Utilization of Preclinical Xenograft Data in Predicting Human Imetelstat Target Tissue Concentrations

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OBJECTIVES

- To better understand imetelstat tissue distribution in tumor and bone marrow from xenograft mouse studies.
- To establish a target tissue pharmacokinetic model to simulate imetelstat human tumor and bone marrow concentration profiles.

INTRODUCTION

Imetelstat is a first-in-class, lipidated 13-mer oligonucleotide *thio*-phosphoramidate telomerase inhibitor with anti-tumor activity. It has a sequence that is complementary to the hTR template region in telomerase. It inhibits telomerase by acting as an active site competitive inhibitor of the enzyme in the sub-nanomolar and sub-micrormolar range in biochemical and cell-based assays, respectively.[1] Imetelstat has demonstrated telomerase inhibitory and cancer growth inhibitory effects in both in vitro and in vivo preclinical models.[1-4] Imetelstat is currently in Phase II clinical development. Its distribution in tissues, specifically tumor and bone marrow, was studied in a xenograft mouse model established using a human OVCAR-5 cell line. Data from xenograft studies indicated imetelstat tumor and bone marrow concentrations were sustained during the terminal phase. This suggests that further evaluation of imetelstat distribution in patient tumor and bone marrow is critical for understanding the determinants for drug tissue accumulation in order to facilitate clinical understanding of drug concentration in the target tissues of hematological and solid tumor malignancies.

Human tumor and bone marrow samples are difficult to obtain. It is impractical to assay serial human tissue concentrations as a function of time. Single time point tumor or bone marrow samples may be obtained during biopsy or surgical procedures. The timing of the samples are difficult to control. Therefore, the purpose of this study is to develop a target tissue pharmacokinetic model, to predict overall imetelstat human tumor or bone marrow concentration-time profiles from a single tissue sample at a random time point. Thereby provide a prediction for overall tumor/bone marrow drug exposure in patients. PK data from preclinical xenograft mouse studies and modeling are presented.

METHODOLOGY

- Xenograft mouse study: Nude mice received a tumor challenge of 3 million OVCAR-5 cells injected subcutaneously into contralateral flanks. Mice were treated when tumors reached ~200 mm^3 in size, with imetelstat 3 times per week via IP bolus injection at 15, 30, 60 and 90 mg/kg. Plasma, tumor and bone marrow samples were collected at various time points post last drug treatment. Tumor samples were flash frozen immediately after collection, and were homogenized in M-Per buffer with Omni hand held homogenizer (Kennesaw, GA). Bone marrow samples were collected from both femurs by PBS flushing, and bone marrow cells were lysed in M-Per buffer. All samples were subjected to a novel extraction procedure using a biotinylated capture probe before imetelstat concentration determination with ion-pairing reversed-phase LC/MS/MS. Imetelstat concentration in bone marrow is presented as total concentration, which accounts for both the cellular and non-cellular fractions.
- Target tissue pharmacokinetic model: Data from preclinical xenograft mouse studies was used in developing a pharmacokinetic model to describe imetelstat tissue distribution in tumor and bone marrow. All programs were written in WinNonlin (Pharsight Corp) custom models.
- Human plasma from solid tumor (ST) patients: Human plasma data was obtained from solid tumor patients in a Phase I clinical trial after a 2-hr IV infusion of imetelstat at 9.4 mg/kg. Imetelstat plasma concentrations were determined by a LC/MS-based assay.
- Human bone marrow from multiple myeloma (MM) patients: Bone marrow samples were collected from imetelstat treated MM patients enrolled in a Phase I clinical trial. The pre- and post-treatment samples were enriched for mononuclear cells, then separated into CD138+ and CD138- cells. Cell lysate was prepared from CD138- cells in M-Per buffer and imetelstat bone marrow concentrations were determined using a LC/MS-based assay. Imetelstat concentration in human bone marrow is presented as cellular concentration in Figure 5.

RESULTS AND DISCUSSION

Data from Xenograft Mouse Study

- Imetelstat concentrations in plasma, tumor and bone marrow were quantified in xenograft mouse samples. For simplicity, detailed concentration-time profiles are only shown for 30 and 90 mg/kg dose groups in Figures 1 and 2.

Figure 1. Imetelstat Levels in Xenograft Mouse (30 mg/kg).

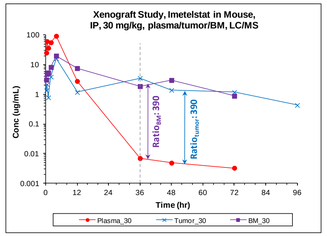
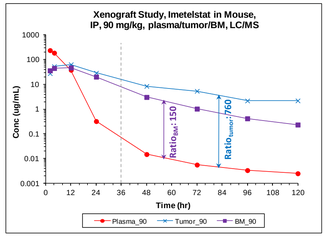


Figure 2. Imetelstat Levels in Xenograft Mouse (90 mg/kg).



- Imetelstat distributes rapidly into xenograft tumor and bone marrow.
- Imetelstat concentrations in tumor and bone marrow were at higher levels than that in plasma when equilibrium was established after 24 hrs following distribution between tissue and plasma.
- Tissue : plasma ratio changes in the first 24 hrs, and maintains a constant ratio only after equilibrium is achieved during the terminal phase. This was observed in all dose groups, and it is in agreement with literature reports on other oligonucleotides.[5-7]
- The absolute concentration ratio between tissue and plasma during the terminal phase varies among different dose groups.
- No saturation in exposure was observed in tumor and bone marrow at the highest dose of 90 mg/kg.

Target Tissue PK Modeling

- Xenograft datasets were used in building a preclinical pharmacokinetic model to describe imetelstat tissue distribution in tumor and bone marrow. Imetelstat concentration-time profiles in plasma and tissue were modeled simultaneously. Plasma concentrations followed a biphasic decline, and were modeled as a two compartment open model with a first order input. Tissue compartments (effect compartment, tumor or bone marrow) were modeled as a linked compartment from the central compartment.[8] Imetelstat transfers from the central to the tissue compartment via a rate constant, ke0 (Scheme I). Simulated plasma and tissue profiles at 30 mg/kg in xenograft mouse can be seen in Figure 3.

Scheme I. Tissue PK Model.

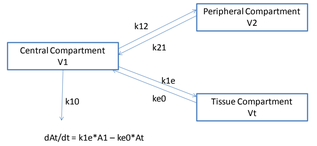
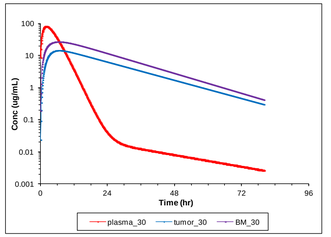


Figure 3. Target Tissue PK Model in Xenograft Mouse (30 mg/kg).



Human Prediction

- Human tumor concentration-time profile following continuous IV infusion was similarly modeled with only the adjustment of the input function. The rate constant from central to tissue compartment, ke0, was assumed to be similar between human and xenograft mouse. Using this model, together with human plasma data at 9.4 mg/kg, imetelstat tissue concentration profiles in human tumor and bone marrow were simulated as shown in Figure 4.
- Bone marrow concentration-time profile was predicted for a MM Phase I patient, based on the observed plasma data (Figure 5). The predicted imetelstat concentration at 24 hrs after dosing is 6.4 ug/g, which is in concordance with the observed value of 5.5 ug/g.

Figure 4. Target Tissue PK Model in Human (9.4 mg/kg).

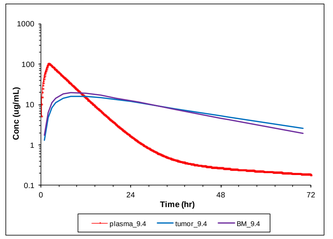
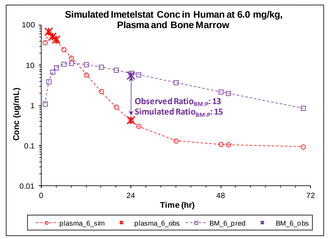


Figure 5. Simulated Human Bone Marrow Profile (6 mg/kg).



CONCLUSIONS

1. A target tissue pharmacokinetic model was established to describe imetelstat tumor and bone marrow distribution profiles in xenograft mice.
2. The current model will serve as a starting point for human target tissue prediction. It will allow the estimation of maximum target tissue concentration (C_{max}) and overall target tissue exposure (AUC) from sparse clinical sampling.
3. Once optimized and validated through observed clinical tissue measurements, the model may be used to predict human target tissue PK and guide clinical trial dosing and dosing regimen(s).
4. Single patient bone marrow data was in good concordance with the model.
5. Additional patient plasma, tumor and bone marrow data will be valuable for confirmation and refinement of the current target tissue PK model. Human tumor has greater variability than preclinical xenograft models. Therefore, inter-tumoral variability will need to be assessed by sampling various tumors from the clinical setting.
6. The model's application to other tumor types (e.g. primary brain tumor) will be assessed from patient samples derived from future studies.

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