FPN: 476P

FIRST-IN-HUMAN, DOSE ESCALATION PHASE I TRIAL OF INTRATUMORAL CYPEP-1 IN PATIENTS WITH ADVANCED SOLID TUMORS

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ABSTRACT

Background: CyPep-1 is a synthetic tumor membrane-targeting 27-D-amino acid alpha-helical peptide. Preclinical studies with CyPep-1 have demonstrated immunogenic cell death and synergism with anti-programmed cell death 1 (anti-PD1) antibodies. The dose escalation part of this study assessed CyPep-1's safety profile and determined its recommended Phase II dose (RP2D) for further development.

Methods: Patients with assessable and injectable, non-ulcerating cutaneous or subcutaneous tumor deposits were eligible. Three pre-defined dose levels (DL) of CyPep-1 were studied: 0.5, 2 and 5 mg/mL. CyPep-1 was administered intratumorally (IT) every 14 days for 3 doses in volumes up to 4 mL per administration, depending on tumor size. Preferentially, IT administration was pursued in a single lesion, but up to 3 lesions could be injected. Pharmacokinetic (PK) blood samples were collected pre-dose as well as 15, 30, 60 and 240 minutes post-dose following the first IT administration. Cytokines, T-cell receptor (TCR) clonality and blood cell immunophenotyping were assessed pre-dose as well as on days 15 and 36 post-dose. Pre- and post-treatment biopsies from an injected lesion were analyzed. The dose limiting toxicity (DLT) observation period was 6 weeks. Response assessment was performed every 8 weeks.

Results: Fourteen patients were enrolled, twelve completed the DLT period: 3 in DL1, 3 in DL2 and 6 in DL3. All patients had exhausted standard of case (SoC) therapy. No DLT was observed. One patient discontinued treatment after three IT administrations due to ulceration of the injected lesion. Injection site reactions were noted in 79% of patients (11/14), mainly mild pain (related to IT administration). No grade 3 or higher treatment related adverse events (AEs) were observed. PK: concentrations below limit of quantification in 5 patients (10 ng/mL). Six patients had detectable levels that peaked at 15 minutes. Highest concentration observed in 1 patient was 226 ng/mL. Tumor biopsy analysis revealed increased cell death and necrosis (range 10-80%) in 5 of 6 patients at DL3. TCR clonality: increased clonality in peripheral blood after treatment. Two patients had immune response evaluation criteria in solid tumors (iRECIST) stable disease (SD) lasting >8 months.

Conclusions: IT administration of CyPep-1 is well tolerated at the RP2D of 5 mg/mL. PK data showed minimal systemic exposure with IT injection. Histopathologic examination showed local oncolytic effect, and TCR clonal changes were consistent with systemic immune activation.

BACKGROUND AND RATIONALE

Mode of action

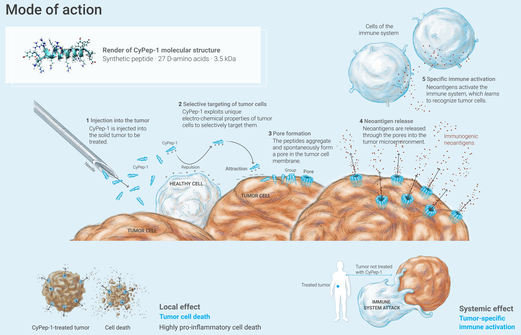


Figure 1. CyPep-1 is a synthetic 27 D-amino acid tumor membrane-targeting peptide that is refractory to proteolytic degradation. Its 3D structure was predicted by the AlphaFold protein structure database (DeepMind.com). Anti-tumor effect: normal cells have a positive membrane charge. In general, cancer cells show a membrane lipid asymmetry where the negatively-charged phospholipid, phosphatidylserine (PS), is present in the outer leaflet.[1] Cancer cells are also negatively-charged based on their metabolic characteristics (i.e. the Warburg effect, glycolysis and lactate production).[2] CyPep-1 binds to the negatively-charged cancer cells, forming pores that lead to the release of tumor neo-antigens, systemic immune activation and cancer cell death.

METHODS

CICILIA – CyPep-1 Injections in Cancer Inducing Lymphocyte Infiltrate Accumulations

Figure 2. First-in-human, 3+3 dose escalation study design with biweekly IT injection up to 4 mL.

Key eligibility criteria:

- Metastatic cancer refractory to SoC treatment, with no limit to the number of treatment lines
- Presence of lesions amenable to IT administration
- The Eastern Cooperative Oncology Group (ECOG) scale of performance status (PS) ≤1 and normal organ function
- No autoimmune diseases or requirement of pharmacologic doses of corticosteroids

RESULTS

Study population

Cohort	Subject identifier	Sex	Age (years)	ECOG PS	Anatomic origin and histology	Metastasis	Size of injected lesion (mm)
Cohort 1 (N=3, 0.5 mg/mL)	01-004	Male	70	0	Pleural epithelioid mesothelioma	Pleura, lung, lymph nodes (LNs)	85
	02-001	Female	49	1	Gallbladder adenocarcinoma	Gallbladder, omentum, subcutaneous	21
	02-003	Male	53	1	Unknown primary adenocarcinoma	Subcutaneous, LNs	15, 35, 18
Cohort 2 (N=5, 2 mg/mL)	01-005	Male	74	0	Pleural biphasic mesothelioma	Pleura, skin	22
	01-006	Male	64	1	Pleural mesothelioma	Pleura, chest wall, mediastinum, liver	118
	02-004	Female	53	1	Breast lobular carcinoma	Chest wall, LNs	23
	02-005	Female	55	1	Pancreas adenocarcinoma	Pancreas, peritoneum, abdominal wall	20, 19
	05-001	Male	77	1	Bone chondrosarcoma	Subcutaneous, LNs, intrapelvic soft tissue	280
Cohort 3 (N=6, 5 mg/mL)	01-007	Female	60	0	Malignant melanoma	Skin, LNs, liver	27
	01-008	Male	51	0	Malignant melanoma	Skin, LNs, abdominal wall, lung, adrenal, liver	56
	02-006	Male	66	1	Pulmonary carcinoid	Skin, pancreas, lung, peritoneum, pleura, bone	48
	02-007	Male	54	1	Penile squamous cell carcinoma	Perineum, LNs, lung	53
	02-008	Female	62	1	Anal carcinoma	LNs, lung, liver	16
	04-002	Male	53	1	Colon mucinous adenocarcinoma	Peritoneum, lung, liver	100

Table 1. Baseline demographic and disease characteristics of participating patients.

Safety results

Preferred terms	Toxicity grade	Cohort 1 (N=3, 0.5 mg/mL) E, n (%)	Cohort 2 (N=5, 2 mg/mL) E, n (%)	Cohort 3 (N=6, 5 mg/mL) E, n (%)	Total (N=14) E, n (%)
Injection site oedema	1			1, 1 (16.7%)	1, 1 (7.1%)
Injection site pain	1		2, 1 (33.3%)	5, 3 (60.0%)	18, 6 (100%)	25, 10 (71.4%)
	2			1, 1 (20.0%)	1, 1 (16.7%)	2, 2 (14.3%)

Event in preferred terms	Cohort 1 (0.5 mg/mL)	Cohort 2 (2 mg/mL)		Cohort 3 (5 mg/mL)		Total (E)	Duration (E)				Analgesics given due to AEs (E)
	Grade 1 (E)	Grade 1 (E)	Grade 1 (E)	Grade 1 (E)		<24 h	1-3 days	>3 days	NA	
Injection site pain	2	5	1	18	26	14	8	1	4	10
Injection site edema	0	0	0	1	1	0	0	1	0	0
Skin wound	0	0	0	1	1	0	0	1	0	0

Table 2. Overview of treatment-related local reactions – Phase I.

E events.

Cohort	Subject identifier	Worst severity (CTCAE grade)	Preferred term (n)
Cohort 1 (0.5 mg/mL)	01-004	3	Upper respiratory tract infection (1)
		3	Dysphagia (3)
		3	Esophageal obstruction (1)
	02-001	4	Gamma-glutamyltransferase increased (1)
	02-003	1	Epistaxis (1)
		1	Injection site pain (2)
Cohort 2 (2 mg/mL)	01-005	2	Cough (1)
		2	Cancer pain (1)
		2	Dyspnea (1)
	01-006	3	Cancer pain (1)
	05-001	3	Rectal hemorrhage (1)
	02-004	3	Anemia (1)
	02-005	3	Vomiting (1)
Cohort 3 (5 mg/mL)	01-007	1	Injection site pain (4)
		1	Alopecia (1)
		1	Vitiligo (1)
	01-008	2	Injection site pain (1)
		2	Fatigue (1)
		2	Anemia (1)
	02-006	3	Cancer pain (1)
		3	Hypoxia (1)
	02-007	1	Injection site pain (3)
		1	Myalgia (1)
		1	Post-procedural complication (1)
	02-008	2	Hyponatremia (1)
	04-002	3	Anemia (2)
		3	Pyelonephritis (1)
		3	Small intestinal obstruction (1)

Table 3. Worst AEs per subject by common terminology criteria for adverse events (CTCAE) grade – Phase I.

Pharmacokinetics

Cohort	Subject identifier	Scheduled time (h)					
		Pre-dose	15 min	30 min	1 h	2 h	4 h
Cohort 1 (0.5 mg/mL)	01-004	<LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ
	02-001	<LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ
	02-003	<LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ
Cohort 2 (2 mg/mL)	01-005	<LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ
	01-006	<LLOQ	11.8	LLOQ	LLOQ	LLOQ	LLOQ
	02-004	<LLOQ	33.7	LLOQ	LLOQ	LLOQ	LLOQ
	02-005	<LLOQ	14.3	LLOQ	LLOQ	LLOQ	LLOQ
Cohort 3 (5 mg/mL)	05-001	<LLOQ	226.0	72.7	24.1	13.4	10.2
	01-007	<LLOQ	19.7	LLOQ	<10	LLOQ	LLOQ
	02-006	<LLOQ	164.0	88.3	15.9	11.7	LLOQ
	02-007	<LLOQ	LLOQ	LLOQ	LLOQ	LLOQ	LLOQ

Table 4. Pharmacokinetic characteristics of participating patients. t_{max} was based on 6 patients in the range of 0.22 to 0.5 h. Half-life ($t_{1/2}$) was 0.56 h. **Lower limit of quantification (LLOQ) was 10 ng/mL.**

Pharmacokinetics – continued

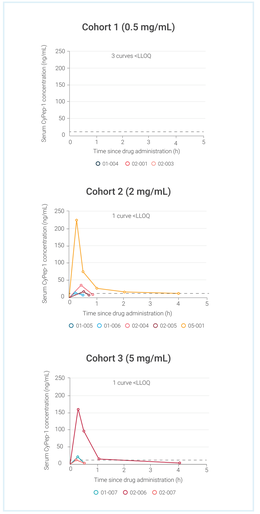


Figure 3. Serum samples were collected at different time-points following CyPep-1 injection. By liquid chromatography (LC)-tandem mass spectrometry (MS), CyPep-1 was only detected in cohorts 2 and 3. The serum concentration reached peak levels >15 min after CyPep-1 administration. This is in line with observations obtained from preclinical studies.

LLOQ was 10 ng/mL.

Tumor biopsy

Cancer cell death and immune cell infiltration

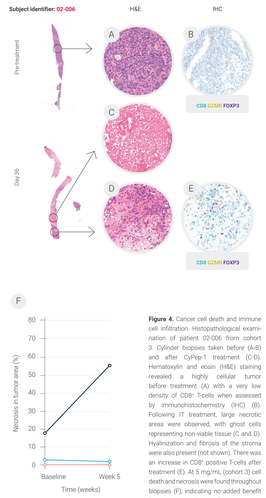


Figure 4. Cancer cell death and immune cell infiltration. Histopathological examination of patient 02-006 from cohort 3. Cylinder biopsies taken before (A-B) and after CyPep-1 treatment (C-D). Hematoxylin and eosin (H&E) staining revealed a highly cellular tumor before treatment (A) with a very low density of CD8+ T-cells when assessed by immunohistochemistry (IHC) (B). Following IT treatment, large necrotic areas were observed, with ghost cells representing non-viable tissue (C and D). Hyalinization and fibrosis of the stroma were also present (not shown). There was an increase in CD8+ positive T-cells after treatment (E). At 5 mg/mL (cohort 3) cell death and necrosis were found throughout biopsies (F), indicating no added benefit from increasing the dose further.

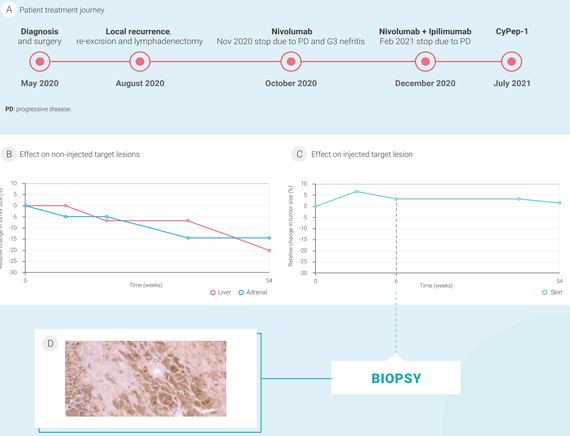
Clinical activity

Figure 5. Swimmer plot (response evaluation criteria in solid tumors [RECIST] 1.1).
ICI immune checkpoint inhibitor; **OS** overall survival.

Clinical activity – continued

Figure 6. Tumor burden iRECIST for the study populations (cohorts 1-3).

IMP: investigational medicinal product.



PD: progressive disease.

Figure 7. Clinical findings for a melanoma patient refractory to checkpoint inhibitors (subject identifier 01-008). Biopsy at week 6 shows necrosis surrounded by immune cell infiltrate. TCR-seq of peripheral blood reveals 16.5% increase in clonality.

CONCLUSIONS

01 IT administration of CyPep-1 was well tolerated at the RP2D of 5 mg/mL.

02 No immune-related serious adverse events (SAEs) were reported.

03 PK data showed minimal systemic exposure with IT injection.

04 Histopathologic examination showed anti-tumor effect, and TCR clonality changes were consistent with systemic immune activation.

05 The current study will be followed by extension cohorts of both monotherapy and combination therapy with an anti-PD1 checkpoint inhibitor.

DISCLOSURES

Lars Prestegarden declares financial interests, stock ownership, and full-time employment at Cytovation ASA.
Cesar Pico declares financial interests and full-time employment at Cytovation ASA.

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