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Target-inhibition of Galectin-3 by Inhaled TD139 in Patients with Idiopathic Pulmonary Fibrosis

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ABSTRACT

Galectin-3 (Gal-3) is a pro-fibrotic β-galactoside-binding lectin that plays a key role in the pathogenesis of idiopathic pulmonary fibrosis (IPF) and IPF exacerbations. TD139 is a novel and potent small molecule inhibitor of Gal-3.

A randomised, double-blind, multi-centre, placebo-controlled, phase I/IIa study was conducted to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of inhaled TD139 in 36 healthy subjects and 24 patients with IPF (NCT02257177). Six dose cohorts of six healthy subjects were evaluated (4:2 TD139:placebo ratio) with single doses of TD139 (0.15 mg to 50 mg) and three dose cohorts of eight patients with IPF (5:3 TD139:placebo ratio) with once daily doses of TD139 (0.3 mg to 10 mg) for 14 days.

Inhaled TD139 was well tolerated with no significant treatment-related side effects. TD139 was rapidly absorbed, with mean T_{max} values ranging from 0.6 h to 3 h and a $T_{½}$ of 8 h. The concentration of TD139 in the lung was >567-fold higher than in the blood, with systemic exposure predicting exposure in the target compartment. Gal-3 expression on alveolar macrophages was reduced in the 3 mg and 10 mg dose groups compared to placebo, with a concentration-dependent inhibition demonstrated. Inhibition of Gal-3 expression in the lung was associated with reductions in plasma biomarkers centrally relevant to IPF pathobiology (PDGF-BB, PAI-1, Gal-3, CCL18 and YKL-40).

TD139 is safe and well tolerated in healthy subjects and IPF patients. It was shown to suppress Gal-3 expression on BAL macrophages and, in a concerted fashion, decrease plasma biomarkers associated with IPF progression.

INTRODUCTION

Idiopathic Pulmonary Fibrosis (IPF) is a progressive, irreversible, ultimately fatal lung disease characterized by a progressive decline in lung function. The median survival is 3 years and incidence is increasing. The underlying pathogenesis of IPF is unknown, however, it most likely arises as a result of repeated alveolar epithelial cell injury and subsequent aberrant healing. It is considered likely that multiple intrinsic and environmental triggers might lead to IPF (1, 2). Two anti-fibrotic therapies have gained regulatory approval for use in IPF; pirfenidone, which has an uncertain mechanism of action and nintedanib, which is a mixed tyrosine kinase inhibitor (3, 4). Both drugs have moderate efficacy, but 20-30% of patients discontinue treatment or have dose-limiting side-effects. Thus, safe, effective treatments for IPF are urgently required. Numerous early phase clinical trials have been performed in IPF, but few of these have progressed to phase 2b or 3 trials. One of the reasons for this attrition from early- to late-phase trials is the lack of confidence that the study drug engages with its target in the human disease state. This issue is not unique to IPF.

Galectin-3 (Gal-3) is a central regulator of fibrosis in the lung with expression upregulated in the bronchoalveolar lavage (BAL) fluid and serum of IPF patients, and further elevation observed during exacerbations (5, 6). The pro-fibrotic function of Gal-3 is multifactorial due to its ability to cross-link and promote signalling via multiple cell surface receptors including integrins and growth factor receptors (7), such as transforming growth factor-β (TGFβ) (6, 8), vascular endothelial growth factor (VEGF) (9) and platelet-derived growth factor (PDGF) receptors (10, 11). Constitutive global deficiency of Gal-3 leads to attenuated fibrosis in murine models (6, 12-14).

TD139 is a 3,3'-Bis-(4-aryltriazol-1-yl) thio-digalactoside Gal-3 inhibitor with high affinity for the Gal-3 carbohydrate recognition domain that has shown efficacy in murine models of lung fibrosis (6, 15). The anti-fibrotic potential of TD139 centres around the inhibition of the recruitment and expansion of Gal-3 secreting macrophages that drive local myofibroblast activation (14, 16, 17). TD139 has been shown pre-clinically to exhibit effects on all of the key IPF cell types: modulating macrophage phenotype/Gal-3 expression and fibroblast activation, reducing the effects of key pro-fibrotic growth factors that act on myofibroblasts and inhibiting epithelial-mesenchymal transition (EMT) (6, 16, 18-20).

The aim of this study was to investigate the safety, pharmacokinetic (PK) and pharmacodynamic (PD) profile of TD139 when administered via a dry powder inhaler in healthy subjects and IPF patients.

MATERIALS AND METHODS

Study Design

This study was a randomised, double-blind, multi-centre, placebo-controlled dose-escalation study investigating the safety, tolerability, PK and PD properties of TD139, a Gal-3 inhibitor administered by inhalation, in healthy subjects (part 1) and patients with IPF (part 2). Part 1 was completed at Simbec Research Ltd (Merthyr Tydfil, UK) and part 2 was undertaken at four hospitals in the UK (Edinburgh Royal Infirmary; Royal Brompton Hospital, London; Royal Victoria Infirmary, Newcastle upon Tyne; and the Royal Devon and Exeter NHS Trust). Independent ethics committee approval was obtained prior to initiation of the study. Screening in study centres was performed within 28 days prior to randomisation and first dosing (day 1).

In part 1 of the study following an initial screening process, subjects were randomly assigned in a 4:2 ratio to receive a single dose of TD139 (0.15 mg, 1.5 mg, 3 mg, 10 mg, 20 mg or 50 mg) or placebo via a dry powder Plastiape™ (Berry Bramlage, Lohne, Germany) inhaler (randomisation code generated using the PROC PLAN procedure of SAS). All subjects, site and study sponsor personnel were blinded to the study group assignments throughout the study. TD139 or placebo was administered in the morning of day 1 fasted (after an overnight fast of at least 10 h). Subjects were discharged 24 h post-dose (day 2) provided there were no safety concerns and returned for 3 out-patient visits on days 3, 8 and 14 post-dose, with a post-study follow-up between days 26 to 30. Safety and PK assessments were made at pre-determined time-points.

In part 2 of the study following an initial screening process, patients with an MDT confirmed diagnosis of definite or probable IPF according to current international consensus criteria (1) were randomly assigned in a 5:3 ratio to receive a dose of TD139 (0.3 mg, 3 mg or 10 mg) or placebo via a dry powder Plastiape™ (Berry Bramlage, Lohne, Germany) inhaler once daily for 14 days (randomization code generated using the PROC PLAN procedure of SAS). All patients, investigators, sites and study sponsor personnel were blinded to the study group assignments throughout the study. Patients underwent study-specific assessments on days 2, 3, 7, 14 and 15 and a post-study assessment was carried out 26-30 days after the last day of the study period. These included; vital signs (supine blood pressure, pulse, O_2 saturation, oral temperature and respiratory rate), spirometry, 12-lead ECG, physical examination, laboratory safety screen (haematology , biochemistry, urinalysis and urinary drugs of abuse screen) and blood sampling for PK measurements. All adverse events (AE) and concomitant medications were recorded from the time of the screening visit until the post-study follow-up visit. Broncho-alveolar lavage (BAL) was performed at day -1 (24 h before first dose) and at day 14 (2 h after the last dose). The BAL procedure was performed as per international guidelines (21). Briefly, 240 mL of 0.9% saline at room temperature was instilled in 40 mL aliquots into the right middle lobe and aspirated by suction after each aliquot.

Following completion of dosing for the final subject in each dosing cohort, an interim dose review meeting was held with a safety committee consisting of principal investigator, medical monitor, pharmacokineticist and statistician to review the safety, tolerability and PK data (study design schematic shown in figure 1). In order to conduct the analyses required for dose-review decisions the study statistician and

pharmacokineticist were unblinded at the end of each cohort. The primary endpoint was the safety and tolerability of single or multiple doses of TD139 in healthy subjects or IPF patients, respectively. Secondary endpoints included evaluation of the PK properties of TD139, expression of Gal-3 in lung and blood, changes in lung macrophage phenotype and plasma biomarkers following treatment.

Study participants

The healthy subject population comprised of 36 male subjects aged between 23 and 53 years. The patient population comprised 24 patients aged between 45 and 85 years with a consensus multi-disciplinary diagnosis of IPF based on the American Thoracic Society, the European Respiratory Society, the Japanese Respiratory Society and the Latin American Thoracic Association (ATS/ERS/JRS/ALAT) criteria (1). Subjects had a forced vital capacity (FVC) ≥ 45% predicted; a forced expiratory volume in 1 second (FEV1)/FVC ratio ≥ 0.7; oxygen saturation >90% on air; a diffusing capacity (DLCO) >25% predicted; and were judged by investigators to be safely able to undergo BAL prior to drug treatment and again after 14 days treatment. Patients receiving oral corticosteroids or any approved or investigational antifibrotic therapies for IPF within 4 weeks of initial screening were excluded from the study. The baseline demographics are shown in Table 1 and a full list of inclusion and exclusion criteria are presented in supplementary table 1.

Study drug

TD139 was administered once daily via a dry powder inhaler. For part 2 a dose range between 0.3 mg and 10 mg once daily was predicted to provide pharmacologically relevant exposures based on *in vitro* and *in vivo* pharmacology studies.

Pharmacokinetics

Full plasma PK profiles were conducted on days 1 (first day of dosing) and 14 (last day of dosing). Plasma, BAL alveolar macrophage and BAL fluid concentrations of TD139 were analysed using a validated analytical method based on protein precipitation, followed by high performance liquid chromatography/mass spectrometry (MS) analysis. Standard non-compartmental methods were applied to derive PK parameters using Phoenix® WinNonlin® version 6.3 software (Certara UK Ltd., Sheffield, UK)). Reported parameters were maximum concentration (C_{max}), minimal concentration (C_{min}), terminal elimination half-life ($T_{1/2}$) and area under the concentration time curve (AUC) from time of dosing extrapolated to infinity (AUC_{0-inf}). Drug compartment measurements in BAL alveolar macrophage and fluid were determined as described previously (22). Epithelial lining fluid (ELF) concentrations of TD139 were calculated as described previously (23) with urea measured using a QuantiChrom™ urea assay kit (BioAssay Systems, CA, USA).

Pharmacodynamic endpoints

BAL cells were separated from fluid by centrifugation and cells were collected, stained with antibodies (supplemental methods) then fixed before analysis using a LSRFortessa™ flow cytometer (Becton Dickinson, NJ, USA). Data were analysed using FlowJo software (Becton Dickinson, NJ, USA). Macrophages were identified as having high side scatter properties and HLA-DR positivity. Gal-3 expression (mean fluorescence intensity, MFI) was determined from the macrophage gate (see supplemental figure S1 and S2). A range of pre-selected plasma biomarkers known to be involved in Gal-3 pathways and/or suggested to be putative biomarkers in IPF

were also investigated (see supplemental methods and table S2). Samples were analysed by magnetic Luminex assay (R&D Systems, MN, USA) or ELISA.

Statistical analysis

For part 2 of the study, placebos were combined from the 3 dose-specific cohorts into a pooled control group (n=9). For alveolar macrophage Gal-3 expression and the plasma biomarkers an analysis of covariance (ANCOVA) was performed for percentage of baseline ((day14/day1) x100). The ANCOVA model included effects for treatment group and baseline (day1) value. The least square means (LS-Mean) for each treatment group, the difference in LS-Means for each active dose compared to control with associated 95% 2-sided confidence interval as well as the p-value for treatment difference were obtained. As some imbalance was seen in demographic and baseline characteristics between the treatment groups a further ANCOVA model was fitted including effects for age, weight and FEV1 at baseline. These covariates were not found significant in any model and did not change the interpretation of the results, so were removed from final models. A Bayesian approach was taken to use these results to quantify the evidence for positive effect of drug for each biomarker. Under the assumption of a non-informative prior for the treatment difference, the posterior probability of a treatment effect greater than zero (percentage of baseline<100%) is equivalent to 1-the one-sided lower p-value (H_1: $\mu_{Drug} < \mu_{Placebo}$) from this ANCOVA model. An additional analysis including dose as a continuous covariate was also performed. Pearson correlation coefficient and associated p-value were calculated for the percentage of baseline and day 14 values between plasma biomarkers and alveolar macrophage Gal-3 expression. If appropriate, a Fisher's exact test was performed for whether a subject had a % baseline less than 100 for the plasma biomarker and alveolar macrophage Gal-3 expression (Y/N) vs treatment (placebo *vs.* 10 mg). The analysis is exploratory, and there was no pre-planned adjustment for multiple testing; the study was not powered for this. Alveolar macrophage Gal-3 expression was considered the primary exploratory analysis with the pre-selected biomarkers secondary. A Bonferroni correction for multiple testing of the secondary endpoints comparing the 10 mg group to placebo gives a critical value of 0.0063. Data shown in text is the difference in LS-means with (95% CI) and p-value unless otherwise stated. All analyses were performed using SAS version 9.4. A concentration–response model was fitted to the alveolar macrophage concentration of TD139 for each subject and the alveolar macrophage surface Gal-3 level (% of baseline) using Prism 8.0 (GraphPad Software, San Diego, CA, USA) to generate an E_{max} and IC_{50} value.

RESULTS

The enrollment and flow of healthy subjects and IPF patients through part 1 and part 2 of the study is detailed in the CONSORT (Consolidated Standards of Reporting Trials) diagram (figure 1). Subject disposition and demographics are presented in table 1. A small amount of imbalance was observed in age, weight, BMI, and FEV, however this was found not to impact the analysis results.

Overall, TD139 was considered safe and well tolerated at single doses up to 50 mg and multiple inhaled doses up to 10 mg. In part 1, a total of 28 mild treatment-emergent adverse events (TEAEs) were reported by 15 (41.7%) subjects during the study (table 2). The most commonly occurring TEAE associated with TD139 was mild dysgeusia (distortion of sense of taste, 36.1 %) but with no serious tolerability issues. Cough was reported although the incidence was low (11.1 %). There were no other TEAEs of note and in general there were no dose-related trends observed. In addition, there were no clinically significant safety findings or treatment, or dose-related changes observed in biochemistry, haematology, vital sign or 12-lead ECG data. In part 2, there were no treatment or dose-related trends observed in any TEAE profile, biochemistry, haematology, vital signs or 12-lead ECG data following treatment with TD139. Twenty patients had at least 1 TEAE (table 2), however there were no withdrawals as a result. Most TEAEs were unrelated to the study drug and only 2 patients had a TEAE (diarrhoea, dysgeusia and oropharyngeal pain) considered possibly related to study drug. There was one post-treatment severe AE of pneumonia reported in the 10 mg TD139 IPF group. This patient received all 14 doses of study drug with the treatment course recorded as uneventful and pneumonia diagnosed 4 days after the last dose of TD139 following hospital admission. The pneumonia worsened leading to death 32 days later. Infection was regarded as possibly related to the BAL procedure but unrelated to study drug by the study investigators.

Plasma concentrations of TD139 in healthy subjects and IPF patients are presented in figure 2a and 2b, respectively, and derived PK parameters shown in table 3. TD139 absorption was non-linear, increasing with dose and between day 1 and day 14. The PK profile of TD139 in IPF patients in this study was comparable to healthy volunteers, however the exposure at the highest 10 mg dose was much greater in patients with IPF, compared with the same dose in healthy subjects. Thus, a 3.3-fold increase in dose to 10 mg in patients with IPF resulted in a disproportionately high increase in exposure on day 1, with a 5-fold increase in C_{max} and a 13-fold increase in $AUC_{0\text{-}inf}$. After 14 days' dosing, 10 mg values were 5- and 8-fold greater, respectively, with ~50% accumulation. With a $T_{½}$ of ~8 h and a dosing interval of 24 h, the expected accumulation would be ~14%. These data suggest that TD139 is retained in the lungs but to a lesser extent in IPF patients compared with healthy subjects and may be saturable, with a slightly greater accumulation in the plasma than expected over time and a much higher systemic exposure with increasing dose. TD139 concentration was assayed in alveolar macrophages derived from patients' day 14 BAL, in the ELF and plasma (figure 2c). There was a dose-dependent correlation between TD139 concentrations measured between the 3 compartments (figure 2d and e). The concentration of TD139 in BAL macrophages was between 567- and 1,930-fold higher than from systemic exposure at 2 h post-dosing on day 14.

Gal-3 expression on BAL macrophages was reduced by administration of TD139 (figure 3a and supplemental figure S3). The percentage of baseline at day 14 differed between the 3 mg (-38.66% (-69.59 to -7.73); p=0.017) and 10 mg (-44.63 (-80.44 to -8.81); p=0.0173) dose groups compared with placebo. A concentration-dependent reduction in cell surface Gal-3 was observed in BAL macrophages, with a near maximal effect observed in the 10 mg TD139 cohort (Figure 3b and c). The concentrations required to induce this effect were several orders of magnitude greater than that present in the systemic circulation (figure 3b).

Circulating Gal-3 plasma concentrations were reduced in the highest dose group (10 mg) *vs.* placebo (-67.63 (-126.86 to -8.40); p=0.0275) and correlated with the change in expression of Gal-3 on BAL macrophages at day 14 (figure 4). Fisher's exact test of whether a subject has % baseline < 100 for Gal-3 plasma and Gal-3 on BAL macrophages compared for treatment groups, found a difference between the 10 mg *vs.* placebo groups (p=0.031).

Plasma from patients at day -1 and day 14 was analysed for the expression of a panel of pre-specified inflammatory and fibrosis biomarkers. A disease relevance score was retrospectively assigned to these biomarkers to rank them for importance in IPF progression (table 4 and supplemental table S2). When comparing placebo with the 10 mg TD139 group after adjusting for baseline values, five of the eleven high relevance plasma markers (PDGF-BB, PAI-1, Galectin-3, CCL18 and YKL-40) with a link to IPF pathogenesis were reduced (p-value<0.05). The Bayesian probability that the treatment effect is greater than zero (% baseline < 100) in the 10 mg group is > 98% (figure 4a, table 4 and supplemental table S2). Another three biomarkers with medium or high ranking (MMP-8, PDGF-AA, MMP-1) were impacted by treatment with > 90% probability (Table 4). Similar results were found when dose was included as a continuous covariate (supplementary table S4). There was also a good correlation between the change in lung Gal-3 concentration and the change in plasma levels of the high relevance biomarkers in the 10 mg group (figure 4b). Overall, the differential impact of TD139 was predominantly on pro-fibrotic *vs.* pro-inflammatory markers. In addition, there was no significant change in any biomarker between day -1 and day 14 in the placebo group (table 4).

DISCUSSION

This is the first study to evaluate the pharmacology of a novel therapeutic targeting Gal-3 inhibition in IPF. In addition, it is also the first inhaled therapeutic to be investigated in an IPF clinical study. A large body of evidence points to Gal-3 being an important mediator of fibrosis across multiple organs and pre-clinical work with TD139 has demonstrated the anti-fibrotic potential of targeting Gal-3 in lung fibrosis (6, 19). An initial study in healthy subjects (part 1) demonstrated that single doses of TD139 between 0.15 and 50 mg were well tolerated. Reported adverse events were all judged to be mild and inhaled administration of TD139 in this healthy subject study demonstrated a favorable PK profile that supported undertaking a multiple ascending dose study in individuals with IPF (part 2).

Daily inhaled administration of TD139 for 14 days via dry powder inhaler was safe and well tolerated by patients with IPF and the adverse events observed were mild in severity. None of the drug-related adverse events resulted in discontinuation of treatment. The SAE in this study was deemed to be a fatal acute exacerbation of IPF (AE-IPF). The subject experienced new respiratory symptoms 2 days after the second BAL and was initially treated for presumed infection but subsequently went on to develop AE-IPF according to the then relevant 2011 ATS/ERS criteria. Molyneaux *et al* have recently reported on the safety of BAL in IPF in a large prospective cohort. However, we are aware that there are published data that indicate that BAL could cause AE-IPF but this is uncommon or rare (24). Repeat BAL could plausibly increase this risk, if for example the first BAL induced a 'priming' environment that could trigger an AE-IPF with a second BAL. A number of studies, including this one, have successfully repeated BAL in IPF subjects with a cumulative experience of >80 subjects and no reports of procedure related adverse events see (25, 26). The 14-day interval between BALs in this study was determined in part to mitigate against this hypothetical situation.

Inhaled administration of TD139 resulted in measurable, dose-dependent levels of the drug in plasma, ELF and alveolar macrophages. The PK profile of TD139 in individuals with IPF was consistent and characterised by low inter- and intra-patient variability. The PK profile in subjects with IPF was comparable to the profile in healthy subjects, although the exposure in IPF was much higher at the 10 mg dose. The $AUC_{0\text{-}inf}$ values in IPF patients receiving 10 mg after 14 days was almost equivalent to the 50 mg values in healthy subjects receiving a single dose. Loss of alveolar barrier integrity with increased epithelial permeability is well described in the fibrotic lung (27), that may account for the high systemic levels observed in IPF patients with TD139. The variability of the plasma levels at day 1 was larger than at day 14 for all three dose-levels, indicating that patients may achieve a more consistent and reproducible drug exposure as they become accustomed to using the inhaler. Drug levels in the lung determined in ELF and alveolar macrophages showed a strong correlation with plasma levels, allowing a model for achieved lung exposure to be built based on the levels in the systemic circulation. This, combined with the plasma profile observed for TD139, suggested a sustained release of TD139 from the lung into the systemic compartment, demonstrating an extended lung retention. This would be predicted to achieve a prolonged inhibition of Gal-3 in the lung over a 24 h dosing period.

The Gal-3 levels on alveolar macrophages were inhibited by TD139 in a concentration-dependent manner, with the PK/PD relationship defined in the target compartment and the 10 mg dose causing almost maximal inhibition. In addition, the highest dose of TD139 also decreased circulating Gal-3 that correlated with a reduction in several fibrosis-related biomarkers. Four highly relevant disease biomarkers, PDGF (28-31) , CCL18 (32, 33), PAI-1 (34-38) and YKL-40 (39-41) have been shown to have prognostic significance in IPF and have well described effects on myofibroblast activity *in vitro*. These biomarkers were reduced from baseline for the 10 mg dose group compared to placebo and offer a less invasive measure of PD effects going forward into the next phase of clinical development for TD139. The partial reduction in BAL macrophage Gal-3 in the 0.3 and 3 mg groups was associated with a reduction in some biomarkers *e.g.* PDGF-BB in plasma. Due to the pleiotropic nature of Gal-3 in fibrotic disease and the many signalling pathways it influences it could be hypothesised that some could be more sensitive to Gal-3 modulation than others. This could result in maximal downstream inhibition of biomarkers such as PDGF-BB with only a partial inhibition of Gal-3.
In a post-hoc analysis of the ASCEND and CAPACITY trials, both YKL-40 and CCL18 were prognostic for progression in the test cohort but only CCL18 was consistently prognostic for a change in FVC in both the test and replication cohorts (42). However of note there was no association between pirfenidone treatment and the longitudinal concentration of any biomarker (42). In another study comparing treatment naïve IPF patients with those on anti-fibrotic treatment (pirfenidone or nintedanib), CA-125, CXCL13, MMP7, YKL-40 and OPN predicted differential transplant free survival in treated patients but at higher thresholds than treatment naïve individuals (43). There is therefore substantial evidence that several biomarkers are related to disease severity and prognosis, particularly YKL-40 and CCL-18, but no conclusive study that relates biomarker changes in currently approved anti-fibrotic therapy to survival.

The limitations of this study are small sample size, short duration of treatment and mild severity of disease. It is therefore encouraging that TD139 has demonstrated evidence of shifts in these biomarkers despite these limitations.

In conclusion, inhaled dosing of TD139 for 14 days to individuals with IPF has been shown to be safe and well-tolerated. TD139 effectively engages with Gal-3 in the alveolar space and is associated with favorable shifts in mediators that plausibly drive lung fibrosis. These data provide the information necessary for selecting the optimal dosing strategy for TD139 and strongly support performing longer and larger trials in individuals with IPF to assess anti-fibrotic efficacy and the effect on lung function. A Phase IIb study with TD139 in individuals with IPF is now underway (Galactic-1 NCT03832946).

TABLES

TABLE 1 Baseline demographics

Part 1 – Healthy subjects

	TD139						Placebo	Total
	0.15 mg	**1.5 mg**	**3 mg**	**10 mg**	**20 mg**	**50 mg**		
Subjects	4	4	4	4	4	4	12	36
Age years	42.3 ± 9.0	29.8 ± 6.8	39.3 ± 10.2	29.8 ± 2.1	30.8 ± 9.5	32.8 ± 9.9	35.6 ± 7.5	34.6 ± 8.4
BMI kg.m^{-2}	25.6 ± 5.1	30.3 ± 2.7	25.9 ± 3.5	25.1 ± 2.7	23.8 ± 1.2	25.7 ± 1.4	25.6 ± 2.4	25.9 ± 3.1
Height m	1.84 ± 0.12	1.75 ± 0.10	1.77 ± 0.07	1.75 ± 0.03	1.73 ± 0.05	1.85 ± 0.11	1.79 ± 0.07	1.78 ± 0.08
Weight kg	86.0 ± 15.5	93.1 ± 12.8	80.8 ± 9.2	76.0 ± 5.9	71.4 ± 1.5	88.2 ± 13.4	81.8 ± 8.8	82.3 ± 11.0

Part 2 – IPF patients

	TD139			Placebo	Total
	0.3 mg	**3 mg**	**10 mg**		
Subjects	5	5	5	9	24
Age years	69.0 ± 6.3	73.6 ± 5.9	79.2 ± 2.8	72.9 ± 4.6	73.5 ± 5.8
Sex					
Male	4 (80.0)	5 (100.0)	5 (100.0)	9 (100.0)	23 (95.8)
Female	1 (20.0)	0 (0)	0 (0)	0 (0)	1 (4.2)
BMI kg.m^{-2}	28.8 ± 1.7	25.0 ± 1.4	26.3 ± 2.2	28.4 ± 2.4	27.3 ± 2.4
Height m	1.73 ± 0.06	1.77 ± 0.05	1.74 ± 0.09	1.78 ± 0.05	1.76 ± 0.06
Weight kg	86.3 ± 3.9	78.5 ± 5.7	80.1 ±10.6	90.2 ± 7.4	84.8 ± 8.5
FVC % predicted	108.0 ± 19.6	81.8 ± 16.0	98.0 ± 13.1	91.7 ± 14.0	94.3 ± 16.8
FEV1 % predicted	104.0 ± 19.6	88.4 ± 16.8	106.4 ± 17.7	94.9 ± 13.6	97.9 ± 16.8

Data are presented as n, mean ± SD or n (%). All subjects in part 1 were male. BMI, body mass index; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 second.

TABLE 2 Summary of treatment-emergent adverse events

Part 1 – Healthy subjects

	TD139						Placebo	Total
	0.15 mg	**1.5 mg**	**3 mg**	**10 mg**	**20 mg**	**50 mg**		
Number of TEAEs	0	0	2	7	7	10	2	28
Number (%) of subjects reporting ≥1:								
TEAE	0	0	2 (50.0)	3 (75.0)	4 (100.0)	4 (100.0)	2 (16.7)	15 (41.7)
Serious TEAE	0	0	0	0	0	0	0	0
TEAE Leading to Withdrawal	0	0	0	0	0	0	0	0
Number (%) of subjects with TEAE by severity:								
Mild	0	0	2 (50.0)	3 (75.0)	4 (100.0)	4 (100.0)	2 (16.7)	15 (41.7)
Number (%) of subjects with TEAE by relationship to study IMP:								
Almost Definite	0	0	1 (25.0)	3 (75.0)	4 (100.0)	4 (100.0)	2 (16.7)	14 (38.9)
Probable	0	0	0	0	1 (25.0)	0	0	1 (2.8)
Possible	0	0	0	2 (50.0)	1 (25.0)	3 (75.0)	0	6 (16.7)
Unlikely	0	0	1 (25.0)	0	0	0	0	1 (2.8)

Part 2 – IPF patients

	TD139			Placebo	Total
	0.3 mg	**3 mg**	**10 mg**		
Number of TEAEs	9	10	13	23	55
Number (%) of subjects reporting ≥1:					
TEAE	4 (80.0)	5 (100.0)	4 (80.0)	7 (77.8)	20 (83.3)
Serious TEAE	0	0	1 (20.0)	0	1 (4.2)
TEAE Leading to Withdrawal	0	0	0	0	0
Number (%) of subjects with TEAE by severity:					
Mild	2 (40.0)	4 (80.0)	1 (20.0)	4 (44.4)	11 (45.8)
Moderate	2 (40.0)	1 (20.0)	2 (40.0)	3 (33.3)	8 (33.3)
Severe	0	0	1 (20.0)	0	1 (4.2)
Number (%) of subjects with TEAE by relationship to study IMP:					
Almost Definite	0	0	0	0	0
Probable	0	0	0	0	0
Possible	1 (20.0)	1 (20.0)	0	0	2 (8.3)
Unlikely	1 (20.0)	1 (20.0)	2 (40.0)	2 (22.0)	6 (25.0)
Unrelated	2 (40.0)	3 (60.0)	2 (40.0)	5 (55.6)	12 (50.0)

Data are presented as n (%). A subject with multiple occurrences of an adverse event is counted only once within each level of severity or relationship. TEAE, treatment-emergent adverse event; IMP, investigational medicinal product.

TABLE 3 Summary of plasma derived pharmacokinetic parameters

Part 1 – Healthy subjects

	TD139					
	0.15 mg	**1.5 mg**	**3 mg**	**10 mg**	**20 mg**	**50 mg**
Subjects	4	4	4	4	4	4
C_{max} (ng.mL^{-1})	BLQ	2.37 ± 1.48	8.21 ± 2.67	22.6 ± 8.69	81.1 ± 27.0	125.9 ± 43.3
$T_{1/2}$ (h)	ND	1.13 ± 0.63	3.00 ± 0.82	1.19 ± 0.94	1.31 ± 1.80	0.81 ± 0.80
$AUC_{0\text{-}inf}$ (ng.h.mL^{-1})	ND	17.9 ± 14.0	70.8 ± 22.7	184.4 ± 64.5	543.8 ± 113.6	1161.1 ± 532.0

Part 2 – IPF patients

	TD139		
	0.3 mg	**3 mg**	**10 mg**
Subjects	5	5	5
C_{max} (ng.mL^{-1})			
Day 1	0.55 ± 0.02	10.6 ± 11.1	56.3 ± 19.3
Day 14	0.86 ± 0.25	16.5 ± 8.34	82.8 ± 11.8
C_{min} (ng.mL^{-1})			
Day 1	BLQ	1.06 ± 0.43	12.9 ± 7.88
Day 14	BLQ	1.48 ± 0.51	14.7 ± 8.64
$T_{1/2}$ (h)			
Day 1	ND	6.36 ± 2.09	9.69 ± 1.70
Day 14	ND	6.91 ± 1.40	8.20 ± 2.26
$AUC_{0\text{-}inf}$ (ng.h.mL^{-1})			
Day 1	ND	69 ± 50	921 ± 479
Day 14	ND	149 ± 47	1150 ± 462

Data are presented as mean ± SD. C_{max}, maximum plasma concentration, C_{min}, plasma concentration at 24 h post-dose; $T_{1/2}$, plasma half-life; AUC_{inf}, the area under the curve (AUC) extrapolated to infinity from dosing time, based on the last observed concentration at 24 h post-dose; BLQ, below limit of quantification (0.5 ng/ml); ND, not determined due to insufficient data.

TABLE 4 Summary of statistical analysis of plasma pharmacodynamic biomarkers

Biomarker	LS-Mean %baseline (SEM)				Difference in LS-Means: 10mg *vs.* placebo (95% CI)	2-sided P-value (10mg *vs.* placebo)	*Bayesian probability of effect (10 mg)
	Placebo	0.3 mg	3 mg	10 mg			
PDGF-BB	275.2 (42.2)	90.5 (55.2)	103.7 (54.1)	35.7 (54.2)	-239.5 (-384.8, -94.2)	0.003	99.86
PAI-1	197.6 (32.7)	84.6 (48.8)	92.4 (44.5)	45.8 (43.7)	-151.8 (-265.3, -38.2)	0.012	99.43
Galectin-3 (BAL)	81.7 (9.0)	67.9 (12.1)	43.0 (11.7)	37.1 (13.5)	-44.6 (-80.4, -8.8)	0.017	99.14
Galectin-3	121.0 (15.6)	113.9 (21.2)	119.5 (21.3)	53.4 (23.5)	-67.6 (-126.9, -8.4)	0.028	98.63
CCL18 (PARC)	109.5 (8.8)	89.6 (11.8)	98.9 (12.0)	74.6 (11.8)	-35.0 (-65.8, -4.2)	0.028	98.59
YKL-40 (CHI3L1)	80.4 (7.9)	84.1 (12.5)	50.2 (18.6)	51.6 (10.4)	-28.9 (-55.9, -1.9)	0.037	98.13
MMP-8	176.9 (39.3)	138.0 (52.8)	115.2 (52.7)	51.5 (56.1)	-125.4 (-271.3, 20.5)	0.088	95.60
PDGF-AA	213.3 (45.6)	61.7 (60.6)	117.1 (62.1)	83.6 (61.2)	-129.7 (-295.3, 35.8)	0.117	94.13
HGF	110.3 (12.6)	100.3 (17.5)	103.2 (16.9)	73.2 (18.8)	-37.2 (-85.5, 11.2)	0.124	93.79
MMP-1	164.2 (42.0)	67.3 (56.3)	107.1 (59.1)	62.1 (58.1)	-102.1 (-251.4, 47.3)	0.169	91.55
CCL2 (MCP-1)	161.1 (29.6)	126.7 (39.3)	93.7 (38.7)	89.4 (43.5)	-71.7 (-187.1, 43.7)	0.209	89.55
CCL5 (RANTES)	129.9 (25.7)	62.3 (37.1)	115.1 (35.1)	75.8 (35.8)	-54.1 (-147, 38.8)	0.238	88.10
MIF	194.1 (62.2)	123.6 (83.6)	95.9 (83.9)	73.9 (83.7)	-120.2 (-339.2, 98.9)	0.265	86.74
MMP-7	166.4 (44.6)	79.3 (59.2)	82.3 (58.4)	84.4 (58.7)	-82.0 (-237.9, 73.9)	0.285	85.76
Pentraxin-3 (PTX3)	136.5 (41.8)	51.8 (68.6)	74.2 (53.1)	67.0 (56.1)	-69.6 (-220.3, 81.2)	0.343	82.88
Osteopontin	118.1 (17.9)	106.2 (23.9)	84 (23.7)	91.3 (23.4)	-26.8 (-88.0, 34.3)	0.370	81.50
Periostin	98.4 (3.7)	114.0 (5.3)	99.3 (5.2)	93.1 (5.0)	-5.4 (-18.8, 8.0)	0.412	79.42
Galectin-1	106.4 (12.4)	93.1 (16.7)	120.2 (16.6)	89.7 (16.6)	-16.6 (-60.0, 26.7)	0.431	78.45
IL-13	105.2 (6.6)	87.4 (8.8)	100.4 (9.5)	97.6 (8.8)	-7.6 (-30.4, 15.2)	0.496	75.22
SP-D	114.7 (12.0)	109.6 (16.6)	111.7 (18.2)	118.7 (17.3)	4.0 (-41.0, 49.1)	0.854	42.67
IL-1ra	92.0 (15.0)	93.2 (20.3)	98.7 (20.2)	98.0 (20.8)	6.0 (-47.5, 59.4)	0.818	40.89
TIMP-1	102.2 (4.1)	88.6 (5.8)	107.0 (6.0)	106.0 (5.5)	3.8 (-10.6, 18.2)	0.583	29.17

Results from an analysis of covariance model including effects for treatment group and baseline value with the Bayesian probability of effect of 10 mg TD139 group *vs.* placebo shown*. For the following biomarkers, the majority of values were below the lower level of quantification and

therefore were not analysed: amphiregulin, CCL26 (eotaxin), CXCL1 (GROα), CXCL10 (IP-10), EGF, IL-8, IL-10, IL-25, IL-33, IFNγ and TNFα. BAL, bronchoalveolar lavage.

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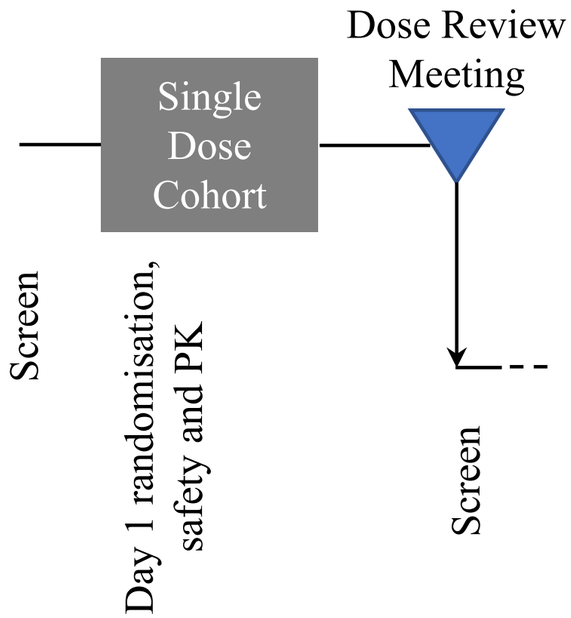
Part 1 – Healthy subjects

a)



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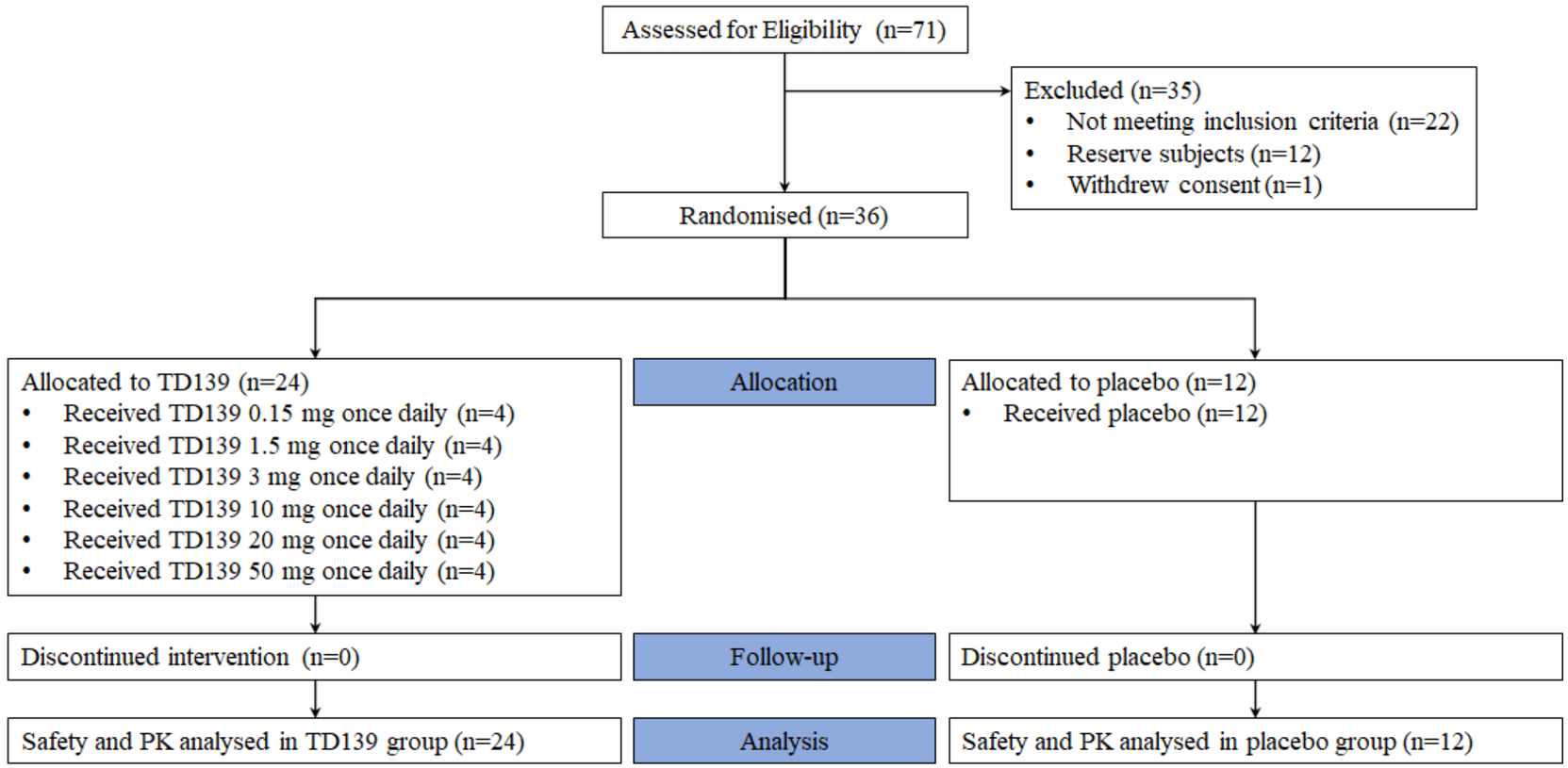
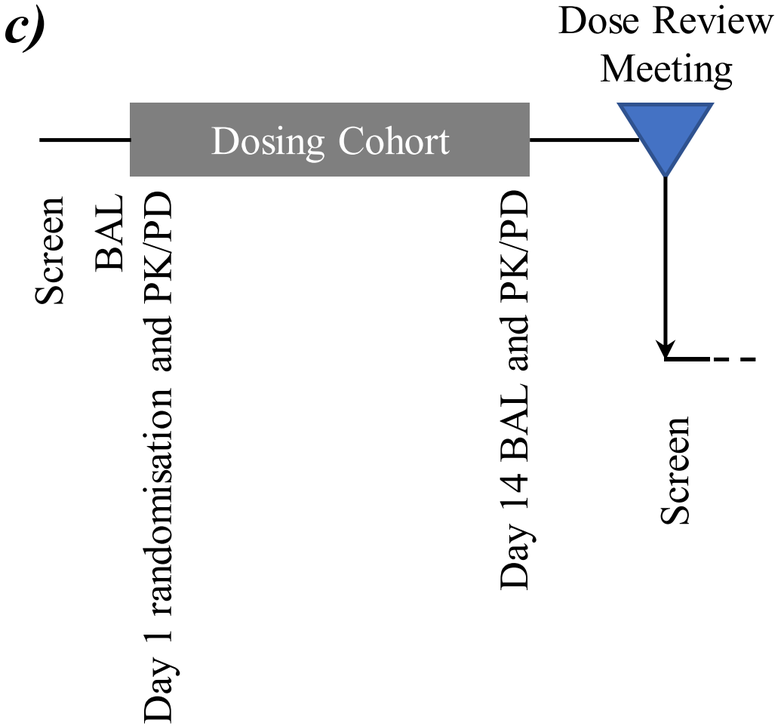


Part 2 – IPF patients

c)



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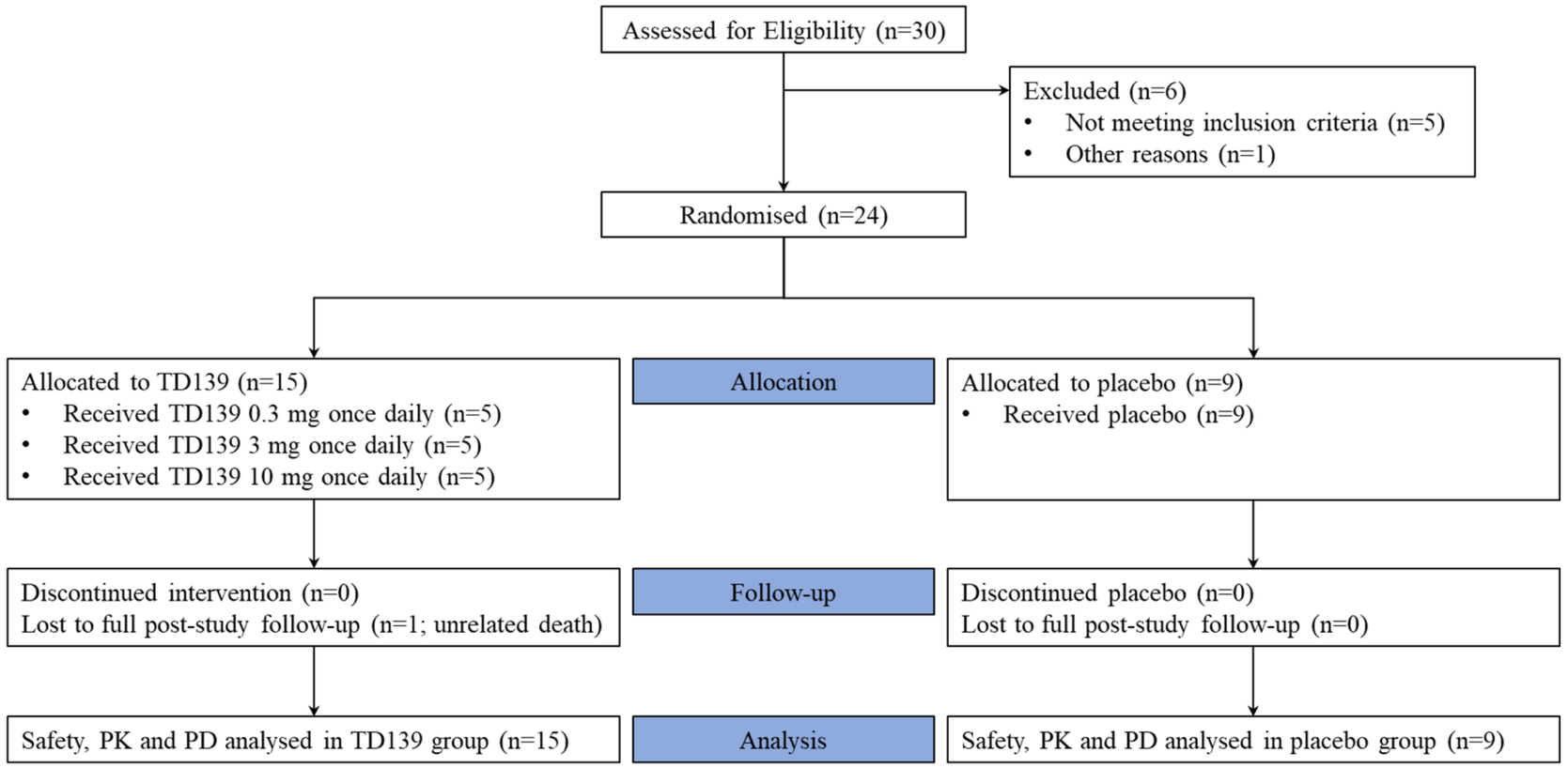

Figure 1. Schematic of the study design and CONSORT (Consolidated Standards of Reporting Trials) diagrams. Part 1 Healthy subjects a) Each single dose cohort consisted of 6 healthy subjects randomised 4:2 to active and placebo. A minimum of 5 patients' data was required in each cohort prior to data review. b) the CONSORT diagram for part 1. Part 2 IPF patients c) Each repeat dose cohort consisted of 8 subjects randomised 5:3 to active and placebo. A minimum of 7 patients' data was required in each cohort prior to data review. d) the CONSORT diagram for part 2. BAL, bronchoalveolar lavage; PK, pharmacokinetics; PD, pharmacodynamics.

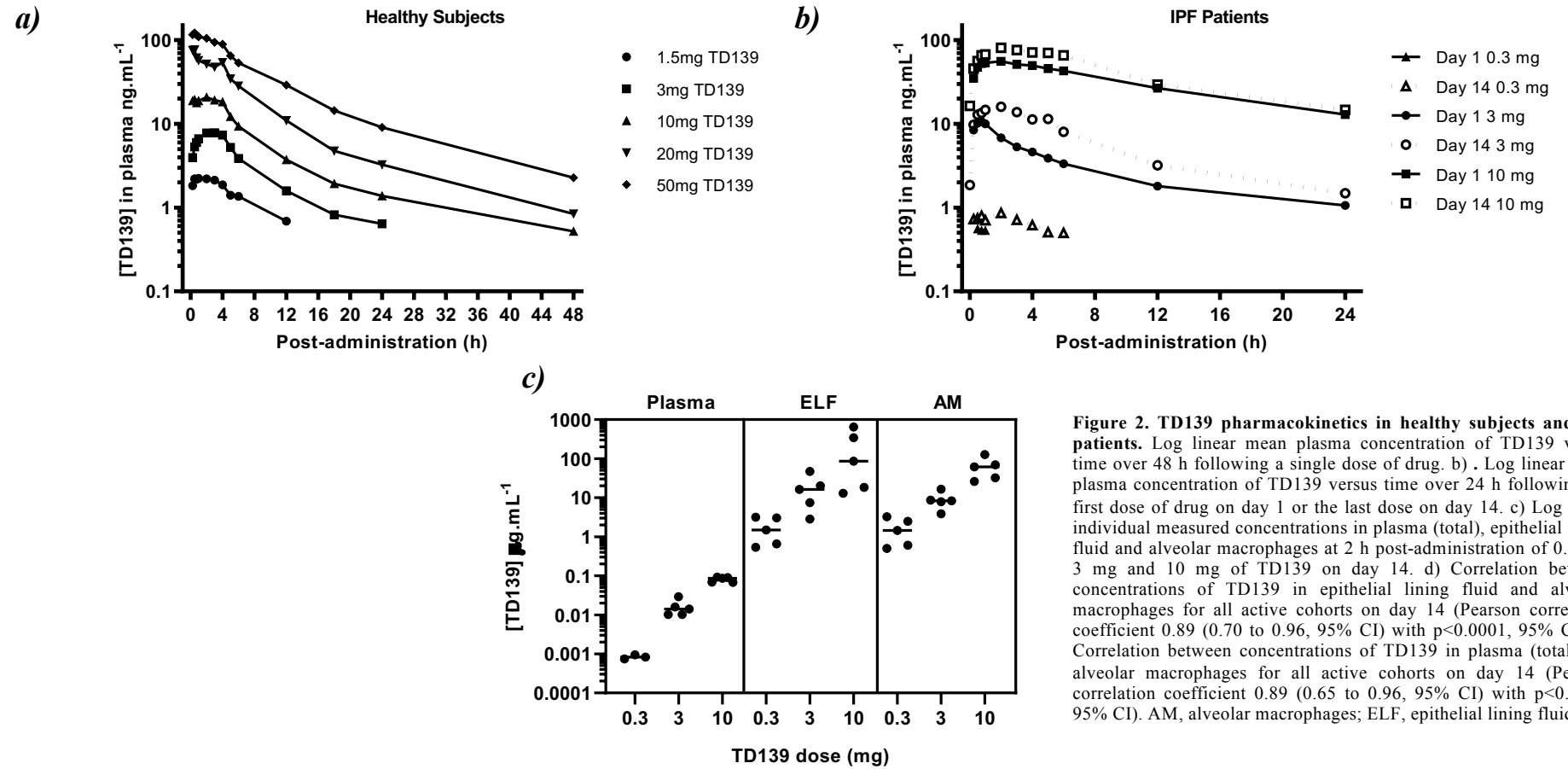

Figure 2. TD139 pharmacokinetics in healthy subjects and IPF patients. Log linear mean plasma concentration of TD139 versus time over 48 h following a single dose of drug. b) **.** Log linear mean plasma concentration of TD139 versus time over 24 h following the first dose of drug on day 1 or the last dose on day 14. c) Log linear individual measured concentrations in plasma (total), epithelial lining fluid and alveolar macrophages at 2 h post-administration of 0.3 mg, 3 mg and 10 mg of TD139 on day 14. d) Correlation between concentrations of TD139 in epithelial lining fluid and alveolar macrophages for all active cohorts on day 14 (Pearson correlation coefficient 0.89 (0.70 to 0.96, 95% CI) with p<0.0001, 95% CI). e) Correlation between concentrations of TD139 in plasma (total) and alveolar macrophages for all active cohorts on day 14 (Pearson correlation coefficient 0.89 (0.65 to 0.96, 95% CI) with p<0.0001, 95% CI). AM, alveolar macrophages; ELF, epithelial lining fluid.

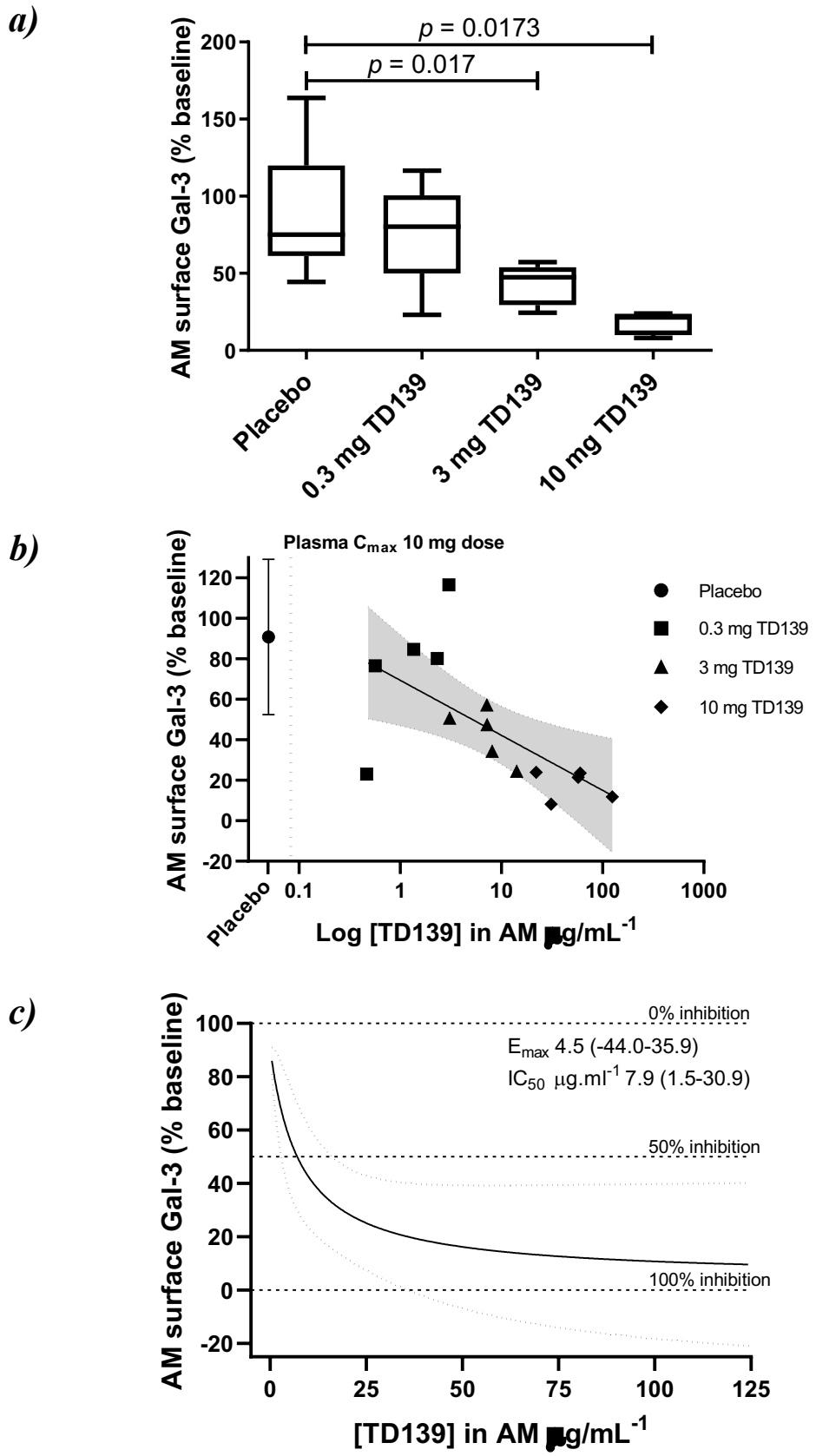

Figure 3. Galectin-3 changes in alveolar macrophages. a) Boxplot of TD139 dose-dependent effect on percentage change from baseline in surface macrophage galectin-3 levels (median, min to max). p-value from ANCOVA model adjusted for baseline. b) Concentration-dependent effect between TD139 in the alveolar macrophages and percentage of baseline in surface macrophage galectin-3, with plasma C_{max} at 10 mg dose (dotted line) shown for comparison (black line and confidence band; Pearson correlation coefficient -0.63 (-0.86 to – 0.18, 95% CI) with p = 0.011; individual subject data are labelled by dose). c) Fitted maximum effect model of surface macrophage galectin-3 (percentage of baseline) *vs.* macrophage TD139 concentration (black line and confidence band). AM, alveolar macrophages.

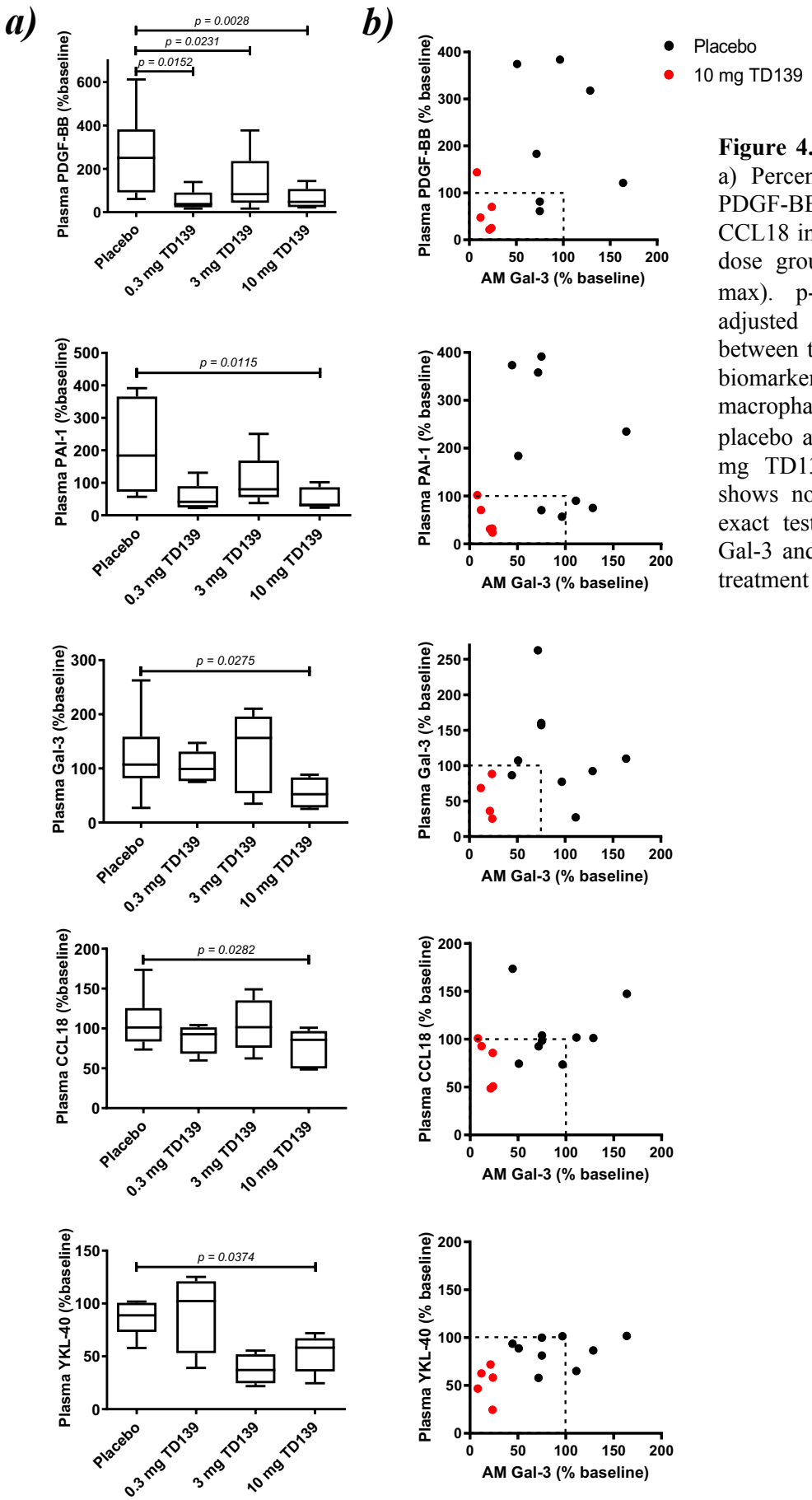

Figure 4. Biomarker changes in plasma. a) Percentage of baseline at day 14 for PDGF-BB, PAI-1, Gal-3, YKL-40 and CCL18 in 0.3 mg, 3 mg and 10 mg TD139 dose groups or placebo (median, min to max). p-value from ANCOVA model adjusted for baseline. b) Correlation between the percentage of baseline in each biomarker *vs.* the change in lung macrophage Gal-3 expression in the placebo and 10 mg TD139 groups (red 10 mg TD139, black placebo). Dotted line shows no change from baseline. Fisher's exact test for %baseline<100 for plasma Gal-3 and AM Gal-3 expression (Y/N) *vs.* treatment group (placebo, 10mg) p=0.031.

SUPPLEMENTAL

METHODS

Flow cytometry

BAL cells were separated from fluid by centrifugation and BAL cells were collected, stained with antibodies then fixed before analysis using a LSRFortessa™ flow cytometer (Becton Dickinson, NJ, USA). Data were analysed using FlowJo software (Becton Dickinson, NJ, USA). After exclusion of doublets and debris a sequential gating strategy was employed to identify cell populations based on surface marker expression. The antibodies used were Gal-3-FITC, CD19-PE, HLA-DR PEcy7, CD14-APC, CD16-pacific-blue, CD11b perCPcy5.5, CD62L-PE-cy7, CD4-AF488, CD56-PE, CD147-perCPcy5.5, CD25-APC, CD8-AF700, CD44-PAC blue, CD3-PE-cy7, CD163-APC, CD206-PE, CD209-perCP5.5 all from Biolegend except Gal-3-FITC which was from Cedarlane. Macrophages were identified as having high side scatter properties and HLA-DR positivity. Gal-3 expression (mean fluorescence intensity, MFI) was determined from the macrophage gate. Macrophage subsets were classed as M_1, M_{2a} and M_{2c} based on the relative expression of CD206 and CD163 as follows; (M_1 $CD206^-/CD163^-$, M_{2a} $CD206^+/CD163^-$, M_{2c} $CD206^+/CD163^+$). In the HLA-DR-ve gate the relative % of neutrophils ($CD19^-/CD16^+$), B cells ($CD19^+$), natural killer (NK) cells ($CD56^+$), NK T-cells (NKT) cells ($CD56^+/CD3^+$), CD4 T cells ($CD3^+/CD4^+$) and CD8 T cells ($CD3^+/CD8^+$) and regulatory T cells (Tregs) ($CD3^+/CD4^+/CD25^+/CD147^-$) were recorded.

Plasma pharmacodynamic biomarkers

The following pre-specified biomarkers were evaluated in samples of plasma based on the basis that these proteins are known to be involved in Gal-3 pathways and/or suggested to be putative biomarkers in IPF: amphiregulin, chemokine (C-C motif) ligand 2 (CCL2) [monocyte chemoattractant protein 1], CCL5 [Regulated on Activation, Normal T Expressed and Secreted], CCL18 [pulmonary and activation-regulated chemokine], CCL26 [eotaxin], chemokine (C-X-C motif) ligand 1 (CXCL1) [growth-regulated oncogene-α], CXCL8 [interleukin-8 (IL-8)], CXCL10 [IP-10], epidermal growth factor (EGF), Galectin-1 (Gal-1), Gal-3, hepatocyte growth factor (HGF), interferon-γ (IFNγ), IL-1 receptor antagonists (IL-1ra), IL-10, IL-12, IL-13, IL-25, IL-33, macrophage migration inhibitory factor (MIF), matrix metalloproteinase-1 (MMP-1), MMP-7, MMP-8, osteopontin, periostin, plasminogen activator inhinitor-1 (PAI-1), pentraxin-3 (PTX3), surfactant protein D (SP-D), tissue inhibitor of metalloproteinases 1 (TIMP1), tumor necrosis factor-α (TNFα), vascular endothelial growth factor (VEGF) and YKL-40 [chitinase-3-like protein 1]. A disease relevance score was retrospectively assigned to these biomarkers to rank them for importance in IPF progression (supplementary table S2).

RESULTS

BAL cell classification and quantification

HLA-DR positive alveolar macrophages were subclassified into M_1, M_{2a} and M_{2c} based on CD163 and CD206 expression (supplemental figure S2). There was a trend for an increase in the relative percentage of M_{2c} cells and corresponding decrease in the percentage of M_{2a} cells following treatment with TD139 in the high dose group. Although baseline frequencies showed some variation between groups there was no change in the abundance or frequencies of CD4+ or $CD8^+$ T cells, Tregs, NK, NKT, B cells, neutrophils or monocytes or the

percentage of inducible or resident monocyte populations with TD139 in any dose groups (supplemental figure S4).

SUPPLEMENTAL TABLE 1 Inclusion and exclusion criteria

Part 1 – Healthy subjects

Inclusion criteria

- Healthy male subjects aged between 18 and 55 years of age.
- Male subject willing to use a condom, if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the subject) from the Day 1 dose of study medication until 3 months afterwards.
- Subject with a body weight of at least 50 kg and a body mass index (BMI) within the range of 18-35 kg/m^2. BMI = Body weight (kg) / [Height (m)]^2.
- Subject with no clinically significant abnormal serum biochemistry, haematology and urine examination values within 28 days of the Day 1 dose of study medication.
- Subject with a negative urinary drugs of abuse screen, determined within 28 days of the Day 1 dose of study medication, (N.B. a positive alcohol result may be repeated at the discretion of the Investigator).
- Subject with negative human immunodeficiency virus (HIV) and hepatitis B surface antigen (Hep B) and hepatitis C virus antibody (Hep C) results.
- Subject with no clinically significant abnormalities in 12-lead ECG determined within 28 days of the Day 1 dose of study medication.
- Subjects were non-smokers or former smokers (having ceased smoking for at least 6 months).
- Subjects with no clinically significant impairment in oxygen saturation.
- Subject satisfied a medical examiner about their fitness to participate in the study.
- Subject provided written informed consent to participate in the study.
- Subject was available to complete the study (including all follow up visits).
- Healthy male non-smoker or a former smoker (having ceased smoking for at least 6 months) aged between 18 and 55 years, weighing ≥ 50 kg with a body mass index (BMI) of 18-35 kg/m2.
- No history of hypersensitivity (anaphylaxis, angioedema) to any drug, or allergy, significant adverse reaction to nicotine, cholinergic drugs, drugs with a similar chemical structure, or drugs similar to the investigational drug.

Exclusion criteria

- A clinically significant illness or surgery within 8 weeks prior to the Day 1 dose of study medication.
- Significant medical history that, in the Investigator's opinion, may have adversely affected participation.
- History of allergy or significant adverse reaction to drugs similar to the investigational drug, to nicotine, or to cholinergic drugs or to any drugs with a similar chemical structure.
- History of hypersensitivity (anaphylaxis, angioedema) to any drug.
- Use of any drug known to induce or inhibit hepatic drug metabolism, within 30 days prior to the Day 1 dose of study medication.

- Use of medications known to prolong QT/QTc interval within 14 days prior to the Day 1 dose of study medication.
- Any clinically significant findings of physical examination or laboratory findings at screening.
- A clinically significant history of drug or alcohol abuse.
- Receipt of regular/over-the-counter medication within 14 days of the Day 1 dose of study medication that may have had an impact on the safety and objectives of the study (at the Investigator's discretion).
- Evidence of renal, hepatic, central nervous system, respiratory, cardiovascular or metabolic dysfunction.
- Inability to communicate well with the Investigator (i.e., language problem, poor mental development or impaired cerebral function).
- Participation in a New Chemical Entity clinical study within the previous 4 months or a marketed drug clinical study within the previous 3 months. (N.B. washout period between studies is defined as the period of time elapsed between the last dose of the previous study and the first dose of the next study).
- Donation of 450 mL or more blood within the previous 3 months.

Part 2 – IPF patients

Inclusion criteria

- males or females of non-childbearing potential with IPF.
- age between 45 and 85 years of age.
- FVC $\geq$ 45% predicted and an FEV1/FVC ratio $\geq$ 0.7.
- Oxygen saturation >90% by pulse oximetry while breathing ambient air at rest.
- diffusing capacity (DLCO) >25%.
- a clinical diagnosis consistent with IPF prior to screening (based on ATS/ERS/JRS/ALAT consensus criteria confirmed at a multidisciplinary team meeting where the HRCT findings will have been discussed with a radiologist).
- able to undergo bronchoalveolar lavage (BAL).
- able to provide written informed consent to participate in the study.
- human immunodeficiency virus (HIV) and hepatitis B surface antigen (Hep B) and hepatitis C virus antibody (Hep C) negative confirmed at screening.
- no clinically significant abnormalities in 12-lead electrocardiogram (ECG) determined within 28 days of the first dose.
- negative urinary drugs of abuse screen, determined within 28 days of the first dose.

Exclusion criteria

- Any condition that makes the patient at unacceptable risk for bronchoscopy.
- Active cigarette smoking (defined as smoking more than 3 cigarettes daily within the last 6 months).
- Presence of a significant co-morbidity felt to limit life expectancy to less than 12 months.
- HRCT pattern showing emphysema more than the extent of fibrosis of the lung area conducted within 12 months of Day 1.
- Evidence of renal, hepatic, central nervous system, or metabolic dysfunction.
- Evidence of poorly controlled diabetes mellitus (defined as a HbA1c of > 59 mmol/mol [7.5%]).

- Use of systemic immunosuppressants within 30 days of dosing.
- Subjects currently receiving oral corticosteroids, cytotoxic drugs (e.g. chlorambucil, azathioprine, cyclophosphamide, methotrexate), antifibrotic drugs (e.g. pirfenidone), vasodilator therapies for pulmonary hypertension (e.g. bosentan), unapproved (e.g. INF-γ, penicillamine, cyclosporine, mycophenolate) and/or investigational therapies for IPF or administration of such therapies within 4 weeks of initial screening. A current inhaled steroid dose of ≤1000 micrograms beclomethasone dipropionate (BDP) equivalent per day is acceptable if the dose is anticipated to remain stable during the study.
- History of malignancy, including carcinoma during the preceding five years.
- History of, or current asthma.

Participation in a clinical study of an unlicensed drug in the previous 4 months, or a marketed drug study within the previous 3 months.

N.B. washout period between trials defined as the period of time elapsed between the last dose of the previous study and the first dose of the next study.

SUPPLEMENTAL TABLE 2 – Plasma pharmacodynamic biomarker relevance criteria

Biomarker	***Bayesian probability of effect (10 mg)**	**Disease Relevance Criteria**				
		1	**2**	**3**	**4**	**5**
PDGF-BB	99.86	(1)	(2,3)	(2,4)	(2,3)	(5)
PAI-1	99.43	(6,7)	(8-10)	(7,8,11)	(8,9)	(12)
Galectin-3	98.63	(13)	(13,14)	(13,14)	(13,14)	
CCL18 (PARC)	98.59	(15)	(15,16)	(17)	(15)	(18)
YKL-40 (CHI3L1)	98.13	(19)	(19-21)	(19)	(19-21)	
MMP-8	95.60		(22)	(22)	(22)	
PDGF-AA	94.13	(1)	(23)	(4)	(23)	(5)
HGF	93.79			(24)		
MMP-1	91.55		(25,26)	(25)	(25,26)	
MIF	<90.0		(27)	(27,28)	(27)	
CCL2 (MCP-1)	<90.0		(29)	(30)	(29)	(18)
TIMP1	<90.0		(26,31)	(26)	(26,31)	(5)
MMP-7	<90.0	(25)	(25,26)	(25)	(25,26)	
IL-13	<90.0		(32)	(33)	(32)	(18)
SP-D	<90.0	(34)	(34,35)	(35)	(34,35)	
CCL5 (RANTES)	<90.0		(36)	(36)	(36)	
Osteopontin	<90.0		(37)	(37)	(37)	
Galectin-1	<90.0		(38,39)	(39)	(38,39)	
Periostin	<90.0		(40,41)	(40,42)	(40,41)	
Pentraxin-3 (PTX-3)	<90.0		(43)		(44)	
IL-1ra	<90.0			(45)		

The plasma biomarkers measured were given a disease relevance score by applying 5 criteria: (1) correlation with disease outcome, (2) validated fibrosis effector mechanism *in vitro* and *in vivo*, (3) elevated systemically or in the lung of IPF patients, (4) expressed in key disease cells (fibroblasts/macrophages) and (5) link to the mechanism of action of Nintedanib (approved IPF treatment). A biomarker was considered high

relevance if 4-5 criteria were met; medium relevance if 2-3 criteria met, and low if 0-1 criteria met (see supplemental references for supporting evidence). Results from an analysis of covariance model including effects for treatment group and baseline value with the Bayesian probability of effect of 10 mg TD139 group *vs.* placebo are shown*.

SUPPLEMENTAL TABLE 3 Plasma pharmacodynamic biomarker raw data

Biomarker	Day	Mean (SD) pg/ml			
		Placebo	0.3 mg	3 mg	10 mg
CCL2 (MCP-1)	1	111.3 (79.75)	112.2 (37.78)	164.5 (67.02)	357.1 (375.36)
	14	148.7 (85.53)	133.8 (15.06)	137.6 (63.21)	270.3 (348.45)
CCL5 (RANTES)	1	81617.7 (82526.36)	125883.5 (63253.82)	53815.5 (21806.74)	41872.9 (21417.78)
	14	83297.5 (87964.32)	47658.1 (27017.8)	75675.6 (92820.57)	33994.2 (29435.9)
CCL18 (PARC)	1	88328.6 (39842.99)	91072.2 (15471.37)	68752.8 (17747.82)	80185.3 (38709.52)
	14	88335.6 (35582.91)	79733.8 (25166.88)	73022.7 (33487.12)	53456.8 (11339.99)
Galectin-1	1	56708.6 (28698.58)	48231.4 (20322.42)	53988.6 (11006.04)	55630.8 (24962.77)
	14	52118.8 (16994.43)	41996.4 (7357.87)	60416.7 (17348.65)	48409.7 (21339.06)
Galectin-3	1	10900.4 (10123.84)	12934.3 (3948.52)	8269.5 (2684.8)	10447.8 (6157.76)
	14	8938.9 (3208.78)	13290.4 (4951.24)	9298.5 (3499.54)	4486.3 (697.25)
Galectin-3 (BAL)	1	16475.9 (5917.25)	15310.4 (15593.37)	19995.8 (8088)	30449.8 (6574.47)
	14	13590.2 (5754.29)	7794.8 (2990.48)	8058.6 (3001.05)	5237 (2033.86)
HGF	1	143.1 (66.58)	105.7 (15.28)	140.3 (29.60)	244.7 (172.4)
	14	144.2 (49.74)	109.8 (23.24)	159.1 (110.2)	131.2 (44.47)
IL-1ra	1	1335.8 (2143.31)	580.8 (340.16)	764.4 (216.85)	2401.1 (3495.04)
	14	559.4 (221.94)	548.3 (279.69)	709.8 (338.05)	1992.9 (2627.15)
IL-13	1	1350.5 (235.89)	1385.7 (253.53)	1784.3 (751.45)	1368.5 (178.84)
	14	1458.1 (493.82)	1219.4 (155.7)	1476.6 (140.99)	1358.8 (149.34)
MIF	1	54503.1 (54799.79)	69021.4 (27636.58)	47342.3 (30164.83)	70206.1 (64466.35)
	14	53472.9 (44175.64)	65871.3 (42284.53)	38884 (35236.82)	39660.6 (57202.1)
MMP-1	1	1189.2 (1137.8)	1187.8 (1196.68)	1858.7 (798.94)	741.6 (237.64)
	14	1842.5 (2072.58)	535.1 (449.22)	1494.4 (647.67)	537.5 (257.55)
MMP-7	1	3951.3 (8150.31)	560.7 (259.3)	1991.7 (2160.04)	1171.7 (887.93)
	14	6056.7 (14116.38)	447.6 (205.21)	1849.2 (2244.12)	1130.6 (939.62)
MMP-8	1	10716.6 (28261)	1412.8 (422.24)	1969.4 (844.47)	139102.5 (306988.65)
	14	2222.3 (1575.24)	1911.5 (1577.82)	2263.3 (2428.78)	44436.7 (97465.67)

Osteopontin	1	68739.2 (57730.49)	34241.8 (13563.92)	38808.4 (22367.52)	60286 (48443.67)
	14	68125.8 (74480.11)	37756 (20451.51)	31032.4 (13230.48)	48630.5 (37737.51)
PAI-1	1	50298.9 (43195.44)	97468.4 (52279.19)	41170 (9420.67)	50442.3 (30219.02)
	14	80727.1 (55809.77)	45736.7 (29863.26)	45744.4 (44222.1)	23122.2 (12659.84)
PDGF-AA	1	2140.6 (1391.8)	1996.4 (1004.22)	4207 (3264.35)	4019.9 (1276.63)
	14	4104 (3200.22)	1299.7 (754.57)	4075.9 (5511.46)	2556.7 (987.79)
PDGF-BB	1	2760.4 (2897.46)	3624 (2279.74)	1791.6 (912.36)	1749.9 (659.84)
	14	3667.3 (2678.96)	1519 (1034.92)	2201.9 (2935.52)	1131.4 (1190.78)
Periostin	1	134749.5 (23951.64)	109819 (15283.82)	143649.3 (21734.75)	117090.8 (31288.75)
	14	131484 (24870.01)	125952.3 (17487.63)	142019.1 (30390.08)	108663.7 (24686.82)
Pentraxin-3 (PTX3)	1	1379 (2796.23)	170.3 (335.06)	938.4 (738.13)	7055.8 (13193.33)
	14	480.4 (402.83)	116.3 (214.7)	629.9 (581.55)	2534.7 (4451.2)
SP-D	1	58685.4 (33447.61)	35595.9 (11189.97)	84308.4 (38808.22)	28901.4 (12165.25)
	14	68522 (43801.19)	38987.4 (10734.36)	91473.6 (43437.58)	30347 (3552.88)
TIMP1	1	44543.7 (8450.21)	38898.1 (3364.27)	52018.3 (1712.3)	43880.4 (14514.68)
	14	44090.5 (6162.09)	38084.3 (4735.81)	49880.6 (1032.27)	45458.7 (11908.04)
YKL-40 (CHI3L1)	1	141225.5 (100517.87)	50518.5 (26360.63)	417206.4 (144925.81)	146407.5 (30068.05)
	14	110951.3 (78338.33)	45140.2 (34041.85)	146819.5 (40114.19)	79975.5 (38590.77)

For the following biomarkers, the majority of values were below the lower level of quantification and therefore were not analysed: amphiregulin, CCL26 (eotaxin), CXCL1 (GROα), CXCL10 (IP-10), EGF, IL-8, IL-10, IL-25, IL-33, IFNγ and TNFα. BAL, bronchoalveolar lavage.

SUPPLEMENTAL TABLE 4 Plasma pharmacodynamic biomarker analysis including dose as a continuous covariate

Dependent	**Estimate**	**SE**	**t-statistic**	**p-value**
PDGF-BB	-18.06	7.43	-2.43	0.024
PAI-1	-12.25	5.42	-2.26	0.035
Galectin-3 (BAL)	-4.19	1.72	-2.44	0.024
Galectin-3	-6.45	2.60	-2.48	0.022
CCL18 (PARC)	-2.88	1.39	-2.08	0.050
YKL-40 (CHI3L1)	-2.88	1.20	-2.41	0.026
MMP-8	-11.55	6.37	-1.81	0.084
PDGF-AA	-8.04	7.91	-1.02	0.321
HGF	-3.44	2.15	-1.6	0.125
MMP-1	-7.11	6.69	-1.06	0.301
CCL2 (MCP-1)	-6.35	5.18	-1.23	0.234
CCL5 (RANTES)	-3.93	4.35	-0.9	0.376
MIF	-9.92	9.51	-1.04	0.309
MMP-7	-5.22	6.87	-0.76	0.456
PTX-3	-5.14	6.72	-0.76	0.455
Osteopontin	-2.40	2.67	-0.9	0.379
Periostin	-1.15	0.63	-1.81	0.084
Galectin-1	-1.17	1.94	-0.6	0.552
IL-13	-0.17	1.04	-0.16	0.876
SP-D	0.56	1.84	0.31	0.763
IL-1ra	0.60	2.32	0.26	0.799
TIMP-1	0.82	0.69	1.2	0.244

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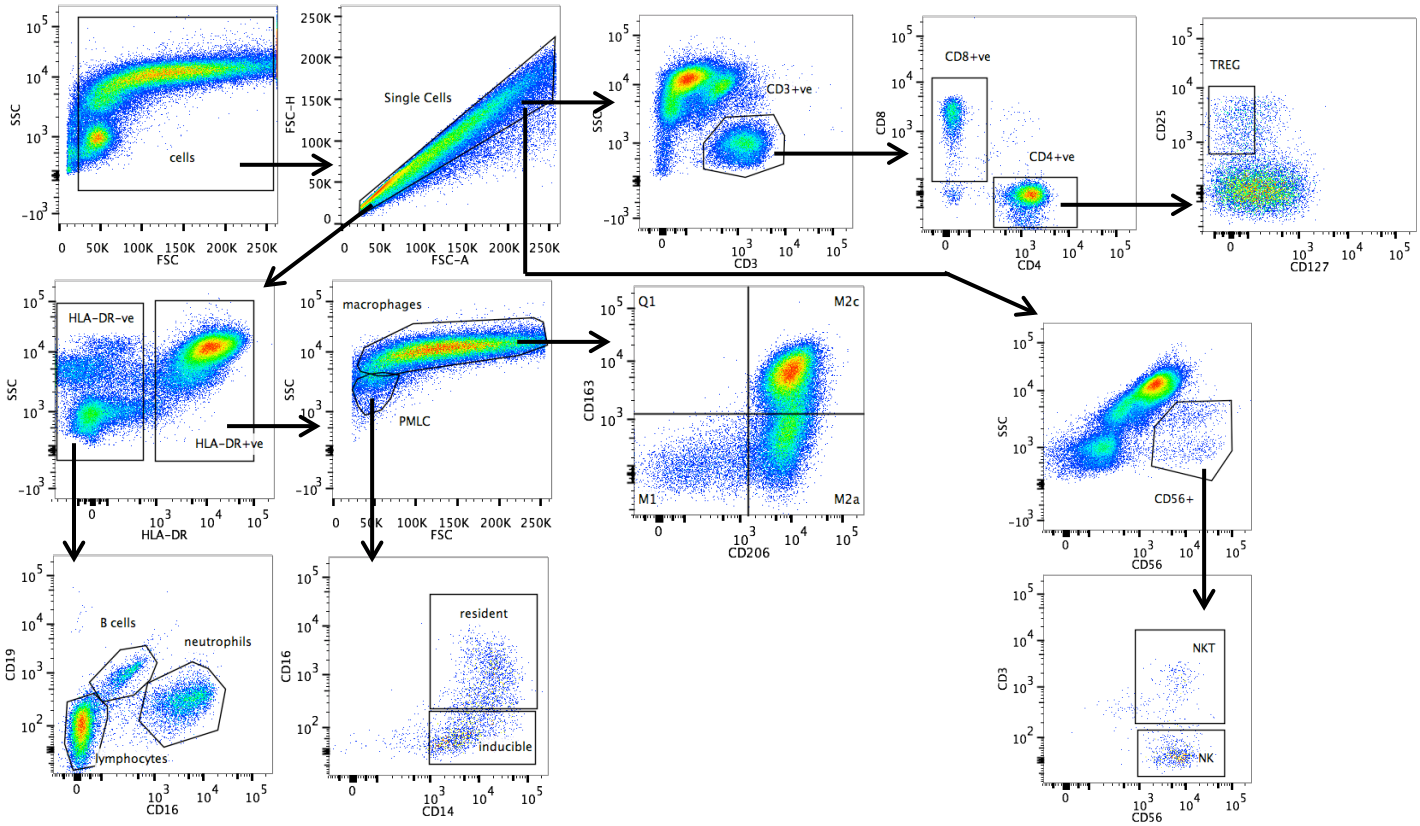

Figure S1. Flow cytometry analysis of BAL cells: gating strategy. After exclusion of doublets and debris, a sequential gating strategy was then employed to first identify populations expressing specific markers: $CD3^+/CD4^+$ and $CD3^+/CD8^+$ T cells. Tregs ($CD3^+/CD4^+/CD25^+/CD147^-$). HLA-$DR^+$ cells were subdivided into alveolar macrophages (high side scatter $CD163^+/CD206^+$ (M_{2c}), $CD163_{lo}/CD206^+$ (M_{2a}) and $CD163_{lo}/CD206_{lo}$ (M_1)) and monocytes (HLA-DR^+ low side scatter) were classified as resident ($CD14^+/CD16^+$) and recruited ($CD14^+/CD16^-$). Neutrophils ($HLA\text{-}DR^-/CD16^+$), B cells ($HLA\text{-}DR^-/CD19^+$), NK ($CD56^+/CD3^-$), NKT cells ($CD56^+/CD3^+$).

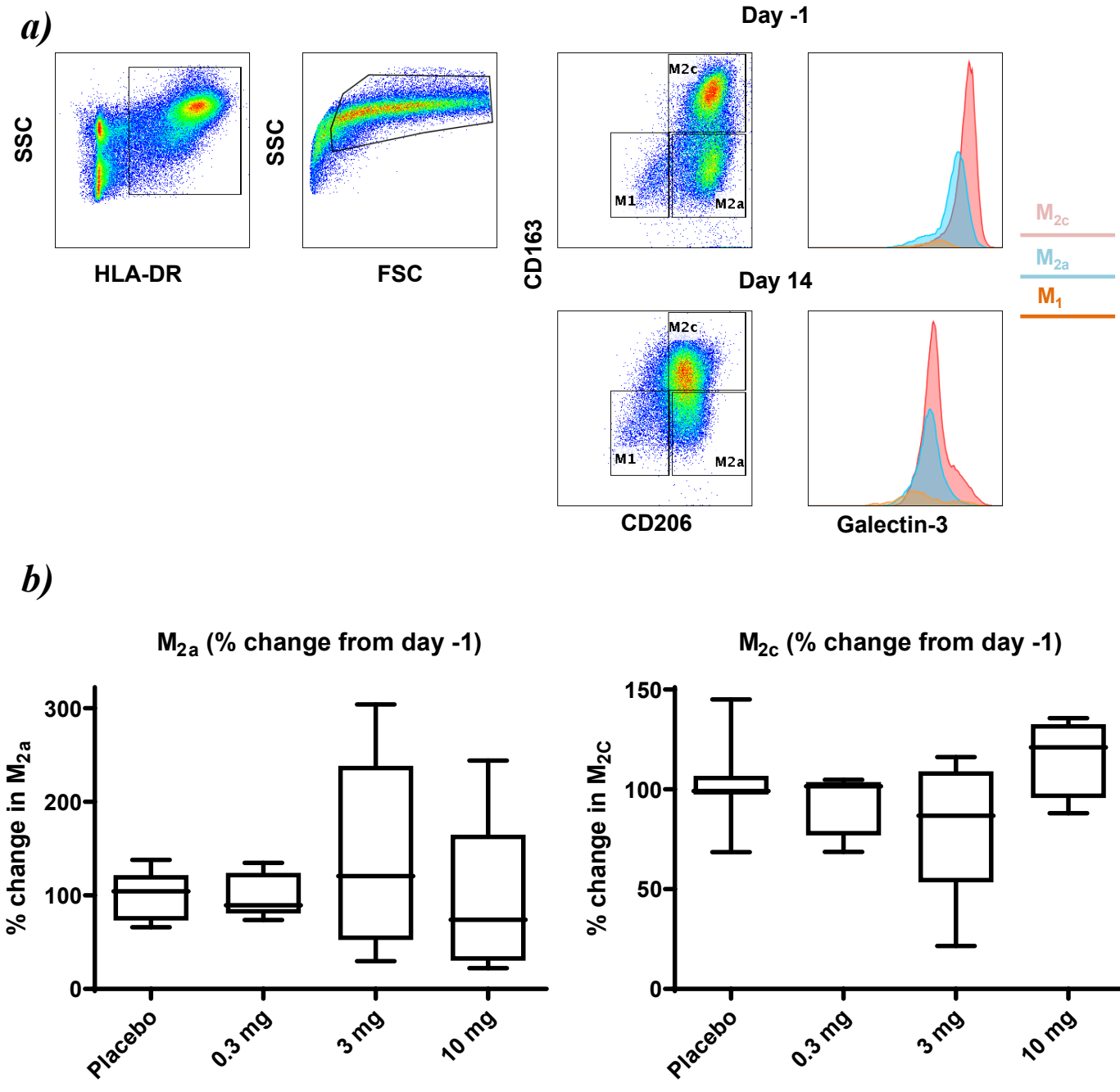

Figure S2. Flow cytometry analysis of BAL cells: Alveolar macrophage gating strategy. a) Representative alveolar macrophage gating strategy in a single patient pre-dose at day-1 and at day 14 at 2 h post-dose following 10 mg TD139 administration for 14 days. HLA-DR$^+$ macrophages were classified as M_1, M_{2a} or M_{2c} based on CD206 and CD163 expression. Histograms show Galectin-3 expression on the different subsets M_{2a} pink, M_{2c} blue and M_1 orange on day -1 and day 14. b) % change in prevalence of M_{2a} and M_{2c} subsets from day -1 to day 14 in the different dose groups.

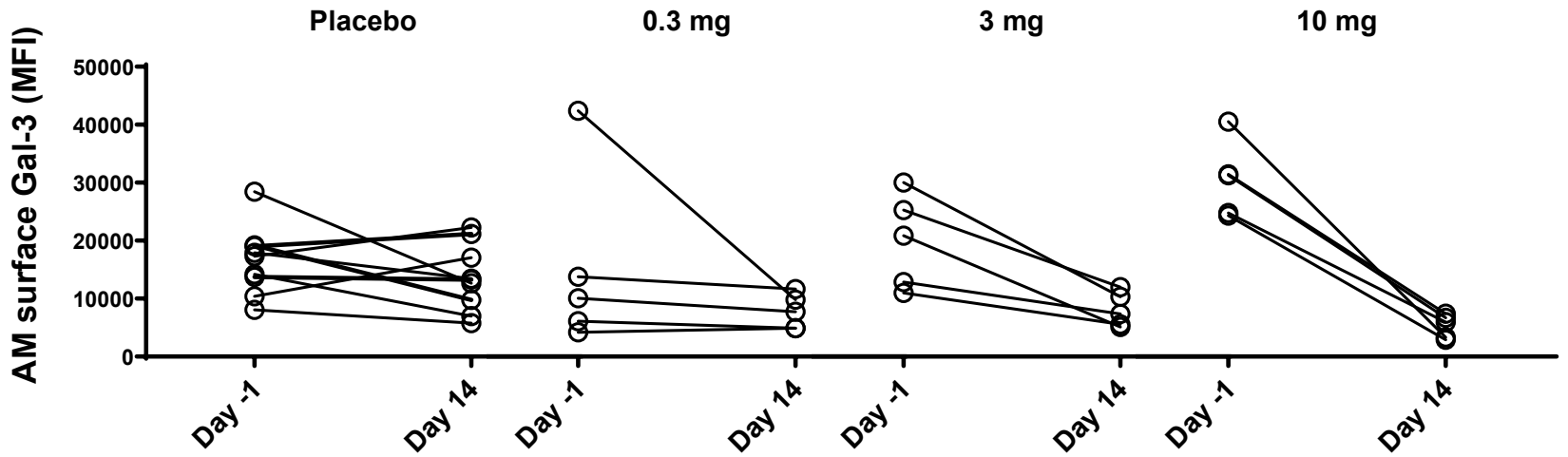

Figure S3. Galectin-3 changes in alveolar macrophages. Absolute change in surface macrophage galectin-3 on day -1 pre-dose or at 2 h post-administration of placebo, 0.3 mg, 3 mg and 10 mg of TD139 on day 14. AM, alveolar macrophages; MFI, mean fluorescence intensity.

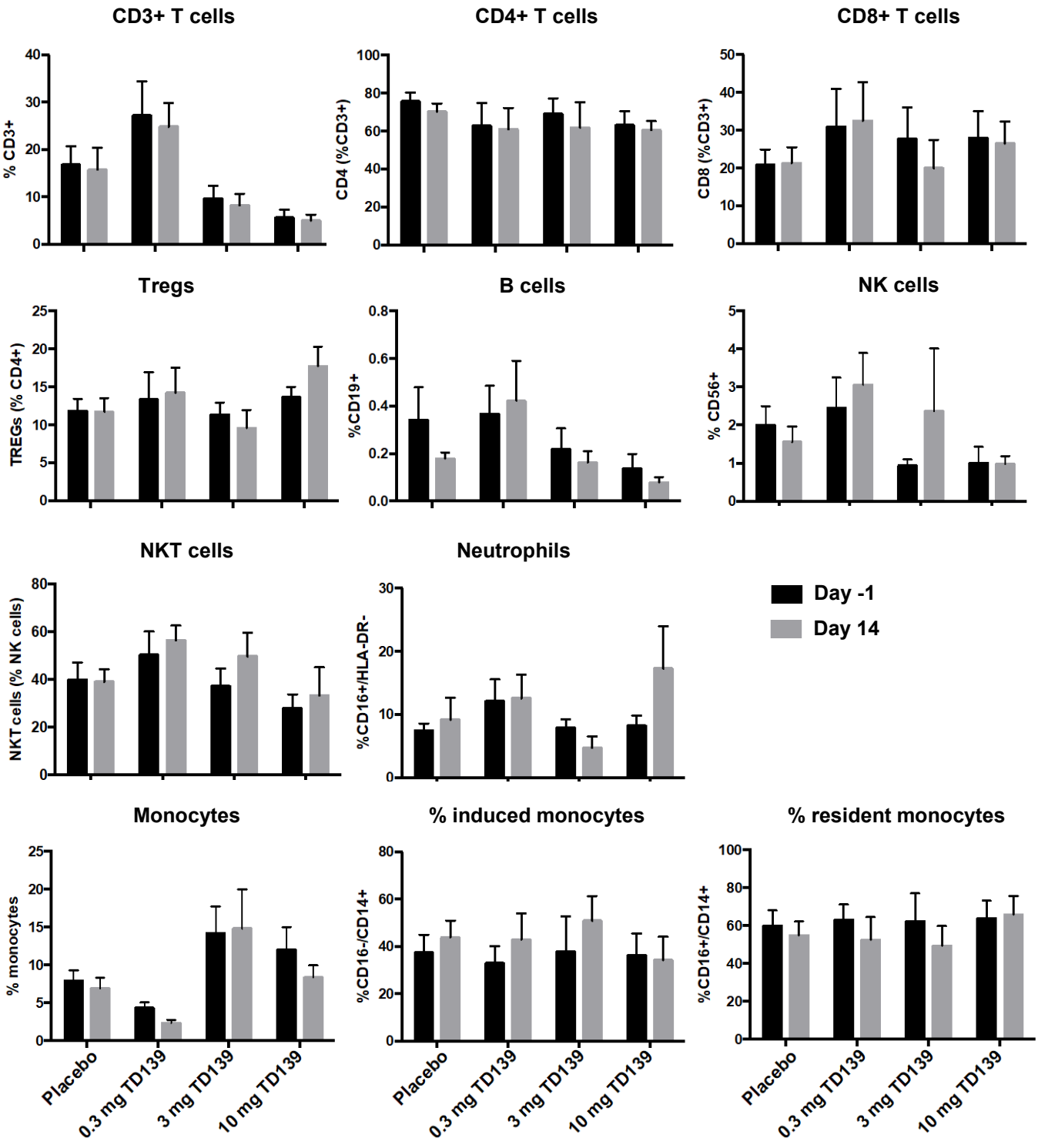

Figure S4. Flow cytometric analysis of immune cells in BAL. Cell types in BAL were identified as described in supplemental methods and outlined in figure S1. Results represent mean ± SEM from day -1 and day 14 samples from placebo and 0.3 mg, 3 mg and 10 mg TD139 groups.