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ORIGINAL ARTICLE

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TSH receptor specific monoclonal autoantibody K1-70™ targeting of the TSH receptor in subjects with Graves' disease and Graves' orbitopathy—Results from a phase I clinical trial

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Abstract

Objectives: In Graves' disease (GD), autoantibodies to the thyroid stimulating hormone receptor (TSHR) cause hyperthyroidism. The condition is often associated with eye signs including proptosis, oedema, and diplopia (collectively termed Graves' orbitopathy [GO]). The safety profile of K1-70™ (a human monoclonal TSHR specific autoantibody, which blocks ligand binding and stimulation of the receptor) in patients with GD was evaluated in a phase I clinical trial.

Patients and Study Design: Eighteen GD patients stable on antithyroid drug medication received a single intramuscular (IM) or intravenous (IV) dose of K1-70™ during an open label phase I ascending dose, safety, tolerability, pharmacokinetic and pharmacodynamic (PD) study. Immunogenic effects of K1-70™ were also determined.

Results: K1-70™ was well-tolerated in all subjects at all doses and no significant immunogenic response was observed. There were no deaths or serious adverse events. Increased systemic exposure to K1-70™ was observed following a change to IV dosing, indicating this was the correct dosage route. Expected PD effects occurred after a single IM dose of 25 mg or single IV dose of 50 mg or 150 mg with fT3, fT4, and TSH levels progressing into hypothyroid ranges. There were also clinically significant improvements in symptoms of both GD (reduced tremor, improved sleep, improved mental focus, reduced toilet urgency) and GO (reduced exophthalmos measurements, reduced photosensitivity).

Conclusions: K1-70™ was safe, well tolerated and produced the expected PD effects with no immunogenic responses. It shows considerable promise as a new drug to block the actions of thyroid stimulators on the TSHR.

KEYWORDS

autoimmune diseases, Graves' disease, Graves' ophthalmopathy, hyperthyroidism, thyroid diseases

1 | INTRODUCTION

Graves' disease (GD) is a common autoimmune condition characterised by the presence of autoantibodies to the thyroid stimulating hormone receptor (TSHR) which mimic the actions of TSH and cause hyperthyroidism.[1,2] Current therapies for GD usually target the ability of the thyroid to produce thyroid hormones and include antithyroid drugs (ATD) and destruction of the thyroid using radioiodine or surgical thyroidectomy.[3] However, they often have variable and unsatisfactory outcomes,[3] highly dependent on the treatment modality used.[4] In a study of 549 GD patients, 21% were still positive for TSHR autoantibodies (TRAb) after 5 years of continuous therapy with ATD.[5]

Standard treatment for moderate to severe Graves' orbitopathy (GO) includes systemic steroids and/or surgical interventions as appropriate, but is often unsatisfactory.[6,7]

New therapies for GD and GO are emerging and one of these uses the human monoclonal TSHR autoantibody K1-70™ to block TSHR activation.[8] Others include drugs such as rituximab,[9] tocilizumab[10] and teprotumumab[11] and immunotherapy with TSHR peptides.[12]

K1-70™ binds specifically to the TSHR with high affinity (4×10^{10} mol/L)[8] and prevents receptor binding by TSH and by patient TRAb thus blocking the stimulating actions of these ligands. This effect has been demonstrated in vivo[13] and in preclinical studies[14] where K1-70™ was effective in lowering T3 and T4 levels and increasing TSH. Results from these preclinical studies[14] provided the basis for the design of the first in man phase I clinical trial we now describe.

2 | MATERIALS AND METHODS

2.1 | Study drug

Recombinant human K1-70™ immunoglobulin G (IgG) drug was produced in CHO cells under current good manufacturing practice and formulated at 10 mg/ml in 25 mmol/L sodium citrate; 75 mmol/L sodium chloride; 50 mmol/L glycine; 0.02% (wt/vol) polysorbate 80; pH 6.0 and designated for both intramuscular (IM) and intravenous (IV) administration. Stability studies support a shelf-life of vialed K1-70™ of 60 months at 2–8°C.

2.2 | Study objectives

An open label study in which each subject received a single dose of K1-70™ (IM or IV) was carried out. The dose was increased for each study group of 3 subjects (cohorts 1–6). Subjects diagnosed with GD were selected for the study as the risk associated with healthy subjects becoming hypothyroid was deemed unacceptable.

The primary objective was to determine the safety and tolerability of IM and IV administration of K1-70™ in subjects with GD. The secondary objectives were to determine the pharmacokinetic (PK) and the pharmacodynamic (PD) effects and to evaluate the immunogenic potential of K1-70™. Exploratory objectives were to investigate the correlation between baseline TRAb levels and change in PD markers and to evaluate any effect of K1-70™ on GO.

2.3 | Statistical analyses

Summary statistics for continuous variables consisted of the number of nonmissing observations, means, standard deviations (*SD*s), minimum, median and maximum values for observed baseline and changes from baseline at each timepoint. The correlation between baseline TRAb levels and change from baseline in each of the thyroid function parameters was assessed across all cohorts by visit using a Spearman's rank order correlation. The correlation coefficient (rs) was reported. The correlation between any changes from baseline TSH and change from baseline in both fT3 and fT4 was also assessed across all cohorts by visit using a Spearman's rank order correlation with rs reported in all cases.

Medical history and treatment emergent adverse events (TEAEs) were coded to system organ class and preferred term, using the Medical Dictionary for Regulatory Activities (MedDRA) version 19.0. Prior and concomitant medications were coded using the World Health Organization Drug Dictionary (WHO Drug) version March 2016.

2.4 | Study design and recruitment

The study was carried out at two clinical sites in the United Kingdom in compliance with the Declaration of Helsinki[15] and the International Conference on Harmonisation.[16] It was registered with clinicaltrials.gov[17] and approved by the appropriate Research Ethical Committees and the UK regulatory authorities. All participating subjects were fully apprised of the study drug, study objectives, design and potential risks and were required to give their written informed consent.

Male or female subjects with GD between 18 and 75 years of age were eligible for the study. Pregnant subjects were excluded. Patients taking antithyroid medication at a stable dose for at least 6 weeks who were clinically and biochemically euthyroid or hyperthyroid at screening and Day −2 (or Day −1) or not treated with anti-thyroid medications due to intolerable side effects for at least 6 weeks and who were clinically and biochemically hyperthyroid at screening and Day −2 (or Day −1) were eligible. Subjects with GO with a clinical activity score (CAS) greater than 3/7 and with evidence of optic neuropathy and/or corneal breakdown at screening were excluded in view of the risk (albeit unlikely) of K1-70™ causing unexpected deterioration of GO.

The study was designed and approved for 6 cohorts of 3 subjects each with an additional 3 subjects per cohort if required i.e. for a minimum of 18 and up to a maximum of 36 subjects. The provision for additional subjects was included in the protocol so that it could be adapted in case serious adverse events (SAEs) or other safety concerns occurred. However, the trial was completed successfully with 18 subjects as there were no SAEs or safety concerns.

A starting dose of 0.2 mg IM K1-70™ was calculated from in vivo pharmacology data[13] according to the US Food and Drug Administration (FDA) guidance.[18] K1-70™ was administered to three subjects in each cohort using a sentinel approach and in the absence of SAEs the dose was escalated for the next cohort following review of the available safety, tolerability, PK, antidrug antibody (ADA) and PD data.

Subjects were followed up postdosing as summarised in Figure 1. A full range of safety and tolerability assessment at entry and throughout the follow-up including injection site assessment, vital signs, physical examination, electrocardiography, clinical laboratory evaluations (serum biochemistry, haematology, coagulation, urinalysis, serology, hormone panels) pregnancy tests and eye examination were carried out.

Full eye examinations (including CAS) were performed by a registered optometrist (or suitably qualified member of the ophthalmology team) at screening and on Days 14, 42, 70, and 100. Abbreviated eye examinations were performed by the Study Physician on Days 1, 3, 7, 14, 21, 28, 42, 56, 70, and 100. Eye examinations were carried out in accordance with European Group on Graves' Orbitopathy Guidelines for the management of Graves' Orbitopathy (EUGOGO) recommendations.[6]

All observations, results of examinations and safety tests, PD markers, PK and ADA results, concomitant medications and adverse events (AEs) were recorded using an electronic case report form.

Serum concentrations of K1-70™ were measured by Labcorp using a PK enzyme-linked immunoassay (ELISA) from RSR Ltd.[19]

Any specific antibodies to K1-70™ IgG produced in response to administration of K1-70™ drug were detected using an ADA assay specially developed by Labcorp using a Meso Scale Discovery platform. ADA values were classified as negative or positive after a two-stage screen for negative/positive results. The confirmatory cut point was 19.3% in keeping with recently updated FDA guidance.[20]

Thyroid function tests were performed by the Doctors Laboratory Ltd. and TRAb were measured by Dr Schottdorf Laboratory (Augsburg, Germany) using the Roche Elecsys® Anti-TSHR platform (cut off for positivity 1.5 U/L).

2.5 | Study subjects and dosing regimen

A total of 47 subjects with GD were screened, of whom 20 were eligible for the study. Two withdrew before dosing and the remaining eighteen were enrolled and dosed with K1-70™. K1-70™ was administered by IM injection to 12 subjects; cohort 1 (0.2 mg/dose), cohort 2 (1 mg/dose), cohort 3 (5 mg/dose) and cohort 4 (25 mg/dose). Six subjects received K1-70™ by IV infusion; cohorts 5 (50 mg/dose) and 6 (150 mg/dose). There were 3 subjects in each cohort. All eighteen subjects completed a 100-day follow-up.

The dosing regimen was amended during the study due to insufficient systemic exposure in earlier cohorts and the route of K1-70™ administration changed to IV with the conservative K1-70™ systemic exposure limit of 0.031 mg/ml replaced with either a Cmax of 0.850 mg/ml or an AUC of 83100 μg·h/ml.

Demographic data for patients in the study is shown in Table 1.

The baseline CAS of participants was between 0 and 1/7, with baseline exopthalmometry measurements ranging from 13 to 24 mm.

Ranges of baseline thyroid function parameters are shown in Table 1.

At predose 6/18 patients were negative for TRAb (<1.5 IU/L) and 9/18 patients were positive.

3 | RESULTS

3.1 | Safety and tolerability

K1-70™ was safe and well-tolerated at all doses and by all patients in the study. A total of 86 TEAEs were reported by the 18 subjects (Table 2), of which 22 (25.6%) were considered as possibly related to study treatment. No TEAE was determined as directly related to the study drug K1-70™. TEAEs were mild or moderate and there were no serious TEAEs, no TEAEs that led to discontinuation, dose

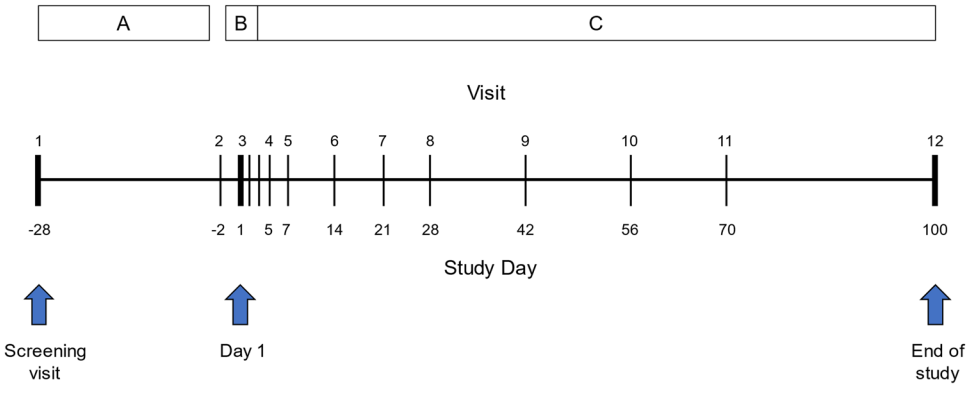

FIGURE 1 Time course and patient visit schedule

TABLE 1 Demographic characteristics of the study population ($n = 18$) at screening

Variable	Value
Age (years), mean ± *SD*	45.9 ± 13.5
BMI (kg/m^2), mean ± *SD*	26.1 ± 4.1
Female sex, *n* (%)	13 (72%)
Presence of eye disease, *n* (%)	8 (44%)
TSH (mIU/L), median (min–max), ref: 0.27–4.20	0.40 (0.01–3.79)
fT4 (pmol/L), median (min–max), ref: 12.0–22.0	16.65 (11.50–43.30)
fT3 (pmol/L), median (min–max), ref: 3.10–6.80	5.25 (3.60–16.50)
TRAb (IU/L), median (min–max), positive ≥1.5	3.78 (0.30–19.40)
Patients taking ATD	
Carbimazole, *n* (%)	15 (83%)
Propylthiouracil, *n* (%)	2 (11%)
Block and replace, *n* (%)	1 (6%)
Clinical status	
Euthyroid with ATD, *n* (%)	12 (67%)
Hyperthyroid, *n* (%)	6 (33%)

Note: At dosing all patients were on antithyroid drug (ATD) treatment. A total of 12/18 patients were euthyroid and 6/18 were hyperthyroid at screening, 15 patients were on carbimazole, two on propylthiouracil and one on "block and replacement" treatment with carbimazole and thyroxine. A total of 6/18 had negative TRAb at dosing and these subjects might have been in remission.

All study subjects were required to have a body mass index of between 18.5 and 35.0 kg/m^2 (revised to <32.0 kg/m^2 as a COVID-19 mitigation measure).

Due to the COVID-19 pandemic, the final subject of cohort 5 had their Day 70 full eye examination cancelled and their Day 100/end of study visit eye examination took place on Day 164.

reduction, or dose interruption and no TEAEs with fatal outcome were observed.

There were 11 TEAEs in cohort 1, 8 in cohort 2, 10 in cohort 3, 10 in cohort 4, 32 in cohort 5 and 15 in cohort 6. The high number of TEAEs in cohort 5 was largely due to a relatively high number of AEs (19) being reported by a single subject (501). However AEs experienced by subject 501 were classified as mild or moderate in nature only with all but one resolved by the end of the study. Furthermore, no AE reported by subject 501 was considered directly related to the study drug with only 8 of their AEs being classified as possibly related to the study drug. Overall, there was no indication of a relationship between the dose of K1-70^{TM} administered and the number of TEAEs experienced by study subjects over the course of the trial.

The most common treatment-related TEAEs by preferred term were fatigue (in 6/18 subjects), lethargy (3/18), and diarrhoea (5/18). All other treatment-related TEAEs were single reports. No SAEs were experienced by the subjects and no trial stopping criteria were met.

3.2 | Pharmacokinetics and immunogenicity

Fifteen subjects (83.3%) had ADA negative results throughout the study. Positive results for low levels of ADA were observed in two subjects administered 0.2 mg K1-70^{TM} IM and one subject administered 1.0 mg K1-70^{TM} IM. However, 2/3 of these subjects tested positive for ADA before the administration of K1-70^{TM} and no ADA response was observed for any of the other 15 patients (including cohorts receiving much higher doses of K1-70^{TM}).

Serum K1-70^{TM} levels following administration of K1-70^{TM} are shown in Figure 2. In cohort 1 (0.2 mg K1-70™ IM), serum concentrations of K1-70^{TM} were below the lower limit of quantitation of 10 ng/ml at most timepoints.

Median K1-70^{TM} half-life was seen to increase with increasing doses of K1-70^{TM} (Figure 2): from 262.0 h (approximately 11 days) after 1.0 mg IM to 447.0 h (approximately 19 days) after 25.0 mg IM. The median half-life after 150 mg IV was 522.0 h (21.8 days) and 467.0 h (19.5 days) after 50 mg IV. Median time of the maximum observed serum concentration (Tmax) after the end of the 50 and 150 mg IV infusions was 2.5 and 1.6 h, respectively, and much sooner than that following IM administration (Figure 2).

3.3 | Pharmacodynamic effects on GD and GO

Little or no PD effect was observed at lower doses of K1-70^{TM} (0.2–5 mg IM) while a marked PD effect was observed in subjects administered higher doses (25 mg IM, 50 mg IV, and 150 mg IV) with a decrease in fT3 and fT4 levels and an increase in TSH levels. For both routes the PD effect was most evident on study Day 28 when 11/18 (61%) patients were in a hypothyroid state (Figure 3 and Table 3A). For higher dose cohorts 4–6, 9/9 subjects (100%) progressed from either the euthyroid state at dosing (3/9 subjects) or the hyperthyroid state at dosing (6/9 subjects) to the hypothyroid state on or before Day 28 postdose. TRAb and TSH levels pre-dose and at Day 28 are shown in Table 3B.

The PD effect of K1-70^{TM} was also noted from patient and clinician reported symptom improvements (Table 3C) and this was unexpected for a phase I safety study. For example, with subject 501 (50 mg of K1-70^{TM} IV) exophthalmos improved on Days 14 to 14 mm in the right eye from 19 mm at screening and to 16 mm in the left eye from 19 mm at screening. Shortly after (24 h onwards) K1-70^{TM} IV infusion she reported improvement in conjunctival redness and gaze-evoked pain, clearer vision, decreased photosensitivity (no longer needed to use sunglasses indoors) and resolution of her "gritty eyes" sensation. Her CAS improved slightly from 1/7 (both eyes) to 0/7 (both eyes) by Day 3 postdosing. She also reported improved toilet urgency, improved mental focus and an improvement in general aches and pains. The improvements lasted through the follow-up, beyond the end of the 100-day study period and enabled the patient to undergo eye lid surgery. She commented that she felt like "a new woman" and had applied for a job. For subject 502 (50 mg of K1-70^{TM} IV) exophthalmos in the right eye decreased from 23 mm at

TABLE 2 Treatment emergent adverse events (TEAEs) in cohorts 1–6 following K1-70™ administration

	Cohort 1 (0.2 mg IM, *n* = 3)	Cohort 2 (1.0 mg IM, *n* = 3)	Cohort 2 (5.0 mg IM, *n* = 3)	Cohort 4 (25 mg IM, *n* = 3)	Cohort 5 (50 mg IV, *n* = 3)	Cohort 6 (150 mg IV, *n* = 3)
Total number of TEAEs	11	8	10	10	32	15
Total with serious TEAEs	0	0	0	0	0	0
Total with TEAEs leading to discontinuation	0	0	0	0	0	0
Total with TEAEs leading to dose reduction	0	0	0	0	0	0
Total with TEAEs leading to drug interruption	0	0	0	0	0	0
Total with TEAEs leading to death	0	0	0	0	0	0
Severity of TEAEs[a]						
Mild	10	7	8	10	20	10
Moderate	1	1	2	0	12	5
Severe	0	0	0	0	0	0
Relationship of TEAEs to study treatment[a]						
Not related[b]	9	6	9	9	23	8
Related[c]	2	2	1	1	9	7

Abbreviations: IM, intramuscular; IV, intravenous; n, number of subjects.

[a]If a subject experienced more than one TEAE, the subject was counted once at the most severe or most related event.

[b]Not related TEAEs are the total number of TEAEs classified as not related to study drug and TEAEs classified as unlikely related to study drug.

[c]Related TEAEs are those TEAEs classified as possibly related to study drug (no TEAEs were classified as related to study drug).

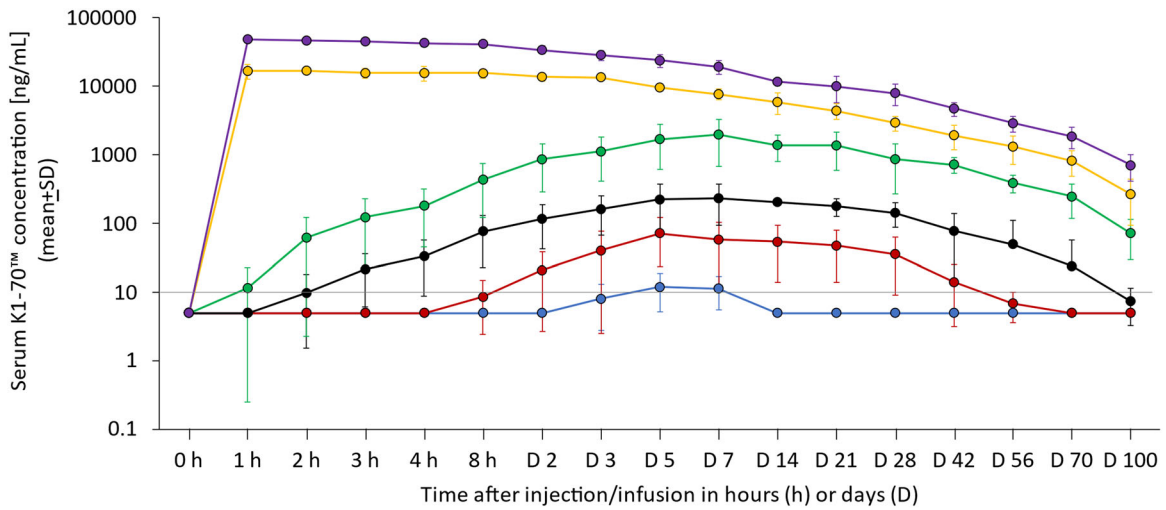

FIGURE 2 Serum concentrations of K1-70™ (logarithmic scale) in all cohorts following intramuscular (IM) or intravenous (IV) administration. The grey line indicates the lower limit of quantitation value of 10 ng/ml. Vertical bars indicate the standard deviation (*SD*). The timepoints shown are measured from the end of IM injection or IV infusion. Cohort 1 (0.2 mg K1-70™ IM). Cohort 2 (1.0 mg K1-70™ IM). Cohort 3 (5.0 mg K1-70™ IM). Cohort 4 (25 mg K1-70™ IM). Cohort 5 (50 mg K1-70™ IV). Cohort 6 (150 mg K1-70™ IV)

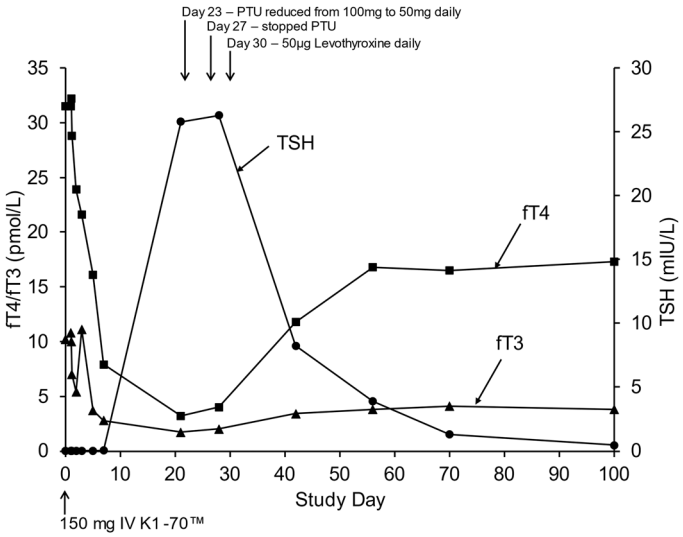

FIGURE 3 Pharmacodynamic effect on fT4, fT3, and TSH levels in study subject 602 following intravenous infusion of 150 mg K1-70™. The patient was taking 50mg propylthiouracil (PTU) twice daily at dosing. The lower limit of normal concentrations for fT4 (■–■) = 3.1 pmol/L, for fT3 (▲–▲) = 12 pmol/L. For TSH (•–•) the upper limit of normal concentrations = 4.2 mIU/l. TSH, thyroid stimulating hormone

screening to 17 mm at the end of the study, and in the left eye from 21 mm at screening to 17 mm at the end of the study. Subject 502 also reported improvement in GO symptoms within 24 h of K1-70™ infusion with fluid bag reduction under the right eye and improved eye focus. On Day 2 after 150 mg IV of K1-70™, subject 603 reported that her eyes felt less puffy and watery as well as an improvement in general aches and joint pains. Her exophthalmos reduced from 24 mm bilaterally predose to 21 mm (left eye) and 20 mm (right eye) on Day 42 of the study. These improvements continued until the end of the study.

TRAb assays by Roche Elecsys® measured K1-70™ plus endogenous patient TRAb and the values increased rapidly following administration of K1-70™. In higher dose cohorts, TRAb levels remained high throughout the study period (Tables 3A and 3B). There were no clear changes from baseline TRAb levels in cohorts 1 and 2. In cohort 3 mean (*SD*) TRAb values were 4.0 (3.3) IU/L at baseline and 25.1 (14.2) IU/L on Day 7, remained similar to 25 IU/L until Day 21 and returned to near baseline by Day 56 (8.5 [4.9] IU/L). In cohorts 4, 5, and 6 mean (*SD*) TRAb levels were above the measuring range of the assay (40 IU/L) on Day 7 and remained above measuring range until Day 42 (cohort 4), Day 70 (cohort 5) or Day 100 (cohort 6). These results are in agreement with the serum concentrations of K1-70™ only which were determined by PK K1-70™ ELISA and shown in Figure 2.

4 | DISCUSSION

Our phase I clinical trial demonstrates K1-70™ to be safe and well tolerated in all subjects at all doses, thus the safety endpoint of the study was met. The risk-benefit ratio was favourable and is expected to remain favourable for later phases of drug development.

No deaths, other SAEs or AEs which were significant were reported. None of the reported TEAEs were deemed as directly related to the study drug and all reported AEs were mild or moderate in nature. In particular, there were no treatment-related TEAEs such as nausea, vomiting, or allergic reactions following either IM or IV K1-70™ administration. Hypothyroidism was the expected PD effect of K1-70™ and was managed by standard care (adjustment of subject ATD dose and levothyroxine). It was not considered an AE.

The commonly reported TEAEs particularly with higher doses of K1-70™ were fatigue and lethargy. These TEAEs were not unexpected as fatigue and lethargy are well-documented symptoms of hypothyroidism.[21]

Physical examinations were found to be normal during the course of the study, no significant injection site reactions were observed and there were no clinically significant abnormal laboratory findings for any subject following the administration of K1-70™ at any of the visits.

Pharmacokinetic analysis following IV administration of K1-70™ demonstrated the expected systemic exposure (which was improved compared to IM administration) and therefore the IV route will be appropriate for subsequent studies. The half life of K1-70™ given IV was about 500 h (as expected for human IgG).[22] Consequently the drug was still detectable at the end of the study in patients receiving 50 mg or 150 mg IV.

No significant immune response to administered K1-70™ was observed in any subject in the study including those receiving IV doses of 50 mg or 150 mg K1-70™. This apparent immune tolerance is likely due to K1-70™ being a fully human antibody.[8]

PD effects were observed after a single dose of K1-70™ with subjects showing progression to the hypothyroid state most prominent by study Day 28 (Figure 3 and Tables 3A and 3B). PD effects were also evident in patient reported symptom improvements for both GD and GO. In particular, clinically significant reductions in exophthalmos measurements (>2mm) were observed in addition to other reported improvements (Table 3C). Many improvements remained after the end of the study.

The effect of K1-70™ on thyroid function in patients with GD may vary depending on: (a) the levels of TRAbs; (b) the relative

TABLE 3A TSH, fT3, fT4, and TRAb levels at baseline and at study Day 28 for cohorts 4, 5, and 6

	Cohort 4 25.0 mg IM *n* = 3	Cohort 5 50.0 mg IV *n* = 3	Cohort 6 150.0 mg IV *n* = 3
TSH (mIU/L)			
Baseline, mean (*SD*)	1.63 (1.40)	0.47 (0.79)	0.34 (0.57)
Median	2.32	0.02	0.01
Min, max	0.02, 2.55	0.01, 1.38	0.01, 1.00
Change from baseline to Day 28			
Mean (*SD*)	2.70 (2.38)	14.95 (16.19)	10.65 (13.83)
Median	2.31	11.98	5.67
Min, max	0.55, 5.25	0.46, 32.42	0.01, 26.30
fT4 (pmol/L)			
Baseline, mean (*SD*)	18.26 (3.29)	14.80 (3.05)	29.40 (13.87)
Median	17.00	13.90	31.50
Min, max	15.80, 22.00	12.30, 18.20	14.60, 42.10
Change from baseline to Day 28			
Mean (SD)	−2.57 (2.25)	−3.97 (6.15)	−21.60 (15.33)
Median	−1.80	−3.80	−27.50
Min, max	−0.80, −5.10	2.10, −10.20	−4.2, −33.10
fT3 (pmol/L)			
Baseline, mean (*SD*)	4.83 (0.49)	4.93 (1.02)	10.30 (6.45)
Median	4.60	4.50	10.20
Min, max	4.50, 5.40	4.20, 6.10	3.90, 16.80
Change from baseline to Day 28			
Mean (*SD*)	−0.53 (0.40)	−1.57 (1.40)	−7.50 (6.38)
Median	−0.30	−1.50	−8.20
Min, max	−0.30, −1.00	−0.20, −3.00	−0.80, −13.50
TRAb (IU/L)			
Baseline, mean (*SD*)[a]	1.55 (1.00)	2.58 (2.54)	9.23 (8.45)
Median	1.24	1.19	9.20
Min, max	0.74, 2.66	1.03, 5.51	0.80, 17.70
Change from baseline to Day 28[b]			
Mean (*SD*)	38.45 (1.00)	37.42 (2.54)	30.77 (8.45)
Median	38.76	38.81	30.80
Min, max	37.34, 39.26	34.49, 38.97	22.30, 39.20

Abbreviations: IM, intramuscular dose; IV, intravenous dose.

[a]At time of dosing, 2 subjects in cohort 4, 2 subjects in cohort 5 and 1 subject in cohort 6 had undetectable endogenous TRAb levels (a further subject in cohort 3 was also TRAb negative).

[b]Due to effect of K1-70™ in the TRAb assay.

TABLE 3B TRAb and TSH levels predose and at Day 28

Subject number	Predose TRAb (IU/L)	TRAb status	TSH (mIU/L)	Day 28 TRAb[a] (IU/L)	TSH[b] (mIU/L)
402	0.74	Negative	0.02	>40	0.57
602	0.80	Negative	0.01	>40	**26.3**
501	1.03	Negative	0.02	>40	**12.0**
503	1.19	Negative	0.01	>40	0.47
301	1.24	Negative	2.00	21.3	3.28
401	1.24	Negative	2.32	>40	**4.63**
411	2.66[c]	Positive	2.63[c]	>40	**7.80**
103	2.89	Positive	0.01	4.4	0.01
311	2.99	Positive	2.62	11.7	2.64
102	3.19	Positive	0.01	2.31	0.23
203	3.71	Positive	3.81	9.95	**14.3**
101	3.86	Positive	0.31	3.12	0.73
502	5.51	Positive	1.38	>40	**33.8**
202	6.64	Positive	0.08	4.99	**48.4**
302	7.69	Positive	0.01	26.9	0.01
601	9.20	Positive	0.01	>40	0.01
201	10.0	Positive	0.01	12.7	0.01
603	17.7	Positive	1.00	>40	**6.67**

[a]TRAb levels at Day 28 are elevated because of the effect K1-70™ in the TRAb assay.

[b]In **bold**, TSH levels above the normal range (0.27–4.2 mIU/L).

[c]Data from screening. Predose data unavailable.

concentrations and activities of blocking and stimulating TRAbs present in patients' sera at the same time; (c) interaction of endogenous TSH with the TSHR in the thyroid and (d) the ability of the thyroid gland affected by autoimmune destruction or inflammation to respond to the stimulating activities of TSH and TRAb.[1,2,8] In our study 6/18 (33.3%) of subjects (all 18 on ATD)

TABLE 3C Patient reported improvements/outcomes

Study Subject	K1-70™ dose	Reported improvements/outcomes	Maximum exophthalmos reduction (mm) RE (Day)	LE (Day)
401	25 mg IM	Improved eyelid closure	1 mm (Day 14)	2 mm (Day 14)
402	25 mg IM	Reduced photosensitivity	2 mm (Day 42)	1 mm (Day 14)
		Reduction in 'gritty eyes' sensation		
		Improved sleep		
501	50 mg IV	Reduced conjunctival redness	5 mm (Day 14)	6 mm (Day 70)
		Reduced gaze-evoked pain		
		Clearer vision		
		1 point CAS improvement (both eyes)[a]		
		Improvement in mental focus		
		Reduced photosensitivity (still ongoing at end of study)		
		Reduced toilet urgency		
		Improvement in aches & pains		
		Reduction in "gritty eyes" sensation		
		Symptoms improved enough to seek employment again		
502	50 mg IV	Reduction in fluid under eye	8 mm (Day 70)	4 mm (Day 70)
		Improvement in vision focus		
601	150 mg IV	Improvement in stool consistency	No change	
		Improvement in appetite		
		Improvement in hand tremor		
603	150 mg IV	Improvement in eye puffiness	4 mm (Day 42)	4 mm (Day 70)
		Reduced watering of eyes		
		Improvement in aches & pains (particularly joints)		

Note: The maximum exophthalmos reduction is the difference (in mm) between the largest and smallest exophthalmos measurement observed during the course of the study.

Abbreviations: IM, intramuscular dose; IV, intravenous dose; LE, left eye; RE, right eye.

[a]The change in CAS score of one point was not considered clinically significant.

had undetectable predose TRAb levels, and might have been in remission. Statistical analysis showed weak correlations between predose endogenous TRAb levels and changes in TSH from baseline at all study timepoints (rs range: −0.338–0.152). Moderate correlations between predose TRAb levels and changes in fT4 from baseline were observed 2 h postdose (rs = 0.546), and on Day 100 (rs = 0.444). The correlation between predose TRAb levels and changes in fT3 from baseline was moderate at Day 100 (rs = 0.470). No other trends between predose TRAb levels and PD effects were observed.

When TSH levels at Day 28 were compared with predose TRAb positivity or negativity, 3/6 TRAb negative and 5/12 TRAb positive subjects had TSH values in the hypothyroid range (Table 3B). Consequently in this study with a small number of patients the effect of K1-70 on TSH levels at Day 28 did not appear to differ between TRAb negative and TRAb positive (at dosing) subjects.

Also K1-70™ has been administered safely and effectively to a female US patient with follicular thyroid cancer complicated by GD, high levels of TRAb and severe GO in a single-patient expanded access study.[23] K1-70™ had a markedly beneficial effect on her GO and multiple doses (ranging from 17 to 120 mg IM or IV over 27 months) did not result in her having any drug related AEs or SAEs. Furthermore she had no detectable ADA response.

Several antibody drugs have been trialled for GD/GO therapy,[3] but none (except K1-70™) target the TSHR directly. Teprotumumab, a fully human monoclonal insulin-like growth factor 1 (IGF1) receptor inhibitory antibody[24] blocks signalling through the IGF1 receptor complex thus acting as an antiinflammatory agent. In 2020, teprotumumab was licensed by the US FDA for exclusive use in the treatment of GO.[25] However, relatively large doses of teprotumumab are needed to be effective (in the region of 10 g per patient per 6 month course) and commonly reported side effects include nausea,

diarrhoea, muscle spasms, dry skin, infusion reactions and hypoglycaemia.[26,27] Also ototoxicity leading to permanent hearing impairment has been reported[28–30] as has rapid progressive cognitive decline (resolved following plasmapheresis).[31]

In contrast to teprotumumab and other antibody drugs for GD and/or GO therapy,[3] K1-70™ targets the TSHR directly.[2,8] Specific high affinity binding of K1-70™ to the receptor blocks binding by patient TRAb thus preventing them from stimulating the receptor.[8,13,14]

In our study on patients stable on ATD treatment, the impact of K1-70™ administration on their TSH, fT4, and fT3 levels confirm the drug acts effectively on TSHRs in patients' thyroids. Progression from hyperthyroidism or euthyroidism (at the time of dosing) to the hypothyroid state occurred in 12/18 (67%) of subjects, while in higher dose cohorts 9/9 subjects (100%) experienced hypothyroidism irrespective of their thyroid status at dosing. Also the beneficial effects of K1-70™ on patients' eye signs supports the suggestion[23] of a key role for TRAb stimulation of orbital TSHRs in GO.

Our phase one study results are preliminary and must be extended in further clinical trials which should include GO Patient Reported Outcomes using a suitable Quality of Life questionnaire such as GO-QoL, and Clinician Reported Outcomes for parameters such as eyelid aperture, exophthalmos measurements, extraocular muscle function, visual function and soft tissue changes as recently proposed.[32] Furthermore, future clinical trials should be designed to explore repeat dosing of K1-70™ in individual patients and assessment of efficacy compared to current standard care. However, the clear PK/PD relationship we observed with single doses of K1-70™ (25 mg IM, 50 mg IV, and 150 mg IV), the beneficial effects on GO and minimal AEs are promising findings. They suggest that K1-70™ has considerable potential as a new, safe and effective TSH receptor specific drug.

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CONFLICT OF INTERESTS

Jadwiga Furmaniak, Jane Sanders, and Bernard Rees Smith are Directors of AV7 Limited. Jadwiga Furmaniak, Jane Sanders, Paul Sanders, Yang Li, and Bernard Rees Smith are employees of RSR Ltd. which is a manufacturer of diagnostic kits to measure autoantibodies including autoantibodies to the TSH receptor.

AUTHOR CONTRIBUTIONS

Study design: Jadwiga Furmaniak, Jane Sanders, Paul Sanders, and Bernard Rees Smith. *Compilation and analysis of data*: Jadwiga Furmaniak, Jane Sanders, Paul Sanders, and Yang Li. All authors contributed to interpretation of the results. *Manuscript writing*: Paul Sanders, Jadwiga Furmaniak, and Bernard Rees Smith who had unrestricted access to the data. *Data validation*: Jadwiga Furmaniak, Jane Sanders, Paul Sanders, and Yang Li. The article was reviewed and approved by all authors. All authors made the decision to submit the manuscript for publication and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. All authors accessed all the data reported in the study.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Rees Smith B, McLachlan SM, Furmaniak J. Autoantibodies to the thyrotropin receptor. *Endocr Rev*. 1988;9(1):106-121.
2. Furmaniak J, Sanders J, Núñez Miguel R, et al. Mechanisms of Action of TSHR Autoantibodies. *Horm Metab Res*. 2015;47(10):735-752.
3. Lane LC, Cheetham TD, Perros P, Pearce SHS. New therapeutic horizons for Graves' hyperthyroidism. *Endocr Rev*. 2020;41:873-884.
4. Nalla P, Young S, Sanders J, et al. Thyrotrophin receptor antibody concentration and activity, several years after treatment for Graves' disease. *Clin Endocrinol*. 2019;90:369-374.
5. Bandai S, Okamura K, Fujikawa M, Sato K, Ikenoue H, Kitazono T. The long-term follow-up of patients with thionamide-treated Graves' hyperthyroidism. *Endocr J*. 2019;66:535-545.
6. Bartalena L, Baldeschi L, Boboridis K, et al. The 2016 European Thyroid Association/European Group on Graves' orbitopathy guidelines for the management of Graves' orbitopathy. *Eur Thyroid J*. 2016;(5):9-26.
7. Genere N, Stan MN. Current and emerging treatment strategies for Graves' orbitopathy. *Drugs*. 2019;79:109-124.
8. Evans M, Sanders J, Tagami T, et al. Monoclonal autoantibodies to the TSH receptor, one with stimulating activity and one with blocking activity, obtained from the same blood sample: monoclonal autoantibodies to the TSH receptor. *Clin Endocrinol*. 2010;73:404-412.
9. Stan MN, Salvi M. Rituximab therapy for Graves' orbitopathy—lessons from randomized control trials. *Eur J Endocrinol*. 2017;176:R101-109.
10. Venkiteshwaran A. Tocilizumab. *Mabs*. 2009;1:432-438.
11. Winn BJ, Kersten RC. Teprotumumab—interpreting the clinical trials in the context of thyroid eye disease pathogenesis and current therapies. *Ophthalmology*. 2021:28. doi:10.1016/j.ophtha.2021.04.024
12. Pearce SHS, Dayan C, Wraith DC, et al. Antigen-specific immunotherapy with thyrotropin receptor peptides in Graves' hyperthyroidism: a phase I study. *Thyroid*. 2019;29:1003-1011.
13. Furmaniak J, Sanders J, Young S, et al. In vivo effects of a human thyroid-stimulating monoclonal autoantibody (M22) and a human thyroid-blocking autoantibody (K1-70). *Autoimmun Highlights*. 2012;3:19-25.
14. Furmaniak J, Sanders J, Clark J, et al. Preclinical studies on the toxicology, pharmacokinetics and safety of K1-70™ a human monoclonal autoantibody to the TSH receptor with TSH antagonist activity. *Autoimmun Highlights*. 2019;10:11.
15. WMA-The World Medical Association-WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects.

https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects. accessed 12 Aug 2021.
16. ICH E6 (R2) Good clinical practice. European Medicines Agency 2018. https://www.ema.europa.eu/en/ich-e6-r2-good-clinical-practice. accessed 12 Aug 2021.
17. AV7 Limited. K1-70 - A Phase I, Single Ascending Intramuscular Dose or Single Ascending Intravenous Dose, Safety, Tolerability, Pharmacokinetic and Pharmacodynamic Study in Subjects With Graves' Disease. Report No.: NCT02904330. https://clinicaltrials.gov/ct2/show/NCT02904330. accessed 10 Aug 2021.
18. Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. U.S. Food and Drug Administration https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers. accessed 28 Jul 2021.
19. RSR Limited In Vitro Diagnostics. Manufacturer of Medical Diagnostic Devices, Reagents & Kits for the Test and Diagnosis of Autoimmune Diseases. http://www.rsrltd.com accessed 22 Jul 2021
20. https://www.regulations.gov/docket/FDA-2009-D-0539. accessed 25 Aug 2021. 'Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection'.
21. Hypothyroidism. British Thyroid Foundation. https://www.btf-thyroid.org/hypothyroidism-leaflet. accessed 12 Aug 2021.
22. Kang TH, Jung ST. Boosting therapeutic potency of antibodies by taming Fc domain functions. *Exp Mol Med*. 2019;51:138.
23. Ryder M, Wentworth M, Algeciras-Schimnich A, et al. Blocking the thyrotropin receptor with K1-70 in a patient with follicular thyroid cancer, Graves' disease, and Graves' ophthalmopathy. *Thyroid*. 2021;31(10):1597-1602. doi:10.1089/thy.2021.0053
24. Chen H, Mester T, Raychaudhuri N, et al. Teprotumumab, an IGF-1R blocking monoclonal antibody inhibits TSH and IGF-1 action in fibroblasts. *J Clin Endocrinol Metab*. 2014;99(9):E1635-E1640.
25. FDA Approves TEPEZZATM (teprotumumab-trbw) for the Treatment of Thyroid Eye Disease (TED). Horizon Therapeutics plc. https://ir.horizontherapeutics.com/news-releases/news-release-details/fda-approves-tepezzatm-teprotumumab-trbw-treatment-thyroid-eye. accessed 2 Jul 2021.
26. Smith TJ, Kahaly GJ, Ezra DG, et al. Teprotumumab for thyroid-associated ophthalmopathy. *N Engl J Med*. 2017;376:1748-1761.
27. Bednarczuk T, Pearce SH. The knowns and unknowns of teprotumumab for thyroid eye disease. *Lancet Diabetes & Endocrinol*. 2021;9:323-325.
28. Belinsky I, Creighton FX, Mahoney N, et al. Teprotumumab and hearing loss: Case series and proposal for audiologic monitoring. *Opthalmic Plast Reconstr Surg*. 2021;38(1):73-78. doi:10.1097/IOP.0000000000001995
29. Chern A, Glass LRD, Gudis DA. Thyroid eye disease, teprotumumab, and hearing loss: An evolving role for otolarnyngologists. *Otolaryngol Head Neck Surg*. 2021;165(6):757-758. doi:10.1177/01945998211004240
30. Highland J, Gordon S, Reddy D, et al. Ototoxicity and teprotumumab. *Ann Otol Rhinol Laryngol*. 2021. online ahead of print. doi:10.1177/00034894211042740
31. Hoang TD, Nguyen NT, Chou E, et al. Rapidly progressive cognitive decline associated with teprotumumab in thyroid eye disease. *BMJ Case Rep*. 2021;14:e242153.
32. Bartalena L, Wiersinga WM. Proposal for standardization of primary and secondary outcomes in patients with active, moderate-to-severe Graves' orbitopathy. *Eur Thyroid J*. 2020;9:3-16.

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