ERJ Express. Published on December 9, 2010 as doi: 10.1183/09031936.00005610

Antiviral and lung protective activity of a novel RSV fusion inhibitor in a mouse model

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Abstract

Respiratory syncytial virus (RSV) causes bronchiolitis in young children and common colds in adults. There is no licensed vaccine, and prophylactic treatment with palivizumab is very expensive and limited to high risk infants. Ribavirin is used as an antiviral treatment in infants and immunosuppressed patients, and its use is limited due to side effects, toxicity to the recipient and staff and evidence of marginal clinical efficacy. We therefore studied the *in vivo* kinetics, antiviral and protective properties of a novel candidate for RSV disease treatment.

The drug is a small molecule (TMC353121) discovered by screening for fusion inhibitory properties against RSV in a cellular infection model. The pharmacokinetics of TMC353121 was studied in BALB/c mice and antiviral effects determined by testing viral loads in lung tissue by quantitative RT-PCR and plaque assay after intranasal RSV infection.

At doses between 0.25-10 mg/kg, TMC353121 significantly reduced viral load, BAL cell accumulation and the severity of lung histopathological change after infection. Treatment remained effective if started within 48 hours post-infection, but was ineffective thereafter.

Therefore, TMC353121 is a novel potent antiviral drug, *in vivo* reducing RSV replication and inhibiting consequential lung inflammation, with a great potential for further clinical development.

Keywords:

Anti-viral therapy, fusion inhibitor, Respiratory Syncytial Virus

Introduction

Respiratory Syncytial Virus (RSV) is a causative agent of common colds in adults, and is responsible for severe bronchiolitis and viral pneumonia in infants and young children [1;2]. Viral bronchiolitis is one of the leading causes of hospitalization of infants under one year [3], associated with the development of recurrent wheezing or asthma in later childhood [4]. Children born prematurely as well as those with chronic lung disease (CLD) or congenital heart disease (CHD) are at highest risk for severe disease and hospitalization due to RSV. In elderly persons, RSV infection is associated with lower respiratory tract symptoms, leading to complications when concomitant conditions are present [5]. The disease burden of RSV infection in elderly and in adults at risk is similar to that of non-pandemic influenza in a population with high prevalence of vaccination for influenza [6].

Currently, three drugs have been approved for use against RSV infection but the treatment of RSV disease remains mainly symptomatic. Ribavirin is used for serious RSV infections in infants with severe bronchiolitis and in immunocompromised patients. However, its use is limited due to highly variable efficacy and toxicity risks [7]. RespiGam® (not longer available) and Synagis® (palivizumab), polyclonal and monoclonal antibodies respectively, were designed for prophylaxis, but are very expensive and hence, use is limited in many settings by financial considerations [8;9]. Motavizumab (Numax®) – an improved monoclonal antibody under consideration for market approval - is expected to have therapeutic indications similar to palivizumab [10].

Attempts to develop a safe and effective RSV vaccine have been unsuccessful to this date. Formalin-inactivated vaccines did not protect against natural infection and, even led to

enhanced disease [11]. Live-attenuated vaccines have been tried with limited success. Clearly, there is a need for an efficacious, non-toxic and easy to administer drug against RSV infection, with both prophylactic and therapeutic potency.

One potential therapeutic target for an antiviral drug is the RSV entry process into lung epithelial cells. The F (fusion) protein is crucial in this process and thus plays a critical role in establishing an infection. Several small molecule inhibitors of the fusion process have been identified [12-17], but most were discontinued for scientific or strategic reasons [18].

The RSV fusion inhibitor TMC353121 has been developed from the precursor molecule JNJ-2408068 [17] using a molecular modelling approach. It maintains high activity (pEC50=9.9) and low cytotoxicity, while presenting a shorter retention time in the lung ($T_{1/2}$ lung=25h) [19]. The mechanism of action of TMC353121 has been confirmed in time-of-addition and in *in vitro* resistant mutant selection studies.TMC353121 was found to inhibit RSV by preventing both virus-cell fusion and syncytia formation by causing a local disturbance of the natural 6-helix bundle conformation of RSV-F protein [20].

In this article, we show that TMC353121 administered prophylactically or therapeutically, has potent anti-viral properties *in vivo* in a BALB/c mice model and protects against lung infection and virus-induced inflammation.

Materials & Methods

Animals and drug administration

Inbred 8-12 week old female BALB/c mice were purchased from Harlan Olac Ltd (UK), and maintained in pathogen-free conditions. The human RSV A2 strain was plaque-purified and selected in HEp-2 cells. The protocols were carried out under licence from the UK Home Office.

TMC353121 [19;21] was administered intravenously (iv) in saline at doses between 0.25-10 mg/kg, and at various times in relation to the RSV infection (Figure 1A). Mice were infected with 2 x 10^6 PFU of plaque purified human strain RSV A2 (100µl intranasally). Individual body weight was used to monitor animal health and response to infection, and was recorded daily.

RSV detection in the lung

a) qRT-PCR

RNA was extracted from homogenised lung tissue using the RNeasy kit (Qiagen) following the manufacturer's instructions. Total RNA concentration was determined by a spectrophotometer at 260nm, and cDNA was generated using random primers (Promega) and the Omniscript RT-kit (Qiagen).

Viral load measurements were conducted using RT-PCR, specific for the RSV L-gene, using TaqMan chemistry (Applied biosystems) and the ABI Prism 7700 Sequence Detection System. The PCR assay consisted of 50°C for 5min and 95°C for 10min, prior to 40 cycles of 15s at 96°C and 1min at 60°C. QuantiTect Probe PCR master mix (Qiagen), 900 nM forward

primer (GAA CTC AGT GTA GGT AGA ATG TTT GCA), 300nM reverse primer (TTC AGC TAT CAT TTT CTC TGC CAA T) and 100nM probe (*6FAM* – TTTGAACCTGTCTGAACATTCCCGGTT –*TAMRA*) were used. The limit of detection was 10 copies of RSV-L gene. Quantification was performed from standard curves of a pcDNA3 plasmid carrying a fragment of the RSV L-gene. Results are expressed as RSV-L gene copy numbers and transformed in $\log_{10}$ values. The antiviral effect of TMC353121 is expressed as median log reduction versus untreated control ($\Delta\log_{10}$).

b) Plaque assay

Lungs harvested 4 days after virus challenge were homogenized, centrifuged for 4min at 700g, and supernatants were titrated in doubling dilutions on HEp-2 cell monolayers in 96-well plates. Twenty-four hours later, cells were fixed with methanol and incubated with biotinylated anti-RSV antibody (Biogenesis, UK) followed by peroxidase-conjugated Avidin (Sigma). DAB substrate (Sigma Fast Tablets) was added and plaques enumerated by light microscopy. Results are expressed as PFU/ lung and the antiviral effect expressed as $\Delta\log_{10}$ in comparison to control group.

Histopathology

In some studies, unwashed lungs were formalin-fixed and embedded in paraffin, sectioned, and stained with hematoxylin-eosin (H-E). Lungs were evaluated for inflammatory infiltrates and graded similarly to the classification described by Ponnuraj *et al*.[22]. The method was validated in our lab. Three sections from the upper, medium and lower lung were scored blindly in a random order according to the degree of inflammation in interstitium, alveoli, surrounding airways and vessels. A value of 0 (none), 1 (minimal), 2 (mild), 3 (moderate), or 4 (severe) was assigned to each site. The sum of these scores for each animal was used as a

total lung inflammation score. Random slides were scored by a second observer and results compared when sample codes revealed.

BAL cell counting

Bronchoalveolar lavage (BAL) was performed by infusing the lungs with 2% lidocaine in Earle's Balanced Salt Solution (EBSS) containing 2% bovine serum albumin (BSA). Total viable BAL cells were counted from samples diluted in Trypan blue (0.4%), using light microscopy.

For differential counts, 100µl of BAL was spun onto slides at 100g for 5min; slides were dried, fixed with methanol and stained with H-E. 300 cells were counted from each sample and macrophages, polymorphs (paying attention to eosinophils), and lymphocytes were scored.

TMC353121 bioanalysis

The cryotubes for TMC353121 bioanalysis were decontaminated for possible live RSV virus by the addition of three parts of absolute ethanol for one part of sample. Serum and tissue samples were analyzed individually for TMC353121 by means of a qualified research LC-MS/MS method. The limit of detection for TMC353121 was 1ng/g for lung tissue and 0.8ng/ml for serum.

Luminex

Cytokine levels in the BAL were quantified using multiplex immunoassay (MILLIPLEX MAP Kit, Millipore) according to the manufacturer's instructions. BAL samples were assayed with appropriate standards and controls for each cytokine. Beads were added to duplicate samples and the plates were incubated overnight at 4°C. Detection antibodies were

added and plates incubated on a shaker followed by addition of streptavidin-phycoerythrin to each well. After washing, sheath fluid (PBS 1X) was added and the plates were run on Luminex 100^{TM}. The Median Fluorescent Intensity (MFI) was analysed using StarStation and the concentrations for each cytokine calculated. Detection limit for each cytokine was 3.2pg/ml.

Data analysis

Viral loads were expressed as median log viral load. Changes in viral load (Δ log) were calculated as difference between median log viral logs between untreated and treated groups.

GraphPad Prism software was used for One Way ANOVA or Kruskal Wallis test to investigate differences between groups. Comparisons of treatments were conducted using Dunn`s or Tukey`s tests. A trend of a dose-response curve was evaluated using linear trend test. General linear mixed model was used for the analysis of PK data and for body weight change over time. Cytokine concentrations were analysed using One Way ANOVA. Statistical tests used are indicated in figure legends.

Experimental design

The antiviral activity of TMC353121 was analyzed in five studies. The compound was administered either before, or after RSV infection, and immunological parameters tested as outcome of such interventions. Drug was delivered via i.v. slow bolus once daily, as a single dose or as multiple doses for successive days. In some studies, different doses of the drug were administered as one single dose, before RSV challenge. Details of the experimental design are presented in Fig. 1A.

Results

Pharmacokinetics of TMC353121 in BALB/c mouse

TMC353121 was administered once, i.v. at 2.5mg/kg or at 0.25mg/kg. Drug levels were determined in lung tissue, serum (Supplementary Figure1), and BAL fluid (data not shown) at different time points. TMC353121 followed multicompartment pharmacokinetics, with a fast decay in serum within the first hour post i.v. injection, followed by a slower decay. The drug was eliminated quickly from the blood resulting in very low blood levels after 24 hours. Lung concentrations were much higher than serum concentrations and in BAL fluid the drug was just above the limit of detection at 8h post injection (data not shown). Very low drug levels could still be detected in the lung five days post-treatment (data not shown).

TMC353121 prevents virus-induced weight loss

Intranasal inoculation with 2×10^6 PFU of RSV on Day 0 resulted in substantial weight loss on Day 6 and 7 post infection (Fig. 1B). Daily administration of TMC353121 before infection (d-5 to d0) or starting from the day of infection (d0 to d+3) was able to significantly abolish the weight loss effect of RSV infection (Fig. 1B). Importantly, a single dose of TMC353121 was able to completely prevent weight loss. On Day 7, a significantly lower weight was observed in the control than in treated groups, whereas no significant differences were shown between treated groups.

TMC353121 reduces viral load in therapeutic and prophylactic administration

Administration of TMC353121 resulted in reduced viral load compared to untreated controls. The median reduction in viral load was 0.94±0.49log (d-5 to d0) and 1.49±1.88log (d0 to d+3) for repetitive administration, compared with 0.4±0.42log reduction for a single administration (Table 1).

Table 1. Antiviral activity of TMC353121 administered in multiple doses. RSV titres were measured by Taqman PCR for the L gene, four days after infection. TMC 353121 was administered by i.v. bolus, at 10mg/kg dose, on indicated days.

***In vivo* protocol**		**TaqMan Lung**		
Study	**Treatment**	**Median log Viral Load (±SD)**	**Δ log [1] (±SD)**	**P [2]**
Study 1				
TMC353121	d-5 to d 0	5.69(±0.54)	0.94(±0.49)	>0.05
	d 0	6.23(±0.47)	0.4(±0.42)	>0.05
	d 0 to d+3	5.14(±2.07)	1.49(±1.88)	**<0.05**
V	d-5 to d+3	6.63(±0.51)	0	NA
Study 2				
TMC353121	d 0 to d+3	4.92(±0.2)	0.76(±0.2)	>0.05
	d+1 to d+3	4.98(±0.2)	0.7(±0.2)	>0.05
	d+2 to d+3	4.64(±1.28)	1.03(±1.28)	**<0.05**
	d+3 to d+3	5.55(±0.55)	0.12(±0.54)	>0.05
V	d 0 to d+3	5.67(±0.5)	0	NA

NA- not applicable, [1] the median log reduction in lung was calculated versus untreated control. . [2] *P* values for differences between viral loads for untreated versus treated groups were calculated using Kruskal Wallis with Dunn`s post-test. 5-6 mice per group were tested.

The second study analyzed the efficacy of TMC353121 in different therapeutic regimens. The compound was administered daily with delays in relation to RSV challenge (Study 2,

Table1, Fig.1A). In this Study, lower level of infection was observed (control group: 5.67±0.5log vs. 6.63±0.51log), in comparison to Study 1; however 0.7-1log reduction in viral load was observed when drug was administered with increasing delay, up to Day 2 after RSV challenge.

TMC353121 has significant antiviral activity in a wide dose range in BALB/c mice

The antiviral efficacy of TMC353121 was explored in a wide dose range in prophylactic regimens with the drug administered as a single dose; 60min prior to RSV challenge (Studies 3 and 4, Table 2).

Table 2. Antiviral activity of different doses of TMC353121 administered once. RSV titer was measured by Taqman PCR and plaque assay four days after infection. TMC 353121 was administered once by i.v. bolus, 60min prior to RSV challenge.

***In vivo* protocol**			**TaqMan**		**Plaque assay**	
Study	**Dose**	**Median log Viral Load (±SD)**	**Δlog[1]**	**P [2]**	**Δlog [1]**	**P [2]**
Study 3						
	10mg/kg	4.65(±0.55)	1.02 (±0.18)	**<0.01**	0.82 (±0.12)	**<0.001**
	5mg/kg	4.98(±1.28)	0.69 (±0.28)	**<0.05**	0.69 (±0.09)	**<0.001**
	2.5mg/kg	4.91(±0.2)	0.75(±0.23)	**<0.01**	0.56 (±0.06)	**<0.001**
	0mg/kg	5.67(±0.5)	0	NA	0	NA
Study 4						
	10mg/kg	4.97(±0.21)	0.58 (±0.69)	>0.05	0.73(±0.05)	**<0.001**
	2.5mg/kg	5.00(±0.19)	0.55 (±0.29)	>0.05	0.59(±0.13)	**<0.001**
	1mg/kg	5.25(±0.29)	0.31 (±0.19)	>0.05	0.56(±0.09)	**<0.001**
	0.25mg/kg	4.99(±0.69)	0.56 (±0.21)	>0.05	0.53(±0.04)	**<0.001**
	0mg/kg	5.55(±0.17)	0	NA	0	NA

NA-not applicable, [1] the median viral log reduction in lung was calculated versus untreated control. [2] P values were calculated using one way ANOVA with Tukeys` multiple comparison tests. 4-6 mice per group were tested.

In both studies, similar titres of RSV viral load were recovered in untreated controls (5.67±0.5log vs.5.55±0.17 log). Based on qRT-PCR, TMC353121 administration within the dose range 0.25-10 mg/kg resulted in 0.5- 1 Δlog viral load reduction compared to untreated groups. These differences were significant for all doses in Study 3, but not in Study4 (Table 2). However, by the plaque assay, all analysed doses of TMC353121 in both studies showed statistically significant reductions in PFU/ lung.

TMC353121 reduces lung inflammation

TMC353121 reduced BAL cell influx in all tested doses. The reduction in cellular infiltration was attributed mainly to macrophages and lymphocytes (Fig 2A). For the dose range 1-10mg/kg, the level of total cell accumulation in BAL was similar to that in non-infected mice whereas the total amount of BAL cells was roughly doubled in RSV-infected untreated mice compared with RSV-infected treated mice (Fig. 2A).

Lung sections were stained with H-E to assess the localization and extent of cell infiltrates. A total histopathological score of 12 for untreated mice indicated a clinically significant RSV infection leading to accumulation of inflammatory cells in the bronchiolar and alveolar space (Fig. 2C). The total score and scores in various compartments (interstitial, alveolar, peribronchiolar, perivascular) of treated mice were significantly reduced compared with the RSV-infected, untreated control group and showed values similar to the non-infected control group (Fig. 2C). Examples of tissue sections stained for histopathology evaluation are presented in Fig. 2B. There was no visual difference between the non-infected control group and the infected, treated group. The improved lung histopathology in the presence of TMC353121 treatment was confirmed in another study, for doses lower than 10mg/kg dose (data not shown).

TMC353121 significantly reduced levels of tested chemokines and cytokines in the BAL (Fig.3). In animals TMC353121-treated and RSV-infected, very significant reduction in inflammatory cytokines (such as TNF-α, IL-6, IL-1β) and chemokines (IP-10, KC, MIP1α, MCP) in BAL was observed 24h later. The same set of cytokines/chemokines was investigated four days later, but there were no more significant differences, and some cytokines were below the limit of detection (Supplementary Fig.2). IP-10 and KC were also present in serum 24h post RSV infection, and were significantly reduced in animals receiving TMC353121 (data not shown).

Discussion

Our results demonstrate potent antiviral activity of the fusion inhibitor TMC353121 in the murine model of RSV infection. The drug was well tolerated, and prevented weight loss characteristic of RSV infection in mice [23]. In these studies, optimal recovery of body weight was seen even after single drug administration (Figure 1B).

Pharmacokinetic studies showed that TMC353121 was present in the serum at concentrations above the target inhibitory concentration (7ng/ml) for the first 24 hours for the 2.5mg dose and less for the 0.25mg/kg dose (Supplementary Figure 1). The targeted plasma concentration was calculated based on the EC_{50} value of 0.07pg/ml obtained in the *in vitro* assay [24], assuming 100% protein binding. The compound is quickly eliminated from the body; however its high potency seems to balance rapid elimination.

The BALB/c mouse model is semi-permissive for RSV, with limited viral replication in lung epithelial cells. In BALB/c mice, RSV first infects epithelial cells in nasal cavities [25] and spreads to the lung where it peaks around Day 4 after infection. Optimally, RSV fusion inhibitors should be present at the time of infection to block virus entry to the cell. The results presented here for TMC353121 in therapeutic regimens (Study 2, Table 1) demonstrate that the drug is able to reduce viral replication when administered two days after RSV challenge, confirming that sustained viral replication does occur in this murine model and can be inhibited even by delayed drug administration. In comparison, other F protein fusion inhibitors such as BMS-433771, and RFI-641 were not effective when used therapeutically in the murine model [13;26], although RFI-641 showed efficacy in the African Green Monkey when administered this way [27].

TMC353121 reduced viral load in a broad dose range: 0.25-10mg/kg, when measured by Taqman and plaque assay. Both methods quantify RSV in different ways; TaqMan RT-PCR measures the number of RSV-L-gene copies (present in both infectious virus and in defective particles), while the plaque assay quantifies fully functional infectious virions. This RT-PCR method does not distinguish between virus used for the challenge and newly produced viral particles therefore additional information from the functional assay is pivotal in accurate data interpretation.

In our study, infected and treated mice showed reduced cell influx into BAL fluid compared to non-treated controls, and a dose-dependent reduction in lymphocyte numbers ($P<0.05$ for linear trend, Figure 2A). Lung histopathology was analysed at the time of the highest lung inflammation in murine model [28] characterized by a pneumonia [25;29] with macrophages and lymphocytes infiltrating perivascular and peribronchiolar tissues [25]. TMC353121-treated mice demonstrated histopathology sections similar to those of normal mice (Figure 2B and 2C).

The protective effect of TMC353121 on lung histopathology correlates with the reduction of viral replication. Although one would consider this type of correlation obvious, it is not always the case. Notably, the anti-F protein monoclonal antibody palivizumab significantly reduces viral load but does not improve lung histopathology and lung inflammation in the cotton rat RSV model [30]. In the same study, Numax showed better antiviral activity and improved lung histopathology than palivizumab. Discrepancy between our and others results may be explained by different mechanisms of action of the compounds. Complexes formed by antibodies with viruses must be cleared by APCs and this process initiates immune pathways leading to release of chemokines attracting other cells (mainly lymphocytes) to the lung. This is reflected in histopathology as cell infiltrates are visible while no RSV particles

can be detected. In contrast, TMC353121 blocks fusion of virus with a target cell and this process is not associated with release of chemokines, therefore other cells are not attracted to the lungs.

As we show here, RSV infection within hours leads to release and production of chemokines and inflammatory cytokines (Group V in Figure 3) resulting in increased numbers of macrophages, lymphocytes and neutrophils. In our model, reduction in IP-10, IL1β, KC, MIP1-α, MCP-1, IL-6 and TNF-α levels in mice treated with TMC353121 (Figure 3) coincides with reduced numbers of BAL cells; mainly in macrophage and lymphocyte subsets (Figure 2A), suggesting pivotal role of chemokines in BAL cell recruitment at early stage of lung inflammation. These mediators return to levels not different from those in normal animals within four days in both non-treated and drug-treated groups (Supplementary Fig.2).

Neonatal immune system is inherently biased towards T-helper-2 responses and RSV infection early in life may further enhance these responses. The ideal intervention for RSV infection would be preventive, but the options are currently limited. The significant antiviral activity, particularly when used in therapeutic regimen and the protective effect on lung inflammation make TMC353121 a promising candidate for treatment of bronchiolitis and respiratory complications caused by RSV infection. Further research is required to better understand the protective mechanisms and to validate this molecule for RSV treatment.

Acknowledgements

The work was sponsored by the Flemish Community IWT 070005 project. B.W. and P.O. were supported by the Wellcome Trust grant nr 071381/Z/03/Z. The authors thank Dr F. Culley for the critical reading of the manuscript.

Figure legends

Fig.1 (A) Protocols used for testing anti-viral properties of TMC353121. "x" indicates i.v. injection of TMC353121 or vehicle (V) used as a negative control. In different experiments, between 5 and 7 mice were used per group. Infection and harvests indicated by arrows. **(B) Example body weight change after RSV infection. Means ±SD are presented**. Control group ("V") received vehicle whereas the other groups received TMC353121 in a single (10 mg/kg injected 60 min before infection), or multiple doses (daily). Five mice per group were used. * denotes significant difference of p <0.05 compared with V group, at day 7, based on One Way ANOVA, Tukey`s comparison test. No significant differences were observed between treated groups at day 7.

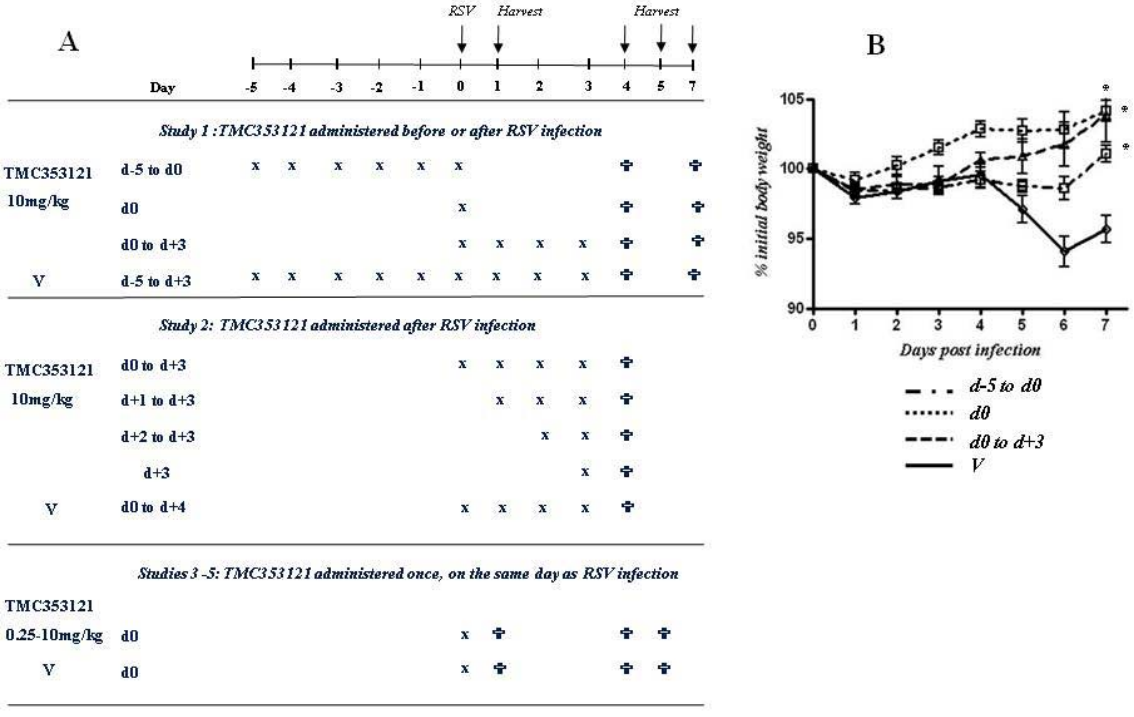


Fig.1

Fig.2 (A) BAL cell influx is inhibited in TMC353121-treated, RSV infected animals. Mice were culled on day four post infection and viable BAL cells were counted using Trypan blue exclusion and differential counts were performed after H+E staining of cytospins. Mice (5 per group) received one i.v. injection 1h prior to RSV infection containing TMC535121 at indicated dose, or vehicle control. NM- non-infected mouse. Mean values ± SE are shown. * denotes significant difference of p <0.05 between total cell numbers. Multiple comparison was done with One Way ANOVA with Dunnett test. Linear Trend for lymphocytes accumulation vs. TMC doses was assessed with One Way ANOVA with post test for linear trend: p<0.05. **(B) Significantly reduced lung cellular infiltrates in TMC353121-treated, RSV infected animals.** Representative slides for H+E staining of lung sections. B_1: Non-infected lung, B_2: RSV-infected lung, B_3: TMC353121-treated (10mg/kg) and RSV-infected lung. Lungs were analysed 5 days post-infection. Figures underneath indicate how the particular views were graded. Details of scoring system are described in Materials and Methods. **(C) Lung histopathological score in a representative experiment.** Lung sections were stained with H&E to assess cell infiltrate and their localization. Lungs were graded according to a scheme similar to that described by Ponnuraj *et al* [22]. In this experiment, each group consisted of two mice. Three lung sections (upper, medium and lower horizontal segments) were evaluated per lung. NM- non-infected, non-treted mice. Median of total histopathological score and tissue specific scores are shown. Multiple comparisons between groups were done using Kruskal Wallis with Dunn`s test. ** denotes significant difference of p<0.005. *P* values for 10-mg/kg groups versus the 0-mg/kg group (saline) are shown. Such an assessment was performed in four more studies using 2-5 mice per group.

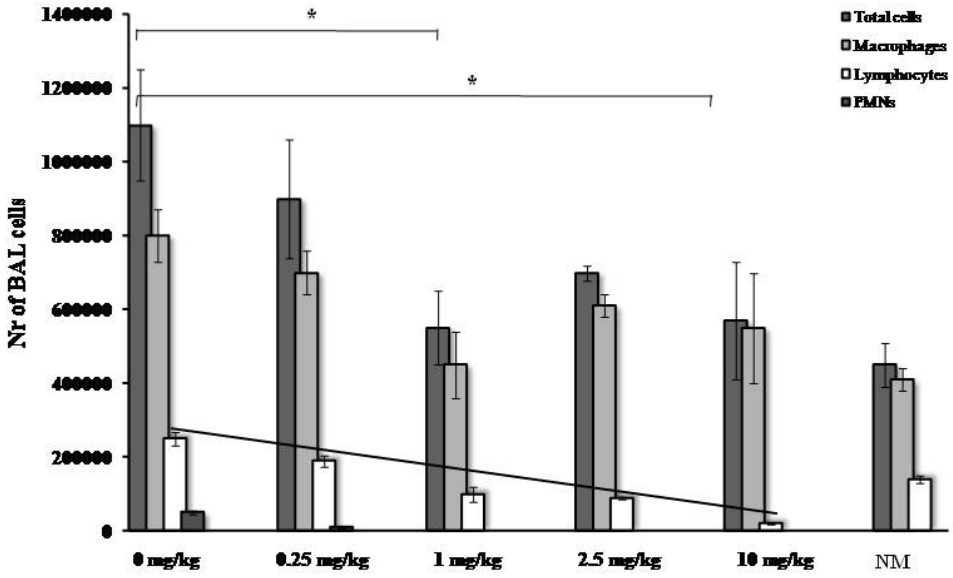
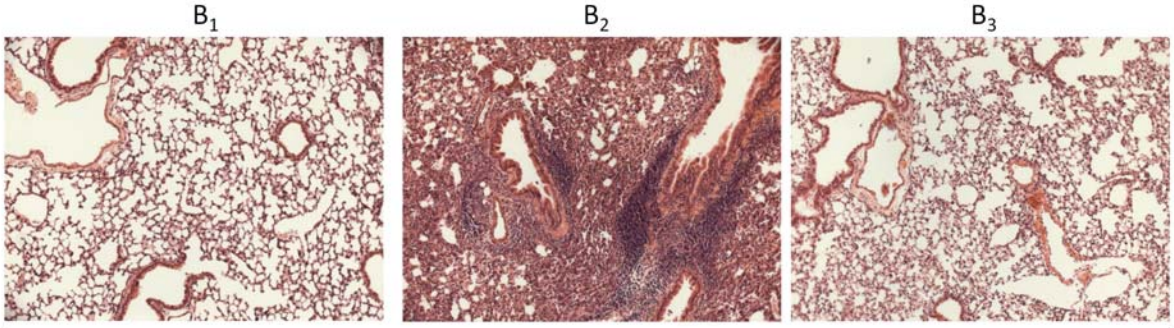

1400000
1200000
1000000
800000
600000
400000
200000
0
Nr of BAL cells
*
*
Total cells
Macrophages
Lymphocytes
PMNs
0 mg/kg
0.25 mg/kg
1 mg/kg
2.5 mg/kg
10 mg/kg
NM

Fig.2 A



Group	B1	B2	B3
Total score	2	14	4
Individual scores	(Interstitial=1, Perivascular = 0, Alveolar=1, Peribronchiolar=0)	(Interstitial=4, Perivascular = 4, Alveolar= 3, Peribronchiolar=3)	(Interstitial=2, Perivascular = 0, Alveolar=2, Peribronchiolar=0)

Fig.2 B

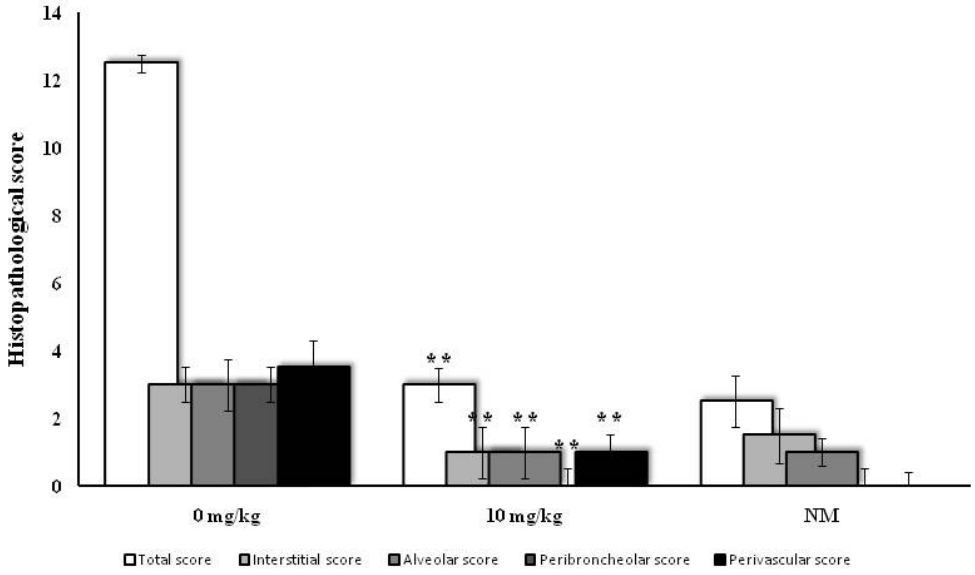

Histopathological score
14
12
10
8
6
4
2
0
**
**
**
**
**
0 mg/kg
10 mg/kg
NM
Total score
Interstitial score
Alveolar score
Peribroncheolar score
Perivascular score

Fig.2 C

Fig.3. Early chemokines and cytokines are inhibited in TMC353121-treated, RSV infected animals. BAL cell supernatants obtained from mice culled one day after RSV infection were analysed for the presence of chemokines and cytokines using Luminex system. In this experiment: in RSV group N=4, in RSV+TMC353121 group N=5, and in NM group N=3. NM-non-infected, non-treated mice. Mean values± SE are shown. * denotes significant difference of $p<0.05$ as analysed using One Way ANOVA with Tukey's test.

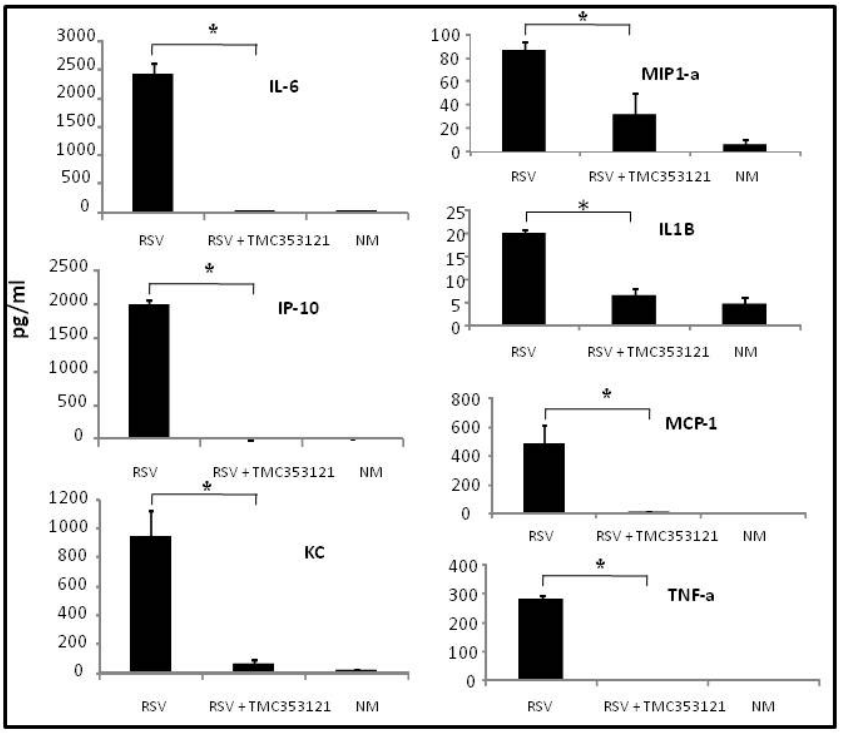


Fig.3

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