INTERMITTENT DOSING OF IMETELSTAT SODIUM, A TELOMERASE INHIBITOR, INDUCES DRUG EXPOSURE CONSISTENT WITH IN VIVO TUMOR GROWTH INHIBITION

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introduction

- Preservation of telomere length is crucial to cell survival.
- Telomerase is a promising target for novel cancer therapeutics.
- The 2009 Nobel Prize for Medicine was awarded for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase**.**
- Imetelstat (GRN163L) is the only telomerase inhibitor in clinical development.

Telomeres

- TTAGGG hexa-nucleotide repeats that cap the ends of chromosomes and prevent chromosomal fusion.[1]
- Shorten by 50-200 basepairs (bp) per cell division.[2-3]
- Critically short telomeres may cause growth arrest and senescence.[4]

Telomerase

- Consists of at least two essential components, the RNA template (hTR) and the catalytic subunit (hTERT).
- Prevents telomere loss by countering the loss by the direct addition of TTAGGG repeats to the chromosome ends.[1]
- Repressed in most somatic cells[5] and downregulated in hematopoietic and other somatic stem cells.[6]

Telomerase and cancer

- Immortalization is invariably accompanied by constitutive activation of telomerase and preservation of telomeres.[7-8]
- Telomerase is highly activated in cancer stem cells, which have been shown to be sensitive to telomerase inhibition in several models.[9]

Imetelstat

- 13-mer thio-phosphoramidate oligonucleotide with a 5′ palmitoyl "tail".
- Its sequence overlaps the template region of telomerase.
- Designed to inhibit intracellular telomerase by binding to the RNA template in the active site of the enzyme.[10]
- Has demonstrated telomerase inhibitory and cancer growth inhibitory effects in both in vitro and in vivo preclinical models.[10-13]

References

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purpose

- Increase exposure of patients with solid tumors to levels of imetelstat that induce telomerase inhibition and are consistent with efficacy in preclinical models.
- Initial dosing: 2 hour once-weekly infusions of imetelstat
  - The dose limiting toxicity (DLT) was thrombocytopenia and was often seen after the first 3 weeks.
  - The maximum tolerated dose (MTD) was exceeded at 4.8 mg/kg.
  - This dose was below the minimally efficacious dose seen in mouse models.
- Alternative "intermittent" regimen of imetelstat
  - 2-hour infusions on Days 1 and 8 of a 21-day cycle
- Plasma samples were collected to test pharmacokinetic effects, and tissue samples were collected to test pharmacodynamic effects.

methods

- Design: Phase I, sequential cohort, 3+3 dose escalation, multicenter trial
- Patient population: Adults with refractory, advanced solid tumors
  - Inclusion criteria: Patients with malignancy that is evaluable or measurable; refractory or not amenable to standard therapy, Karnofsky status ≥ 70%, anticipated life expectancy ≥3 months.
  - Exclusion Criteria: Primary malignancy or active metastasis in CNS; Hematologic malignancies; Hemoglobin <9.0 g/dL; ANC <1,500 /mm^3; Platelet count <100,000 /mm^3; significant serum chemistry abnormality (bilirubin, AST, ALT, albumin, creatinine)
- Treatment: Successive cohorts at 4.8, 6.0, 7.5, 9.4 and 11.7 mg/kg imetelstat, given by 2 hr. i.v. infusions on Day 1 and Day 8 of 21-day cycles with delays up to 2 weeks allowed between cycles.
- Endpoints: Safety (primary), pharmacokinetics and efficacy (secondary)
- Dose Limiting Toxicity (DLT): grade 4 non-hematologic toxicity, grade 3 non-hematologic toxicity lasting >3 days or not medically-controllable, grade 4 neutropenia lasting >5 days, ≥grade 3 neutropenia associated with fever, grade 4 thrombocytopenia, grade ≥3 coagulation abnormality lasting >1 day, inability to complete cycle 1 or treatment delays of >2 weeks from next scheduled dose due to any toxicity thought to be associated with study drug.

results

Baseline Characteristics

- Results are presented for patients receiving the intermittent treatment regimen with infusions on Days 1 and 8 of the 21-day cycle. 31 patients were enrolled on this schedule, and 25 had data reported by the cut-off date of September 25, 2009.
- Most patients (22/25; 88%) received prior cytotoxic regimens (mean 4.0), and 15 (60%) had prior irradiation. See Table 1.

Table 1. Baseline Patient Characteristics

No. of Patients	25	Primary Tumor Site	
Male	12	GI	15
Female	13	Lung	3
Age: Median (years)	65.0	Skin	2
Range	29,77	Other	5
Karnofsky Status		Prior Cancer Therapies	
90-100	5	Single Agent	20
70-80	20	Combo Therapy	22
Stage		Rad Therapy	15
Stage 3b	1	Surgery for Primary Dx	25
Stage 4	24		

Dosing and Disposition

- 25 patients received at least 1 infusion of imetelstat.
- One patient in Cohort 9 is currently in treatment, and 21 of 24 patients discontinued the study due to disease progression.

Table 2. Disposition

Cohorts*	6	7	8	9	10	Total
Dose (mg/kg)	4.8	6.0	7.5	9.4	11.7	--
# of Patients	6	4	3	6	6	25

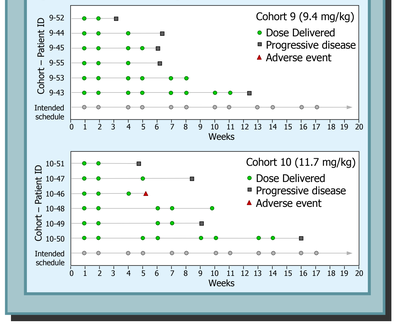
*Cohorts 1-5 were treated during the first phase of this study on a once-weekly schedule.

results

Dose Escalation

- Patients in Cohort 9 (9.4 mg/kg) were generally able to receive doses of imetelstat according to the pre-defined schedule (Figure 1). Patients received a median of 1.75 cycles prior to disease progression.
- Dose escalation proceeded to Cohort 10 (11.7 mg/kg) (Figure 1). The majority of patients had delays in treatment due to hematologic toxicities, and thus this dose was considered to exceed the MTD.
- Additional patients have recently been enrolled into Cohort 9 in order to further evaluate the dosing schedule

Figure 1. Timeline of Dosing Events in Cohorts 9 and 10.



Safety

- Infusions of imetelstat were generally well tolerated. Adverse events (AE) that were considered to be CTC Grade 3+ were reported in 21/25 (84%) patients. See Table 3.
- No dose limiting toxicities (DLT) were observed in Cohorts 6-9.
- Transient (<24hr) prolongation of aPTT occured without clinical sequelae.
- Cytopenias were noted at all dose levels but were more severe at the highest dose levels.

Table 3. Non-Hematologic adverse events CTC grade 3+ in >1 patient†

Cohorts	6	7	8	9	10	
Dose (mg/kg)	4.8	6.0	7.5	9.4	11.7	Total
# of Patients	6	4	3	6	6	25
Reported at least 1 AE	5 (83%)	4 (100%)	3 (100%)	3 (50%)	6 (100%)	21 (84%)
Activated partial thromboplastin time prolonged	3 (50%)	1 (25%)	3 (100%)	2 (33%)	4 (67%)	13(52%)
Blood Alkaline phosphatase increased	1 (17%)	1 (25%)	0	0	0	2 (8%)
Gamma-glutamyltransferase increased	1 (17%)	1 (25%)	0	0	0	2 (8%)

†Adverse events in 1 patient only included blood fibrinogen decreased, bone pain, chest pain, chills, convulsion, dyspnea, fatigue, hemorrhage, hyperglycemia, hypokalemia, prothrombin time prolonged, pulmonary embolism, rbc sedimentation rate increased, respiratory distress, and superior vena caval occlusion.

results

Safety continued

- Hematologic events are shown in Table 4.

Table 4. Hematologic adverse events by grade‡

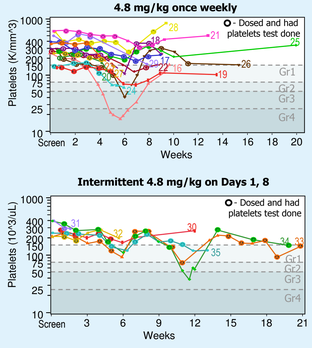
Cohorts	6	7	8	9	10	
Dose (mg/kg)	4.8	6.0	7.5	9.4	11.7	Total
# of Patients	6	4	3	6	6	25
Thrombocytopenia						
Grade 2	1	0	0	2	2	5
Grade 3	1	0	0	1	1	3
Grade 4	0	0	0	0	1	1
Anemia						
Grade 2	0	3	1	1	3	8
Grade 3	0	0	0	1	1	2
Grade 4	0	0	0	0	1	1
Neutropenia						
Grade 2	0	0	0	1	2	3
Grade 3	1	0	1	1	1	4
Grade 4	0	0	0	1	0	1

‡At the time of enrollment, 13 patients had grade 1 anemia, and 1 patient had grade 2 anemia.

Platelet Levels

- Grade 3 and 4 thrombocytopenia was seen with the weekly administration of imetelstat at 4.8 mg/kg. See Figure 2.

Figure 2. Platelet levels vs time with 4.8 mg/kg at different schedules



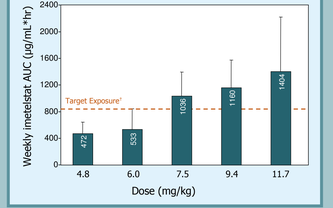
- Intermittent dosing ameliorated the thrombocytopenia observed with the weekly schedule

results

Patient Pharmacokinetics

- Plasma samples were obtained prior to and following infusions for determination of imetelstat concentration by a Hybridization-ELISA assay.
- Patient weekly plasma AUC values were calculated, and mean values for each dose group are shown in Figure 3.
- The exposure in patients (AUC values at 7.5mg/kg and above) is higher than the exposure associated with tumor growth inhibition in xenograft models.

Figure 3. Imetelstat Weekly AUC-inf in patients (columns) and in xenograft mice (dashed line)

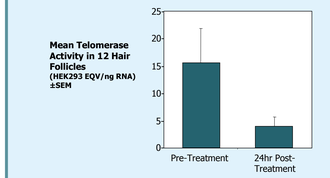


†Target exposure is based on 15mg/kg tiw dosing in mice, which was associated with both a ≥50% inhibition of telomerase in tumors and a concomitant inhibition of tumor growth.

Telomerase Activity: Pharmacodynamics

- Hair follicles were collected pre- and post-treatment and analyzed for telomerase activity using the TRAP assay (Telomere Repeat Amplification Protocol).
- There was a trend toward inhibition at 24-hours post-treatment in the 9 patients with hair follicle data. The inhibition was more pronounced in patients with the strongest baseline activity.
- Data from Patient 7-39 is presented in Figure 4. Telomerase activity was normalized against the RNA level for for the 12 follicles at the pre- and 24-hours post-treatment time-points.

Figure 4. The average RNA normalized telomerase activity of hair follicles pre- and post-imetelstat treatment (6 mg/kg) in Patient 7-39‡



‡Hair follicles were lysed in M-Per lysis buffer to make a soluble extract for the Gel TRAP assay. Total RNA was measured from the same extracts for normalization. The CyS labeled telomerase products were resolved in acrylamide gels and the gels scanned by gel imager.

summary

- An intermittent dosing schedule of imetelstat allows for exposures to be achieved which are consistent with efficacy in the preclinical models.
- Preliminary pharmacokinetic assessment of the intermittent dosing schedule demonstrates achievement of imetelstat plasma levels that are consistent with efficacy in the preclinical models.
- Hematologic toxicity was dose-limiting with the weekly dosing schedule but may be mitigated with the intermittent dosing schedule; other observed toxicities are either mild or infrequent.
- Preliminary pharmacodynamic (PD) evidence of target inhibition has been observed in selected patient tissues such as hair follicles. PD activity in other tissues is being evaluated.
- The 9.4 mg/kg dose given on an intermittent dosing schedule (Days 1 and 8 of a 21-day cycle) is the recommended dose for single agent phase II studies of imetelstat.
- A 28-day schedule is also under evaluation.

Acknowledgements

- The authors gratefully acknowledge Deena Gruver and Shuling Hwang for their important contributions and dedicated work throughout this study.
- The authors would like to acknowledge the patients and their families who participated in this study, the investigators and study coordinators who made this study possible.
- Funding provided by Geron

Poster (09-A-374-AACR) presented at the EORTC-NCI-AACR symposium on "Molecular Targets and Cancer Therapeutics" in Boston, MA, USA, 15-19 November 2009