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Akt Activation With IPL344 Treatment for Amyotrophic Lateral Sclerosis: First in Human, Open-Label Study

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ABSTRACT

Introduction/Aims: Akt intracellular signal transduction pathway dysfunction has been reported in people with amyotrophic lateral sclerosis (ALS) providing a novel target for intervention in this devastating progressive disease. This first-in-human study evaluated the safety, tolerability, and preliminary efficacy of the Akt pathway activator, IPL344, in people with ALS.

Methods: Nine participants with ALS and a progression rate >0.55 points/month on the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) received open-label IPL344 treatment (once-daily) for up to 36 months. Safety was assessed through adverse event (AE) reporting. Plasma neurofilament light chain (NfL) concentrations were measured before and after treatment. Clinical outcomes were compared to historical data.

Results: The mean $\pm$ SD duration of IPL344 follow-up was 14.0 ± 12.5 months. One participant developed drug hypersensitivity, two had central venous catheter-related AEs, and two had serious pneumonia AEs. The unadjusted mean $\pm$ SE slope of decline in ALSFRS-R was -0.53 ± 0.15 (48% slower progression vs. historical controls, $p = 0.028$). Adjustment for disease stage and rate-indicating covariates indicated a 64% slower ALSFRS-R progression ($p = 0.034$), with increased rather than reduced body weight ($p = 0.02$). Eight of nine IPL344-treated participants had a significantly improved slope compared to the median slope of a matched control group ($p = 0.04$). Plasma NfL concentrations were lowered by 27% ($n = 6$). Unadjusted median survival for participants in the IPL344 group was 43.4 months [95% CI: 20.5, NA] compared with 19.1 months [17.4, 23.0] in the historical control group.

Discussion: These preliminary data indicate that IPL344 was safe and well-tolerated, and possibly effective. Our findings may merit further investigation in a larger placebo-controlled clinical trial.

Abbreviations: AE, adverse event; ALS, amyotrophic lateral sclerosis; ALSFRS-R, Amyotrophic Lateral Sclerosis Functional Rating Scale; CVC, central venous catheter; FRS, functional rating scale; FVC, forced vital capacity; ITT, intent to treat; NfL, neurofilament light chain; PAV, permanent assisted ventilation; SAE, serious adverse event; SVC, slow vital capacity.

Part of this work was presented as poster at the 2022 Annual NEALS meeting and virtual 33rd international symposium on ALS/MND December 2022.

1 | Introduction

While the etiology of amyotrophic lateral sclerosis (ALS) is not fully understood, accumulating evidence has indicated that deficits in the DNA damage response (DDR), which is closely related to RNA preprocessing and nuclear TAR DNA binding protein 43 (TDP-43) loss of function, have a major role in ALS [1]. The DDR has also been shown to directly activate the pro-survival Akt intracellular signal transduction pathway [2], which plays a vital role in maintaining cellular homeostasis and is of critical importance with regard to protein-life cycle signaling and cell metabolism [3–5]. Akt pathway dysfunction is common to many age-related neurodegenerative diseases [6, 7], and studies have shown it is downregulated in motor neurons and skeletal muscles in animal models and in post-mortem samples from people with ALS [8–10]. In preclinical models, down-regulation of Akt is an early feature, observed even at presymptomatic stages [11–13]. Other studies in human ALS have shown that higher Akt levels are associated with slower disease progression and better overall survival [14, 15].

IPL344 is a soluble, proline-rich heptapeptide (H-Leu-Pro-Pro-Leu-Pro-Tyr-Pro-OH) that was selected for mimicking damaged DNA and activates the Akt pathway [16]. In preclinical studies, IPL344 exhibits potent anti-apoptotic activities, both in vitro in cell culture and in vivo in a mouse model of lethal irradiation [16]. Treatment of *SOD1G93A* transgenic mice by daily bolus injections after symptom onset slowed the progression of the disease and extended survival [17]. The aim of this first-in-human study was to evaluate the safety, tolerability, and preliminary efficacy of IPL344 in people with ALS treated for up to 36 months. Consistent with its proof-of-concept design, we included several methods of clinical assessment and statistical analysis to guide the selection of potential endpoints for future clinical trials.

2 | Methods

2.1 | Study Conduct

This 28-day study with a long-term safety extension was conducted from August 1, 2018 to August 1, 2022 at a single center (Neuromuscular unit, Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, Israel). The Israeli Ministry of Health approved the study. Ethics approval was given by the Hadassah Medical Center Ethical Committee, and the trial was executed in accordance with the declaration of Helsinki and International Conference on Harmonization Good Clinical Practice Guidelines. Site medical staff were trained and certified in outcomes assessment by the Barrow Neurological Institute Clinical Research Organization (BNI CRO). Both phases of the study were registered at ClinicalTrials.gov (28-day dose escalation phase: NCT03652805; extension phase: NCT03755167). Data from an early additional participant treated under a similarly designed compassionate use protocol (December 2014 to January 2017) are also included. All participants provided written informed consent before entering the study.

2.2 | Study Population

Full details of the inclusion and exclusion criteria of the 28-day study with long-term safety extension and other key protocol details are given in the Supporting Information. In brief, adult men or women (aged 18–80 years) were eligible if they fulfilled criteria for clinically probable or definite ALS [18], with a recent (within past 6 weeks) Amyotrophic Lateral Sclerosis Functional Rating Scale [19] (ALSFRS-R) score > 20 *and* a documented disease progression rate averaging > 0.55 ALSFRS-R points/month over ≥ 4 months prior to the latest ALSFRS-R test. Eligible participants weighed between 50 and 100 kg and had a BMI of 18.5–31 kg/m^2. In addition, they had a documented forced vital capacity (FVC) or slow vital capacity (SVC) ≥ 60% of predicted and were medically able to undergo placement and maintenance of a central venous catheter (CVC). Those taking riluzole had to be on a stable dose for ≥ 30 days prior to the first IPL344 treatment (Day 1 of the study) and were expected to remain at that dose until the final study visit. No participant was taking either edaravone or other ALS-specific medications.

Key exclusion criteria included the permanent use of invasive or noninvasive ventilation, or any concurrent therapy, condition, or circumstance that, in the judgment of the investigator, might contraindicate or increase the risk to the participant or decrease the chance of obtaining satisfactory data to achieve the objectives of the study. The participant treated under a compassionate use protocol had ALSFRS-R scores and respiratory volume below the inclusion criteria (15 rather than > 20 ALSFRS-R and 43% rather than ≥ 60% SVC) but complied with all other criteria.

2.3 | Determination of Dosing

Preclinical pharmacokinetic studies in rats and dogs showed the drug is detectable in plasma for less than an hour after injection (data not shown) thereby initially (at the time of study) precluding any formal pharmacokinetic determination of dose. The dose for the first participants in the clinical study was therefore estimated based on experience with preclinical mouse models [17] and the participant treated under a compassionate-use protocol. This dose was increased after seven participants to explore a higher dose, but study recruitment was discontinued due to the COVID-19 pandemic after introducing a single participant at a higher dose.

2.4 | Study Design

The participant treated under a compassionate use protocol received once-daily infusions of IPL344 via a peripheral IV catheter, which was initiated at 0.88 mg/kg, titrated to 2.6 mg/kg by Week 6, and increased to 3.3 mg/kg at Month 4 and 4.13 mg/kg at Month 11. Participants enrolled in the formal study received once-daily infusions of IPL344 via a CVC using a syringe pump (infusion rate 350 mL/h). On Day 1, treatment with IPL344 was initiated in the clinic at 1.7 mg/kg for the first 7 participants and was escalated (increments of 0.5 mg/kg every 3–4 days starting Day 4) based on tolerability to a maximal dose of 3.2 mg/kg (see Supporting Information for

protocol). The eighth participant was initiated at 2.7 mg/kg (maximal dose of 4.5 mg/kg). For safety, dose escalations were performed in the medical center; otherwise, drug administration was performed daily at home during the first 28 days by a nurse who also trained the caregivers to administer treatment during the extension phase. During the extension treatment, participants were monitored at home by a nurse every 2–3 weeks between the study visits held according to the protocol (see Supporting Information).

All participants completed a baseline visit (up to 10 days prior to first dose) and were followed for outcomes and clinical care every 1–4 months. Dates of tracheostomy, permanent assisted ventilation, and death were collected after study termination.

2.5 | Study Assessments

The primary safety endpoint was the frequency and severity of adverse events (AEs), including clinically significant abnormalities after study drug administration based on vital signs, 12-lead electrocardiograms, physical examinations, clinical laboratory values, and evaluation of complications at the injection site. AEs considered to be related to underlying disease progression were not included in the safety reporting. Study visits were at dose escalation in the 28-day dose escalation phase, monthly until Month 6, and then every 2 or 3 months thereafter. After study termination, participants were followed up for survival, PAV, and tracheostomy.

Clinical outcomes included the rate of progression on ALSFRS-R and SVC, weight, and survival. Participant blood samples were collected before and at different time points following IPL344 treatment, and the separated serum and plasma components were stored immediately at −80°C. Plasma neurofilament light chain (NfL) concentrations were evaluated using a Simoa HD-1 Analyzer (Simoa Accelerator Laboratory, Quanterix, Lexington, MA) with an analytical limit of detection of 0.038 pg/mL.

2.6 | Statistical Analyses and Natural History Control

We recognize the exploratory nature of any efficacy analysis on small datasets and, with this in mind, we used four analytic approaches suitable for small studies to analyze the rate of progression on ALSFRS-R versus historical controls [20] with the goal of maximizing information for future work. There was no sample size calculation for this first in human study, and our statistical plan included all available data until September 2023.

1. Rates of ALSFRS-R change were first analyzed without correction for any covariates and compared against published historical control data [20] obtained from the open access Pooled Resource Open-Access ALS Clinical Trials (PRO-ACT) Database [21]. This published control data is a set of 16 historical studies that were selected for a study duration of ≥ 6 months. This method provides 95% probability limits for a new ALS treatment study as a function of the standard error and the treatment effects of that study, accounting for the observed study-toto-study variability [20].
2. The slope of ALSFRS-R was also compared to the entire PRO-ACT database using a linear random effects model with an intercept and slope variable for each covariate, with correction for common covariates that are prognostic and commonly used in ALS prediction methods [22, 23] (baseline scores, bulbar onset and ΔFRS [defined as baseline ALSFRS-R-48, divided by months from symptom onset to baseline]). An adjusted slope was calculated from this model by predicting the average ALSFRS-R slope that the patients in the PRO-ACT database would have had, had they been treated with IPL344 and similarly, had they been treated with placebo [24].
3. All nine of the IPL344 treated individuals were matched with patients from the PRO-ACT database using slope and disease severity. Matched groups included all records with at least two ALSFRS-R observations (including baseline) that had a similar baseline ALSFRS-R score (≤ 3 point difference vs. the matched patient) and also had a ΔFRS slope (from onset to baseline) within 20% limits. In the absence of lead-in data for PRO-ACT controls, the ΔFRS approximates the main inclusion criterion of a pretreatment slope > 0.55. As ΔFRS and baseline ALSFRS-R were both matched, the duration from onset was approximately matched. Data points after month 15 were excluded from the analysis (study and control) to avoid any influence from patients with an atypically long follow-up duration. On average, each IPL344 treated participant was matched with 116 PRO-ACT participants. If there were fewer than 10 matches based on the primary criteria, additional matches were sought (by using the second datapoint available for each patient in the control group as the baseline value for ALSFRS-R, and then the third one and so on), until at least 10 matches were found. This was done for patients 0 and 6. Once matched, linear regression analysis was performed to find the slope of the ALSFRS-R scores for each patient.
4. The ALSFRS-R slope was also compared for each participant before and after treatment to determine if there is an improvement after treatment began. For this analysis, pretreatment ALSFRS-R slopes were measured using data from medical records for durations of about 6 months. In a single case, when treatment started as soon as 8 months after onset, the ΔFRS was used. We used a random effects model assuming each participant has a random slope and intercept at the start of their observation and a random offset of this slope during treatment. The treatment effect was defined as the mean value of the offset in slopes from the observed ALSFRS-R rate of progression compared to the prediction from pre-treatment values assuming linear progression.

Rates of change in weight (assessed using a chair scale) and SVC were analyzed and compared against the PRO-ACT database in a similar manner to the ALSFRS-R, adjusting for ΔFRS and bulbar onset. The patients from PRO-ACT included in each one of those comparisons were all those recorded in PRO-ACT having at least one datapoint for the specific

endpoint and all the covariates included in the model, thus bulbar/nonbulbar designation, ALSFRS-R at first treatment, and date of onset. Changes in NfL plasma levels were evaluated descriptively.

Since the studies contained in the PRO-ACT database rarely included sufficiently long post-treatment follow-up, survival of the participants during treatment and posttreatment follow-up was analyzed using a Cox model (robust variance [25]) and compared against the placebo group of a single placebo-controlled ceftriaxone study [26] that uniquely included a long duration of both treatment and follow-up such that the control data could be used as a benchmark for our study. Analysis incorporated covariates (bulbar onset, El-Escorial criteria, ΔFRS, and baseline ALSFRS-R scores) measured in both studies. In all analyses, tracheostomy and permanent assisted ventilation [PAV] were considered death events. In one participant, the tracheostomy that occurred 30 months after IPL344 treatment termination was done proactively together with the installation of PEG (to avoid future further surgery). PAV for that participant was not used until data lock (15 more months) and was not considered a death event. All analyses were conducted as intended to treat (ITT), regardless of treatment length.

3 | Results

3.1 | Participant Disposition and Baseline Characteristics

Nine participants (five males, four females), including the participant treated under a compassionate use protocol, completed dose escalation, and the mean ± SD (median) duration of IPL344 treatment was 14.0 ± 12.5 (9.7) months. One participant had a tracheostomy involving PAV during the study and continued to receive the drug afterward (participant request). No other participants received PAV during IPL344 treatment in this study. The post-tracheostomy data were only considered for the safety assessment. The main reasons for study discontinuation were withdrawal of consent ($n = 4$) due to perceived lack of efficacy, preference to spend more time with family, travel difficulties during the COVID-19 pandemic, and enrollment in another study.

Baseline characteristics for the nine participants are presented in Table 1 and compared with unmatched participant level data from the PRO-ACT database of patients treated with placebo who were selected for each one of the comparisons for

TABLE 1 | Baseline characteristics.

	ALSFRS-R analysis			SVC analysis		Survival analysis	
	IPL344 ($N = 9$)	**PRO-ACT placebo ($N = 2380$)**	***p***	**PRO-ACT placebo ($N = 460$)**	***p***	**Ceftriaxone placebo ($N = 173$)**	***p***
Age (years)	58.5 (12.6)	$n = 1654$ 56.1 (11.5)	0.300	$n = 460$ 57.3 (11.2)	0.460	54.9 (10.3)	0.217
Sex, (female), *n* (%)	4 (44%)	$n = 2380$ 900 (37.8%)	—	$n = 460$ 166 (36%)		72 (42%)	—
Weight (kg)	71.5 (14.4)	$n = 2122$ 79.7 (21.2)	0.259	$n = 457$ 77.8 (16.0)	0.373	78.9 (16.1)	0.306
Months from onset	23.2 (13.2)	$n = 2380$ 18.1 (9.7)	0.150	$n = 460$ 14.9 (5.2)	0.084	18.7 (8.1)	0.178
Time from onset to diagnosis (months)	7.3 (4.8)	$n = 2264$ 10.3 (7.2)	0.117	$n = 460$ 8.0 (4.6)	0.917	11.0 (6.9)	0.060
ALSFRS-R total score	30.1 (7.6)	$n = 2380$ 37.6 (5.5)	0.024	$n = 460$ 38.1 (5.6)	0.020	36.9 (5.4)	0.035
ΔFRS (points/month)	−0.98 (0.57)	$n = 2380$ −0.71 (0.53)	0.212	$n = 460$ −0.74 (0.53)	0.403	−0.70 (0.52)	0.200
FVC%	72% (18%)	$n = 1186$ 83% (16%)	0.106	NR	NR	88% (17%)	0.037
SVC%	74% (20%)	$n = 451$ 91% (16%)	0.041	$n = 451$ 91% (16%)	0.082	NR	—
Bulbar onset, *n* (%)	2 (22%)	$n = 2380$ 503 (21.1%)	0.180	—	—	35 (20.3%)	0.180
Using riluzole, *n* (%)	8 (89%)	NR	—			NR	—

Note: Data are mean (SD) unless otherwise indicated; *p* values are for IPL344 versus the relevant placebo group.
Abbreviations: ALSFRS-R, Revised Amyotrophic Lateral Sclerosis Functional Rating Scale; FVC, forced vital capacity; SVC, slow vital capacity.

having the minimally required data ($n = 2380$ for ALSFRS-R assessment, $n = 460$ for SVC assessment, and $n = 2165$ for weight assessment). The ceftriaxone study placebo included 173 participants and is also shown. Compared with the unmatched historical control groups, study participants had similar age, sex, weight, and bulbar onset, but more advanced disease (as evidenced by ALSFRS-R total scores and %SVC/FVC).

3.2 | Safety

The early participant treated under compassionate use developed drug hypersensitivity (rash and tachycardia) after 13.5 months of IPL344 treatment (last 3 months at a dose of 4.1 mg/kg) that was successfully treated by a desensitization process (replacing bolus infusion with drop-by-drop administration at an accelerated rate and increasing quantities), and treatment continued at a reduced dose of 2.37 mg/kg for an additional 12 months without further issue.

No other drug-related serious adverse events (SAEs) were reported, and no participants discontinued treatment due to drug-related AEs. Two participants reported CVC-related AEs (local itching, secretion, and vasovagal syndrome) that recovered spontaneously or were locally treated with betamethasone. One participant died 1.5 months after enrollment due to pneumonia, considered not related to the study drug. This participant had an SVC > 60% and an oxygen saturation of 94% during screening but lost 15% of SVC by baseline and had 85% oxygen saturation upon first treatment, which heralded his severe respiratory decline and poor clinical status before treatment initiation. Another participant, after being treated with IPL344 for 10 months, was hospitalized due to pneumonia and later atelectasis, both SAEs considered not related to the study treatment. This participant had a tracheostomy and continued with IPL344 treatment until Month 36.

3.3 | Effects on Disease Progression as Assessed by the ALSFRS-R

In the unadjusted model, compared to the published 16 studies control set, study participants showed a mean ± SE slope of decline in ALSFRS-R of -0.53 ± 0.15, which is outside of the 95% probability limits calculated from the 16 placebo PRO-ACT studies [20] (Figure 1a,b) and suggests slower progression for participants treated with IPL344 compared to the historical controls. ALSFRS-R declined in the treated study participants 48% more slowly compared with historical controls from the PRO-ACT database (two-sided p value of 0.03).

In the adjusted model, (including all participants with covariates for ALSFRS-R at baseline, bulbar onset and ΔFRS) mean [95% CI] adjusted slopes were − 0.39 [−0.96; 0.18] for IPL344 versus −1.09 [1.12; −1.06] for the natural history control group. This equates to a 64% slower progression of ALSFRS-R ($p = 0.03$).

Table 2 shows the results of the matched analysis (comparing each participant's slope to the median slope of a PRO-ACT matched group). Each placebo participant is ranked among all the matched participants based on the slope of the ALSFRS-R, with smaller declines in ALSFRS-R ranked higher than larger declines. This rank helps to understand the relative change of ALSFRS-R scores for each IPL344 treated individual compared to the individual participants in his or her matched control group. Only one IPL344 treated participant had a slope worse than the median slope for the matched group. This would occur by chance in 0.02 of the cases, equivalent to a two-sided p value of 0.04. The median slope difference between each participant and their matched PRO-ACT control group equates to a 58% reduction.

Compared to participants own pretreatment ALSFRS-R data, using the random effects model, and assuming each participant has a random slope and intercept at the start of their observation and a random offset of this slope during treatment and linear progression, the pretreatment slope was -1.13 ± 0.18 compared to the posttreatment slope of -0.37 ± 0.12, equating to a 67% slower progression of ALSFRS-R with the caveat that this assessment is purely descriptive (Figure 2).

3.4 | Effects on Other Outcomes Versus the PRO-ACT Placebo Group

When adjusted for ΔFRS and bulbar onset, IPL344 treated participants had an adjusted mean SVC change of −1.6% per month compared to −2.8% per month in the historical placebo group; however, this difference was not statistically significant ($p = 0.15$). ALSFRS-R total respiratory function subscores remained unchanged in all but one participant during the entire treatment period as measured prior to death/tracheostomy. As described above, the exception was the single participant whose accelerated respiratory deterioration started between screening and study initiation and who died within 1.5 months of enrollment. All three participants who were free of noninvasive ventilation (NIV) assistance at SVC < 60% during treatment remained free of NIV for at least a further 25 months (data not presented).

On average, analysis of adjusted slopes, corrected for ΔFRS and bulbar onset as covariates, indicated that participants treated with IPL344 gained weight (+0.47 kg/month), while those in the historical PRO-ACT placebo control lost weight (−0.39 kg/month); the difference was statistically significant ($p = 0.02$) (Figure 3).

3.5 | NfL Plasma Level

Of the eight participants with available blood samples, NfL concentrations were reduced during IPL344 treatment in all but one participant (Figure 4, Table S1). The single participant who showed an increase in NfL concentration had (as described above) low oxygen saturation upon treatment initiation and died within 1.5 months. Six participants had blood sampling past the initial 28-day dose-escalating study. In these participants, plasma NfL concentrations were reduced from an average of 79.7 pg/mL at baseline to 58.6 pg/mL following treatment; a mean reduction of 27%.

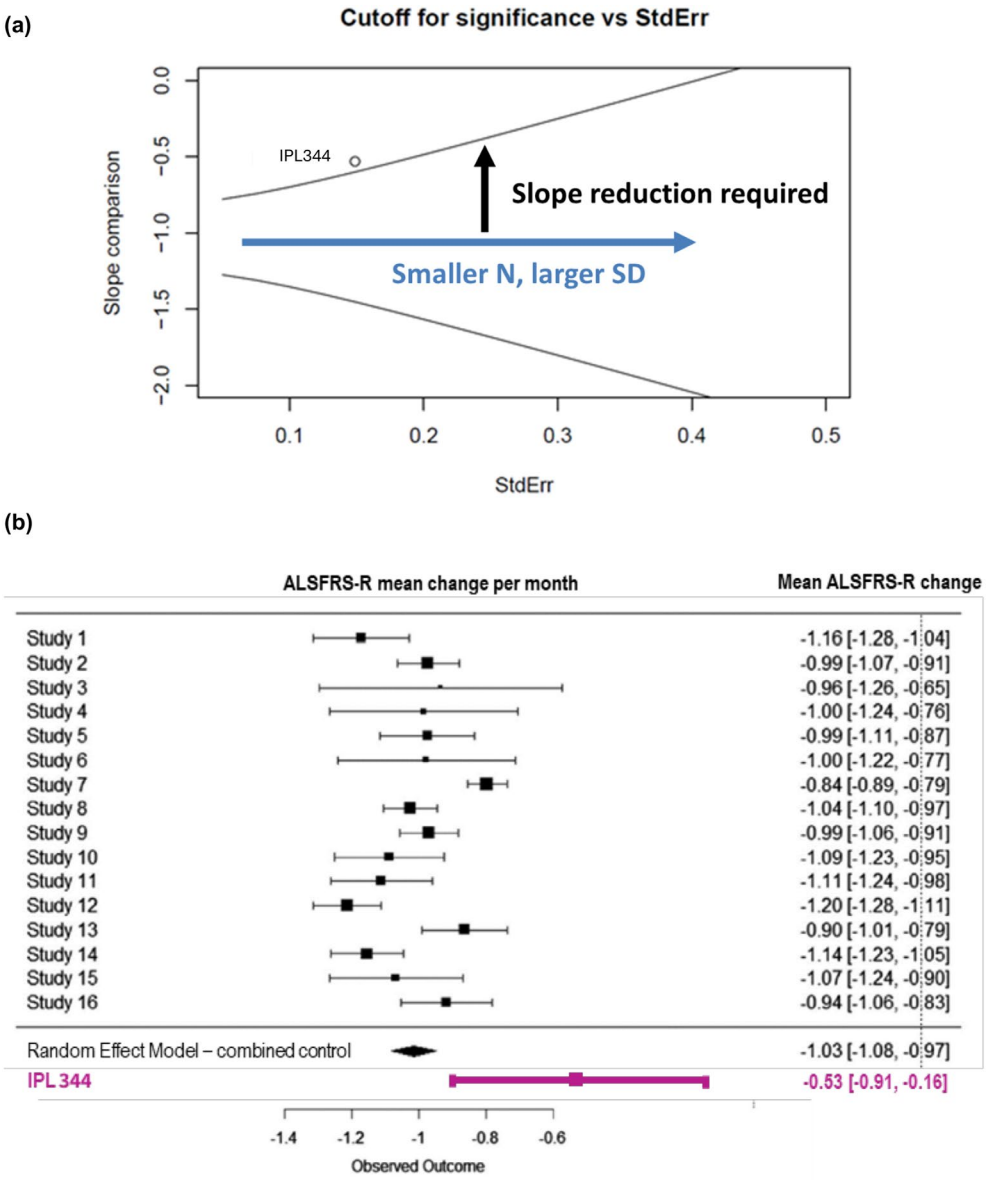


FIGURE 1 | Analysis of ALSFRS-R slopes for IPL344 treated participants with ALS versus historical control of 16 pre-selected studies [27] from the PRO-ACT database (a) unadjusted analysis of IPL344 study significance using 95% tolerance limits. (b) Unadjusted ALSFRS-R slope during treatment compared to all 16 individual control studies. (a) 16 studies were previously analyzed to determine the average rate of change in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) and the variation in the average rate of change from study to study. This analysis was used to generate 95% tolerance limits for a new study as a function of the standard error of the treatment effect for that study. The ALSFRS-R slope in the IPL344 study was above these tolerance limits, which implies significant superiority versus historical controls, accounting for study-toto-study variability in the mean slope of ALSFRS-R [27]. (b) Also, following the methods and charts described by Schoenfeld et al. [27], the IPL344 study is compared to the Forest Plot of the 16 control groups, presenting the mean and error bars for the mean change in ALSFRS-R per month for each one of the studies and the combined data.

3.6 | Mortality Analysis Compared to Ceftriaxone Study

Despite the later disease stage and tendency for faster disease progression, unadjusted median [95% CI] survival for participants in the IPL344 group was 43.4 months [20.5, NA] compared with 19.1 months [17.4, 23] in the placebo group of the ceftriaxone study [26]. Figure 5 shows the Kaplan–Meier curve for the IPL-344 study compared to survival in the placebo group of the ceftriaxone study. Analysis using a Cox model, incorporating disease stage and rate-indicating covariates measured in both studies, found a nonsignificant hazard ratio of 0.43 [95% CI, 0.14, 1.35] versus placebo ($p = 0.15$).

4 | Discussion

Results from this first-in-human study demonstrate that treatment with IPL344 was generally safe. While no conclusions of efficacy can be made from this small study, the reduced slope of decline of ALSFRS-R versus historical controls and other clinical outcomes sets expectations for future clinical trials.

TABLE 2 | IPL344-treated participants compared with matched controls.

Participant	Control	Baseline ΔFRS (points/month)		Baseline ALSFRS-R		Follow-up duration		Analysis Reduction in ALSFRS-R per month			
#	*N*	IPL344	Matched control[a]	IPL344	Matched control[a]	IPL344	Matched control median	IPL344	Matched control[a]	% slope reduction vs. matched control	Rank of IPL344 treated vs. control
0	10	−1.33	−1.3	16	18	13.1	8.0	0.01	0.5	98.0%	60%
1	53	−0.67	−0.66	27	27	1.1	10.1	−0.07	0.82	108.5%	96%
2	80	−1.56	−1.49	29	29	1.7	9.2	2.97	1.55	−91.6%	18%
3	42	−2.11	−1.96	31	31	9.7	8.7	0.81	1.91	57.6%	95%
4	248	−1	−0.95	35	35	4.3	9.6	1.16	1.28	9.4%	60%
5	193	−0.35	−0.36	37	38	4.8	11.6	0.2	0.68	70.6%	83%
6	12	−0.55	−0.57	21	22	10.3	8.0	0.48	0.84	42.9%	67%
7	113	−1.47	−1.43	30	31	12.9	9.6	0.82	1.54	46.8%	81%
8	291	−0.38	−0.4	37	38	13.1	11.4	0.18	0.71	74.6%	85%
ITT ($N=9$)	116	−1.0	−1.0	29.2	29.9	7.9	9.6	0.73	1.09	Median 57.6%	Median 81%
Participants with ≥ 2 months treatment ($N=7$)	128	−1.0	−1.0	29.5	30.4	9.5	9.6	0.52	1.07	Median 57.6%	Median 81%

[a]Data are median.

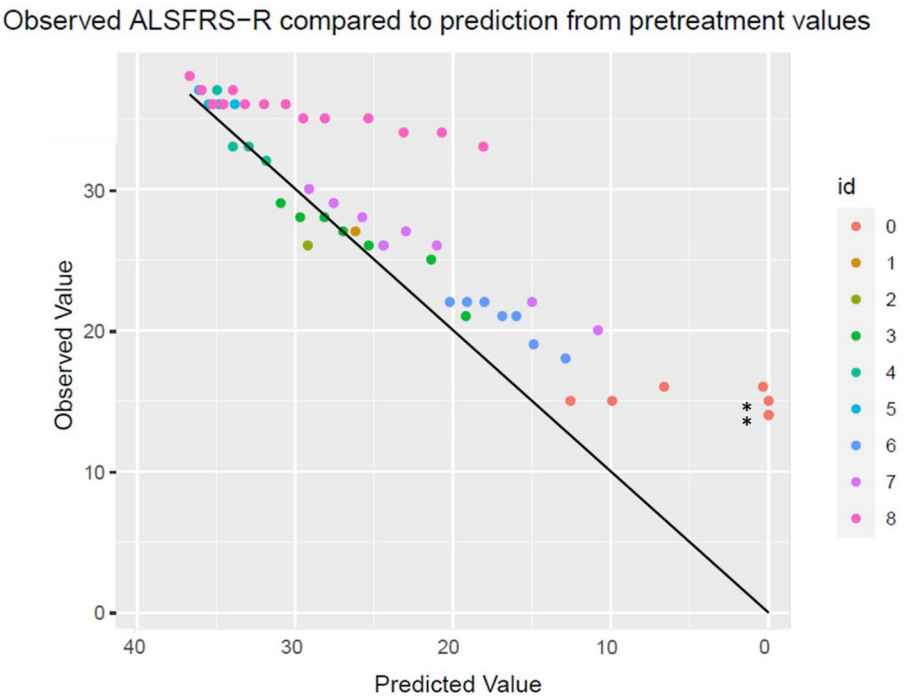


FIGURE 2 | Comparison of observed individual ALSFRS-R measurements with the predicted value of the combined pretreatment slope. Pretreatment records encompass a period of about 6 months before study initiation. The magnitude of treatment benefit is represented by the extent that the participant ALSFRS-R values were above the "without treatment" reference line. Each participant measurement is labeled as a dot with a different color. Note that, by definition, progress along the *x*-axis is determined as time span multiplied by pretreatment slope. While time differences between participants 1 and 8 were similar during study participation, the dots spread proportionally to participants' pretreatment rate: The faster the disease progression, the more distributed the dots. Observed values for periods that would have predicted ALSFRS-R values < 0 are marked with an asterisk*.

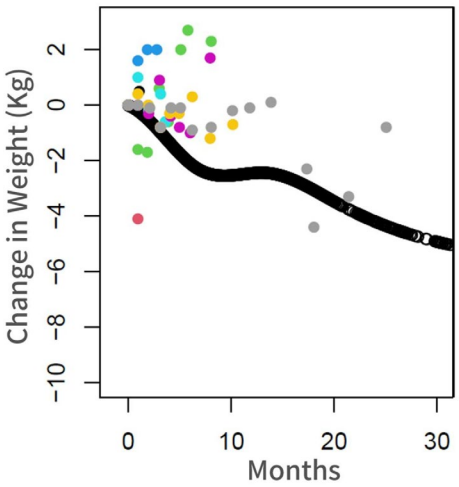
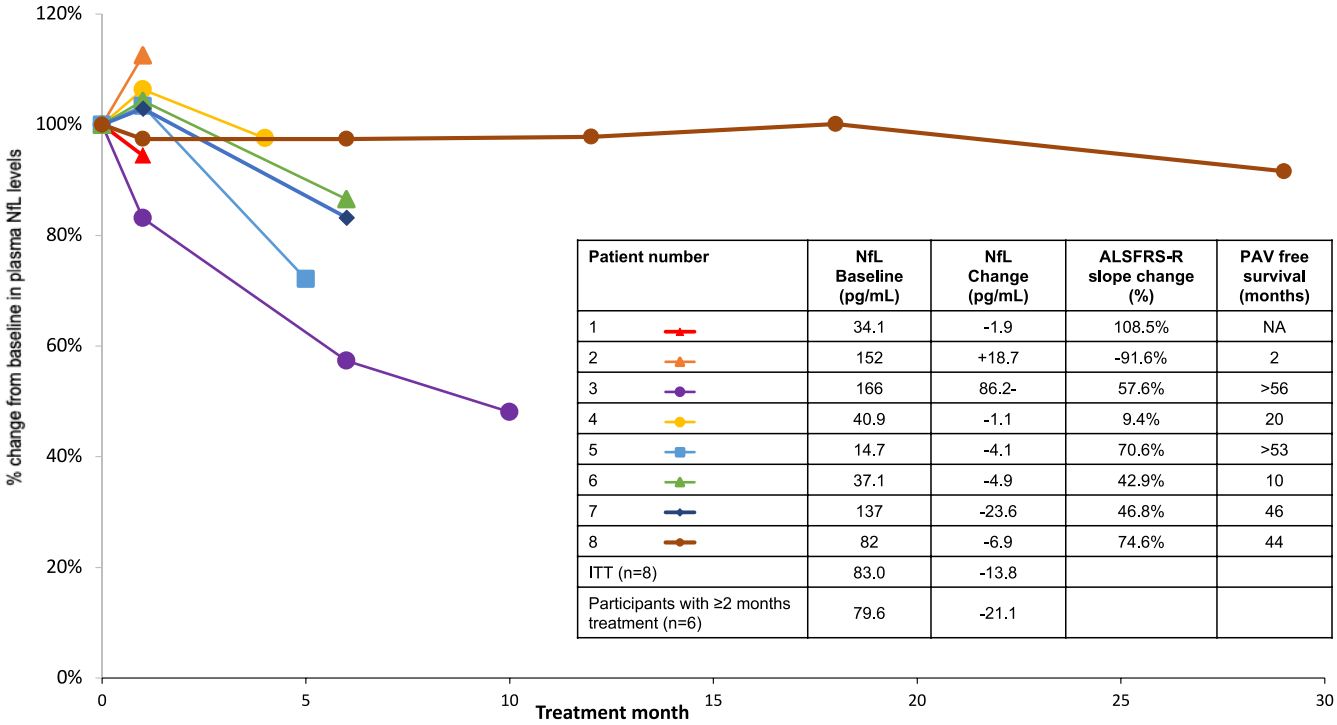


FIGURE 3 | Adjusted treatment effect ($N = 8$) of weight loss/gain compared to historical PRO-ACT controls. The PRO-ACT database [28] was analyzed to determine the change in weight from baseline during treatment. Individual IPL344-treated participants are distinguished by colors, while the black line represents smoothed PRO-ACT placebo values (data are smoothed using a spine with five knots).

Treatment with IPL344 was well tolerated, with one drug-related SAE reported, namely, drug-hypersensitivity (rash and tachycardia) after ≥ 1 year of treatment in the participant treated under a compassionate use protocol. The relatively sparse AE reporting in this study likely reflects the fact that treatment-emergent AEs deemed related to "ALS progression" were not included in the AE reporting for this study. Nevertheless, as expected, two additional participants had SAEs of pneumonia leading to death or tracheostomy—neither of which was considered related to study treatment. It is estimated that around 15% of people who receive CVC have complications [29]. In our study, two participants reported CVC-related mild adverse events, which recovered spontaneously or were treated with betamethasone.

Given the small number of participants, we used several statistical methods to analyze the rate of change in ALSFRS-R score. The first was the analysis previously proposed by Schoenfeld [20] to compare the ALSFRS-R slope in this study with studies recorded in PRO-ACT. This method quantifies inter-center and inter-study variability to determine whether ALSFRS-R progression is reduced more than expected. It shows that the magnitude of ALSFRS-R slope reduction is beyond that expected by inter-study variation, despite the small number of participants. Significant slope reductions were also shown when using either matched controls or corrections for covariates.

A slower symptomatic decline (48%–64% depending on method used) was supported by time-to-event observations during the follow-up period, including 24-month extension of median PAV-free survival and at least 25 months of NIV-free survival for the three participants who were free of NIV assistance at SVC < 60% (NIV initiation is on average, 5.2 months after FVC 60% [30]).



Patient number	NfL Baseline (pg/mL)	NfL Change (pg/mL)	ALSFRS-R slope change (%)	PAV free survival (months)
1	34.1	-1.9	108.5%	NA
2	152	+18.7	-91.6%	2
3	166	86.2-	57.6%	>56
4	40.9	-1.1	9.4%	20
5	14.7	-4.1	70.6%	>53
6	37.1	-4.9	42.9%	10
7	137	-23.6	46.8%	46
8	82	-6.9	74.6%	44
ITT (n=8)	83.0	-13.8		
Participants with ≥2 months treatment (n=6)	79.6	-21.1		

FIGURE 4 | Change in plasma NfL versus baseline over time. Percentage change in plasma NfL concentrations for individual IPL344-treated participants is distinguished in the chart by colors. The table insert includes the absolute baseline value and change in NfL plasma concentration (pg/mL) as well as the % ALSFRS-R slope reduction versus the median of the matched PRO-ACT control and duration of PAV free survival from treatment initiation (months) for that participant. Average absolute NfL values and % change for the eight participants (ITT) and for the six participants with ≥2 months treatment are also presented.

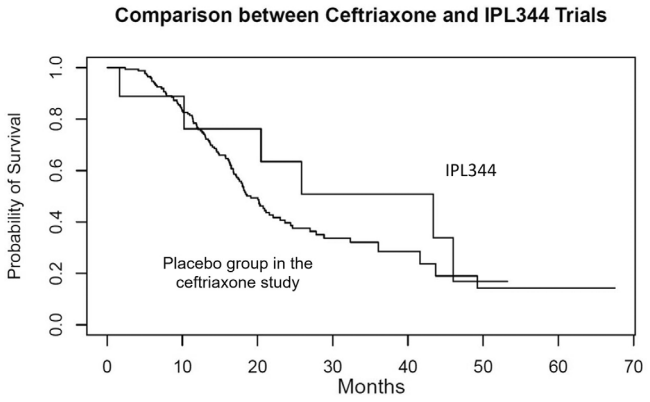


FIGURE 5 | Mortality analysis compared to the ceftriaxone study. Kaplan–Meier curve for the study compared to the survival of the placebo group in the ceftriaxone study.

The preservation of participants' body weight during treatment in this study aligns with the slowed weight loss observed in the pre-clinical SOD1 model study with IPL344 [17] and is notable given the significant reduction of body weight reported for people with ALS [31], and its correlation with survival [27]. This observation might be attributed to the improvement of Akt/GSK3 pathway activity [28, 32, 33]. Overall, NfL levels were either reduced or maintained across follow-up. In established disease, NfL levels remain relatively stable [9] or slightly increase (7%–20%) [34, 35]. The observed decrease in plasma NfL concentrations—which tended to relate to ALSFRS-R slope reduction—is compatible with an effect on neurodegeneration.

This study has several important limitations, including its small sample size, use of retrospective medical records for pre-treatment slope estimation, the assumption of linearity in the ALSFRS-R slope analyses, and lack of placebo control. The statistical methods used here do not discount the possibility of a placebo effect in the IPL344 treated group, as they cannot account for the different certainty levels between open-label study participants who are certain that they are receiving active treatment and participants of a blinded placebo-controlled study (as those used for historical controls). Our analysis plan was based on including the entirety of data available. Thus, we included data from the early participant treated under a compassionate protocol whose ALSFRS-R scores and respiratory volume were below the formal inclusion criteria but whose dosing was in line with the 8th patients in the study who received higher dosing before the COVID pandemic prevented further enrollment to higher dose levels. Problems with historically controlled evaluations include a stronger placebo effect, as well as potential cultural and geographic differences in supportive care and participant characteristics that cannot be controlled for statistically.

This was the first human study conducted with IPL344 and, as such, allowed for several learnings that can be incorporated into future studies. While the rapid plasma clearance of IPL344 precluded the ability to base dosing on pharmacokinetic data, it is not considered detrimental to the therapeutic effect of daily treatment. In addition to the immediate activation of Akt in vitro [19], recent preliminary data now show that DDR-related proteins (including those that activate the Akt pathway) are activated a few minutes after IPL344 treatment and that

DDR functions remain partially active for up to 24 h (unpublished observations). Given that IPL344 induces DNA repair functions, it might be helpful to monitor in future treatments changes in human endogenous retrovirus HML-2, a marker related to TDP43 dysfunction and DNA instability in ALS [36]. Assays to show long-term target engagement (by showing Akt activation) are now in development, and preliminary unpublished results indicate that gradual recovery of Akt activation takes several weeks of treatment. We also learned that the good safety and tolerability profile of IPL344 allows for a less frequent visit schedule than implemented in the present study.

In summary, this small open-label study suggests that IPL344 is well tolerated. Participants in this study showed slower symptomatic decline compared to historical controls and no weight loss. Overall, our observations suggest further assessment of IPL344 may be warranted.

Author Contributions

Marc Gotkine: investigation, conceptualization, writing – original draft. **David A. Schoenfeld:** conceptualization, methodology, formal analysis, writing – review and editing, visualization, software, validation. **Ilana Cohen:** conceptualization, writing – original draft, visualization, supervision, resources, project administration. **Jeremy M. Shefner:** writing – review and editing, visualization, data curation. **Yossef Lerner:** investigation, writing – review and editing. **Irun R. Cohen:** conceptualization, writing – review and editing. **Colin Klein:** investigation, writing – review and editing. **Eran Ovadia:** conceptualization, funding acquisition, writing – original draft, visualization, project administration, supervision, resources, data curation. **Merit E. Cudkowicz:** writing – review and editing, visualization.

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Ethics Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

Marc Gotkine reports Research funding and consultancy for Immunity Pharma. David A. Schoenfeld was the primary data analyst acting as a consultant for Immunity Pharma, which owns the patent rights for IPL344. He reports personal consulting fees for Immunity Pharma, Brainstorm Inc., and Amylyx Inc., and has a use patent for Arimoclomol. Ilana Cohen and Eran Ovadia are employees and shareholders of Immunity Pharma, which owns the patent rights for IPL344 that was used in this study. Jeremy Shefner reports grants from NINDS, ALS Association, AB Sciences; Acorda Therapeutics; Alector; Amylyx; Biogen; Cytokinetics Incorporated; Ionis; Mitsubishi Tanabe Pharma America; Quralis; PTC; Sanofi; Wave; Myolex and safety monitoring board membership for Swanbio and Braingate. He reports personal fees for consultancy from Amylyx, Cytokinetics, Denali, GSK, Mitsubishi Tanabe Pharma America, Neurosense, Orthogonal, Pinteon, RRD, Acurastem, Revalasio, Apellis, Novartis, Sanofi, and Immunity Pharma and hold stocks in Aural Analytics. Yossef Lerner has nothing to disclose. Irun R. Cohen is a shareholder of Immunity Pharma and is a Professor Emeritus in the Weizmann Institute, which owns patent rights for IPL344 that was used in this study. Colin Klein has nothing to disclose. Merit E. Cudkowicz reports consultancy for Immunity Pharm Ltd., Novartis, Roche, Cytokinetics, Transposon, Quralis, Regeneron, AB Sciences, Vector Y, Servier/Adiv, Inflectis, and Pasithea. She reports board membership for Praxis. She has received grant research support from UCB, Biohaven, Clene Nanomedicine, Prilenia, Seelos, Calico, Denali, ALSA, MDA, ALS Finding a Cure, ALS One and NINDS.

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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Supporting Information

Additional supporting information can be found online in the Supporting Information section.