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#62

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

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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, **Decoy20** retains 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 [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**)

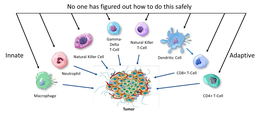


Figure 1. **Decoy20** is designed to activate both innate and adaptive pathways using a Pulse-Prime approach

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 multi-dose expansion, multicenter Phase 1 trial of **Decoy20** in patients with advanced/metastatic solid tumors
- Primary objectives: safety/tolerability
- Secondary objectives: anti-drug immunogenicity, pharmacokinetics, and anti-tumor activity
- Exploratory objective: systemic immune activation via immune biomarkers
- Eligibility: measurable tumors relapsed or refractory to standard therapies
- Single-Ascending Dose (SAD) cohort evaluations precede Multiple Dose (MD) cohorts
- 3+3 design
- Starting dose: 1-hour i.v. 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
- Plasma biomarkers: Luminex platform (**Table 4**)
- Blood immune cell profiling: Epiontis platform (**Figure 3**)

Enrollment

- 2 cohorts
  - Each received a single dose of **Decoy20**
    - **Decoy20** dose $7x10^7$: 1F/3M, median age 48
    - **Decoy20** dose $3x10^7$: 5F/2M, median age 61
- Demographic and tumor specifics in **Table 1** below

Table 1. Participant and Disease Characteristics

Participant	Dose	Age/Sex	1° Disease Site	Prior Systemic Therapy
1	$7x10^7$	45/F	Tongue adenoid cystic CA	Lenvatinib
2		71/M	Piriform sinus squamous cell CA	5-FU/carboplatin/docetaxel; cetuximab; pembrolizumab; pembrolizumab/trastuzumab; DF1001/nab-paclitaxel
3		48/M	Appendiceal adeno CA	FOLFOX; FOLFIRI/bevacizumab; Q702 (AxlMer/CSF1R TKI); VMD928 (TrkA inhibitor)
4		47/M	MSS colon CA	FOLFOX; FOLFIRI/bevacizumab
5	$3x10^7$	48/F	Anal squamous cell CA	cisplatin; 5-FU/mitomycin; docetaxel/cisplatin/5-FU; durvalumab/investigational vaccine; nivolumab
6		65/F	Anal squamous cell CA	chemoradiotherapy; carboplatin/paclitaxel; nab-paclitaxel; KZR261 (Sec61 translocon inhibitor)
7		59/M	MSS colon CA	FOLFOXIRI; FOLFIRI/Bevacizumab x 2 courses
8		71/F	Ovarian carcinosarcoma	carboplatin/paclitaxel/bevacizumab; gemcitabine/docetaxel
9		65/F	MSS rectal CA	FOLFOX/bevacizumab; primary surgery and hepatic metastatectomy; capecitabine/bevacizumab; 5-FU/bevacizumab; FOLFIRI/bevacizumab; pelvic XRT/5-FU
10		61/M	Cholangiocarcinoma	gemcitabine/cisplatin; gemcitabine/cisplatin/durvalumab; pemigatinib
11		44/F	Mucinous ovarian CA	carboplatin/paclitaxel; carboplatin/paclitaxel/olaparib; capecitabine; pembrolizumab; toripalimab

CA, carcinoma; MSS, microsatellite stable.

Safety Results

Safety Summary: Safety data reveal limited high-grade and short-duration AEs. The most commonly observed related AEs (see **Table 2**) among 11 participants were: lymphopenia (n=9), increased AST (n=5), fatigue (n=4), increased ALT (n=4), hypotension (n=4), chills (n=4), and vomiting (n=4). Resolution of related AEs of hypotension was within hours with i.v. fluids or no intervention. Transaminase elevations peaked at grade 3, resolved within 72 hours (except for an ALT increase in 1 patient with resolution at 1 week), and are expected for **Decoy20** due to the presence of LPS and given the presumed rapid passive hepatic clearance. Frequently occurring grade ≥3 related AEs (see **Table 3**) were limited in number: lymphopenia (n=9) and increased AST (n=3), both of which resolved within 72 hours. One grade 5 AE of hypotension (not related to **Decoy20**) occurred in a participant with disease progression and acute renal failure.

Table 2. All Treatment-Related Adverse Events

	$7x10^7$ Decoy20 (n=4)				$3x10^7$ Decoy20 (n=7)			
Preferred Term	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
ALT increased	1	1			1		1	
Arthralgia						1		
AST increased			2		1	1	1	
Back pain					1			
Conjugated bilirubin increased					1			
Alkaline phosphatase increased	1							
Total bilirubin increased					1			
Bradycardia			1					
Chills	2	1			1			
Constipation					2			
Decreased appetite	2							
Fatigue	2				1	1		
Fibrin D-Dimer increased					1	1		
Headache		1			1			
Herpes simplex reactivation		1						
Hiccups					1			
Hypoalbuminemia					1			
Hypophosphatemia		1			2			
Hypotension		2				2		
Infusion-related reaction		1	1					
INR increased					1			
Leukopenia					1		1	
Lymphopenia				4			2	3
Malaise			1		1			
Myalgia					1			
Nausea	2				1	1		
Neutrophil count decreased						1		
Peripheral edema					1			
Pain–extremity						1		
Platelet count decreased					2	1		
Prothrombin time prolonged					1			
Pyrexia	2				1			
Rash–maculo-papular					1			
Sinus tachycardia	1				1			
Vomiting		2			1	1		

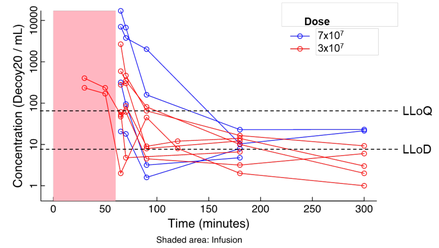
Table 3. All AEs Grade 3 or Higher Irrespective of Relatedness

Yellow Highlight Denotes Related

	$7x10^7$ Decoy20 (n=4)		$3x10^7$ Decoy20 (n=7)		
Preferred Term	**Grade 3**	**Grade 4**	**Grade 3**	**Grade 4**	**Grade 5**
Acute kidney injury			1		
ALT increased			1		
AST increased	2		1		
Bradycardia	1				
Dyspnea			1		
Failure to thrive				1	
Fatigue			1		
Hematuria			1		
Hyperkalemia				1	
Hyponatremia			1		
Hypotension					1
Infusion-related reaction	1				
Leukopenia			1		
Lymphopenia		4	2	3	
Malaise	1				
Venous stenosis			1		

Results (Pharmacokinetics)

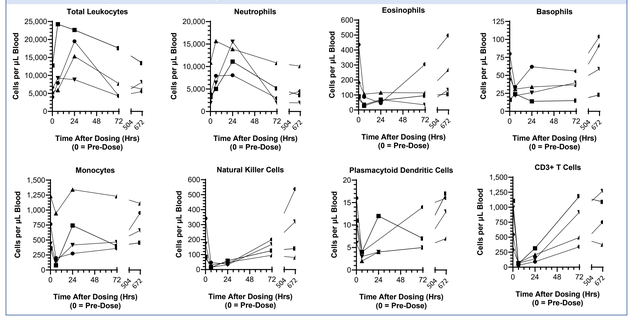
Figure 2. Pharmacokinetic analysis confirms rapid disappearance of systemically administered Decoy20 from blood. A ddPCR method with a lower limit of detection (LLoD) of 8 **Decoy20** /mL blood and a lower limit of quantitation (LLoQ) of 74 **Decoy20** /mL blood was developed and used to determine **Decoy20** 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 (Participants 1-9). Timepoints were added at 30 minutes and 50 minutes (during infusion) and 120 minutes after the start of the infusion for Participants 10 and 11. 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.



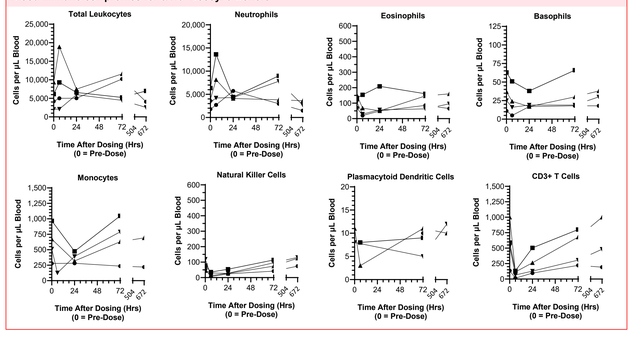
Results (Immune Cell Profiling)

Figure 3. Blood immune cell profiling demonstrates immune cell trafficking after Decoy20 administration. Epiontis platform analysis was carried out pre-dose and at 6, 24, 72 hours and 4 weeks after **Decoy20** administration. A rapid increase in neutrophils was accompanied by decreases in most other leukocytes over 6 to 24 hours, with most cell types returning to baseline within about 72 hours. Figures represent the four participants in the $7x10^7$ dose cohort or the first four participants in the $3x10^7$ dose cohort.

Blood immune cell profiles for $7x10^7$ Decoy20 Cohort



Blood immune cell profiles for $3x10^7$ Decoy20 Cohort



Results (Plasma Biomarkers)

Biomarker analysis confirms broad, but transient, induction of plasma cytokines, chemokines, and biomarkers associated with innate and adaptive immune responses. Luminex analysis was carried out at pre-dose, 0.5, 1, 2, 4, 6, 24, 48, 72 hours, and 4 weeks after end of infusion. Most induced analytes peaked within 2-6 hours and resolved within 24-72 hours. Data in **Table 4** represent the maximum fold induction or reduction.

Table 4. Plasma Biomarkers

	Cohort $7x10^7$ participants 1-4	Cohort $3x10^7$ participants 5-11
	Entries are maximum induction or reduction (↓) ≥3-fold N = No change ≥3-fold Empty cell = No participants with ≥3-fold change	
Biomarker		
April	5, N, N, 5	↓5, 3, N, 23, N, N, N
BAFF	10, 4, N, N	
BLC (CXCL13)	6, ↓4, N, 8	N, N, N, 5, N, N, N
CD30	3, N, N, 4	N, N, N, 4, N, N, N
ENA-78 (CXCL5)	N, N, 5, N	↓9, 4, N, N, 4, ↓6, N
Eotaxin (CCL11)	9, N, 7, 5	N, 3, 3, 19, 4, N, N
Eotaxin-2 (CCL24)	N, ↓5, N, N	N, 3, N, 34, N, N, ↓9
Eotaxin-3 (CCL26)		
FGF-2	3, 4, N, N	
Fractalkine (CX3CL1)	N, 6, N, N	
G-CSF (CSF-3)	18, 3, 67, 9	5, 3, 7, N, 28, 5, N
GM-CSF	3, N, N, 5	11, 7, N, N, 4, N, N
Gro-alpha (CXCL1)	4, 15, 43, 6	11, 14, 6, 11, 6, 13, 7
HGF	8, N, 63, 21	N, N, N, 4, N, N, 3
IFN-alpha		
IFN-beta	N, N, 4, N	
IFN-gamma	27, N, N, 18	5, N, 4, N, N, N, N
IL-1-alpha	7, 17, 5, 5	N, 6, N, N, 10, 4, N
IL-1-beta	23, 5, 6, 10	5, 13, N, N, 10, 5, 3
IL-1ra (IL-1F3)	519, 431, 279, 575	9, 6, 11, 10, 40, 5, N
IL-2	4, N, N, N	N, 5, N, N, 5, N, N
sIL-2r	133, N, 5, 120	N, N, 5, 3, N, N, ↓4
IL-3		
IL-4	N, N, 4, 5	4, 6, N, 4, 6, N, N
IL-5		
IL-6	83, 5, 71, 39	44, 49, 67, 33, 70, 30, 18
IL-7		N, 3, N, 4, N, N, N
IL-8 (CXCL8)	31, N, 31, 24	43, 49, 29, 87, 45, 9, 17
IL-9	6, 13, N, N	N, 4, N, N, 3, N, N
IL-10	N, 6, 13, 6	5, 14, N, 8, 6, 6, 8
IL-12p40	N, N, 7, 3	4, N, 3, N, N, N, N
IL-12p70	6, N, N, 4	
IL-13		
IL-15	N, N, N, 4	N, 3, N, N, 4, N, N
IL-16	4, ↓5, N, 5	↓6, N, N, 14, N, N, ↓5
IL-17a (CTLA-8)	N, N, 4, 3	N, 4, N, N, 4, N, N
IL-18	8, 4, N, 4	N, N, N, 6, 3, N, N
IL-20		
IL-21	3, N, 7, 4	3, 5, N, 5, 8, 3, N
IL-22		
IL-23		N, N, N, N, ↓4, N, N
IL-27	32, N, 15, 3	4, 7, 5, 4, 11, 3, N
IL-31	14, N, N, 9	N, 8, N, N, 8, N, N
IL-33		3, N, N, N, N, N, N
LIF	6, N, 44, 4	N, 4, N, N, 9, N, N
IP-10 (CXCL10)	28, 3, 28, 28	7, 10, 12, 9, 12, 10, 5
I-TAC (CXCL11)	24, 5, 14, 17	25, 9, 9, 10, 9, 5, 14
MCP-1 (CCL2)	40, 19, 22, 51	10, 12, 29, 22, 24, 17, 4
MCP-2 (CCL8)	15, N, 10, 25	7, 10, 14, 14, 5, 14, 7
MCP-3 (CCL7)	4, 3, N, N	
MCP-4 (CCL13)	6, 7, 11, N	3, 8, 3, N, 9, N, 3
M-CSF	N, 9, 3, N	
MDC (CCL22)	N, ↓4, N, N	↓3, N, N, 3, N, N, N
MIF	N, ↓8, N, 18	↓8, ↓7, N, 85, N, N, ↓5
MIG (CXCL9)	42, N, 6, 24	9, N, N, N, 4, N, 4
MIP-1a (CCL3)	22, N, 34, 27	16, 26, 22, 21, 29, 23, 22
MIP-1b (CCL4)	36, 25, 253, 47	12, 31, 19, 19, 77, 22, 34
MIP-3a (CCL20)	25, 25, 92, 78	111, 86, 50, 82, 84, 31, 43
MMP-1	4, ↓4, N, N	N, 3, N, 24, N, N, ↓3
NGF-beta	6, 3, N, N	
SCF	N, N, 6, 4	↓4, N, N, 15, N, N, N
SDF-1a (CXCL12)	5, ↓4, N, 9	↓5, ↓4, N, 25, N, N, ↓3
TARC (CCL17)	7, 3, 25, N	N, 6, N, N, 3, N, 4
TNF-alpha	17, 18, 95, 3	N, 6, N, N, 24, N, N
TNF-beta	N, N, 3, 6	3, 8, N, N, 6, N, N
TNF-RII	5, N, N, 11	N, N, N, 7, N, N, N
TRAIL (CD253)	N, 6, 8, 7	N, 7, 4, N, 7, N, N
TWEAK		N, N, N, N, N, N, ↓4
TSLP	4, N, 25, 4	N, 5, 4, N, 5, N, N
VEGF-A	38, N, 6, 23	N, 15, N, 16, 7, 14, 4

Some inductions may be underestimates due to pre-dose value being below the lower limit of quantitation.
Yellow highlight indicates molecules associated with anti-tumor immune response (not exhaustive).

Results (Plasma Biomarkers)

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. A single dose of i.v. **Decoy20** produced transient ≥3-fold induction of the cytokines and chemokines highlighted in yellow in **Table 5**.

Table 5. Cytokines and Chemokines

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 this single dose of **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.

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 [3,4]. Adverse effects were generally tolerable and resolved with or without treatment within 30 minutes to 3 days.

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

Six tumor-specific multi-dosing cohorts are currently enrolling 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. Combinations with a PD(L)-1 inhibitor are planned.

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