)	
	Grade 3	Grade 4
Aspartate aminotransferase increased	1	0
Hypertension	1	0
Lymphopenia	0	2

Highlighted AEs were considered related to treatment.

Results (Pharmacokinetics)

Figure 2. Pharmacokinetic analysis confirmed rapid disappearance of systemically administered Decoy20 from blood, providing a mechanistic basis for the "Pulse" hypothesis

A ddPCR method with a lower limit of detection (LLoD) of 8 Decoy20 /mL blood and a lower limit of quantitation (LLoQ) of 74 Decoy20 /mL blood was developed and used to determine Decoy20 levels in participant blood pre-dose and 65, 70, 90, 180, 300 minutes, 24 hours, and 4 weeks after the start of the 60-minute infusion (7 single dose participants at 3x10^7 and 6 single dose participants at 7x10^7 Decoy20). Timepoints were added at 30 minutes and 50 minutes (during infusion) and 120 minutes after the start of the infusion for 4 single dose participants (2 at each dose level) and 2 weekly dose participants at 3x10^7 Decoy20. Profiles are shown for all single dose participants and the first week of dosing for the 2 weekly dosing participants. The 24-hour and 4-week time points are not shown, as all values were below the limits of detection or quantitation. **Decoy20 blood levels dropped rapidly to below the limits of detection or quantitation within 30 to 120 minutes after the end of the infusion,** with significant variability in the pre and post infusion values. Decoy20 exposure was observed with repeat dosing in the 2 weekly dose participants without a consistent increase or decrease in exposure over multiple weeks (data not shown). Collection of an additional timepoint at 15 minutes after the start of infusion is planned.



Results (Plasma Biomarkers)

Table 5. A single dose of 3x10^7 Decoy20 induced transient plasma expression of an extremely broad array of cytokines, chemokines and biomarkers involved in innate and adaptive immune responses

Luminex-based plasma analysis of 71 analytes was carried out at pre-dose, 0.5, 1, 2, 4, 6, 24, 48, 72 hours, and 4 weeks after the end of infusion with 7 participants receiving a single dose of 3x10^7 Decoy20. **Induction of 53 analytes (≥3-fold) was observed.** Data are presented for the 27 analytes most consistently induced. Maximal induction typically occurred within 2-6 hours and resolved within 24-72 hours after the end of the Decoy20 infusion. Data in Table 5 represent the maximum fold induction for each analyte. Three-fold induction is considered to be a minimum threshold for potential biological significance.

	Maximum-Fold Induction (≥3-fold) With a Single Dose of 3x10^7 by Participant #*						
	1	2	3	4	5	6	7
Eotaxin (CCL11)		3	3	19	4		
G-CSF	5	3	7		28	5	
GM-CSF	11	7			4		
Gro-Alpha (CXCL1)	11	14	6	11	6	13	7
IFN-γ	5		4				
IL-1β	5	13			10	5	3
IL-1ra	9	6	11	10	40	5	
IL-2		5			5		
sIL-2r			5	3			
IL-4	4	6		4	6		
IL-6	44	49	67	33	70	30	18
IL-8 (CXCL8)	43	49	29	87	45	9	17
IL-10	5	14		8	6	6	8
IL-21	3	5		5	8	3	
IL-27	4	7	5	4	11	3	
I-TAC (CXCL11)	25	9	9	10	9	5	14
IP-10 (CXCL10)	7	10	12	9	12	10	5
MIG (CXCL9)	9				4		4
MCP-1 (CCL2)	10	12	29	22	24	17	4
MCP-2 (CCL8)	7	10	14	14	5	14	7
MCP-4 (CCL13)	3	8	3		9		3
MIP-1a (CCL3)	16	26	22	21	29	23	22
MIP-1b (CCL4)	12	31	19	19	77	22	34
MIP-3a (CCL20)	111	86	50	82	84	31	43
TARC (CCL17)		6			3		4
TNF-α		6			24		
TRAIL		7	4		7		

*Blank cells indicate no induction or induction <3-fold.

Results (Plasma Biomarkers)

Table 6. Broad, transient plasma cytokine, chemokine and biomarker induction was observed after each weekly dose of 3x10^7 Decoy20 with 2 weekly dosing participants (A and B), consistent with the "Pulse-Prime" hypothesis

Luminex-based plasma analysis of 71 analytes was carried out at pre-dose, 2, 4, 6, and 24 hours after the end of infusion, plus 72 hours (1st dose only). Data (pg/mL, except units/mL for sIL-2r) are presented for the 27 analytes most consistently induced. Yellow highlight indicates induction ≥3-fold. Blank cells indicate no sample received. Participant B exhibited some decreased biomarker induction after the 3rd or 4th weekly dose.

	Eotaxin (CCL11)		G-CSF		GM-CSF		Gro-alpha (CXCL1)		IFN-γ		IL-1β		IL-1ra		IL-2		sIL-2-r		IL-4		IL-6		IL-8 (CXCL8)		IL-10		IL-21		IL-27		I-TAC (CXCL11)		IP-10 (CXCL10)		MIG (CXCL9)		MCP-1 (CCL2)		MCP-2 (CCL8)		MCP-4 (CCL13)		MIP-1a (CCL3)		MIP-1b (CCL4)		MIP-3a (CCL20)		TARC (CCL17)		TNF-α		TRAIL	
Timepoint	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Week 1																																																					
Pre-Dose	8	13	72	38	43	14	9	4	11	11	15	7	746	754	70	10	15160	2459	42	22	62	13	20	3	5	4	15	19	23	10	12	17	13	21	19	10	37	88	5	3	59	98	4	5	13	11	52	11	97	367	15	15	10	29
2 Hours	15	5	81	66	54	14	47	59	11	11	34	44	>ULQ	>ULQ	70	47	11750	2624	70	69	426	234	103	103	14	25	45	37	54	37	47	20	45	49	21	10	297	337	14	15	279	149	51	51	145	191	916	425	258	371	24	28	16	23
4 Hours	9	15	74	103	41	14	15	23	11	23	21	66	>ULQ	>ULQ	58	44	12890	5429	54	195	236	141	30	77	9	16	36	106	47	63	54	107	78	242	8	10	281	727	14	48	171	120	22	58	86	137	866	1315	330	360	18	24	10	42
6 Hours	8	6	47	58	23	14	8	7	11	11	9	7	>ULQ	>ULQ	35	38	9282	2755	26	47	52	22	17	3	4	4	13	14	11	10	25	26	38	102	10	10	214	357	6	12	178	130	9	19	41	44	128	108	418	443	15	15	4	3
24 Hours	12	9	84	59	67	14	13	5	29	146	37	51	6492	>ULQ	106	10	26026	15267	83	78	72	28	32	3	13	7	35	76	60	37	62	358	110	263	155	919	117	465	13	48	67	201	7	10	44	49	94	43	97	422	22	15	22	54
72 Hours	9	5	47	49	39	14	9	7	11	11	12	7	777	1240	73	10	18847	6961	43	32	56	19	22	3	6	4	18	25	24	10	12	20	18	41	17	10	41	77	5	6	53	114	4	3	10	9	58	14	97	397	15	15	9	32
Week 2																																																				
Pre-Dose	6	5	37	44	22	14	5	4	11	11	7	7	598	713	40	10	16041	2254	17	13	34	13	14	3	4	4	10	10	10	10	12	12	12	9	10	10	28	42	3	2	49	77	3	3	9	9	45	11	97	269	15	15	4	3
2 Hours	8	5	76	64	46	14	19	7	11	11	23	18	>ULQ	>ULQ	67	10	7824	2570	50	49	191	113	56	22	11	9	30	11	24	10	23	15	26	15	11	10	488	391	13	21	90	100	53	61	153	165	381	86	97	324	24	15	12	17
4 Hours	15	8	96	59	41	14	14	6	15	17	25	20	>ULQ	>ULQ	58	43	12786	3995	65	56	393	94	40	12	10	4	32	36	56	26	79	68	104	127	31	10	505	606	21	26	134	112	34	44	119	102	1062	807	97	389	18	15	14	25
6 Hours	5	7	35	49	19	14	7	4	11	14	9	7	>ULQ	>ULQ	17	10	5741	2703	24	31	29	23	10	3	4	4	15	18	22	10	34	33	38	107	10	10	159	447	5	13	288	114	10	17	28	43	211	298	496	484	15	15	<3	18
24 Hours	19	7	59	36	41	14	9	4	11	11	19	24	3630	3553	78	10	21626	6640	42	29	33	15	24	3	9	4	22	15	34	11	68	64	76	63	136	10	93	118	9	9	58	105	7	12	39	13	68	11	97	321	15	15	22	41
Week 3																																																			
Pre-Dose	7	4	44	63	25	14	6	4	11	11	8	7	610	698	47	42	11651	3735	24	54	19	16	15	3	4	4	10	16	14	11	12	12	12	10	10	10	28	47	2	3	52	83	4	4	9	9	49	11	97	292	15	15	6	24
2 Hours	11	3	62	34	40	14	20	5	11	11	16	7	>ULQ	4060	49	41	8774	<100	43	13	162	13	50	3	10	4	26	10	30	10	28	12	37	6	11	10	586	259	15	6	75	97	59	42	164	69	548	11	97	315	19	15	14	3
4 Hours		5		46		14		9		11		7		>ULQ		10		<100		12		27		3		4		9		10		30		42		10		488		14		95		25		65		431		340		15		14
6 Hours		5		34		14		4		11		7		>ULQ		10		3896		33		13		3		4		9		10		19		51		10		318		13		104		7		22		178		405		15		24
24 Hours		6		22		14		4		11		7		1625		10		5186		36		16		3		4		19		10		12		27		10		85		6		81		6		9		11		277		15		35
Week 4																																																			
Pre-Dose	3	4	33	51	25	14	7	4	11	11	7	7	552	605	32	10	9925	<100	16	13	23	13	12	3	4	4	10	10	10	10	12	12	8	7	10	10	18	31	2	2	47	88	3	3	9	9	38	11	97	290	15	15	3	3
2 Hours	11	6	80	64	49	14	20	7	11	11	36	7	>ULQ	831	69	10	10398	3100	60	39	227	16	57	3	15	5	36	21	44	15	29	12	68	104	127	31	10	505	606	21	26	134	112	34	44	119	102	1062	807	97	389	18	15	14	25
4 Hours	16	3	100	44	46	14	22	8	20	11	31	7	>ULQ	2700	63	10	12175	2459	69	50	287	14	45	3	19	4	38	11	63	13	80	12	103	9	37	10	460	108	21	4	174	82	37	4	124	9	966	31	231	267	21	15	17	14
6 Hours		3		38		14		5		11		7		3129		10		<100		13		13		3		4		10		10		12		10		10		80		3		84		3		9		11		282		15		3
24 Hours	15	10	101	54	72	64	11	10	11	12	12	7	1914	977	93	49	28675	6440	62	60	89	33	30	3	10	11	22	66	32	20	60	36	52	38	174	205	63	157	9	9	62	83	5	9	34	59	65	11	97	248	15	17	19	68
Week 5																																																			
Pre-Dose	7		46		29		8		11		7		688		56		15054		27		35		21		4		14		18		12		15		18		36		3		57		4		9		58		97		15		9	

>ULQ = result was greater than the upper limit of quantification for the assay.

Results (Serum Biomarkers)

Table 7. Analysis of Decoy20-mediated cytokine induction in serum samples revealed induction of IFN-γ and TNF-α, which was not consistently observed in plasma samples

Electroluminescence (MSD)-based serum analysis of 11 analytes was carried out at pre-dose, 4, and 24 hours after the end of infusion with the same two weekly dosing participants reported in Table 6. Results are pg/mL, with yellow highlight indicating induction ≥3-fold. Several overlapping analytes were consistently observed at higher concentrations with the serum panel. Results for IFN-γ and TNF-α are shown. Similar results were observed with single dose participants (data not shown). LPS was previously reported to induce serum INF-γ and TNF-α. The previous studies with LPS and our results may reflect induction of intracellular cytokine expression and release from cells that lyse during serum preparation. Participant B exhibited some decreased biomarker induction after the 3rd or 4th weekly dose, as reported for the plasma panel.

	IFN-γ		TNF-α	
	A	B	A	B
Week 1				
Pre-Dose	5	14	5	2
4 Hours	87	605	76	44
24 Hours		8780		11
Week 2				
Pre-Dose	5	8	4	2
4 Hours	1217	1590	83	33
24 Hours	140	272	10	6
Week 3				
Pre-Dose	5	7	5	2
4 Hours	1222	1113	87	18
24 Hours		100		4
Week 4				
Pre-Dose	5	5	5	2
4 Hours	1814	147	86	5
24 Hours	36	28	9	3
Week 5				
Pre-Dose	7	5	6	2

*Blank cells indicate that no samples were received.

Cytokines and Chemokines

Table 8. Decoy20 transiently induces 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. **Decoy20** produced transient ≥3-fold induction of the cytokines and chemokines highlighted in yellow.

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-1αβ, 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-1αβ, TNF-α, TFN-β	T-cells (Th1, Th17 or Th2 CD4+ or CD8+) Including CIK, CTL, LAK

Results (Preliminary Activity)

No complete response/partial response has been observed following weekly doses of **Decoy20** administration in 5 evaluable participants who have received Decoy20 on a weekly schedule for 4-8 weeks. One of these participants, with a high PD-L1 expressing SCCHN, had stable disease at the first imaging interval.

Discussion and Conclusions

"Pulse Prime" hypothesis confirmed: Single IV doses of **Decoy20** were 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.

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 IV administration of purified LPS-endotoxin, based on published clinical experience [3,4].

Weekly administration of Decoy20 for at least 4 weeks at 3x10^7 (n=6) revealed no DLTs and confirmed transient (4–24 hour) immune activation with each weekly infusion and rapid Decoy20 clearance, similar to single dose administration. Adverse effects were generally tolerable and resolved with or without treatment within 30 minutes to 3 days.

One participant, with a high PD-L1 expressing SCCHN, had stable disease at the first imaging interval.

Transient systemic immune activation at a tolerable weekly dose supports the pulse-prime hypothesis.

Six tumor-specific weekly-dosing cohorts continue to enroll, including colorectal cancer with liver metastases, hepatocellular, pancreatic adenocarcinoma, non-small cell lung cancer, squamous cell carcinoma of the head and neck, and urothelial carcinoma. A combination with a PD-1 inhibitor is planned.

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Contact Roger Waltzman for questions: roger@indaptusrx.com

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