UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT
Pursuant to Section 13 or 15(d)
of the Securities Exchange Act of 1934

Date of Report (Date of earliest event reported): April 2, 2019

PIERIS PHARMACEUTICALS, INC.
(Exact name of registrant as specified in its charter)

Nevada	001-37471	30-0784346
(State or other jurisdiction of incorporation)	(Commission File Number)	(IRS Employer Identification No.)
255 State Street, 9th Floor Boston, MA		02109
(Address of principal executive offices)		(Zip Code)

Registrant's telephone number, including area code: 857-246-8998

N/A
(Former name or former address, if changed since last report.)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions:

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)

☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)

☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))

☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Indicate by check mark whether the registrant is an emerging growth company as defined in Rule 405 of the Securities Act of 1933 (17 CFR §230.405) or Rule 12b-2 of the Securities Exchange Act of 1934 (17 CFR §240.12b-2).

Emerging growth company ☒

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Item 7.01: Regulation FD Disclosure.

On April 2, 2019, Pieris Pharmaceuticals, Inc. presented preclinical data regarding PRS-342 at the 2019 American Association for Cancer Research Annual Meeting. The poster is furnished as Exhibit 99.1 to this Current Report on Form 8-K and is incorporated by reference herein.

The information set forth under this "Item 7.01. Regulation FD Disclosure," including Exhibit 99.1 attached hereto, shall not be deemed "filed" for any purpose, and shall not be deemed incorporated by reference into any filing under the Securities Act of 1933, as amended, or the Securities Exchange Act of 1934, as amended, regardless of any general incorporation language in any such filing. except as shall be expressly set forth by specific reference in such filing.

Item 9.01 Financial Statements and Exhibits

(d) *Exhibits.*

99.1 Conference Poster, Dated April 2, 2019.

SIGNATURE

Pursuant to the requirements of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

PIERIS PHARMACEUTICALS, INC.

Dated: April 2, 2019

/s/ Allan Reine

Allan Reine

Chief Financial Officer

Costimulatory T-cell engagement by PRS-342, a GPC3/4-1BB bispecific molecule, leads to activation of T cells and tumor growth inhibition in a HCC humanized mouse model

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AACR Annual Meeting 2019
Abstract # 4302

Background

4-1BB (CD137) is a key costimulatory immunoreceptor and a highly promising therapeutic target in cancer. To overcome toxicity and efficacy limitations of current 4-1BB-targeting antibodies, we have developed 4-1BB Anticalin®/tumor-targeting mAb bispecifics that activate T cells in a tumor localized fashion. We have previously reported on the generation and characterization of PRS-343, a clinical-stage 4-1BB/HER2 bispecific molecule, with regard to preclinical proof-of-concept and basic drug-like properties (1). Here, we describe the preclinical dataset for PRS-342, a 4-1BB/GPC3 bispecific based on the Anticalin® technology. GPC3 is an oncofetal protein with high tumor selectivity and high expression in not only hepatocellular carcinomas, but also in a variety of other tumors with high medical need.

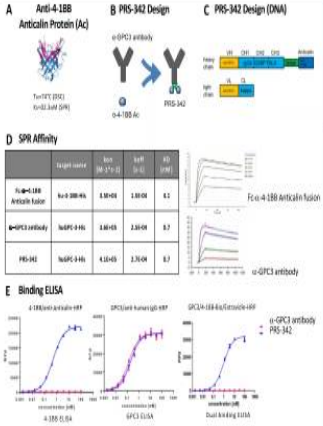
Anticalin® therapeutics are 18 kD proteins derived from human lipocalins. We utilized phage display to generate an Anticalin® protein binding to 4-1BB with high affinity and specificity. The PRS-342 bispecific construct was generated by genetic fusion of the 4-1BB-specific Anticalin® protein to a humanized high affinity GPC3-targeting monoclonal antibody with an engineered IgG4 backbone.

PRS-342 has excellent drug-like properties and can be produced with high yields. PRS-342 was designed to be strictly dependent on tumor binding, which is necessary for clustering of 4-1BB, to elicit 4-1BB costimulation and T-cell activation. This was confirmed using different *in vitro* T-cell costimulation assays based on mixed culture of human T cells and GPC3-expressing tumor cell lines. These data further demonstrate the ability of PRS-342 to bind both targets simultaneously. PRS-342 was also evaluated for activity in a humanized HepG2 mouse xenograft model, with results supporting its differentiated MoA compared to relevant benchmark controls.

Concept: tumor-specific and tumor-localized costimulatory activation of T cells

Concept of costimulatory T-cell engagement by PRS-342: Within a patient's tumor, tumor-specific T cells are bridged with tumor cells by the costimulatory bispecific PRS-342 which simultaneously binds the tumor target GPC3 and the immune receptor 4-1BB. The resulting clustering of 4-1BB provides a local coactivatory signal to the T cell, further enhancing its T-cell receptor (TCR)-mediated activity and leading to tumor destruction. Toxic side effects are expected to be manageable, as PRS-342 does not induce clustering and activation of 4-1BB in the absence of target-positive cells, and healthy tissue is spared by tumor costimulated T cells due to the absence of a primary, TCR-mediated signal.

PRS-342 design, target binding and activity in reporter and T-cell costimulation assay

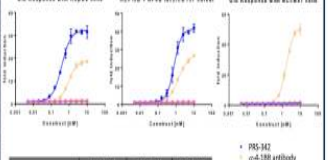


	Target name	kon ($M^{-1}s^{-1}$)	koff (s^{-1})	KD (nM)
Fc-α-4-1BB Anticalin fusion	hu-4-1BB-His	3.3E+05	2.30E-04	0.7
α-GPC3 antibody	huGPC-3-His	3.6E+05	2.2E-04	0.7
PRS-342	huGPC-3-His	4.1E+05	2.7E-04	0.7

PRS-342 Design (A, B, C) and target binding (D, E). (D) shows binding of the Fc-α-4-1BB Anticalin fusion with a KD of 4.1 nM. The GPC3 arm binds with 0.7 nM to GPC3. On and off-rate kinetic binding constant for α-GPC3 antibody and PRS-342 are similar. (E) ELISA data demonstrate PRS-342 binds GPC3 with comparable behavior to the α-GPC3 parental antibody. In a dual binding ELISA setting PRS-342 (4-1BB/GPC3 bispecific), is capable of binding both targets simultaneously.

PRS-342 reporter cell assay

- PRS-342 costimulated T cells in a Jurkat NF-kB reporter cell assay only in the presence of GPC3-positive tumor cell lines.

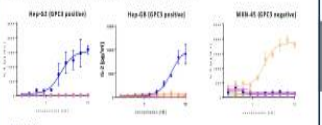


	EC50 [nM] Hep-G2	EC50 [nM] Hep-G8	EC50 [nM] NCI-N87
α-4-1BB antibody	0.94	1.39	1.77
α-GPC3 antibody	-	-	-
PRS-342	0.38	0.78	-

PRS-342 induces 4-1BB clustering and downstream signaling in a Jurkat NF-kB reporter cell line in the presence of GPC3-positive HepG2 and Hep3B cells with low nM EC50 values. GPC3-negative NCI –N87 cell are used as control. Only the 4-1BB antibody can activate the Jurkat NF-kB reporter cell in the absence of GPC3 positive tumor cells.

PRS-342 induces 4-1BB engagement and T-cell activation in a GPC3 dependent manner

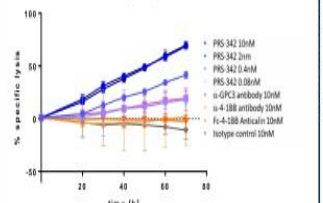
- Pan T cells were coincubated with GPC3$^{+/0}$ Hep-G2, Hep-G8 and MKN-45 cells and PRS-342.
- Supernatant concentrations were determined for IL-2.



IL-2 induced by human Pan T cells costimulated by PRS-342 in the presence of GPC3-positive HepG2 and Hep-G8 cells in a coculture assay. No PRS-342 dependent activation was observed in presence of GPC3-negative MKN-45 cells. IL-2 levels in the culture supernatants were measured by an electrochemiluminescence (ECL) immunoassay.

PRS-342 leads to dose dependent T-cell mediated cytolysis of GPC3 expressing tumor cells

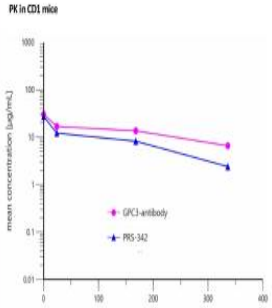
- PRS-342 induced 4-1BB costimulation results in a dose-dependent T-cell killing of GPC3 expressing tumor cells measured with an impedance based method.
- No increase of T-cell mediated killing was observed with equimolar doses of anti-GPC3 antibody, Fc-4-1BB Anticalin fusion, anti-4-1BB-antibody and isotype control.



T-cell mediated cytolysis of GPC3 expressing HepG2 tumor cells was assessed using the xCELLigence RTCA HT system. Non-adherent CD8$^+$ T cells were cocultured with HepG2 cells in presence of test constructs. 4-1BB costimulation of cytotoxic T cells results in an increased cytolysis of HepG2 cells which is measured over time.

Pharmacokinetic profile of PRS-342 in mice

- Preliminary mouse PK was performed in male CD-1 mice to compare PRS-342 with an α-GPC3 antibody.
- PRS-342 has a typical antibody like PK profile in mice comparable to the α-GPC3 antibody used as building block in the bispecific PRS-342 construct.

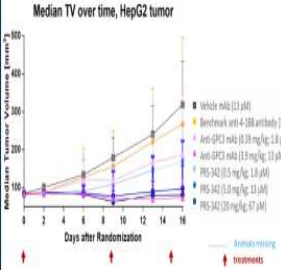


An analysis of the pharmacokinetic properties of PRS-343 as well as of an α-GPC3 antibody was performed in mice. Male CD-1 mice approximately 5 weeks of age (2 mice per timepoint) were injected into a tail vein with a dose 2 mg/kg. Plasma samples from the mice were obtained at the timepoints of 5 min, 24 h, 168 h, and 336 h. Plots of the plasma concentration over time for the anti-GPC3 antibody and PRS-342 are shown. Both the antibody and the bispecific-construct show typical antibody pharmacokinetics profiles.

PRS-342 leads to tumor growth inhibition in a humanized HCC xenograft model

- Immunocompromised mice (NOG) engrafted with GPC3-positive tumor cells (HepG2) were injected with human PBMC and treated weekly with PRS-342 at three dose levels.
- Control molecules were an α-GPC3 antibody (IgG4 variant) in equimolar doses, an α-4-1BB benchmark antibody in equimolar doses, and vehicle control.
- PRS-342 showed dose-dependent tumor growth inhibition (TGI) comparable to α-GPC3 antibody, indicating that TGI is dominated by GPC3 inhibition in this model.

A Median tumor growth inhibition in a humanized HepG2 xenograft model



(A) Immunocompromised female NOG mice carrying established HepG2 xenograft tumors were engrafted with 5 × 10^6 fresh human PBMC, followed by weekly i.p. treatment with PRS-342, α-4-1BB benchmark antibody, α-GPC3 antibody or isotype control at 0.5 mg up to 20 mg/kg doses (i.p.) (Charles River). Mice (n=15 per gr. remained on the study until spontaneous death or if ethical sacrifice was required, read out median tumor growth).

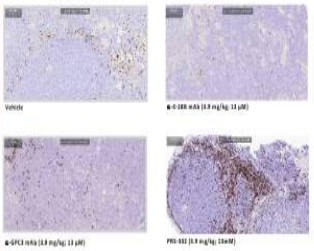
PRS-342 leads to tumor-localized increase of TILs in a humanized HCC xenograft model

- FFPE embedded xenograft tumor were analyzed histologically (HE) and immunohistologically (IHC) for T-cell infiltration.
- Tumor IHC staining for human CD3, CD4 and CD8 shows a dose-dependent increase in the frequency of human tumor associated T cells (TILs) for PRS-342 vs controls, suggesting tumor-localized T-cell activation.

B % TIL frequency hCD3, hCD4 and hCD8 by IHC (necrotic areas are excluded)

Intratumoral T-cell infiltration	% TILs of total tumor area - necrotic area		
TILs	%CD3 T cells	%CD4 T cells	%CD8 T cells
Vehicle control	0.8	0.7	0.4
PRS-342 0.8 µM	6.2	4.7	4.5
PRS-342 13 µM	4.1	3.3	3.0
PRS-342 67 µM	10.7	4.9	7.3
α-GPC3 antibody 0.8 µM	1.6	1.3	0.7
α-GPC3 antibody 13 µM	0.8	0.6	0.5
α-4-1BB antibody 13 µM	0.8	0.7	0.2

C TIL frequency (hCD8$^+$) by IHC



(B) FFPE Xenograft tumors taken from the in vivo study described in (A) were stained for HE (not shown) and for the T-cell marker CD3, CD4 and CD8. Percentage of TILs per tumor area excluding the necrotic area were calculated for all groups (BioSiteHisto). (C) Representative pictures for CD8 staining of Hep-G2 tumors demonstrating significant increased TIL infiltration for PRS-342 tumors compared to all controls (vehicle, α-GPC3 antibody and α-4-1BB antibody).

Summary

- PRS-342 was designed to elicit 4-1BB costimulatory effects in a tumor-localized manner.
- PRS-342 is a 4-1BB/GPC3 bispecific genetic fusion of a high-affinity 4-1BB-binding Anticalin® and a high affinity α-GPC3 antibody.
- PRS-342 has excellent drug like properties and can be produced with high yields.
- PRS-342 has a pharmacokinetic profile comparable to classical antibodies.
- T-cell costimulation by PRS-342 leads to:
  - NF-kB activation in a reporter cell assay.
  - Increased production of IL-2, a pro-inflammatory cytokine associated with anti-tumor immune response in a co-culture assay.
  - Dose dependent cytolysis in impedance based real time killing assay.
  - TIL infiltration in tumors of a HCC xenograft in humanized mice.
- The preclinical studies reported here demonstrate potent T-cell activation that is strictly dependent on the presence of GPC3-positive tumor cells.
- GPC3-dependent activation of tumor-specific T cells is expected to result in an improved safety profile.
- Collectively our *in vitro* and *in vivo* data support the continued development of PRS-342.

References: (1) Cancer Immunol Res 2018;6(11 Suppl):Abstract nr B018.