MAJOR ARTICLE

Efficacy and Safety of Treatment With an Anti-M2e Monoclonal Antibody in Experimental Human Influenza

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(See the editorial commentary by Fry, Zhong, and Gubareva on pages 1033–5.)

Background. The efficacy of TCN-032, a human monoclonal antibody targeting a conserved epitope on M2e, was explored in experimental human influenza.

Methods. Healthy volunteers were inoculated with influenza A/Wisconsin/67/2005 (H3N2) and received a single dose of the study drug, TCN-032, or placebo 24 hours later. Subjects were monitored for symptoms, viral shedding, and safety, including cytokine measurements. Oseltamivir was administered 7 days after inoculation.

Results. Although the primary objective of reducing the proportion of subjects developing any grade ≥2 influenza symptom or pyrexia, was not achieved, TCN-032–treated subjects showed 35% reduction ($P = .047$) in median total symptom area under the curve (days 1–7) and 2.2 log reduction in median viral load area under the curve (days 2–7) by quantitative polymerase chain reaction ($P = .09$) compared with placebo-treated subjects. TCN-032 was safe and well tolerated with no additional safety signals after administration of oseltamivir. Serum cytokine levels (interferon γ, tumor necrosis factor α, and interleukin 8 and 10) were similar in both groups. Genotypic and phenotypic analyses showed no difference between virus derived from subjects after TCN-032 treatment and parental strain.

Conclusions. These data indicate that TCN-032 may provide immediate immunity and therapeutic benefit in influenza A infection, with no apparent emergence of resistant virus. TCN-032 was safe with no evidence of immune exacerbation based on serum cytokine expression.

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Keywords. TCN-032; anti-M2e antibody; influenza viral challenge.

Influenza A infection is a prevalent viral disease for which more effective treatments are needed, particularly for those with serious clinical manifestations and to address emerging strains and drug resistance, which limits the effectiveness of existing therapies. TCN-032 is a fully human monoclonal antibody that targets the ectodomain of the matrix protein 2 (M2e), a highly conserved structure on influenza A virus [1]. M2 forms homotetramers on influenza A viruses and controls viral core pH after viral uptake into host cells during infection. Importantly, the conformational M2e epitope recognized by TCN-032 is present in >99.8% of reported human, avian, and swine influenza A strains (NCBI database). In vivo mouse challenge studies have shown survival benefit with TCN-032 administration in therapeutic settings using highly pathogenic as well as seasonal strains, and coadministration with oseltamivir enhances this benefit [2; unpublished data]. Anti-M2e antibodies such as TCN-032 do not interfere with receptor interactions and therefore are not neutralizing. Furthermore, anti-M2e antibodies do not prevent viral entry or uncoating because they do not block proton

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pump activity. However, they can bind to the M2e protein expressed on the surface of virus-infected host cells and can reduce virus replication by interfering with virus budding [3], by complement-dependent cytotoxicity, or by antibody-dependent cell-mediated cytotoxicity (ADCC) [4]. Uniquely, TCN-032 can bind to the M2e protein expressed on free virus; thus, clearance mechanisms, such as antibody-dependent virolysis, opsonophagocytosis, or viral aggregation, may also contribute to its efficacy. Anti-M2e antibodies have been shown to be highly effective in reducing severity of disease in animal models. Treanor and colleagues [5] showed that passive transfer of 14C2 accelerated viral clearance from the lungs of mice given a sublethal challenge of influenza A virus. Others have replicated these findings, whether by passive transfer of antibody or, more commonly, after vaccination of animals with purified M2e antigen [6–13]. Thus, based on the broad binding capability, TCN-032 may have the potential to serve as a "universal" therapeutic monoclonal antibody against influenza A infection [1, 14].

A phase 1 study of single doses of TCN-032 (1, 3, 10, 20, or 40 mg/kg) given intravenously in healthy human subjects showed that TCN-032 was well tolerated, with most adverse events (AEs) being mild to moderate in severity and unrelated to the study drug [15]. Pharmacokinetic (PK) evaluation indicated a half-life of approximately 15 days with no immunogenicity observed. Given these promising data, the effect of TCN-032 was explored in a therapeutic setting in experimental human influenza.

METHODS

Study Design

This was a randomized, double-blind, placebo-controlled study in healthy volunteers aged 18–45 years with a screening hemagglutination inhibition (HAI) titer to challenge virus of <10. Subjects were admitted to the Retroscreen Virology Ltd quarantine facility on day −2 or day −1 and inoculated intranasally with influenza A/Wisconsin/67/2005 (H3N2), approximately 5.0–5.5 $\log_{10}$ median tissue culture infective dose ($TCID_{50}$)/mL on day 0. A single dose of TCN-032 (40 mg/kg) or placebo was administered intravenously 24 hours after inoculation (day 1). Subjects started a 5-day course of oseltamivir on day 7, were discharged on confirmation of a negative influenza rapid antigen test result (approximately day 9), and returned for final evaluation on day 28. Subjects involved in PK evaluation and immunogenicity testing were also evaluated on day 15. Ethical approval was obtained from the Research Ethics Committee, and informed consent was obtained from all subjects. The study was conducted in accordance with ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines, regional and local regulations, the Declaration of Helsinki, and the study protocol.

Clinical, Virologic, and Immunity Assessments

Subjects used a symptom diary questionnaire 5 times daily to assess influenza symptoms and severities. Symptoms included upper respiratory (runny nose, stuffy nose, sneezing, sore throat, earache), lower respiratory (cough, shortness of breath), or systemic (headache, malaise, muscle and/or joint ache) symptoms. Severity was evaluated on a 4-point scale. Oral temperature was measured, and pyrexia was defined as a temperature ≥37.9°C confirmed by repeated measurement. Mucus weights were evaluated by collecting preweighed packets of paper tissues.

Viral shedding and seroconversion/seroprotection analyses were performed at the translational research laboratory at Retroscreen Virology Ltd. Nasal swab samples taken 3 times daily during quarantine were assessed by means of viral culture and quantitative polymerase chain reaction (qPCR) methods targeting the M1 protein. Viral culture was conducted using Madin-Darby canine kidney (MDCK) cell infection followed by agglutination assay. Viral shedding was determined using a real-time qPCR method. Viral shedding was defined as either viral culture titer >1.5 $\log_{10}$ $TCID_{50}$/mL at least once between days 1 and 7 or ≥2 positive qPCR detections between days 2 and 7. Serum for anti-challenge virus antibodies were collected before inoculation and on day 28 to evaluate seroconversion and seroprotection. Seroconversion was defined as a ≥4-fold increase in HAI titer from prechallenge to day 28 and seroprotection as an HAI titer (geometric mean) of 40 at day 28. Assessment of viral resistance (conducted by Theraclone Sciences) involved comparing the results of genetic sequencing of the M2 gene, TCN-032 binding (on MDCK infected cells), and ADCC susceptibility between the parental strain and the virus isolated from infected TCN-032–treated subjects.

PK and Immunogenicity Assessments

Six TCN-032–treated subjects underwent PK and immunogenicity testing. Serum samples were analyzed at MicroConstants, using a quantitative enzyme-linked immunosorbent assay method. Immunogenicity was evaluated using a semiquantitative electrochemiluminescence method to screen serum for anti-drug (anti–TCN-032) antibodies (Covance Laboratories). Confirmed positive samples were tested for neutralizing activity using a semiquantitative quantitative enzyme-linked immunosorbent assay method.

Safety Assessments

Safety measures included monitoring for AEs and vital signs and performance of clinical laboratory tests, electrocardiography, and spirometry. Influenza symptoms recorded in the symptom diary questionnaire were not recorded as AEs unless their severity and duration were not consistent with the natural history of influenza infection. Any C-reactive protein (CRP) level between 5 and 60 mg/mL or any >15% decline from baseline in spirometric values was categorized as a grade 1 (mild)

AE; greater changes were graded according to clinical judgment. To evaluate the potential for TCN-032 exacerbation of influenza inflammation, a post hoc analysis of serum cytokine expression was conducted. Cytokine levels reported to be elevated during influenza infection (interferon [IFN] γ, tumor necrosis factor [TNF] α, interleukin 8 [IL-8], and interleukin 10 [IL-10]) were assessed with a multiplex electrochemiluminescence-based system.

Study Objectives and Statistical Analyses

The primary objective was to evaluate the effect of TCN-032 compared with placebo in the development of grade ≥2 influenza symptoms or pyrexia from day 1 to day 7 after influenza A viral challenge. Secondary objectives included the effect of TCN-032 compared with placebo on total influenza symptom score area under the curve (AUC; days 1–7); duration, time to peak, and time to resolution of influenza symptoms or pyrexia; total viral shedding (measured by AUC) from the nasal mucosa as determined by viral culture (days 1–7) or qPCR (days 2–7); duration, time to peak, and time to resolution of viral shedding; nasal mucus weights; seroconversion and development of seroprotective titers; and safety, including cytokine release.

A sample size of 64 subjects (32 per arm) would provide 90% power to detect a 60% reduction in the primary end point (ie, proportion of subjects with grade ≥2 symptoms or pyrexia) assuming an event rate of 50% in placebo-treated subjects and a 1-sided α value of 10%. The primary end point was analyzed using the Cochran–Mantel–Haenszel test stratified by quarantine. In this phase 2a exploratory trial, a 1-sided α level of 10% was used to evaluate the resulting *P* value. The AUC analyses for total influenza score and viral shedding and for time to peak, duration, and time to resolution of symptoms were compared using a Wilcoxon rank sum test (1-sided α, 10%). Descriptive statistics were applied for other secondary and safety end points.

Analyses were conducted in 2 populations, the intent-to-treat (ITT) and modified ITT (mITT) groups, both prospectively defined before analysis of the primary end point. The ITT analysis set included all subjects inoculated with virus and randomized to treatment who received the study drug. The mITT analysis set included the ITT population subset with laboratory-confirmed influenza infection, defined as those who shed virus or seroconverted. The primary efficacy parameter was assessed in both ITT and mITT populations. All secondary efficacy end points were assessed in the mITT population. The ITT population served as the safety analysis set. In the PK and post hoc cytokine analyses, all subjects met the definition of laboratory-confirmed influenza infection.

RESULTS

Owing to difficulties in identifying suitable subjects who were serologically naive to the challenge virus and met other study entry criteria, a total of 61 subjects (TCN-032, n = 30; placebo, n = 31) were enrolled in 3 quarantines sessions, including 18–24 subjects per session. All enrolled subjects were inoculated with virus and randomized to study drug treatment. All but 1 TCN-032–randomized subject received study drug, for a total of 60 subjects treated (TCN-032, n = 29; placebo, n = 31), representing the ITT population. The mITT population consisted of 48 subjects (TCN-032, n = 24; placebo n = 24). The median subject age was 30 years (range, 19–45 years). The majority of subjects, 78.3% (47 of 60) were male; 93.3% (56 of 60) were white, 5.0% (3 of 60) Asian, and 1.7% (1 of 60) black.

Table 1. Analysis of the Primary Efficacy Parameter

Analysis Population	Subjects, No. (%)[a] TCN-032 Group	Placebo Group	% Difference	*P* Value[b]
ITT group	10/29 (34.5)	15/31 (48.4)	13.9	.14
mITT group	10/24 (41.7)	13/24 (54.2)	12.5	.21

Abbreviations: ITT, intent-to-treat; mITT, modified intent-to-treat.

[a] Subjects with any grade ≥2 influenza symptom or pyrexia between days 1 and 7.

[b] One-sided *P* value from Cochran–Mantel–Haenszel test (stratified by quarantine for combined analysis).

Efficacy

Analysis of the primary efficacy parameter showed that fewer TCN-032–treated subjects than placebo-treated subjects experienced grade ≥2 influenza or pyrexia, but the differences observed were not statistically significant for either the ITT or the mITT population (Table 1). Nonetheless, among subjects with laboratory-confirmed influenza infection, TCN-032 treatment was associated with greater benefit as measured by total influenza symptoms or pyrexia and by viral shedding. The individual total influenza symptom scores (sum of scores from days 1–7) were lower for TCN-032–treated subjects; they ranged from 0 to 94 for TCN-032–treated and from 0 to 190 for placebo-treated (6 of the latter had scores >100). Accordingly, a 35% reduction in the median total influenza symptom score AUC (days 1–7) was observed among TCN-032–treated compared with placebo-treated subjects ($P = .047$).

Only 2 of 48 subjects with laboratory-confirmed influenza infection had pyrexia; both received placebo. There was no difference between treatment groups in time to peak symptoms, but earlier time to resolution (median difference, 1 day; $P = .06$) and shorter duration of symptoms (median difference, 1.5 days; $P = .11$) was observed with TCN-032 treatment compared with placebo. The median total tissue weight in TCN-032–treated subjects was lower than in placebo-treated subjects (4.65 vs 6.05 g), consistent with the observed lower total symptom scores. The median viral shedding AUC was lower in TCN-032–treated than in

placebo-treated subjects, at both viral culture (difference, 1.32 $\log_{10}$; $P = .21$) and qPCR (difference, 2.2 $\log_{10}$; $P = .09$).

A composite graph of daily influenza symptoms and viral load as determined by qPCR (Figure 1) shows that whereas both symptoms and viral shedding seemed to have the same kinetics in both treatment groups during the first 3 days after viral inoculation, thereafter a prompt decline in viral shedding, mirrored by resolution of symptoms, was observed among subjects treated with TCN-032. In contrast, placebo-treated subjects not only had a higher median daily viral load and higher symptom scores but also experienced a delay in resolution of these parameters.

Viral Resistance

Twelve TCN-032–treated subjects with detectable virus by viral culture on ≥2 samples between days 3 and 7 were assessed for viral resistance [2]. Sequence analysis (454 Next Generation) of the M2 gene derived from the last positive nasal swab samples revealed no changes in the canonical epitope sequence –SLLTE–, as present in the parental virus. No mutations or enrichment of low frequency variations in the M2 protein were observed outside the –SSLTE– epitope compared with parental virus. TCN-032 binding to virus-infected MDCK cells indicated that the half maximal effective concentration (EC_{50}) values for binding were comparable between parental virus (0.27 nmol/L) and virus amplified from the nasal swab samples (0.23–0.49 nmol/L). Similar results were obtained in phenotypic assays using an ADCC surrogate assay (parental virus EC_{50}, 1.52 nmol/L; nasal swab sample amplified virus EC_{50}, 1.17–3.85 nmol/L).

Seroconversion and Seroprotection

Similar proportions of subjects who seroconverted were observed in both groups (TCN-032, 19 of 24 [80%]; placebo, 22 of 24 [92%]). However, seroprotective titers developed in a lower proportion of TCN-032–treated subjects (10 of 24 [42%] vs 20 of 24 [83%] for placebo-treated subjects).

PK and Immunogenicity Assessments

Six TCN-032–treated subjects underwent PK and immunogenicity assessments. After a single intravenous infusion of TCN-032 (40 mg/kg), serum concentrations were measurable on day 28 (27 days after infusion). The maximum serum concentration (C_{max}), time to maximum serum concentration (T_{max}), and area under the serum concentration time curve ($AUC_{0-\infty}$) measures were consistent with those observed in healthy volunteers in the phase 1 study [15]. The half-life (approximately 16 days), CL (0.171 mL/h/kg), and V_{ss} (83.0 mL/kg) were also consistent with those parameters in phase 1. Serum samples for anti-TCN-032 antibody analysis (preinfusion, day 15, and day 28) showed no specific antibodies to TCN-032.

Safety

No treatment-emergent serious AE or AE resulting in discontinuation occurred during the study. The majority of subjects (53 of 60 [88.3%]) experienced ≥1 treatment-emergent AE, and the proportion of subjects with events was slightly lower for TCN-032 (24 of 29 [82.8%]) than for placebo (29 of 31 [93.5%]). All but 1 of the AEs were either mild (grade 1) or moderate (grade 2) in severity. One TCN-032–treated subject experienced severe (grade 3) muscle injury on study day 20, which was considered unrelated to the study drug. The most commonly reported AEs (experienced by >10% of subjects overall) included abnormal spirometric results, epistaxis, and increased levels of alanine transaminase (ALT), CRP, and aspartate transaminase (AST). The proportions of subjects with these findings were similar between treatment groups, with the exception of abnormal spirometric results, which were experienced by more placebo-treated subjects (58.1% vs 41.4% for the TCN-032

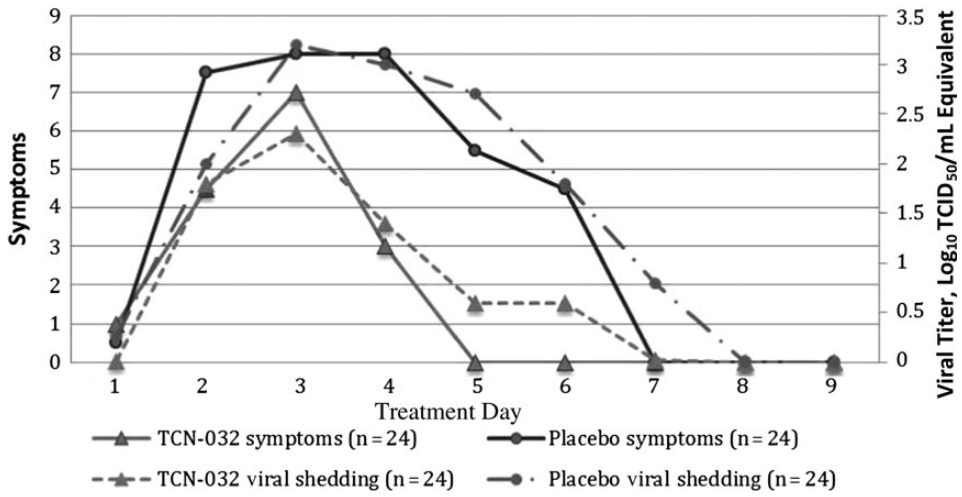

Figure 1. Median total daily influenza symptom scores and viral shedding. Abbreviation: $TCID_{50}$, median tissue culture infective dose.

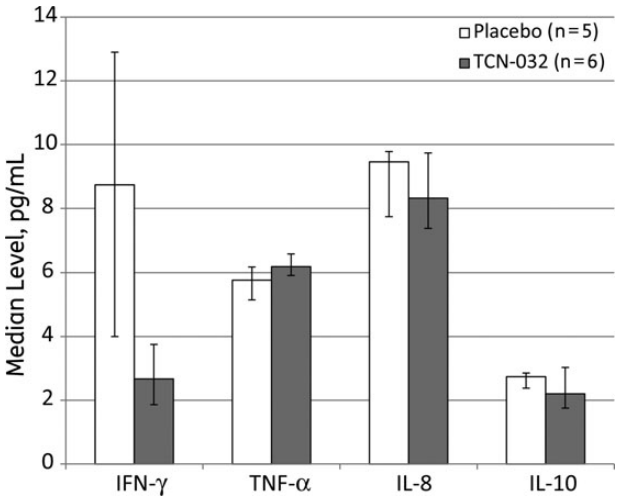

Figure 2. Serum cytokine levels at day 3. Abbreviations: IFN, interferon; IL-8, interleukin 8; IL-10, interleukin 10; TNF, tumor necrosis factor.

group). Although a high proportion of subjects experienced laboratory-related AEs (ie, increased levels of ALT, CRP, AST, or γ-glutamyltransferase), none of these abnormalities were considered clinically significant. Moreover, most of the increases in liver function enzyme AEs reported occurred after initiation of oseltamivir treatment.

Approximately half of the subjects experienced AEs that were deemed related to the study drug, with the proportion of subjects experiencing these AEs slightly lower for TCN-032 than for placebo treatment (12 of 29 [41.4%] vs 16 of 31 [51.6%]). The majority of abnormal spirometric results and increased CRP AEs were attributed to the challenge virus inoculum. The vast percentage of AEs related to abnormal liver function enzymes (ie, increased ALT, AST, and/or γ-glutamyltransferase) were considered related to the challenge virus, study drug, and/or antiviral medication; no differences between the treatment groups were observed.

To assess the potential of immune exacerbation, a retrospective analysis of serum cytokine levels was conducted in a subset of subjects (6 in the TCN-032 and 5 in the placebo group) with laboratory-confirmed influenza infection. Cytokines reported to be elevated in influenza infection (IFN-γ, TNF-α, IL-8, and IL-10) were evaluated on day 3, corresponding to the time of peak influenza symptoms observed during this study. In general, no differences in cytokine levels were detected between the TCN-032 and placebo groups (Figure 2). Notably, based on the interquartile range, lower IFN-γ levels were observed in TCN-032–treated subjects.

DISCUSSION

Given promising preclinical data and the safety observed in the previously completed phase 1 study, the biologic effect of TCN-032 was explored in an experimental human influenza infection trial. In this model, TCN-032 was administered 24 hours after viral inoculation to determine its effect in a therapeutic setting.

In lieu of the conventional virologic end point, a clinical parameter—the occurrence of grade ≥2 influenza symptoms or fever—was considered more appropriate as the primary end point based on the mechanism of TCN-032 action (ie, recruitment of effector cells). Among the potential clinical parameters, the occurrence of any grade ≥2 influenza symptoms or fever was chosen because treatment should mitigate severity of illness and also, importantly, because the historical placebo rate for this model was available to determine the ideal study sample size. Additional end points, such as total symptom scores as well as viral end points, including the emergence of viral escape, were also evaluated as secondary measures of efficacy.

An important population for assessing the efficacy of a novel anti-influenza agent is that of individuals with laboratory-confirmed infection, defined as those who either shed virus or have seroconverted. In these subjects, a reduction in the proportion of subjects who had grade ≥2 symptoms or fever was observed with TCN-032 treatment, but this difference was not statistically significant. Challenges in evaluating this end point included observations of subjects with a low total symptom score who had a single grade 2 event and thus met the primary end point and those with a high total symptom score who did not meet the primary end point because no single symptom was severe enough to be scored as grade 2. However, total daily influenza symptom scores were lower among TCN-032–treated subjects, and the median total influenza symptom score AUC (days 1–7) showed a significant reduction (35%) in TCN-032–treated compared with placebo-treated subjects.

This reduction in total symptoms is similar to what is seen in other human viral challenge models, as reported in the therapeutic setting for oseltamivir (44% reduction) [16] and peramivir (33% reduction) [17]. Consistent with this finding was the reduction in mucus weights observed with TCN-032 treatment. With regard to time to peak, time to resolution, and duration of influenza symptoms, there was no difference in time to peak symptoms, but there was a trend of earlier time to resolution and shorter duration of symptoms with TCN-032 treatment. The lack of difference in the time to peak symptoms is believed to be consistent with the mechanism of TCN-032 requiring recruitment of effector cells to mediate its beneficial effect.

In the virologic evaluations, modest reductions in median AUC by viral culture and qPCR (1.3 and 2.2 logs, respectively) were observed among subjects treated with TCN-032 compared with the placebo group. In concert with amelioration of clinical symptoms, these observations support the antiviral effects of TCN-032. Viral escape analysis showed that the genetic sequence of the TCN-032 epitope of virus isolated from the

nasal swab samples of TCN-032–treated subjects was unchanged compared with the parental virus and that binding of TCN-032 to the isolated virus showed similar EC_{50} values compared with parental virus. Furthermore, TCN-032–induced ADCC of cells infected with isolated virus was similar to that of cells infected with the parental virus. These latter findings suggest that TCN-032 treatment does not induce escape mutations in the first week of infection.

With regard to the immunologic response to challenge virus, similar proportions of subjects were observed to have seroconverted (defined as a ≥4-fold increase in HAI titer) at day 28. However, fewer TCN-032–treated subjects developed seroprotective titers (absolute titers ≥40). Before seroconversion testing it was established that HAI titers were not altered by the presence of increasing amounts of TCN-032 (data not shown). Given the beneficial clinical effects, it is postulated that TCN-032 was more effective in controlling infection, resulting in lessened systemic humoral response.

In the subset of 6 subjects who underwent PK and immunogenicity evaluations, results were consistent with the phase 1 results, with a TCN-032 half-life of approximately 16 days and no anti-drug antibodies observed. Because these subjects also met the definition of laboratory-confirmed infection, it seems that the viral infection did not affect the pharmacokinetics of TCN-032 compared with healthy uninfected subjects.

TCN-032 was safe and well tolerated in this study. Similar proportions of subjects in each arm experienced AEs. The total numbers of AEs reported were also similar, and the majority of AEs were mild (grade 1) to moderate (grade 2) in severity. The most commonly reported treatment-emergent AEs included abnormal spirometric results, reported in more placebo-treated subjects, and laboratory results, notably elevated liver enzymes levels. Of note, these abnormalities may be related to influenza infection after virus inoculation. Furthermore, the majority of elevated liver enzyme levels were observed after oseltamivir initiation, and "liver function tests abnormal" is an associated adverse drug reaction identified during postmarketing approval of this agent. The potential for exacerbation of cytokine release due to the mechanism of action of TCN-032 is an area of concern. Evaluation of selected cytokines reported to have elevated levels during influenza infection (IFN-γ, TNF-α, IL-8, and IL-10) at day 3 (corresponding to the day of peak influenza symptoms observed during this study) showed no differences in these cytokine levels indicative of immune exacerbation with TCN-032 treatment.

The results of this experimental influenza study represent the first demonstration that a nonneutralizing antibody given parenterally may provide immediate immunity and therapeutic benefit in influenza A infection. TCN-032 treatment showed reductions in clinical symptoms and viral shedding, with no apparent emergence of resistant virus. TCN-032 was safe and well tolerated, with no increase in AEs or immune exacerbation based on cytokine measurements. Furthermore, the administration of oseltamivir with TCN-032 did not result in any additional safety observations, suggesting that TCN-032 and oseltamivir may be used safely in combination.

Notes

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Potential conflicts of interest. E. L. R., J. L. M., T. D. K., A. B., and K. M. S. were or are employees of Theraclone Sciences. J. L. M. has US patents issued (8057796, 8114402, and 8460671) and pending (20130158238). D. W. U. and P. F. received personal fees from Theraclone Sciences during the conduct of the study. G. B. is an employee of Retroscreen Virology Ltd.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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