Original Article

Dose-biomarker-response modeling of the anticancer effect of ethaselen in a human non-small cell lung cancer xenograft mouse model

Suo-fu YE1,2, Jian Li1,2, Shuang-min Ji1,2, Hui-hui ZENG1,2,*, Wei LU1,2,*
1State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China; 2School of Pharmaceutical Sciences, Peking University, Beijing 100191, China

Abstract
Thioredoxin reductase (TrxR) is a component of several redox-sensitive signaling cascades that mediate important biological processes such as cell survival, maturation, growth, migration and inhibition of apoptosis. The expression levels of TrxR1 in some human carcinoma cell lines are nearly 10 times higher than those in normal cells. Ethaselen is a novel antitumor candidate that exerts potent inhibition on non-small cell lung cancer (NSCLC) by targeting TrxR. In this study we explored the relationship between the ethaselen dose and TrxR activity level and the relationship between TrxR degradation and tumor apoptosis in a human lung carcinoma A549 xenograft model. BALB/c nude mice implanted with human NSCLC cell line A54 were administered ethaselen (36, 72, 108 mg·kg−1·d−1, ig) or vehicle for 10 d. The tumor size and TrxR activity levels in tumor tissues were daily recorded and detected. Based on the experimental data, NONMEM 7.2 was used to develop an integrated dose-biomarker-response model for describing the quantitative relationship between ethaselen dose and tumor eradication effects. The time course of TrxR activity levels was modeled using an indirect response model (IDR model), in which the influence of the tumor growth rates on K in with the linear correction factor γ1 (0.021 d/mm). The drug binding-inhibition effects on K out was described using a sigmoidal E max model with S max (5.95), SC 50 (136 mg/kg) and Hill’s coefficient γ2 (2.29). The influence of TrxR activity inhibition on tumor eradication was characterized by an E max model with an E max (130 mm 3/d) and EC 50 (0.0676). This model was further validated using a visual predictive check (VPC) and was used to predict the efficacy of different doses. In conclusion, the properties and characteristics of ethaselen acting on TrxR degradation and subsequently resulting in tumor apoptosis are characterized by the IDR model and integrated dose-biomarker-response model with high goodness-of-fit and great predicative ability. This approach shed new light on the detailed processes and mechanism of ethaselen action and may offer a valuable reference for an appropriate dosing regimen for use in further clinical applications.

Keywords: non-small cell lung cancer (NSCLC); thioredoxin reductase; anticancer drug; ethaselen; dose-biomarker-response model; NONMEM

Introduction
Mammalian thioredoxin reductase (TrxR) is a component of several redox-sensitive signaling cascades that mediate some physiological processes, especially cell survival, maturation, growth, migration and inhibition of apoptosis[1–6]. The TrxR1 expression levels in some human carcinoma cell lines are approximately 10 times higher than those in normal cells[7], indicating their close relationships with cell proliferation and progression. In addition, it has been reported that the TrxR activity level is closely associated with the malignancy grade[8].

That is to say that malignant tumors tend to have high activity levels compared to benign hyperplasia. Recent studies have shown that TrxR is a promising target for cancer therapy in a wide range of tumors[9,10]. Ethaselen, as shown in Figure 1, was first synthesized in our group and has now entered a clinical Phase II trial as a novel antitumor candidate that targets TrxR, exhibiting good
antitumor effects in many cancer models[31]. Ethaselen was
designed to inhibit mammalian TrxR activity by selectively
targeting SeCys498/Cys497, the C-terminal active sites of
mammalian TrxR, with high binding potential[12-16]. Ethaselen
can inhibit TrxR activity in several human carcinoma cell lines
and tumor-bearing mouse models[13-16]; in one example, the
antitumor effects on A549, a human non-small cell lung cancer
cell line, appeared to be excellent. In addition, previous stud-
ies have demonstrated that TrxR inactivation correlates with
cell death/apoptosis in the A549 cell line, and the mechanism
is related to inducing mitochondria-dependent apoptosis[37].

A dose-response model is usually an excellent option for
characterizing the relationship between drug effects and doses;
this relationship is crucial for adequate dose selection[18-20]. For
Ethaselen, to make use of the available pharmacokinetic (PK)
measurements in blood samples, complicated extraction meth-
ods and large systems of differential equations are needed to
characterize the Ethaselen dynamics[21]. Taking the difficulty
and complexity of PK measurements into account, we chose a
simpler dose-biomarker-response model that is based on a
pharmacokinetic-pharmacodynamic (PK-PD) model[22], where
the PK component in the unobserved effect compartment is
treated as a latent variable[23].

The dynamic levels of a biomarker are a key indicator
revealing the details and processes of the drug action[24]. Fur-
thermore, by exploring the quantitative relationship between
changes in the biomarker level and in the tumor apoptosis
effects, direct evidence for and detailed information on the
drug actions were obtained, such as the pharmacological
response of the drug, the degree of effects and the time course
of response changes[25]. Previous studies have demonstrated
that the TrxR activity level, which can be specifically inhibited
by Ethaselen, is a potent, effective biomarker for the initiation
and development of cancer[26]. As a result, TrxR activity was
adopted as the biomarker for this pharmacodynamic study.

It has been reported that TrxR is involved in many aspects of
tumor physiology, such as proliferation, apoptosis and metas-
tasis[3-5]. In recent years, an increasing number of TrxR inhib-
itors have received attention and have eventually been demon-
strated as effective[7, 27-29]; Ethaselen is one promising, potent
candidate[30]. However, to the best of our knowledge, no stud-
ies have investigated the quantitative relationship between
Ethaselen administration and tumor eradication, let alone
possible models based on the complicated process and mecha-

nism of Ethaselen action and the TrxR signaling pathway. In
this study, we explored the relationship between Ethaselen
dose and the level of TrxR activity and explored the relation-
ship between TrxR degradation and tumor apoptosis effects
in a human lung carcinoma A549 xenograft model. This is the
first time that an integrated dose-biomarker-response model
has been established to quantitatively describe the relation-
ship between Ethaselen dose, TrxR activity levels and tumor
volume in BALB/c nude mice xenografted with A549 (a non-
small cell lung cancer). Through data-based development
and model-based analysis, this integrated dose-biomarker-
response model sheds new light on the detailed processes and
complicated mechanism of Ethaselen action in vivo and allows
verification of the appropriate dose for clinical treatments.

Materials and methods

Drugs and reagents
Ethaselen (PCT: CN02-00412, purity >99%) was designed and
synthesized in our laboratory (State Key Laboratory of Natu-
rnal and Biomimetic Drugs, School of Pharmaceutical Sciences,
Peking University, Beijing, China). Other chemicals were pur-
chased from Sigma-Aldrich unless otherwise specified.

Cell culture
The human non-small cell lung cancer cell A549 was obtained
from the Cell Center of the Chinese Academy of Medical
Sciences (Beijing, China) and cultured in DMEM (Macgene,
Beijing, China) supplemented with 10% fetal bovine serum,
100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in
a humidified atmosphere of 5% CO₂. Cells in the exponential
growth phase were used for the experiments.

Animals
Five-week-old female BALB/c nude mice were purchased
from the Animal Center of Peking University Health Science
Center. All animal studies and experiments were approved
and conducted strictly under the ethical guidelines issued
by Ethical Committee of Peking University. Animals were
housed under controlled conditions (25±2°C, with 12 h light/
dark cycle). Tumors were established by injecting the right
armpits of mice with human non-small cell lung cancer cells
from the A549 cell line (5×10⁶/0.2 mL per mouse).

TrxR activity assay
TrxR activity was determined by the 5,5’-Dithiobis-(2-nitro-
benzoic acid) (DTNB)-reducing method[30, 31] and expressed
as an increase in the absorbance at 412 nm, which is propor-
tional to the TrxR-dependent reduction of DTNB into TNB.
After tissue homogenization, the same amount of protein was
diluted with an equal volume of 0.1 mol/L PBS and mixed
with the enzymatic reaction solution (100 mmol/L KH₂PO₄/
KH₂HPO₄ (pH 7.0), 5 mmol/L DNTB, 0.2 mmol/L NADPH, 10
mmol/L EDTA, and 0.2 mg/mL BSA). Absorbance was mea-
sured at 412 nm every 5 s for 400 s, and the reaction tempera-
ture was sustained at 37°C.

TrxR activity was calculated with the following formula:

\[ \text{TrxR activity} = \frac{\text{Corrected ΔA412 nm/min} \times \text{Total volume} \times \text{Sample dilution}}{6.35 \text{ (nmol/L)} \times \text{cm}^{-1} \times \text{protein content}} \]  

(Eq 1)

The TrxR activity unit was defined as the nmol value of the
reduced DTNB in units of time. The linear range of this
method was 0-1000 U/mL, and all data detected in the study
were within the linear range.

Pharmacodynamics study based on TrxR activity levels in tumor
tissues
A TrxR activity assay was used to investigate the inhibitory
effect of Ethaselen on the TrxR activity levels in the tumors of A549 xenograft mice. When the tumor volume reached approximately 100 mm$^3$, 128 mice were randomly divided into the following four groups: control group (receiving 0.5% CMC-Na alone, pH 7.4); low-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 36 mg/kg, ig, QD, ×10 d); middle-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 72 mg/kg, ig, QD, ×10 d); and high-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 108 mg/kg, ig, QD, ×10 d). Each group consisted of 33 mice. In each group, before daily dosing (with the first drug administration set as time 0 d), the subcutaneous tumor volume was measured by Vernier calipers and recorded as the pharmacodynamics data. Then, the tumor tissue was resected after decapitating the mice ($n=3$ per day for each group) and stored at -20 °C until further TrxR activity analysis by the DTNB-reducing assays, as described above.

Pharmacodynamics study based on tumor volume

Until the tumor size reached approximately 100 mm$^3$, 28 mice were randomly divided into the following four groups: control group (receiving 0.5% CMC-Na alone, pH 7.4); low-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 36 mg/kg, ig, QD, ×10 d); middle-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 72 mg/kg, ig, QD, ×10 d); and high-dose group (receiving Ethaselen dissolved in 0.5% CMC-Na at a dose of 108 mg/kg, ig, QD, ×10 d). Each group consisted of 7 mice. Ethaselen was dissolved in 0.5% CMC-Na (pH 7.4). The subcutaneous tumor volume was measured using Vernier calipers daily and calculated as shown in Eq.2. Combined with the tumor volume data recorded from the above assays of TrxR activity, the whole tumor volume data set was adopted as the pharmacodynamics data for the dose-response modeling.

\[
\text{Tumor volume (mm}^3\text{)} = \frac{\text{length (mm)} \times \text{width}^2 (\text{mm}^2)}{2} \quad \text{(Eq 2)}
\]

Dose-biomarker-response model

Previous studies have shown that the mechanism of Ethaselen action, including specific binding to the TrxR enzyme, inhibits its activities and subsequently induces tumor apoptosis\[17\]. In addition, it has been reported that the TrxR activity level is closely associated with the malignancy grade\[8\]. As for the human non-small cell lung cancer xenograft mouse model, the malignancy grade can be simplified and represented by the degree of tumor proliferation. Therefore, it was assumed that the TrxR activity levels might be quantitatively related to the tumor growth rates. Finally, an indirect response model and an integrated dose-biomarker-response model, as shown in Figure 2, were established to reveal some aspects of the pharmacodynamics properties of Ethaselen.

Dose-biomarker-response modeling based on TrxR activity levels

The TrxR activity levels were modeled using an indirect response model (IDR model)\[32\]. In this model, $K_{in}$ was influenced by tumor growth rates with a linear relationship, and $K_{out}$ was affected by Ethaselen binding-inhibition after dosing. The latter was characterized by a sigmoidal $E_{max}$ model using an $S_{max}$ value (ie, the maximal TrxR inhibition) and an $SC_{50}$ value (ie, the dose of Ethaselen causing one-half maximal TrxR inhibition). The inhibition effects are described in the block diagram shown in Figure 2 and can be calculated using Eqs 3–4.

\[
\text{Base}_{\text{TrxR}} \times \frac{K_{in}}{K_{out}} \quad \text{(Eq 3)}
\]

\[
\frac{d\text{TrxR}}{dt} = \frac{2\lambda_2 \lambda_3 X}{\lambda_1 + 2\lambda_2 X} - K_{sc} \times \text{TrxR} \times (1 + \frac{S_{max} \times \text{DOSE}^2}{SC_{50} + \text{DOSE}^2}) \quad \text{(Eq 4)}
\]

In Eq 3, TrxR represents the TrxR activity level. $K_{in}$ is the zero order rate constant of the generation of TrxR, and $K_{out}$ is the first order rate constant of the degradation process of TrxR. Here, the Gompertz model was used, with the exponential and linear phases describing the natural tumor growth rates; the total TrxR generation rates were affected by the natural growth rates of the tumor. Additionally, $y_1$ is the linear correction factor, and $y_2$ is the Hill’s coefficient.

Dose-biomarker-response modeling based on tumor volume

The Gompertz model was adopted to apply to the vehicle control group (natural tumor growth)\[33\]. In this model, the following two different phases were suggested: initial exponential growth in the early phase and a subsequent phase of linear growth until reaching a plateau\[34\]. However, because the plateau phase was not achieved over the experimental period, an alternative and simplified model was applied, placing more emphasis on the exponential and linear phases. The tumor natural growth equations (Eqs 5–6) were as follows:

\[
\frac{dX}{dt} = \frac{2 \lambda_2 \lambda_3 X}{\lambda_1 + 2 \lambda_2 X} \quad \text{(Eq 5)}
\]

\[
X(0) = W \quad \text{(Eq 6)}
\]

Where X represents the tumor size and W represents the initial tumor volume just before dosing. Additionally, in this model, $\lambda_1$ and $\lambda_2$ represent the exponential and linear growth rate constants, respectively.

Here, the ratio of TrxR inhibition, $P$, was used as the variable representing the relative TrxR activity inhibition levels. The tumor apoptosis effects caused by TrxR degradation were characterized by a sigmoidal $E_{max}$ model with zero order elimination. The $E_{max}$ value is the maximal tumor eradication effect, and the $EC_{50}$ value is the ratio of TrxR inhibition $P$ causing one-half of the maximal tumor eradication effects that represent the tumor apoptosis effects throughout TrxR degradation. In this study, transit compartments were not used based on the goodness-of-fit with the data. The equations (Eqs 7–9) were as follows:

\[
P = 1 - \frac{\text{TrxR}_{\text{max}}}{\text{TrxR}_{\text{control}}} \quad \text{(Eq 7)}
\]

\[
\frac{dX}{dt} = \frac{2 \lambda_2 \lambda_3 X}{\lambda_1 + 2 \lambda_2 X} \times \frac{E_{max} \times P}{EC_{50} + P} \quad \text{(Eq 8)}
\]

\[
X(0) = W \quad \text{(Eq 9)}
\]
The tumor size data of the vehicle control group, 36 mg/kg Ethaselen group, 72 mg/kg Ethaselen group and 108 mg/kg Ethaselen group were applied to construct the integrated dose-biomarker-response model.

Modeling construction, evaluation, and simulation
All modeling and simulations were performed on NONMEM 7.1.2 (ICON Development Solutions, Ellicott City, MD, USA), Pirana 2.8.0 and Perl-speaks-NONMEM (PsN, Version 3.5.3) with the FOCEI algorithm. All graphs were drawn with the R software. The inter-individual and residual variabilities were estimated and used for evaluating the precision and reliability of the parameters. Selection of the models was based on the change in the objective function value (OFV), value of estimate, relative standard error (RSE), and exploratory analysis of the goodness-of-fit plots. Data for the vehicle control and different treatment groups were used for model development. For model validations, the visual predictive check (VPC) was conducted with 1000 simulations, and the 10th, 50th, and 90th percentiles of the simulations were compared with the observed value. The simulations for the different doses (36, 50, 72, 90, and 108 mg/kg Ethaselen) were performed by fixing all parameters obtained from the integrated model above.

Results
Pharmacodynamic studies
TrxR activities at various time points are shown in Figure 3A. The TrxR activities increase as the tumor grows. After Ethaselen administration, an evident decrease in the TrxR activity levels was observed compared to the control group at each time point. This type of inhibition increased in conjunction with the dose. Compared with the control group, the TrxR activity levels of the high dose group (108 mg/kg) decreased more than the middle dose group (72 mg/kg) and low dose group (36 mg/kg). These results indicated that TrxR inhibition was dose-dependent.

Figure 3B shows the inhibitory effects of Ethaselen (at various doses) on tumor growth in A549 xenograft mice. Compared with the control group, the treatment groups (36, 72, and 108 mg/kg Ethaselen) showed increased inhibition of tumor growth, and the inhibition levels increased with the dose. The tumor volume-time profiles of the different doses were applied to a dose-time-response model as pharmacodynamic data.

Modeling
Modeling based on TrxR activity levels
Time courses of the levels of TrxR activity for all the groups were described by the indirect response model. The observed and model-predicted TrxR activities for each group are graphically presented in Figure 4A–4D, and the estimated parameters are shown in Table 1.

Modeling based on tumor volume
The integrated model, which included the indirect response model for TrxR inhibition, was used to describe the pharmacodynamic profiles of the tumor growth data. The results of the observed and predicted tumor volumes are shown in Figure 4E–4H, and the estimated parameters of the integrated model are listed in Table 1.

Model evaluation and simulation
For the IDR and integrated models, the goodness-of-fit plots are shown in Figure 5 (the IDR model) and Figure 6 (the integrated dose-biomarker-response model), respectively. The dots of the population predicted value (PRED) or individual predicted value (IPRED) versus observed data (OBS) were ran-
domly distributed around \( y=x \). Additionally, the conditional weighted residuals (CWRES) were homogenously distributed around the x axis. The time profiles at the different doses tested by the model were a reasonable fit and the parameters were estimated with good precision.

The VPC results of the IDR and integrated models for the different treatment groups (0, 36, 72, and 108 mg/kg Ethaselen) are shown in Figure 7. The 50th percentiles of the predictions nearly pass through the middle of the observed values, and most observed values were within an 80% confidence interval of the predictions, suggesting that the model accurately fits the current data of TrxR activity and tumor volume with a high predictability.

To better understand the drug action and optimize the therapeutic regimen of Ethaselen treatment, simulations of the TrxR activity-time and tumor volume-time profiles for

Table 1. Estimates of parameters for the integrated dose-biomarker-response model.

| Parameter                      | Definition                                                                 | Estimates | RSE (%) 1 | IV (CV) % 2 |
|--------------------------------|---------------------------------------------------------------------------|-----------|-----------|-------------|
| \( K_{\text{in}} \) (U/mL·d\(^{-1}\)) | TrxR production rate                                                      | 8.27      | 42.4      | 12.4        |
| Base (U/mL)                   | Initial TrxR activity                                                     | 39.7      | 8.60      | 9.6         |
| \( \gamma_1 \) (d/mm)        | Linear correction factor                                                  | 0.021     | 16.5      | NE 3        |
| \( \gamma_2 \) (d\(^{-1}\))  | Hill’s coefficient                                                        | 2.29      | 17.3      | NE          |
| \( S_{\text{max}} \) (µM)    | Maximal TrxR inhibition                                                  | 5.95      | 31.9      | NE          |
| \( E_{C50} \) (mg/kg)        | Dose of ethaselen causing one-half maximal TrxR inhibition               | 136       | 25.2      | NE          |
| \( \lambda_0 \) (d\(^{-1}\)) | Exponential growth rate                                                   | 0.704     | 31.4      | NE          |
| \( \lambda_1 \) (mm\(^3\)/d) | Linear growth rate                                                       | 321       | 11.5      | 32.7        |
| \( W \) (mm\(^3\))          | Initial tumor volume                                                     | 103       | 3.9       | 14.2        |
| \( E_{\text{max}} \) (mm\(^3\)/d) | Maximal tumor eradication effects                                         | 130       | 4.8       | NE          |
| \( EC_{50} \) (d\(^{-1}\))   | Ratio of TrxR inhibition P causing one-half maximal tumor eradication effects | 0.0676   | 23.1      | 19.3        |
| Err\(_{\text{pro}}\)         | Proportional error                                                       | 20.22%    | 13.6      | NE          |
| Err\(_{\text{add}}\) (mm\(^3\)) | Additive error                                                          | 141       | 78        | NE          |

1 Percentage relative standard error.
2 Percentage coefficient of inter-individual variability of the estimate.
3 Inter-individual variability was fixed to zero when CV<0.1%.
a series of different dose regimens (0, 36, 50, 72, 90, and 108 mg/kg) were performed by fixing the parameters. The dose-dependent efficacy in both TrxR activity inhibition and tumor eradication effects was observed, as shown in Figure 8.
Discussion

The present research sheds new light on the dynamic dose-biomarker-response relationships of Ethaselen, a TrxR-specific inhibitor, in a human lung carcinoma A549 xenograft model. Previous studies have demonstrated that many tumor processes are closely related to TrxR, including growth, metastasis and inhibition of apoptosis. Ethaselen is a small molecule that is specifically combined with TrxR and reduces the
TrxR activity levels, exerting good inhibitory effects on several human carcinoma cell lines. However, to the best of our knowledge, no studies have detected TrxR activities after Ethaselen administration, and the dynamic relationship between the degree of TrxR degradation and tumor apoptosis effects remains unclear. The TrxR activity detection results showed that, without drug treatment, TrxR increased with tumor growth in a positive correlation. After the administration of Ethaselen, the TrxR activity levels in the tumor tissues of A549 xenograft mice were inhibited in a dose-dependent manner.

As previously reported and demonstrated, carcinoma cell
proliferation relies on ribonucleotide reductase (RNR) for dNTP biosynthesis, and the reduction of RNR is regulated by TrxR\(^\text{35–37}\). Therefore, the high tumor growth and proliferation rates depend on the increased replication rates and accelerated metabolism of nucleic acid, resulting in elevated TrxR expression, which is a characteristic of many cancers\(^\text{38, 39}\). Clinical observations\(^\text{8, 40, 41}\) also showed that the TrxR activity level is closely associated with the malignancy grade. In this way, malignant tumors with high proliferation rates tend to have higher activity levels than benign hyperplasia. Both the molecular mechanism and clinical observations suggest that the TrxR activities may be proportional to the tumor growth rates. To investigate the changing TrxR activity levels in the tumor, an IDR model was adopted based on the assumption that the rates of the onset and offset of TrxR activity are regulated by an indirect mechanism of action. In addition, from the point of view of goodness-of-fit terms, we explored the importance of the tumor growth rate on the \(K_{\text{int}}\), the zero order rate constant of the generation of TrxR, in the IDR model equation. The IDR model allowed for better adherence to the observed data when considering the influence of the tumor growth rates on the \(K_{\text{int}}\). The model not only reasonably described the dose-time-biomarker relationships between TrxR inhibition and Ethaselen dose, but it also provided the best fit to the observed biomarker data.

TrxR inhibition, tumor eradication effects and their dynamic relationships were illuminated by an integrated dose-biomarker-response model. Two parameters (\(\lambda_0\) and \(\lambda_1\)), representing the exponential and linear phases, characterized the properties of natural tumor growth in the vehicle control group. The outcomes of model fitting showed that the tumor growth model that lacks transit compartments has higher goodness-of-fit with the data compared to adding transit compartments or non-proliferating compartments in the model. This may be due to Ethaselen-induced rapid apoptosis (within 1 h) in A549 cells\(^\text{17}\). The \(E_{\text{max}}\) model was used to describe the drug-elicted effects in the tumor eradication process, which was fit by the zero order elimination beyond the classical first-order elimination based on the goodness-of-fit for the observed data. This model adequately described the antitumor effects of Ethaselen based on the action of Ethaselen, which involves the inhibition of TrxR followed by induction of the mitochondrial apoptosis pathway and eventually results in tumor death\(^\text{17}\). The dose-biomarker-response model reflected the quantitative relationship between TrxR degradation and tumor apoptosis effects. Focusing on the biomarker, the integrated dose-biomarker-response model demonstrated that TrxR activity is an important index for drug action and a key bridge for the dynamic linkage between drug administration and tumor eradication effects, which improves our knowledge of the Ethaselen mechanism of action and will inform future related clinical applications.

The simulation results of the tumor volume in groups after the administration of 50 and 90 mg/kg Ethaselen were conducted well using the integrated dose-biomarker-response model. According to the simulation results, the excellent pre-diction abilities of the dose-biomarker-response model were confirmed, which may provide an easy and effective way to predict tumor apoptosis effects without conducting tumor growth studies and may optimize the experimental design in preclinical and clinical studies.

Although previous studies have demonstrated that several tumor types respond to Ethaselen exposure and TrxR inhibition, this is the first time that the dose-biomarker-response model was constructed in A549 xenograft nude mice with Ethaselen administration. Therefore, the value of TrxR activity as a biomarker must be assessed in more detail in future studies. One consideration is that the TrxR inhibitor Ethaselen might induce tumor apoptosis through some other biomarker responses and signaling pathways. To better understand the dynamic dose-biomarker-response relationship, more mechanistic models need to be constructed and evaluated. Another consideration is that because of the temporary absence of effective Ethaselen plasma concentration measurements, more studies should be performed to explore an effective approach for detecting the Ethaselen plasma concentration and to construct a pharmacokinetic–pharmacodynamic (PK–PD) model, which is a typical and widely applied modeling strategy for acquiring comprehensive, quantitative knowledge of the Ethaselen plasma concentration, TrxR degradation and tumor apoptosis effects in a xenograft model. However, to the best of our knowledge, the analysis presented here is currently the most informative available for developing Ethaselen as a promising, potent anticancer drug.

In conclusion, the relationship between the Ethaselen dose and TrxR activity levels in tumor tissue and the relationship between TrxR activity levels and tumor growth in A549 xenograft mice were appropriately characterized by an integrated dose-biomarker-response model that was reliable and predictable. The outcomes of this study confirmed the mechanism of Ethaselen action and corresponding therapeutic responses, which could improve experimental designs in future preclinical and clinical studies.

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Author contribution

Suo-fu YE, Jian LI, Shuang-min JI, Wei LU, and Hui-hui ZENG designed the research; Suo-fu YE performed the research; Jian LI, Shuang-min JI, and Suo-fu YE analyzed the data; and Suo-fu YE wrote the manuscript.

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