Targeted Oncology (2023) 18:391–402
https://doi.org/10.1007/s11523-023-00962-w

ORIGINAL RESEARCH ARTICLE

A Phase I, First-in-Human Study of PRL3-zumab in Advanced, Refractory Solid Tumors and Hematological Malignancies

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Accepted: 24 March 2023 / Published online: 15 April 2023

Abstract

Background Phosphatase of regenerating liver-3 (PRL-3) is involved in cellular processes driving metastasis, cell proliferation, invasion, motility and survival. It has been shown to be upregulated and overexpressed in cancer tissue, in contrast to low or no expression in most normal tissue. PRL3-zumab is a first-in-class humanized antibody that specifically binds to PRL-3 oncotarget with high affinity and has been shown to reduce tumor growth and increase survival.

Objective In the study, we aimed to determine the safety and efficacy of PRL3-zumab in patients with advanced solid tumors and hematological malignancies.

Methods We conducted a phase I, first-in-human study in advanced solid tumors and hematological malignancies to investigate the safety, tolerability and efficacy of PRL3-zumab. Response rates were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) for solid tumors. For acute myeloid leukemia (AML) patients, bone marrow response criteria based on the European Leukaemia Network (ELN) 2017 guidelines for AML were used. We also explored the pharmacokinetics and pharmacodynamic relationships of PRL3-zumab in patients. This study was registered with ClinicalTrials.gov: NCT03191682.

Results In the dose-escalation cohort, 11 patients with advanced solid tumors were enrolled into the study. An additional 12 patients with solid tumors and four patients with AML were enrolled in the dose-expansion cohort. Maximum tolerability was not achieved in this study, as there were no dose-limiting toxicities. Potential treatment-emergent adverse events were grade 1 increased stoma output and fatigue and grade 2 vomiting. Best response observed was stable disease in three solid-tumor patients (11.1%). The pharmacokinetics of PRL3-zumab were dose proportional, consistent with an IgG type monoclonal antibody.

Conclusions PRL3-zumab, a first-in-class humanized antibody, was safe and tolerable in solid tumors and hematological malignancies.

Key Points

PRL3-zumab is a first-in-class humanized antibody that specifically binds to phosphatase of regenerating liver-3 (PRL-3), an oncotarget that is upregulated and overexpressed in a variety of tumor types and acute myeloid leukemia.

In our phase I study, PRL3-zumab was shown to be safe and tolerable in solid tumors and hematological malignancies.

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1 Introduction

Phosphatase of regenerating liver-3 (PRL-3) is a member of the PRL family of intracellular dual-specificity protein tyrosine phosphatases [1]. PRL-3 localizes to the cytoplasmic face of the plasma membrane and endosomes via its prenylated C-termini [2]. PRL-3 has received considerable interest as a potential therapeutic target in oncology, due to its involvement in cellular processes driving metastasis, including cell proliferation, invasion, motility and survival [3, 4]. Additionally, PRL-3 has been shown to be upregulated and overexpressed in cancer tissues, in contrast to low or no expression in most normal tissues, thus making PRL-3 an attractive protein for targeted cancer therapy [5]. Evidence suggests that PRL-3 promotes multiple stages of malignant transformation via activation of phosphatidylinositol 3-kinase (PI3K)/AKT, extracellular-signal-regulated kinase (ERK) and SRC tyrosine kinase oncogenic pathways through downregulation of phosphatase and tensin homolog (PTEN) and/or activation of upstream receptor tyrosine kinases [6–9]. To date, elevated PRL-3 mRNA or protein levels have been shown to correlate with higher metastatic potential and poor prognosis of numerous cancer types [9–12]. PRL-3 oncotarget is overexpressed in > 80 % of tumors across 11 common cancer types that we have examined, namely, gastric, liver, lung, pancreas, thyroid, kidney, bladder, breast, colon and prostate cancers and acute myeloid leukemia (AML) [13].

As PRL-3 is intracellularly localized, the conventional approach using therapeutic antibodies would seem implausible. However, Zeng et al. [1] reported an unexpected observation that mouse monoclonal antibodies (mAbs) against PRL-1 or PRL-3 were able to prevent the experimental metastasis of cancer cells in *nude* and wild-type C57BL/6 mouse models overexpressing intracellular PRL-1 or PRL-3 oncoproteins [14], which stands as unconventional proof of concept that intracellular antigen can be targetable by antibody [15, 16]. Additionally, a PRL-3 mouse chimeric and humanized antibody (PRL3-zumab) was shown to effectively inhibit the metastatic tumors formed by mouse and human cancer cells that express endogenous PRL-3 and extend survival in mouse metastasis and orthotopic cancer models [5, 13, 17]. The pharmacologically active dose (PAD) for all anti-PRL3 antibodies in all mouse models was 4–5 mg/kg. These results, establishing the potential for therapeutic efficacy of anti-PRL3 treatment, support the hypothesis that this otherwise intracellular protein may be effectively targeted by antibody-based intervention. The mechanism of this phenomenon is yet to be fully elucidated, but may involve permanent or transient externalization of PRL-3, thus making it accessible to classical targeted immunotherapy via natural killer (NK) cell-mediated antibody-dependent cellular cytotoxicity (ADCC) and/or macrophage-mediated antibody-dependent cellular phagocytosis (ADCP) [5, 13].

PRL3-zumab is a first-in-class humanized antibody that specifically binds to PRL-3 oncotarget with high affinity. It does not cross-react with any other proteins and thus has minimal off-target side effects, and PRL3-zumab has been shown to reduce tumor growth and increase survival [4]. In PRL-3-negative gastric cancer mouse models, no response was seen, reflecting the exquisite target specificity of PRL3-zumab. In a total of 175 male Balb/c *nude* mice, administration of PRL3-zumab up to 5 weeks at biweekly intervals demonstrated a lack of any adverse events (AEs) in orthotropic, metastatic and xenograft tumor mouse models. Body weight gain was normal in all PRL3-zumab-treated mice, and increased overall survival was observed. Similarly, there were no adverse findings during the study of PRL3-zumab in cynomolgus monkeys. Taken together, the high expression of PRL-3 in tumor, but not normal tissues, coupled with the high frequency of PRL-3 overexpression observed in gastric cancer and other cancer types, establishes PRL-3 as an ideal tumor-enriched oncotarget whose targeting should have minimal off-target effects [18]. A phase I, first-in-human study in advanced solid tumors and hematological malignancies was undertaken to investigate the safety and efficacy of PRL3-zumab.

2 Methods

2.1 Patient Selection

Patients aged ≥ 21 with histologically or cytologically confirmed, advanced, refractory solid tumors unresponsive to standard anti-cancer therapy or for whom there is no standard therapy available, with or without measurable disease were eligible for the dose-escalation cohort of the study. They were included if they had an Eastern Cooperative Oncology Group performance status of ≤ 2, adequate organ function [creatinine ≤ 1.5 × upper limits of normal (ULN), total bilirubin ≤ 1.5 × ULN], hemoglobin ≥ 9 g/dL, an absolute neutrophil count of ≥ 1500/mL and a platelet count of ≥ 100,000/mL. Key exclusion criteria included prior therapy within 28 days or major surgery within 28 days of study enrollment, unstable cardiac function, uncontrolled active infection or symptoms, symptomatic brain metastasis, any condition requiring the use of corticosteroids that cannot be stopped for the study, and vaccination within 8 weeks of therapy. In the dose-expansion cohort, patients of any solid tumor type were enrolled with measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) [version 1.1 (v1.1)], and a pre-treatment biopsy was mandated. Washout from prior anti-cancer therapy was reduced to 2 weeks in this cohort. All patients had disease

progression on prior line of anti-cancer therapy at study entry. Additionally, in the dose-expansion cohort, patients with newly diagnosed AML but who were unfit for intensive chemotherapy (such as daunorubicin or idarubicin and cytarabine) and a hypomethylating agent or patients with relapsed or refractory AML who progressed on at least one line of anti-cancer therapy and who were deemed unfit for intensive chemotherapy (such as fludarabine, cytarabine and idarubicin) were eligible for the study.

This study was conducted in accordance with the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice guidelines. The study protocol was approved by the Domain Specific Review Board. Informed consent was obtained from all individual patients included in the study. This study was registered with ClinicalTrials.gov (identifier: NCT03191682; date of registration: June 19, 2017).

2.2 Study Design

This was a single-center, phase I, dose-escalation and dose-expansion study evaluating the safety and efficacy of PRL3-zumab as monotherapy in patients with advanced solid tumors and AML who no longer responded to standard therapy or for whom no standard therapy was available. Patients enrolled were dosed once every 2 weeks (Q2W) (± 1 day) in escalating dose cohorts at the dose levels determined in Table 1. The dose-escalation phase was conducted with the initial dose of 0.3 mg/kg and subsequent doses of 0.9 mg/kg, 3 mg/kg and 6 mg/kg. One cycle was 28 days. PRL3-zumab was administered Q2W until disease progression, intolerance of study drug or PRL3-zumab was held exceeding a minimum of 4 weeks. The investigator monitored each patient for the occurrence of AEs. An accelerated dose-titration scheme was adopted, with one to three patients recruited per dose level. Patients were enrolled 1 week apart from the initial dose received and subsequently observed for toxicity. Intra-patient dose escalation was permitted and allowed only once per patient at the discretion of the investigator, if all of the following criteria were met: (1) the patient has received PRL3-zumab on the original dose level for at least 2 cycles; (2) the patient has completed the first imaging assessment; (3) the subsequent higher dose cohort has been cleared without dose-limiting toxicity (DLT) and a slot is available.

Table 1 Dose-escalation schedule

Dose level	Dose (IV, once every 2 weeks)	No. of patients
−1	0.1 mg/kg	Up to 3
1	0.3 mg/kg	Up to 3
2	0.9 mg/kg	Up to 3
3	3.0 mg/kg	Up to 6
4	6.0 mg/kg	Up to 6

1 cycle = 28 days

IV intravenous

Dose-escalation decisions relied on safety and tolerability data reviewed by the Safety Monitoring Committee (SMC), and planned dose escalation continued until the maximum tolerated dose (MTD) was reached, or the SMC determined that the criteria for the recommended phase II dose (RP2D) had been met. The starting dosage was 0.3 mg/kg Q2W based on pre-clinical studies. The PAD in mouse tumor models was 5 mg/kg, and converting this into the human effective dose (HED) by a factor of 12.3 [based on Food and Drug Administration (FDA) Guidance for Industry: "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers"], a PAD of 0.4 mg/kg is expected for humans. Thus, the proposed final starting dose of 0.3 mg/kg was indeed within the PAD range.

Dose escalation was based on whether patients in the first cycle of the cohort experience AEs and/or DLTs that were judged by the investigator as being related to PRL3-zumab exposure and unrelated to underlying conditions and/or concomitant medications. Dose escalation continued as long as no more than one out of six patients in the cohort experienced a DLT. The DLT observation period was determined as the first cycle of treatment (28 days). The SMC met at the completion of a cohort's DLT observation period to confirm the decision to escalate the dose. DLT was defined by the occurrence of any of the following toxicities possibly, probably or definitely related to PRL3-zumab within the first cycle of treatment: grade 4 neutropenia for more than 7 days; grade 3–4 febrile neutropenia; grade 3 thrombocytopenia in the presence of bleeding; grade 4 thrombocytopenia; any observed hematological AEs if they resulted in a treatment delay of more than 14 days (except grade 3–4 lymphopenia); grade 3–4 non-hematological toxicity of any duration (except for grade 3–4 nausea, vomiting or diarrhea if it could not be reduced to grade 2 or less within 2 days with medical management; reversible laboratory abnormalities with no clinical sequelae and/no clinical significance; grade 3 hyperglycemia in patients with diabetes mellitus or decreased glucose tolerance that is controlled pharmacologically; skin toxicity that is adequately controlled with supportive measures; transient, ≤ 24 h of fatigue, headache or nausea that resolves to ≤ grade 1).

In the dose-expansion part of the study, 12 patients with advanced solid tumors and four patients with AML were enrolled to explore the efficacy of PRL3-zumab. The pharmacodynamics (PD) effects of PRL3-zumab were assessed using tumor biopsies, blood samples and imaging. All patients enrolled in the expansion cohort received PRL3-zumab intravenously, once Q2W (± 1 day) at 6 mg/kg over

a period of 30 min–1 h. One cycle was 28 days. Standard monitoring of vital signs per routine was followed. PRL3-zumab was administered Q2W until disease progression, intolerance of study drug or PRL3-zumab was held exceeding a minimum of 4 weeks.

No premedication was required prior to administration of PRL3-zumab. In the event of infusion-related AEs, the investigator, in consultation with the study PI, was able to increase the duration of the infusion over a period of up to 24 h at their discretion or considered suggested premedication with anti-H_1 and anti-H_2 blockers and paracetamol. Treatment with PRL3-zumab was permanently discontinued for any patient experiencing a grade 3 (or greater) acute infusion reaction. Patients who experienced a DLT resulting in treatment delay were able to continue with treatment in the absence of progression of disease as long as the toxicity recovered to the baseline value or lower. The patient would then be treated at the next lower dose level until disease progression or other dose-reducing events. If more than two dose reductions were indicated, the patient was discontinued from PRL3-zumab treatment. Once the dose was reduced, it was not re-escalated.

2.3 Study Objectives and Assessments

The primary objectives were to assess the safety and tolerability and determine the DLTs, MTD, optimum biologic dose (OBD) and RP2D of PRL3-zumab. The secondary objectives were to determine the pharmacokinetic (PK) profile of PRL3-zumab, determine the anti-tumor response and screen for the development of antibodies against PRL3-zumab. Exploratory objectives included assessment of PK and PD relationship for PRL3-zumab and biomarker assessment to identify molecular alterations that may predict efficacy. Safety assessments included history and physical examination, vital sign recordings, review of laboratory test results including electrocardiograms (ECGs) and cardiac function [using echocardiogram or multigated acquisition scan (MUGA)] and AEs. AEs were evaluated using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.03.

Tumor assessments were performed every 8 weeks while on study. Response and progression were evaluated using the RECIST guidelines (v1.1) for solid tumors. Only patients who had measurable disease present at baseline, had received at least one cycle of therapy and had had their disease re-evaluated were considered evaluable for the efficacy endpoint. Patients were also considered evaluable if they exhibited objective disease progression prior to the end of cycle 1. Patients who had lesions present at baseline that were evaluable but did not meet the definitions of measurable disease, had received at least one cycle of therapy and had had their disease re-evaluated were considered evaluable for non-target lesion assessment. The response assessment was based on the presence, absence or unequivocal progression of the lesions. For AML patients, bone marrow response criteria based on the European Leukaemia Network (ELN) 2017 guidelines for AML were used.

For PK analyses in the dose-escalation phase, serum samples were collected at the following time points: cycle 1 pre-dose (0 h); post-treatment at 1 h, 2 h, 4 h, and 6 h; and pre-treatment at cycle 1 day 2, cycle 1 day 3, cycle 1 day 4, cycle 1 day 5, cycle 1 day 8 and cycle 1 day 15, at each dose level tested, i.e., 0.3 mg/kg ($N = 3$), 0.9 mg/kg ($N = 2$), 3 mg/kg ($N = 3$) and 6 mg/kg ($N = 3$). In the dose-expansion study, serum samples were collected at the following time points: cycle 1 pre-dose (0 h); post-treatment at 6 h; and pre-treatment cycle 1 day 3, cycle 1 day 8 and cycle 1 day 15.

2.4 Statistical Analyses

The safety analysis consisted of all patients who received at least one dose of PRL3-zumab. Descriptive statistics based on counts, medians and percentages were used to summarized baseline characteristics and safety analyses for all treated patients and to investigate response outcomes. Descriptive statistics based on mean and standard deviation were used to analyze PK parameters. MTD was defined as the highest dose level where no more than one of six patients experienced DLT. The RP2D was one dose level below the MTD, provided that that dose level was $\leq 25\%$ lower than the highest (intolerable) dose tested. If the projected RP2D was $> 25\%$ lower than the highest dose tested, then an additional cohort of three or more patients was added at a dose that was intermediate between the intolerable dose and the next lower dose. The SMC could elect to terminate dose escalation if an RP2D could be determined by plasma levels of PRL3-zumab that coincided with PD markers that correlated with efficacious exposure levels observed in preclinical tumor models. Such levels of exposure may be considered the OBD.

3 Results

3.1 Demographics and Baseline Characteristics

Patients for the dose-escalation ($N = 11$) and dose-expansion ($N = 12$) cohorts for solid tumors were recruited from March 2017 to May 2018. An additional four patients with AML were enrolled in the dose-expansion cohort from January to November 2020. All patients were recruited and treated at

Table 2: Patient demographics and baseline characteristics

Baseline characteristics	No. (%)	
	Dose-escalation cohort, $N = 11$	Dose-expansion cohort, $N = 16$
Age, years		
Median	63	62.5
Range	36–84	52–75
Sex		
Male	6 (55)	6 (37.5)
Female	5 (45)	10 (62.5)
ECOG performance status		
0	4 (36.4)	4 (25)
1	7 (63.6)	12 (75)
Cancer type		
Breast	5 (45)	2 (12.5)
Lung	–	3 (18.8)
Head and neck	–	–
Colorectal	3 (27)	–
Gastric	1 (9)	–
Liver	1 (9)	–
Pancreas	1 (9)	2 (12.5)
Ovarian	–	2 (12.5)
Gall bladder	–	1 (6.3)
Periampullary	–	1 (6.3)
Salivary gland	–	1 (6.3)
Acute myeloid leukemia		4 (25)
No. of metastatic sites		
1–2	6 (54.5)	6 (37.5)
3 or more	5 (45.5)	6 (37.5)
Not applicable	–	4 (25)
No. of prior treatments		
0–2	–	8 (50)
3 or more	11 (100)	8 (50)

ECOG Eastern Cooperative Oncology Group

the National University Cancer Institute, Singapore. Table 2 summarizes the demographics and baseline characteristics of all patients enrolled in this study.

3.2 Patient Disposition

In the dose-escalation part of the study, three patients were enrolled at dose level 1 (0.3 mg/kg Q2W). One patient discontinued after cycle 1 at the patient's request to withdraw from the study due to intolerable pain from disease. The remaining two patients discontinued treatment after cycle 2 due to disease progression. At dose level 2 (0.9 mg/kg Q2W), two patients were enrolled and completed two cycles of study treatment. They were discontinued at the end of cycle 2 due to disease progression. As there were no safety concerns observed at dose levels 1 and 2, it was decided, after review with the SMC, to proceed with dose level 3 after no DLTs were observed in two patients at dose level 2 as part of the accelerated dose-titration scheme. At dose level 3 (3 mg/kg Q2W), three patients were enrolled. One patient discontinued after cycle 1 due to the patient's request to withdraw from the study. The patient had worsening performance status related to disease and transitioned to hospice care. In the remaining two patients, one patient was discontinued after cycle 2 and another patient after cycle 4 due to disease progression. At dose level 4 (6 mg/kg Q2W), three patients were enrolled and one patient discontinued after cycle 1 due to unequivocal disease progression. In the remaining two patients, one patient was discontinued after cycle 2 and another patient after cycle 4 due to disease progression. In the dose-expansion cohort, 16 patients were enrolled. Six patients were discontinued after cycle 1 due to disease progression (five patients) and delay in treatment for more than 4 weeks (one patient). In the remaining ten patients who continued into cycle 2, nine patients had discontinued at the end of cycle 2 due to disease progression ($N = 7$) and death, which was related to disease and deemed unrelated to the study treatment ($N = 2$). One patient with AML had stable disease post cycle 2 and continued treatment until cycle 4, where treatment was discontinued due to disease progression. Figure 1 summarizes the patient disposition in all cohorts.

3.3 Safety Profile

In the dose-escalation phase with 11 patients, there was a total of 41 treatment-emergent adverse events (TEAEs). Three patients had potentially drug-related TEAEs: one patient (33.3%) experienced grade 1 increased stoma output (0.9 mg/kg Q2W cohort), one patient (33.3%) experienced grade 1 fatigue (3 mg/kg Q2W cohort) and one patient (33.3%) experienced grade 2 vomiting (6 mg/kg Q2W cohort). No grade 3 or 4 potentially drug-related TEAEs were observed. There was one serious adverse event (SAE) with grade 3 gastrointestinal hemorrhage from bleeding gastro-enteral anastomotic ulcers (0.9 mg/kg Q2W cohort), which was unrelated to the study drug. No DLTs were observed.

With 16 patients enrolled in the dose-expansion phase, there was a total of 102 TEAEs (all causality). No patients had potentially drug-related TEAEs. No grade 3 or 4 potentially drug-related TEAEs were observed. Table 3 summarizes the TEAEs in the dose-escalation and dose-expansion phases for both solid-tumor and hematological patients that occurred in $\geq 5\%$ of patients. There were 21 SAEs with grade 3 ascites ($N = 3$, 14.3%), grade 3 abdominal bloating (4.8%), grade 2 dyspepsia (4.8%), grade 4 sepsis (4.8%), grade 3 upper respiratory tract infection (4.8%), grade 3

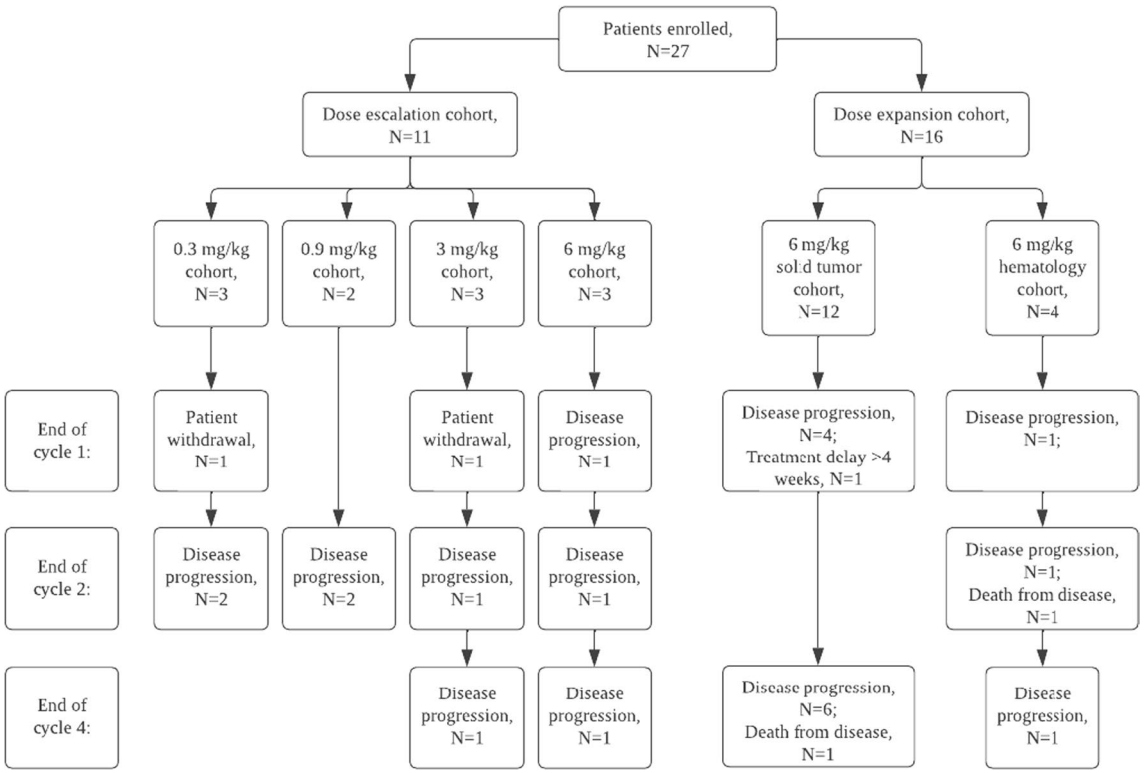

Fig. 1 Flow diagram of study participants

urinary tract infection (4.8%), grade 3 shortness of breath (4.8%), grade 2 fatigue (4.8%), grade 3–4 pancytopenia (19%), grade 3 Sweet's syndrome (4.8%), grade 3 hemorrhagic stroke (4.8%), grade 4 acute kidney injury (4.8%), grade 3 myocardial infarction (4.8%) and grade 3 supraventricular tachycardia (4.8%). All SAEs were unrelated to the study drug. Supplementary Table 1 summarizes the TEAEs from all causalities in the dose-escalation and dose-expansion phases for both solid-tumor and hematological patients that occurred in all patients.

3.4 Efficacy

In the dose-escalation phase, ten (out of 11) patients were evaluable for response. The best overall responses were as follows: stable disease for up to 4 cycles ($N = 2$, 18.2%) and disease progression ($N = 8$, 72.7%). In the patients with stable disease, the maximum number of cycles received was four cycles of treatment. In the dose-expansion phase, 14 (out of 16) patients with solid tumors were evaluable for response. The best overall responses were as follows: stable disease ($N = 1$, 6.3%) and disease progression ($N = 13$, 81.3%). In the patient with stable disease, the maximum number of cycles received was two cycles of treatment. All four patients with AML with myelodysplatic syndrome (MDS) changes had progressive disease. Tables 4, 5 summarizes the best overall responses noted in the study patients.

3.5 Pharmacokinetics

In the dose-escalation phase, serum samples from 11 patients were collected for PK analysis. A two-compartment model analysis with intravenous-infusion input and first-order elimination was fitted to the serum concentration–time data until day 15. The plasma concentration–time curve for the dose-escalation phase is presented in Fig. 2. The average half-life of the β phase ($t_{½,\beta}$) was 6.4 days for the 0.3-mg/kg dose, 12.5 days for the 1-mg/kg dose, 15.8 days for the 3-mg/kg dose and 12.0 days for the 6-mg/kg dose (Table 6). In the dose-expansion study, serum samples were collected from 12 solid-tumor and four hematological-malignancy patients (all receiving 6 mg/kg). A one-compartment model analysis (instead of two compartment; limited by fewer PK sampling time points) with intravenous-infusion input and first-order elimination was fitted to the serum concentration–time data until day 15. The plasma concentration–time curve for the dose-expansion phase is presented in Fig. 3. The average half-life ($t_{½}$) was 8.9 days in solid-tumor patients ($N = 12$) and 7.4 days in hematological patients ($N = 4$) (Table 7). Overall, the mean maximum concentration (C_{max}) and mean area under time–concentration curve (AUC) of PRL3-zumab

Table 3 Safety and tolerability of PRL3-zumab in the dose-escalation and dose-expansion phases

Dose level	Number of study participants (%) with treatment-emergent adverse events from all causalities[a]													
	0.3 mg/kg, $N = 3$		0.9 mg/kg, $N = 2$		3 mg/kg, $N = 3$		6 mg/kg, $N = 3$		6 mg/kg (ST Ex), $N = 12$		6 mg/kg (H Ex), $N = 4$		All patients, $N = 27$	
Grade	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
System organ class														
Gastrointestinal/liver														
Hemorrhage	0	0	2 (100)	1 (50)	0	0	0	0	0	0	0	0	2 (7.4)	1 (3.7)
Vomiting	0	0	0	0	0	0	1 (33.3)	0	2 (16.7)	1 (8.3)	0	0	3 (11.1)	1 (3.7)
Ascites	0	0	0	0	0	0	0	0	3 (25)	3 (25)	0	0	3 (11.1)	3 (11.1)
Abdominal distension	0	0	0	0	0	0	0	0	2 (16.7)	1 (8.3)	0	0	2 (7.4)	1 (3.7)
Weight loss	0	0	1 (50)	0	0	0	0	0	1 (8.3)	0	0	0	2 (7.4)	0
Poor appetite	0	0	0	0	0	0	1 (33.3)	0	0	0	2 (50)	0	3 (11.1)	0
Pain														
Back pain	1 (33.3)	0	0	0	0	0	0	0	1 (8.3)	0	0	0	2 (7.4)	0
Chest wall pain	0	0	1 (50)	0	1 (33.3)	0	2 (66.7)	0	1 (8.3)	0	0	0	5 (18.5)	0
Body pain	0	0	0	0	0	0	0	0	2 (16.7)	0	0	0	2 (7.4)	0
Abdominal pain/discomfort	0	0	0	0	0	0	0	0	3 (25)	0	0	0	3 (11.1)	0
Infection														
Fever	0	0	0	0	1 (33.3)	0	0	0	0	0	4 (100)	1 (25)	5 (18.5)	1 (3.7)
Sepsis	0	0	0	0	0	0	0	0	1 (8.3)	1 (8.3)	4 (100)	4 (100)	5 (18.5)	5 (18.5)
Pneumonia	0	0	0	0	0	0	0	0	0	0	4 (100)	4 (100)	4 (14.8)	4 (14.8)
Urinary tract infection	0	0	0	0	0	0	0	0	0	0	2 (50)	1 (25)	2 (7.4)	1 (3.7)
Respiratory														
Cough	1 (33.3)	0	1 (50)	0	2 (66.7)	0	1 (33.3)	0	5 (41.7)	0	0	0	10 (37)	0
Dyspnea	0	0	1 (50)	0	0	0	0	0	4 (33.3)	1 (8.3)	0	0	5 (18.5)	1 (3.7)
Nervous system														
Stroke	0	0	0	0	0	0	0	0	0	0	2 (25)	1 (25)	2 (7.4)	1 (3.7)
General														
Fatigue	0	0	0	0	1 (33.3)	0	1 (33.3)	0	2 (16.7)	0	1 (25)	1 (25)	5 (18.5)	1 (3.7)
Laboratory parameters														
Gastrointestinal/liver														
Hyperbilirubinemia	0	0	0	0	0	0	1 (33.3)	0	1 (8.3)	1 (8.3)	0	0	2 (7.4)	1 (3.7)
Lipase increased	1 (33.3)	1 (33.3)	0	0	0	0	0	0	1 (8.3)	1 (8.3)	0	0	2 (7.4)	2 (7.4)
Amylase increased	0	0	0	0	0	0	0	0	2 (16.7)	1 (8.3)	0	0	2 (7.4)	1 (3.7)

Table 3 (continued)

Dose level	Number of study participants (%) with treatment-emergent adverse events from all causalities[a]													
	0.3 mg/kg, $N = 3$		0.9 mg/kg, $N = 2$		3 mg/kg, $N = 3$		6 mg/kg, $N = 3$		6 mg/kg (ST Ex), $N = 12$		6 mg/kg (H Ex), $N = 4$		All patients, $N = 27$	
Grade	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4	Any grade	Grade 3/4
Blood system														
Anemia	1 (33.3)	1 (33.3)	0	0	1 (33.3)	0	0	0	1 (8.3)	1 (8.3)	4 (100)	4 (100)	7 (25.9)	6 (22.2)
Leukocytosis	0	0	0	0	0	0	0	0	0	0	1 (25)	1 (25)	1 (3.7)	1 (3.7)
Neutropenia	0	0	0	0	0	0	0	0	0	0	4 (100)	3 (75)	4 (14.8)	3 (11.1)
Thrombocytopenia	0	0	0	0	0	0	0	0	0	0	4 (100)	4 (100)	4 (14.8)	4 (14.8)
Metabolic														
Hypokalemia	0	0	0	0	2 (66.7)	2 (66.7)	0	0	1 (8.3)	1 (8.3)	0	0	3 (11.1)	3 (11.1)
Hyponatremia	0	0	0	0	0	0	0	0	2 (16.7)	2 (16.7)	0	0	2 (7.4)	2 (7.4)

ST Ex solid-tumor, dose-expansion cohort, *H Ex* hematological, dose-expansion cohort

[a]Treatment-related adverse events reported in ≥ 5% of all patients, any grade

increased with increasing dose. The volume of distribution was small, approximately equal to blood volume, and there was no significant effect of sex on the PK of PRL3-zumab.

3.6 Immunogenicity

Immunogenicity to PRL3-zumab was tested on 86 samples taken from 23 patients. Blood samples were taken before each cycle of treatment, as well as at the end of treatment. Induced anti-drug antibody (ADA) incidence was 20%, with four out of 20 patients having at least one time point positive for ADA. Although the majority of ADA-positive patients received higher doses of PRL3-zumab (3 mg/kg and 6 mg/kg), one ADA-positive patient came from the lowest-dose group (0.3 mg/kg). There was no correlation between ADA incidence and clinical outcome.

3.7 Pharmacodynamics

PRL3 status was determined by Western blotting. Table 5 describes the PRL3 status of the tumors in the dose-expansion phase and its correlation with tumor type and best overall response. There were eight PRL3-positive tumors (two ovarian cancers, two non-small cell lung cancers, one breast cancer, one periampullary cancer, one pancreas cancer and one salivary gland adenoid cystic carcinoma) and four PRL3-negative tumors (one gallbladder cancer, one breast cancer, one pancreatic colloid cancer and one non-small cell lung cancer). Amongst the PRL3-positive tumors ($N = 8$), best overall responses included one patient with stable disease, five patients with progressive disease and two patients with non-evaluable disease.

4 Discussion

This is a first clinical experience with PRL3-zumab, a humanized mAb against PRL3 in advanced solid tumors and hematological malignancy. The study established the safety and tolerability of PRL3-zumab in a cohort of patients with various malignancies for a range of doses between 0.3 and 6 mg/kg. MTD was not achieved in this study, as there were no dose-limiting events. The OBD could not be determined as an appropriate biomarker of target engagement, and a mechanism of action could not be deployed in this study. However, drug concentrations achieved in the 6-mg/kg cohort were well above the biologically relevant concentrations for activity in laboratory models.

In general, PRL3-zumab was well tolerated, with few AEs that were considered to be potentially treatment related. We enrolled 11 patients in the dose-escalation cohort, and three events were deemed possibly treatment related: increased stoma output in one patient (9.1%) at

Table 4 Clinical responses in patients treated with PRL3-zumab

	Dose-escalation cohort ($N = 11$)	Dose-expansion cohort (solid tumor) ($N = 12$)	Dose-expansion cohort (hematology) ($N = 4$)	All patients ($N = 27$)
Best overall response, *N* (%)				
CR	0	0	0	0
PR	0	0	0	0
SD	2 (18.2)	1 (8.3)	0	3 (11.1)
PD	8 (72.7)	9 (75)	4 (100)	21 (77.8)
Not evaluable	1 (9.1)	2 (16.7)	0	3 (11.1)

Responses according to RECIST v1.1. All percentages rounded to one decimal place

PD progressive disease, *RECIST* Response Evaluation Criteria in Solid Tumors, *SD* stable disease

Table 5 Summary of tumor type, PRL-3 status and treatment responses

Phase of study	Dose level	Patient ID	Tumor type	PRL-3 status	Best response
Dose escalation	0.3 mg/kg	PRL3-002	Colon	ND	PD
		PRL3-003	Gastric	ND	NE
		PRL3-004	Colon	ND	PD
	0.9 mg/kg	PRL3-005	Pancreas	ND	PD
		PRL3-006	Rectal	ND	PD
	3 mg/kg	PRL3-008	Cholangiocarcinoma	ND	PD
		PRL3-009	Breast	ND	PD
		PRL3-010	Breast	ND	SD
	6 mg/kg	PRL3-011	Breast	ND	SD
		PRL3-012	Breast	ND	PD
		PRL3-013	Breast	ND	PD
Dose expansion	6 mg/kg	PRL3-102	Ovary	Positive[a]	NE
		PRL3-103	Gallbladder	Negative	PD
		PRL3-104	Ovary	Positive[a]	PD
		PRL3-106	Breast	Negative	PD
		PRL3-107	Periampullary	Positive[a]	NE
		PRL3-108	Pancreas	Positive[a]	PD
		PRL3-109	Lung (NSCLC)	Positive[a]	PD
		PRL3-110	Salivary gland	Positive[a]	PD
		PRL3-111	Lung (NSCLC)	Positive[a]	SD
		PRL3-112	Pancreas (colloid)	Negative	PD
		PRL3-113	Breast	Positive[a]	PD
		PRL3-114	Lung (NSCLC)	Negative	PD
		PRL3(1b)-002	AML	ND	PD
		PRL3(1b)-003	AML with MDS changes	ND	PD
		PRL3(1b)-004	AML with MDS changes	ND	PD
		PRL3(1b)-005	AML with MDS changes	ND	PD

AML acute myeloid leukemia, *MDS* myelodysplatic syndrome, *ND* not done, *NE* not evaluable, *NSCLC* non-small cell lung cancer, *PD* progressive disease, *SD* stable disease

[a]In 8 PRL3-positive tumors, best responses were as follows: SD ($N = 1$, 12.5%); PD ($N = 5$, 62.5%); NE ($N = 2$, 25%)

the 0.9-mg/kg dose, grade 1 fatigue in one patient (9.1%) at the 3-mg/kg dose and grade 2 vomiting in one patient (9.1%) at the 6-mg/kg dose. Gastrointestinal hemorrhage in a patient (9.1%) at the 0.9-mg/kg dose was deemed to be due to a bleeding gastro-enteral anastomotic ulcer that was not drug- or malignancy-related. As such, there were no DLTs in cycle 1 for all doses evaluated, allowing dose escalation according to the protocol plan. Beyond cycle 1,

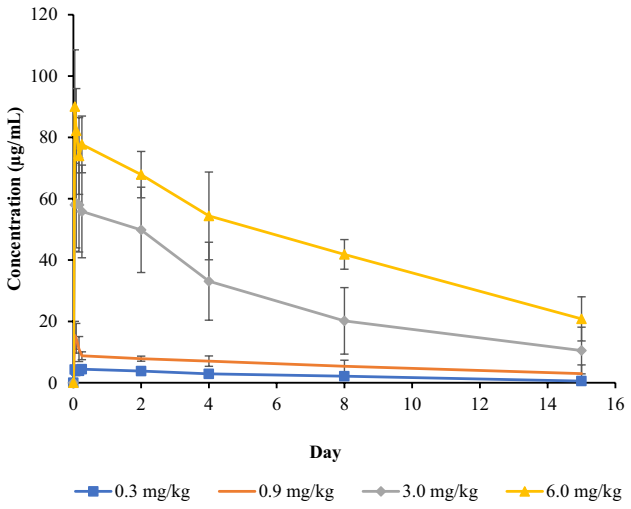

Fig. 2 Mean concentration–time curves of PRL3-zumab, stratified by dose levels in the dose-escalation cohort. Each plot represents the average serum concentration of patients in the dose-level group at a particular time point (mean ± SD): $N = 3$ (0.3 mg/kg); $N = 2$ (0.9 mg/kg); $N = 3$ (3 mg/kg); $N = 3$ (6 mg/kg). *SD* standard deviation

there were no potentially drug-related AEs. No patients required dose reduction due to toxicity. Specifically, there were no hematological events or infusion-related toxicities due to PRL3-zumab. All hematological events observed were related to the underlying disease.

The PK of PRL3-zumab was studied in cycle 1 of treatment. PK were dose proportional, with terminal half-lives (6.4–15.8 days) consistent with IgG type mAbs, and are suitable for 2 weekly administration. Consistent with mAbs, the volume of distribution corresponds approximately to the human blood volume. Higher C_{max} AUC_{0-t} and shorter $t_{½}$ were detected in hematological-malignancy patients compared to solid-tumor patients. Higher distribution of drug to the site of tumor might be the reason for lower C_{max} and AUC in solid-tumor patients. Taken together, these data were used to select an RP2D of 6 mg/kg Q2W for future PRL3-zumab studies.

There were no confirmed complete or partial responses in patients with the various tumor types that received PRL3-zumab in this study. Twenty-four patients were evaluable for response, in whom the best overall response was progressive disease in 21 patients (77.8%), and three patients (11.1%) had stable disease after cycle 2 of treatment: one patient at the 3-mg/kg dose and two patients at the 6-mg/kg dose. The stable disease was not durable, as no patient had treatment beyond 4 cycles; the two patients with stable disease at the 3-mg/kg dose and 6-mg/kg dose, respectively, developed progressive disease after the fourth treatment cycle. The

Table 6 Pharmacokinetic parameters after single-dose injection of PRL3-zumab, stratified by dose level in the dose-escalation phase of the study

PK parameters	0.3 mg/kg ($N = 3$)	0.9 mg/kg ($N = 2$)	3.0 mg/kg ($N = 3$)	6.0 mg/kg ($N = 3$)
C_{max} (µg/mL)				
Mean ± SD	9.04 ± 8.97	17.62 ± 5.01	60.02 ± 15.73	97.64 ± 23.43
Geomean (%CV Geomean)	6.54 (99.26%)	17.26 (28.41%)	58.48 (26.21%)	95.80 (24.00%)
AUC_{0-t} (h*µg/mL)				
Mean ± SD	677.74 ± 116.18	1843.07 ± 640.01	8157.52± 3606.77	14,301.39 ± 885.07
Geomean (%CV Geomean)	670.66 (17.14%)	1786.65 (34.73%)	7645.07 (44.21%)	14,283.23 (6.19%)
AUC_{0-inf} (h*µg/mL)				
Mean ± SD	864 ± 146.18	3686.2± 2741.66	13,752.01 ± 7060.09	25,235.22 ± 5553.13
Geomean (%CV Geomean)	855.74 (16.91%)	31,35.25 (74.38%)	12,119.39 (51.34%)	24,847.94 (22.01%)
$t_{½}$, alpha (day)				
Mean ± SD	0.35 ± 0.55	0.07 ± 0.04	1.41 ± 0.62	0.27 ± 0.40
$t_{½}$, beta (day)				
Mean ± SD	6.41 ± 1.20	12.46 ± 8.95	15.76 ± 15.96	11.97 ± 5.52
CL (L/h)				
Mean ± SD	0.023 ± 0.001	0.017 ± 0.013	0.012 ± 0.004	0.014 ± 0.004
V_{ss} (L)				
Mean ± SD	4.99 ± 1.05	5.47 ± 0.20	5.45 ± 5.12	5.04 ± 0.72
MRT (h)				
Mean ± SD	218.81 ± 38.65	428.09 ± 307.11	458.22 ± 414.8	409.33 ± 184.07

AUC area under time–concentration curve, *CL* clearance, C_{max} maximum concentration, *CV* coefficient of variation, *MRT* mean residence time, *PK* pharmacokinetic, *SD* standard deviation, $t_{½}$ half-life, $t_{½}$ *alpha* half-life of distribution phase, $t_{½}$ *beta* half-life of elimination phase, V_{ss} apparent volume of distribution at steady state

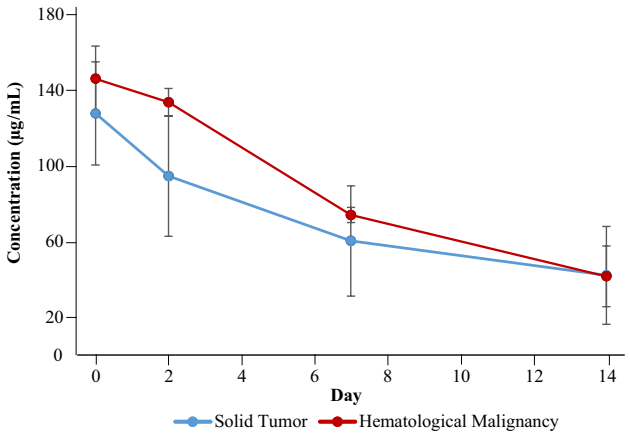

Fig. 3 Mean concentration–time curves of PRL3-zumab (6 mg/kg) in the dose-expansion cohort. Each plot represents the average serum concentration of patients at a particular time point (mean ± SD): $N = 12$ (solid tumor); $N = 4$ (hematological malignancy). *SD* standard deviation

Table 7 Pharmacokinetic parameters after single-dose injection of PRL3-zumab at 6 mg/kg, in the dose-expansion phase of solid-tumor and hematological-malignancy patients

PK parameters	Solid tumor, 6 mg/kg ($N = 12$)	Hematological malignancy, 6 mg/kg ($N = 4$)
C_{max} (µg/mL)		
Mean ± SD	153.09 ± 14.37	124.92 ± 29.43
Geomean	152.59	121.65
(%CV Geomean)	9.42%	23.56%
AUC_{0-t} (µg/mL*h)		
Mean ± SD	28,418.37 ± 2792.47	22,977.75 ± 8883.5
Geomean	28,318.14	21,505.08
(%CV Geomean)	9.86%	38.66%
AUC_{0-inf} (µg/mL*h)		
Mean ± SD	39,110.99 ± 6481.30	38,441.29 ± 26,914.53
Geomean	38,742.47	31,267.85
(%CV Geomean)	16.73%	70.01%
$t_{½}$ (day)		
Mean ± SD	7.4 ± 1.22	8.93 ± 6.38
CL (L/h)		
Mean ± SD	0.01 ± 0.001	0.01 ± 0.01
V_{ss} (L)		
Mean ± SD	2.37 ± 0.22	2.8 ± 0.86
MRT (h)		
Mean ± SD	256.26 ± 42.2	309.26 ± 0.86

AUC area under time–concentration curve, *CL* clearance, C_{max}, maximum concentration, *CV* coefficient of variation, *MRT* mean residence time, *PK* pharmacokinetic, *SD* standard deviation, $t_{½}$ half-life, V_{ss} apparent volume of distribution at steady state

PRL3 status of the tumors in the dose-expansion phase was determined, and its correlation with tumor type and best response analyzed. There was no correlation between PRL3-positive tumors and response. There are currently ongoing studies with PRL3-zumab in gastric cancer, hepatocellular carcinoma and other solid tumors.

In conclusion, this first-in-human study has demonstrated that PRL3-zumab, a humanized mAb against PRL3, is safe and tolerable in advanced solid tumors and hematological malignancy.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11523-023-00962-w.

Declarations

Funding This study was supported by the Singapore Ministry of Health's National Medical Research Council under its NMRC Centre Grant Programme CGAug16M005.

Conflict of interest Qi Zeng is the founder of Intra-ImmuSG Pte. Ltd (IISG), an A*STAR spin-off company that provided PRL3-zumab for this study. Cheng E. Chee, Melissa Ooi, Soo-Chin Lee, Raghav Sundar, Valerie Heong, Wei-Peng Yong, Chin Hin Ng, Andrea Wong, Joline SJ Lim, David SP Tan, Ross Soo, Joshua TC Tan, Song Yang, Min Thura, Abdul Qader Al-Aidaroos, Wee Joo Chng and Boon-Cher Goh declare that they have no conflicts of interest that might be relevant to the contents of this article.

Ethics approval, consent to participate and consent for publication This study was conducted in accordance with the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice guidelines. The study protocol was approved by the Domain Specific Review Board. Informed consent was obtained from all individual patients included in the study. This study was registered with ClinicalTrials.gov (identifier: NCT03191682; date of registration: June 19, 2017).

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Author contributions Conception and design: Cheng E. Chee, Melissa Ooi, Soo-Chin Lee, Raghav Sundar, Valerie Heong, Wei-Peng Yong, Chin Hin Ng, Andrea Wong, Joline SJ Lim, David SP Tan, Ross Soo, Wee Joo Chng, Qi Zeng, Boon-Cher Goh. Provision of study materials or patients: Cheng E. Chee, Melissa Ooi, Soo-Chin Lee, Raghav Sundar, Valerie Heong, Wei-Peng Yong, Chin Hin Ng, Andrea Wong, Joline SJ Lim, David SP Tan, Ross Soo, Wee Joo Chng, Qi Zeng, Boon-Cher Goh. Collection and assembly of data: All authors. Data analysis and interpretation: All authors. Manuscript writing: All authors. Final approval of manuscript: All authors. Accountable for all aspects of the work: All authors.

References

1. Zeng Q, Hong W, Tan YH. Mouse PRL-2 and PRL-3, two potentially prenylated protein tyrosine phosphatases homologous to PRL-1. Biochem Biophys Res Commun. 1998;244(2):421–7.
2. Zeng Q, Si X, Horstmann H, Xu Y, Hong W, Pallen CJ. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome. J Biol Chem. 2000;275(28):21444–52.
3. Saha S, Bardelli A, Buckhaults P, Velculescu VE, Rago C, St Croix B, et al. A phosphatase associated with metastasis of colorectal cancer. Science. 2002;294:1343–6.
4. Al-Aidaroos AQ, Zeng Q. PRL-3 phosphatase and cancer metastasis. J Cell Biochem. 2010;111(5):1087–98.
5. Thura M, Al-Aidaroos AQO, Yong WP, Kono K, Gupta A, Lin YB, et al. PRL3-zumab, a first-in-class humanized antibody for cancer therapy. JCI Insight. 2016;1(9): e87607.
6. Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. Cancer Res. 2007;67(7):2922–6.
7. Walls CD, Iliuk A, Bai Y, Wang M, Tao WA, Zhang ZY. Phosphatase of regenerating liver 3 (PRL3) provokes a tyrosine phosphoproteome to drive prometastatic signal transduction. Mol Cell Proteomics. 2013;12(12):3759–77.
8. Al-Aidaroos AQ, Yuen HF, Guo K, Zhang SD, Chung TH, Chng WJ, et al. Metastasis-associated PRL-3 induces EGFR activation and addiction in cancer cells. J Clin Invest. 2013;123(8):3459–71.
9. Zimmerman MW, McQueeney KE, Isenberg JS, Pitt BR, Wasserloos KA, Homanics GE, et al. Protein-tyrosine phosphatase 4A3 (PTP4A3) promotes vascular endothelial growth factor signaling and enables endothelial cell motility. J Biol Chem. 2014;289(9):5904–13.
10. Park JE, Yuen HF, Zhou JB, Al-Aidaroos AQ, Guo K, Valk PJ, et al. Oncogenic roles of PRL-3 in FLT3-ITD induced acute myeloid leukaemia. EMBO Mol Med. 2013;5(9):1351–66.
11. Hong CW, Zeng Q. Awaiting a new era of cancer immunotherapy. Cancer Res. 2012;72(15):3715–9.
12. Bessette DC, Qiu D, Pallen CJ. PRL PTPs: mediators and markers of cancer progression. Cancer Metastasis Rev. 2008;27(2):231–52.
13. Thura M, Al-Aidaroos AQ, Gupta A, Chee CE, Lee SC, Hui KM, et al. PRL3-zumab as an immunotherapy to inhibit tumors expressing PRL3 oncoprotein. Nat Commun. 2019;10:2484.
14. Guo K, Li J, Tang J, Tan C, Hong C. Targeting intracellular oncoproteins with antibody therapy or vaccination. Sci Transl Med. 2011;3:99ra85.
15. Ferrone S. Hidden immunotherapy targets challenge dogma. Sci Trans Med. 2011;3:99ps38.
16. Lazo JS, Sharlow ER. Drugging undruggable molecular cancer targets. Annu Rev Pharmacol Toxicol. 2016;56:23–40.
17. Guo K, Tang JP, Jie L, Al-Aidaroos AQ, Hong CW, Tan CP, et al. Engineering the first chimeric antibody in targeting intracellular PRL-3 oncoprotein for cancer therapy in mice. Oncotarget. 2012;3(2):158–71.
18. Wang H, Vardy LA, Tan CP, Loo JM, Guo K, Li J, et al. PCBP1 suppresses the translation of metastasis-associated PRL-3 phosphatase. Cancer Cell. 2010;18(1):52–62.