

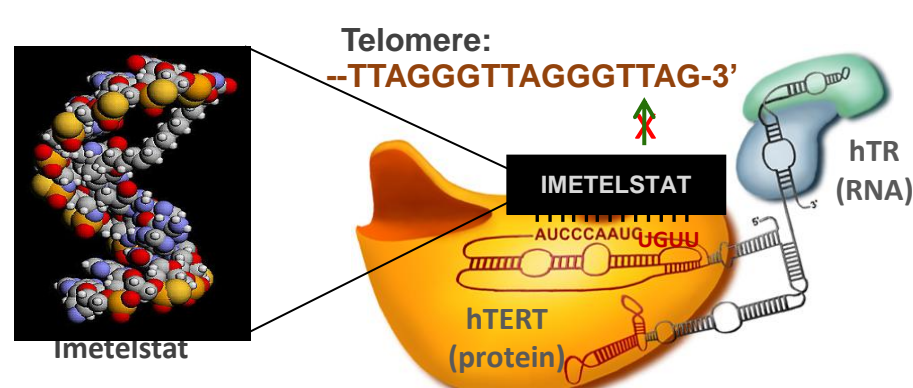
Improved progression-free survival (PFS) in patients with short tumor telomere length: subgroup analysis from a randomized phase II study of the telomerase inhibitor imetelstat as maintenance therapy for advanced NSCLC

Chiappori A⁷, Bassett, K², Burington B², Kolevska T³, Spigel DR⁴, Hager S⁵, Rarick M⁶, Gadgeel S⁸, Blais N⁹, Von Pawel J¹⁰, Hart L¹¹, Wang H², Eng K², Reck M¹², and Schiller J¹

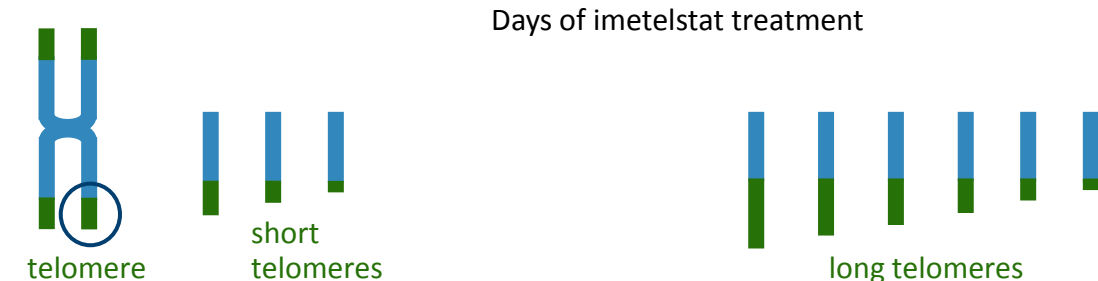
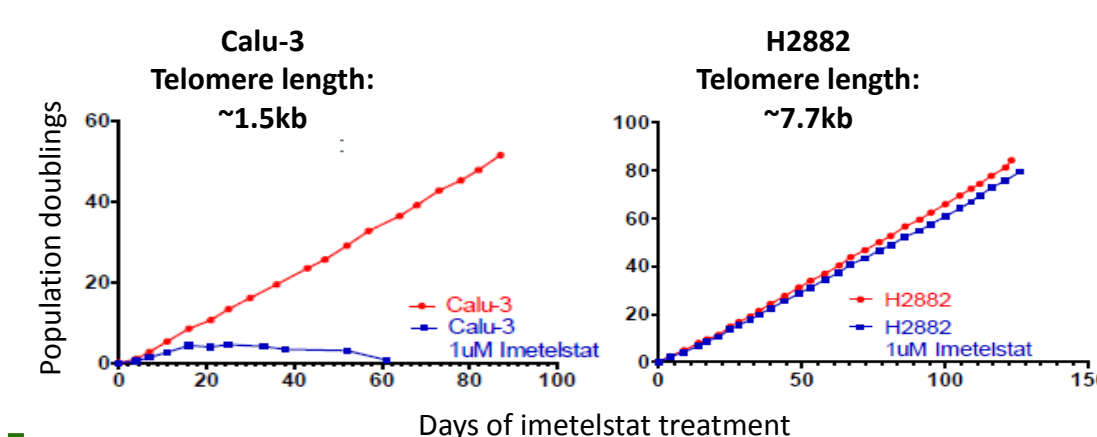
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background & introduction

- Telomeres are protective caps that maintain the stability and integrity of chromosomes. When telomeres reach a critically short length, chromosome ends are exposed to DNA damage mechanisms, which lead to senescence or apoptosis.
- The enzyme telomerase is upregulated in malignant cells and sustains indefinite replication by maintaining telomere length.
- Inhibition of telomerase may be a novel approach to treating cancer by limiting the proliferative capacity of malignant cells.
- Imetelstat, a lipidated 13-mer oligonucleotide, is a potent and specific inhibitor of telomerase.



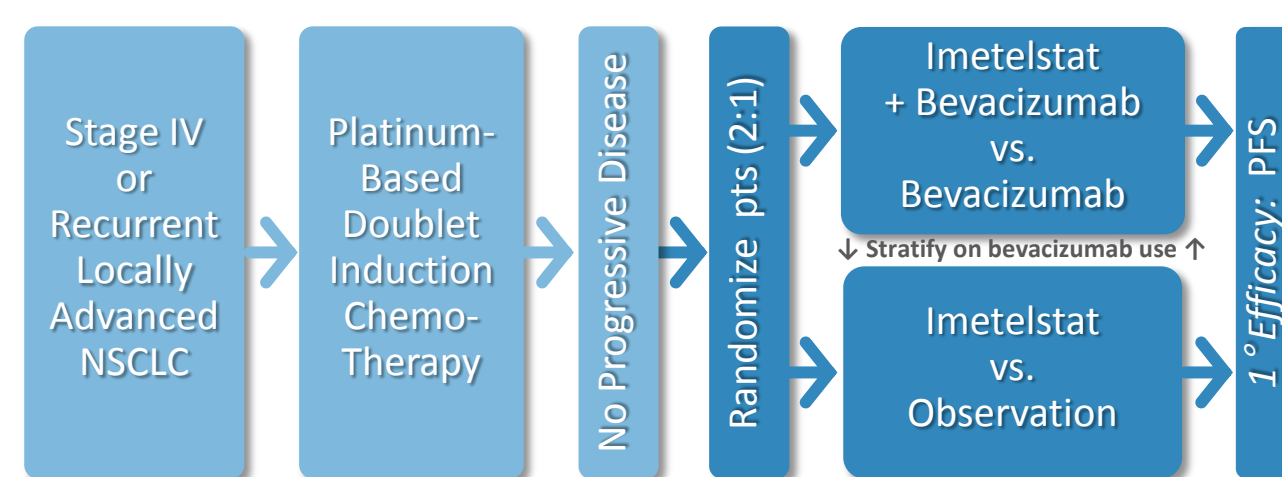
- NSCLC cell lines and other tumor cells with short telomeres appear to be more sensitive to imetelstat in vitro than those with long telomeres.¹ Telomere length varies with tumor type and among individual patients within tumor types. This led to a hypothesis that patients whose tumors have shorter telomeres may be more responsive to imetelstat.



In vitro effects of imetelstat on proliferation of NSCLC cell lines with short and long telomeres.¹

Imetelstat maintenance study design

- A randomized phase II study was conducted to assess whether imetelstat, given as maintenance therapy, prolongs PFS in advanced NSCLC: results for the primary and secondary endpoints are reported separately (²AACR Poster #4660)
- A planned exploratory analysis to determine PFS as a function of tumor telomere length (TL) was performed. Tumor TL was assessed in archival tumor specimens from pts by quantitative PCR (qPCR) and Telomeric Fluorescence In Situ Hybridization (Telo-FISH).



- 114 patients were evaluable for PFS; archival tumor specimens were obtained from 61 patients; 57 values were obtained from the retrospective qPCR assay, 52 values were obtained from the prospective qPCR assay, 59 values were obtained from Telo-FISH.

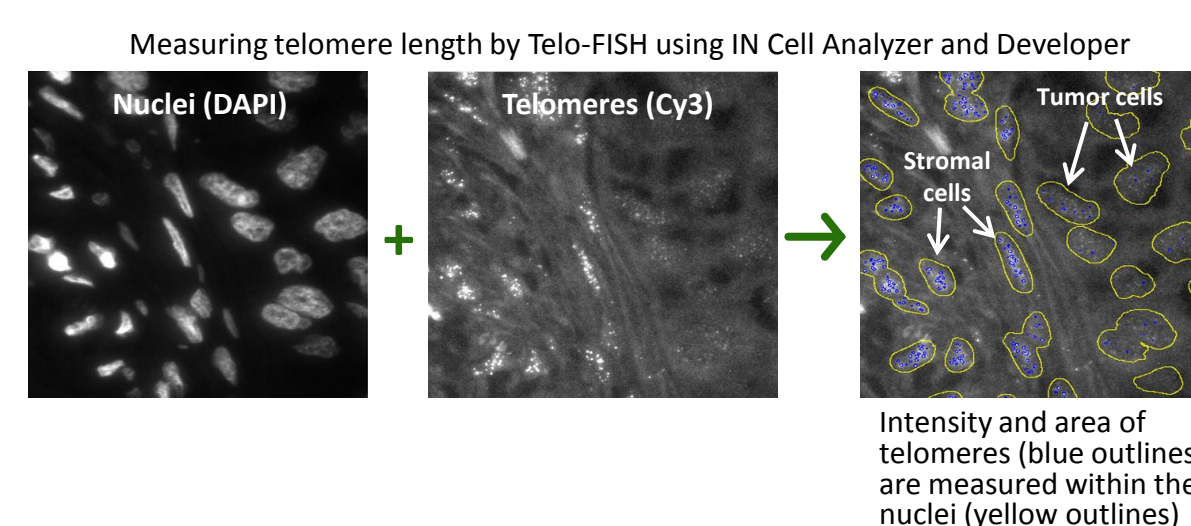
materials & methods

qPCR assay

- 5-micron FFPE sections were obtained from each patient. Telomere length was measured on two sections by a modified, retrospective qPCR assay based on the method of Cawthon R.³ Genomic DNA from FFPE specimen was isolated using FFPE Tissue DNA Extraction Kit (BioChain Institute Inc). DNA concentrations for DNA from FFPE samples were determined by Quant-iT Pico Green dsDNA Assay Kit (Invitrogen).
- All quantitative PCR reactions were carried out using ABI Prism 7900 HT Sequence Detection System (Applied Biosystems). Two PCR reactions were performed for each sample, one to determine the cycles threshold (Ct) value for telomere (T) amplification and the other to determine the Ct value for the amplification of a single copy gene (Acidic ribosomal phosphoprotein P, 36b4). The primer sequences for telomere amplification and 36B4 amplification are described in Cawthon R.³ Each PCR reaction for telomere amplification was performed using 1ng of sample. Relative telomere length was calculated as follows: $(2^{Ct_T/2^{Ct_S}})^{-1} = 2^{-((Ct_T - Ct_S) / 2)} = 2^{-dCt}$.
- The patient samples were run in 7 batches. DOE models were used to adjust for plate effects and sample DNA concentrations. Telomere-length analyses were pre-specified for patients grouped into the shortest 1/2, shortest 1/3 and shortest 1/4 of TL. Results in the group with the shortest 1/4 of TL were similar to the shortest 1/3 TL group, while in the shortest 1/2 group the differential benefit appeared diluted, suggesting that that 50% is too large a group size. Kaplan-Meier and Cox PH estimates were used for time-to-event analyses. Wilcoxon rank sum tests were used to test for differences in baseline characteristics.
- The retrospective assay processed 20 FFPE sections per plate x 2 slides per patient (N=57). The prospective protocol processed 2 sections per patient on a single plate (N=52), with single copy gene (SCG) controls and reference cell lines.

Telo-FISH assay

- Telo-FISH was performed on one FFPE section per patient. Telomere length was measured based on the method of Meeker R.⁴ Cells were hybridized with a telomere-specific probe conjugated to Cy3. Digital images of nuclei and telomeres were obtained using IN Cell Analyzer 2000 (GE Corp). Fluorescent intensities and areas of individual telomeres were measured using IN Cell Developer Toolbox 1.9 (GE Corp). Telomere length was calculated using the equation:
- By Telo-FISH, PFS benefit was associated with the log₂ intensity-to-area ratio: $1.376 \times \log_2(\text{intensity}) - 6.215 \times \sqrt{\text{area}}$, where "intensity" is defined as the intensity of the telomere and "area" is defined as the area of the telomere.



baseline characteristics

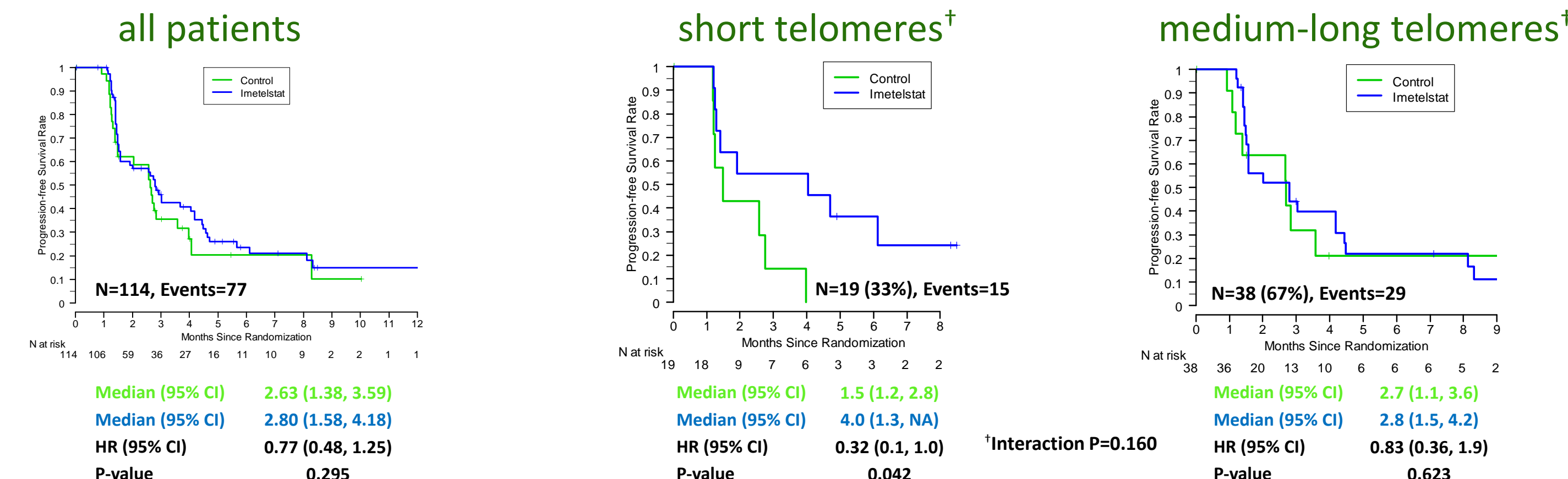
Baseline characteristic	Short Telomere N=19 (33%)	Medium-Long Telomere N=38 (67%)	P
Age (Median)	65.0	62.5	0.165
EGFR Mutant	1 (5.3%)	5 (13.2%)	0.360
Squamous Histology	5 (26.3%)	8 (21.1%)	0.655
Adenocarcinoma	12 (63.2)	28 (73.7%)	0.413
Induction Cycles (Median)	4	4	0.947
Induction Partial Response (vs. SD)	9 (47.4%)	11 (28.9%)	0.170
Days from End Induction to Randomization	34	28	0.030
ECOG 1 (vs. 0)	8 (42.1%)	25 (65.8%)	0.088
Imetelstat Tx	11 (57.9%)	26 (68.4%)	0.433
Bevacizumab Use	5 (26.3%)	10 (26.3%)	1

Note that multivariate adjustment for available prognostic factors does not substantially alter the results of subsequent analyses (not shown).

promising initial prediction of PFS benefit by retrospective batched qPCR assay

Primary PFS

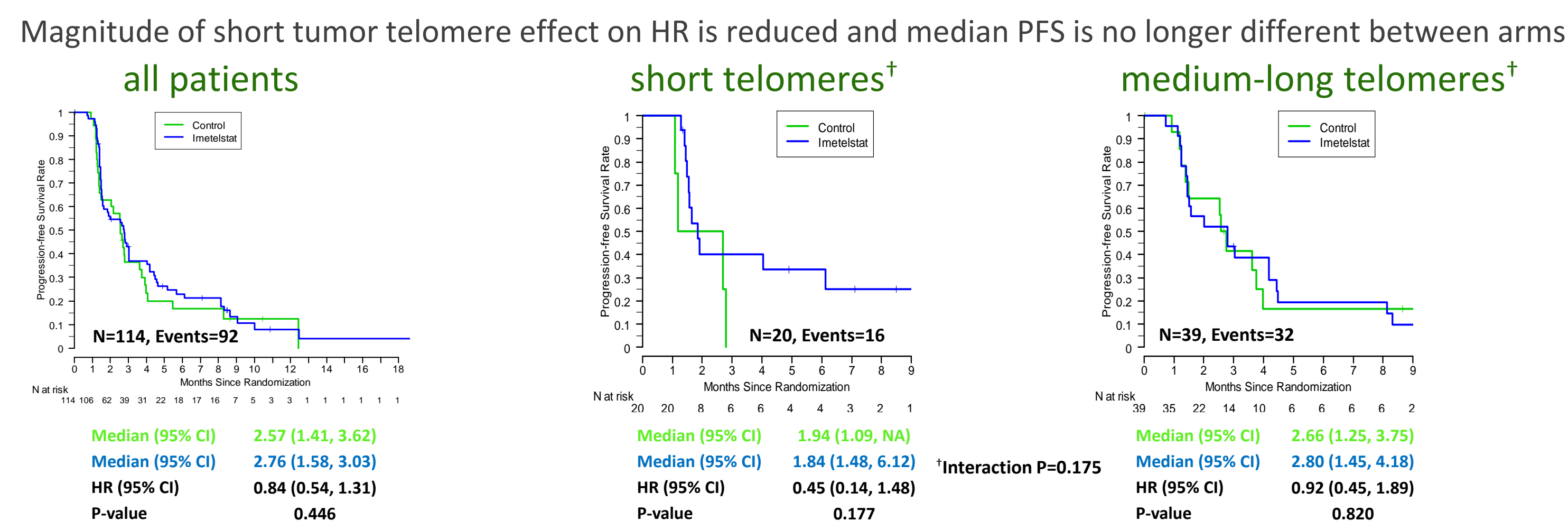
July 9, 2012
median FU=2.8mn



prospective Telo-FISH assay is predictive of PFS

Updated PFS

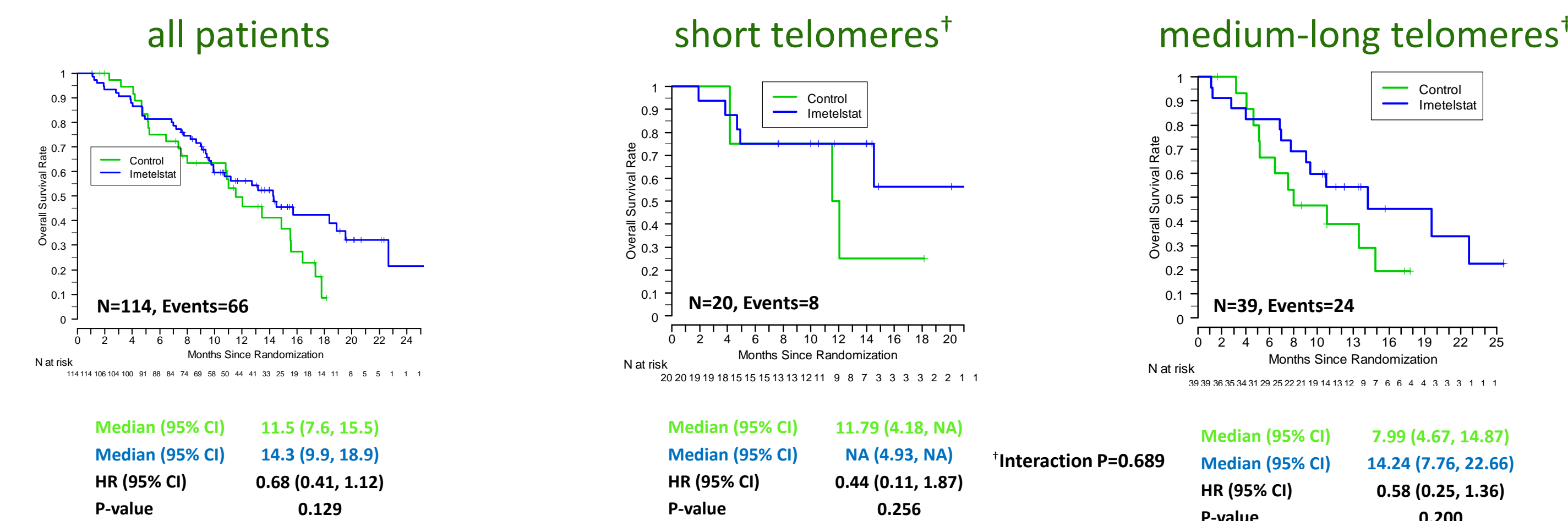
Feb. 11, 2013
median FU=2.6mn



prospective Telo-FISH assay is not predictive for overall survival

Mature OS

Feb. 11, 2013
median FU=10.5mn

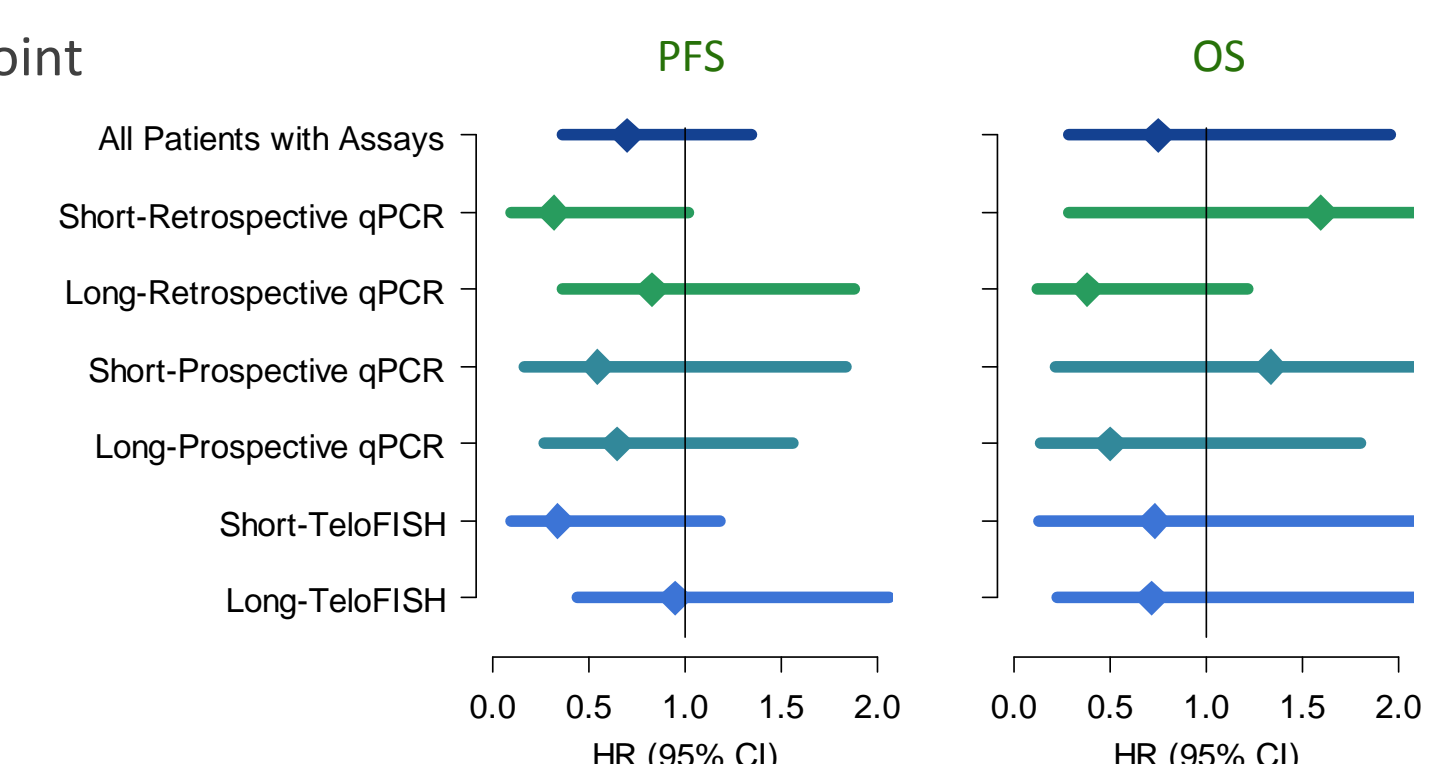


sub-group hazard ratios

For retrospective qPCR, prospective qPCR and prospective Telo-FISH

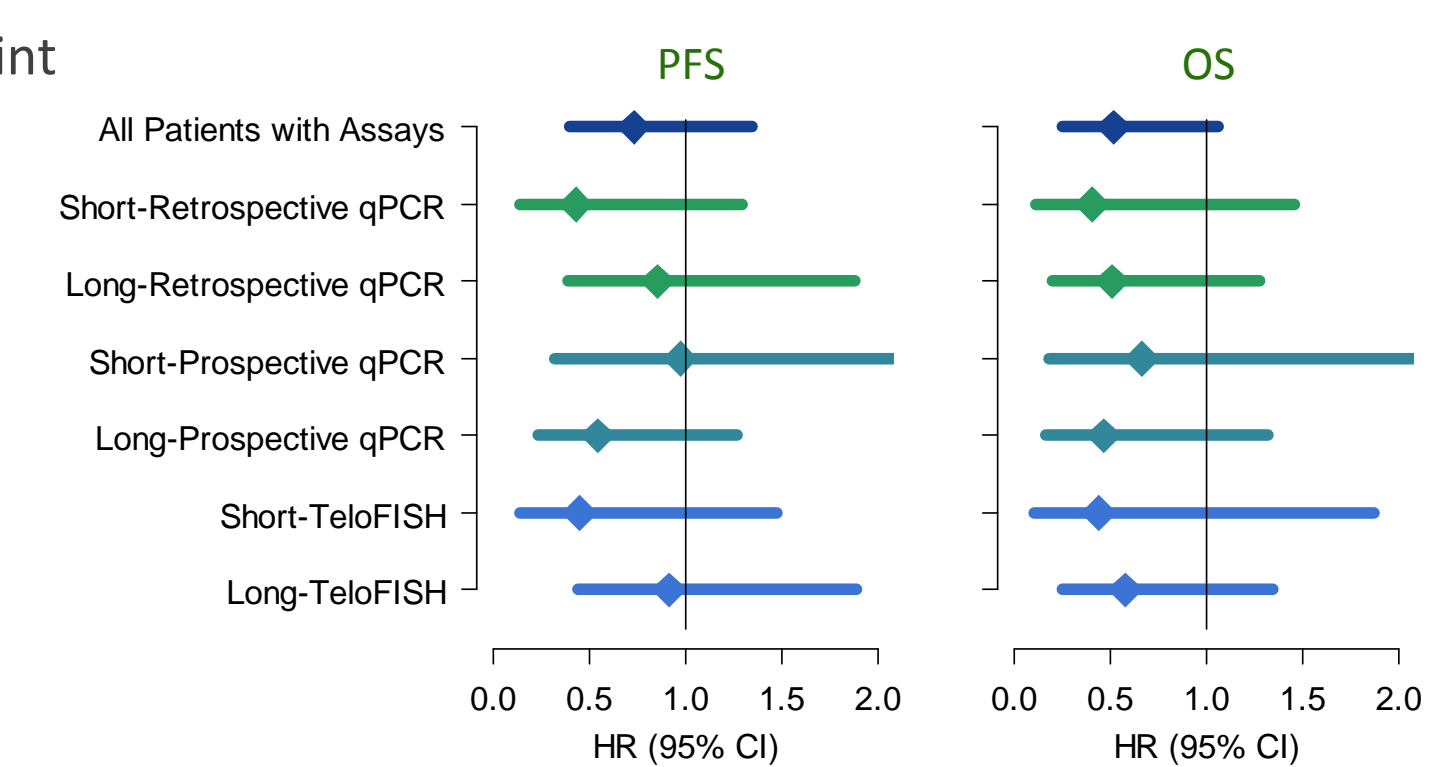
July 9, 2012:

Primary PFS Time Point



February 11, 2013:

Mature OS Time Point



conclusions

- In the original primary analysis of PFS, imetelstat given as maintenance therapy appeared to prolong PFS in patients with advanced NSCLC whose tumors have short telomeres, as measured by a retrospective qPCR assay.
- Results for Telo-FISH, a prospective assay, show a trend toward increased PFS benefit in the short telomere subgroup.
- Mature OS analyses suggest a survival benefit in patients with both short telomeres and medium-long telomeres. These data are not consistent with the hypothesis that clinical benefit from telomerase inhibition is potentially greater in patients with tumors possessing short telomeres.
- The impact of these findings on the development of imetelstat in solid tumors with short telomeres is being evaluated.

references

- Frink R et al, 2010 AACR Annual Meeting: Abstract #3577
- Chiappori A et al, 2013 AACR Annual Meeting: Abstract #4660
- Cawthon R et al, 2009 Nucleic Acids Res. 37(3): e21
- Meeker A et al, 2002 Am J Path 160(4): 1259

AACR abstract #2376

We thank all of the patients, caregivers, principal investigators and staff who have participated in this study.

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analyses

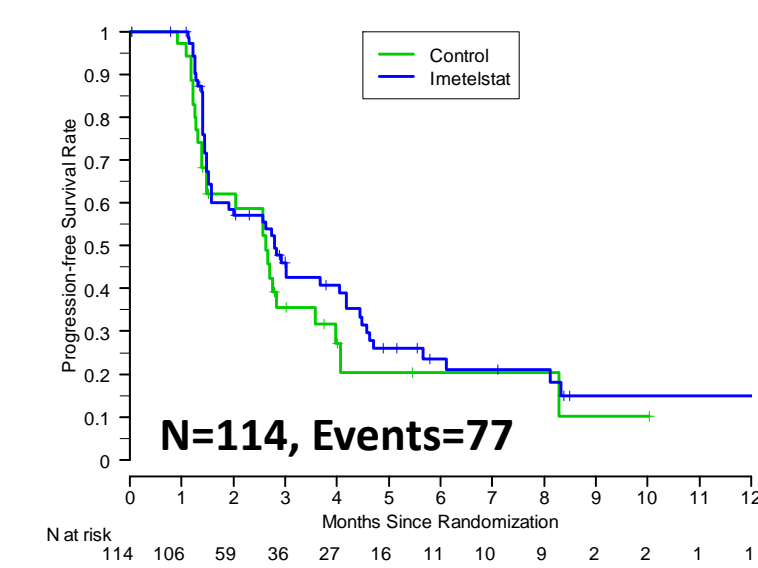
Primary PFS

- July 9, 2012
- median follow-up = 2.8 months

Legend:

- Control
- Imetelstat

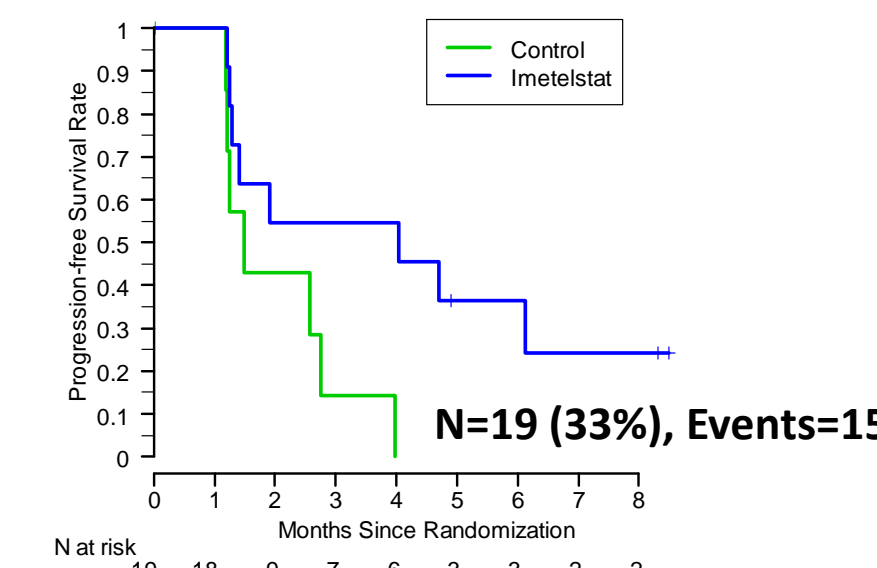
overall patient population



Median (95% CI)
 Control: 2.63 (1.38, 3.59)
 Imetelstat: 2.80 (1.58, 4.18)
 HR (95% CI): 0.77 (0.48, 1.25)
 P-value: 0.295

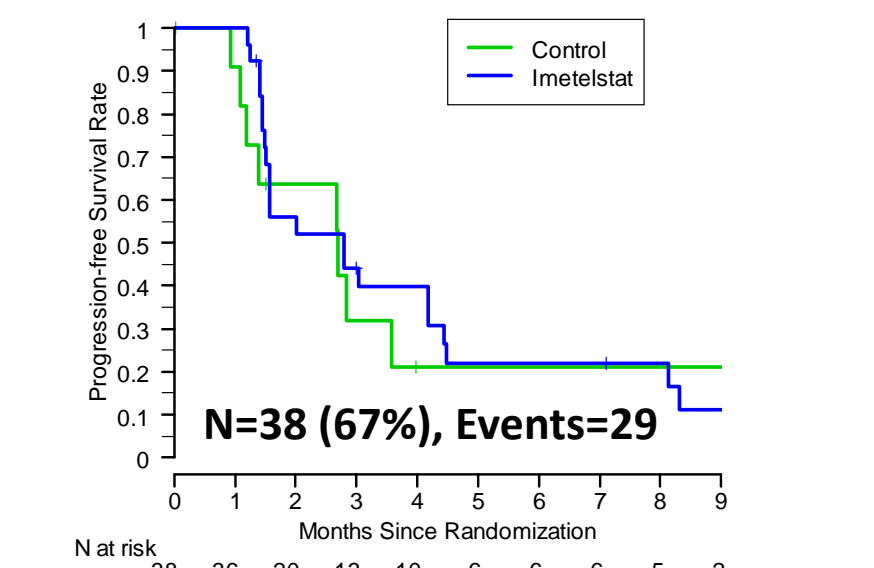
retrospective qPCR assay

short telomeres[†]



Median (95% CI)
 Control: 1.5 (1.2, 2.8)
 Imetelstat: 4.0 (1.3, NA)
 HR (95% CI): 0.32 (0.1, 1.0)
 P-value: 0.042

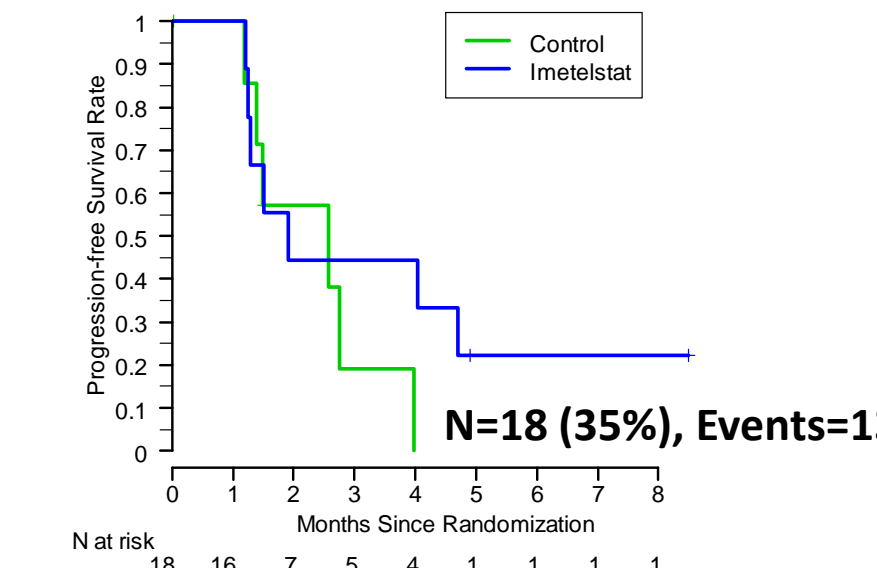
medium-long telomeres[†]



Median (95% CI)
 Control: 2.7 (1.1, 3.6)
 Imetelstat: 2.8 (1.5, 4.2)
 HR (95% CI): 0.83 (0.36, 1.9)
 P-value: 0.623

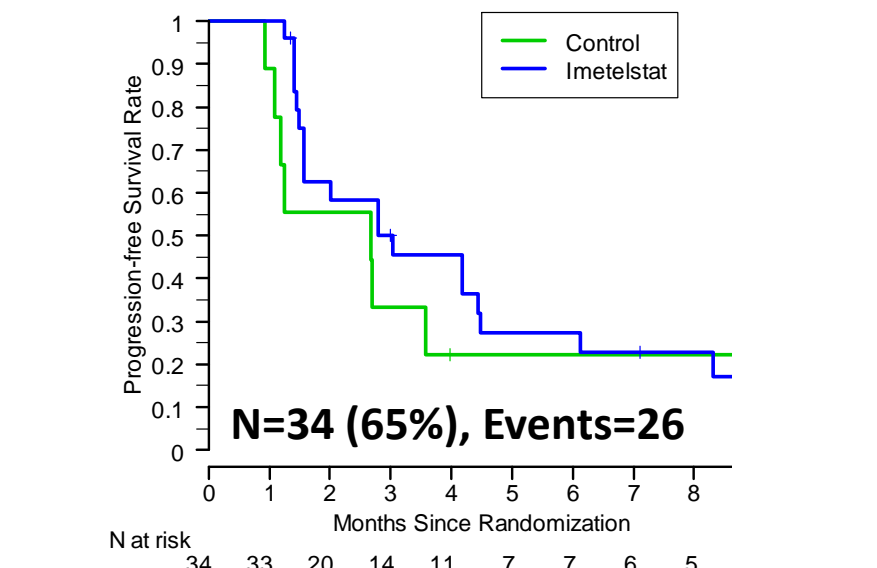
prospective qPCR assay

short telomeres[†]



Median (95% CI)
 Control: 2.57 (1.18, NA)
 Imetelstat: 1.91 (1.22, NA)
 HR (95% CI): 0.55 (0.17, 1.84)
 P-value: 0.325

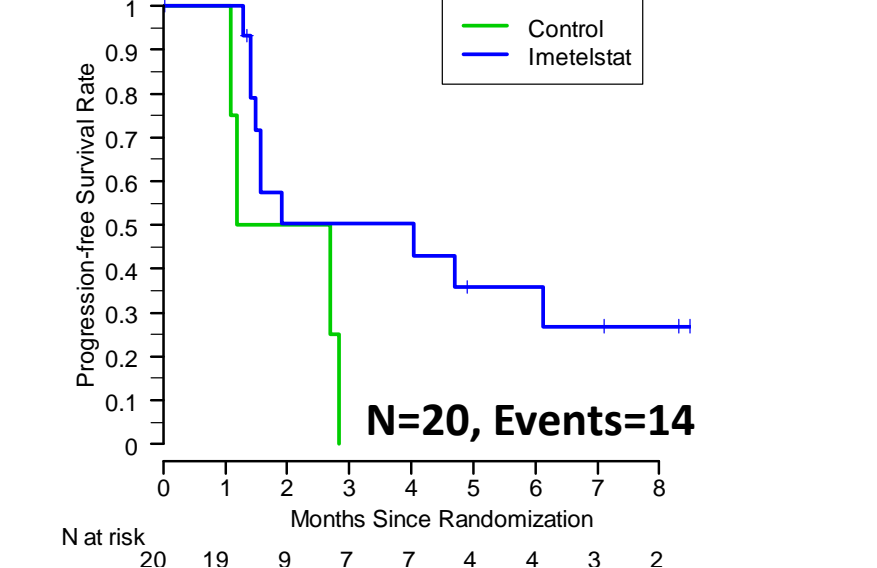
medium-long telomeres[†]



Median (95% CI)
 Control: 2.66 (0.92, NA)
 Imetelstat: 3.03 (1.58, 4.47)
 HR (95% CI): 0.65 (0.27, 1.56)
 P-value: 0.334

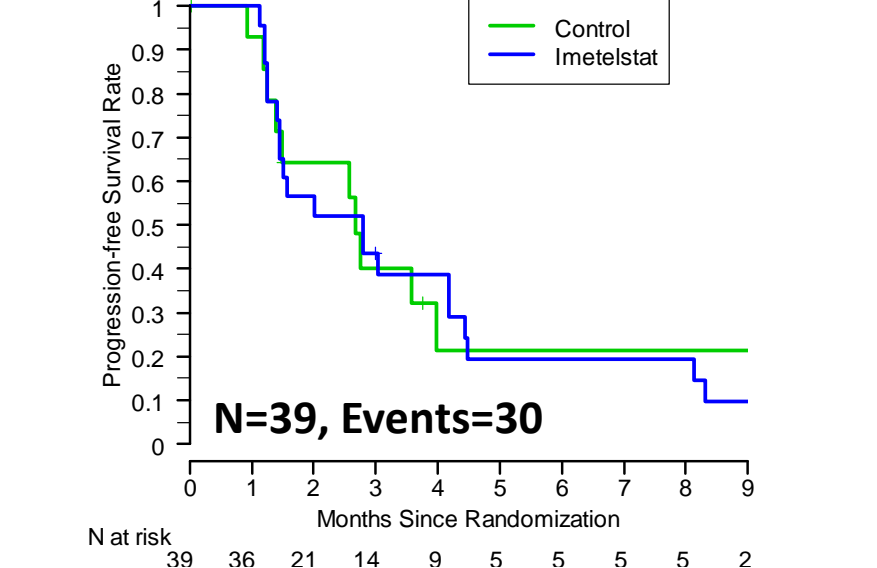
prospective TeloFISH assay

short telomeres[†]



Median (95% CI)
 Control: 1.94 (1.09, NA)
 Imetelstat: 4.05 (1.41, NA)
 HR (95% CI): 0.34 (0.1, 1.18)
 P-value: 0.067

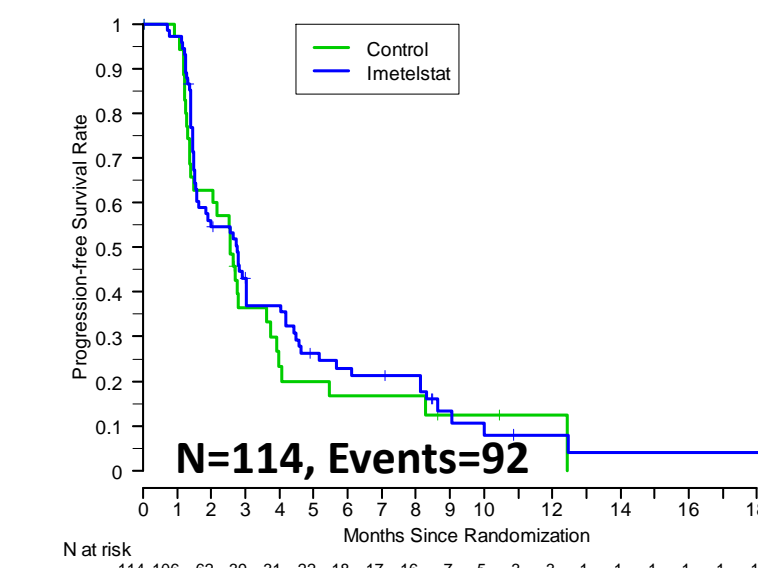
medium-long telomeres[†]



Median (95% CI)
 Control: 2.66 (1.25, 3.98)
 Imetelstat: 2.8 (1.45, 4.18)
 HR (95% CI): 0.95 (0.44, 2.06)
 P-value: 0.88

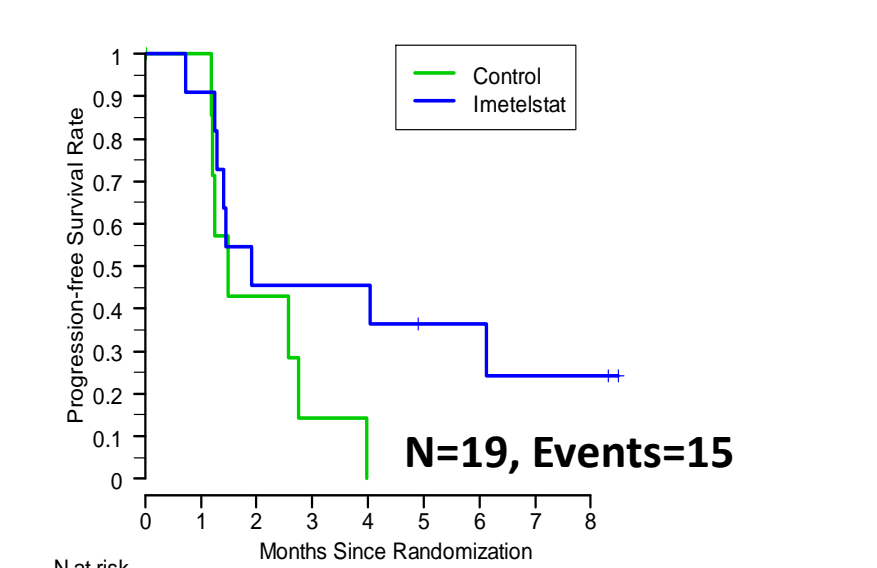
Updated PFS

- February 11, 2013
- median follow-up = 2.6 months



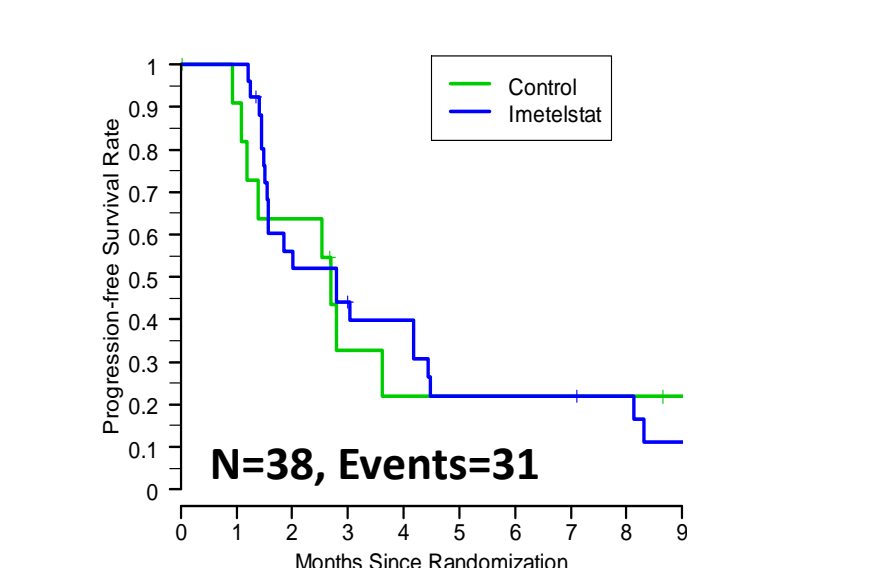
Median (95% CI)
 Control: 2.57 (1.41, 3.62)
 Imetelstat: 2.76 (1.58, 3.03)
 HR (95% CI): 0.84 (0.54, 1.31)
 P-value: 0.446

short telomeres[†]



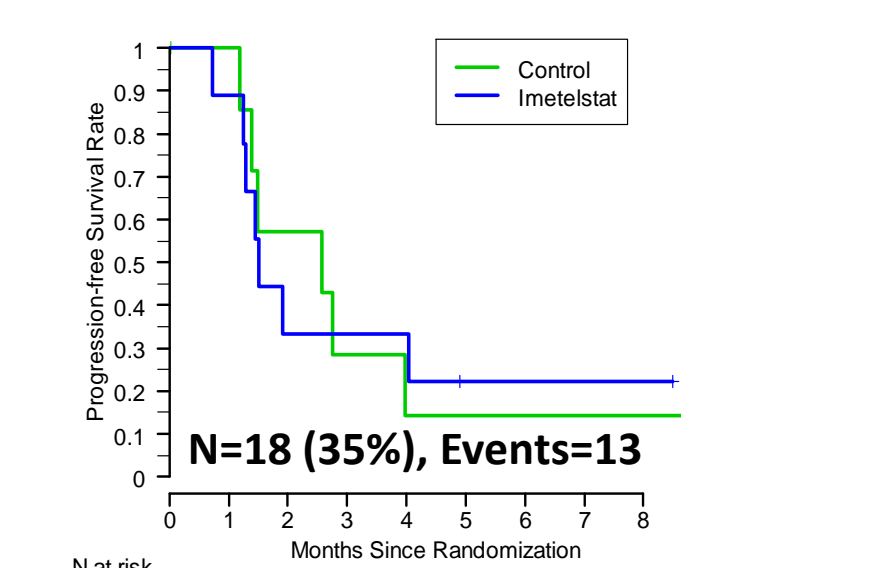
Median (95% CI)
 Control: 1.48 (1.18, 2.76)
 Imetelstat: 1.91 (1.25, NA)
 HR (95% CI): 0.43 (0.14, 1.3)
 P-value: 0.124

medium-long telomeres[†]



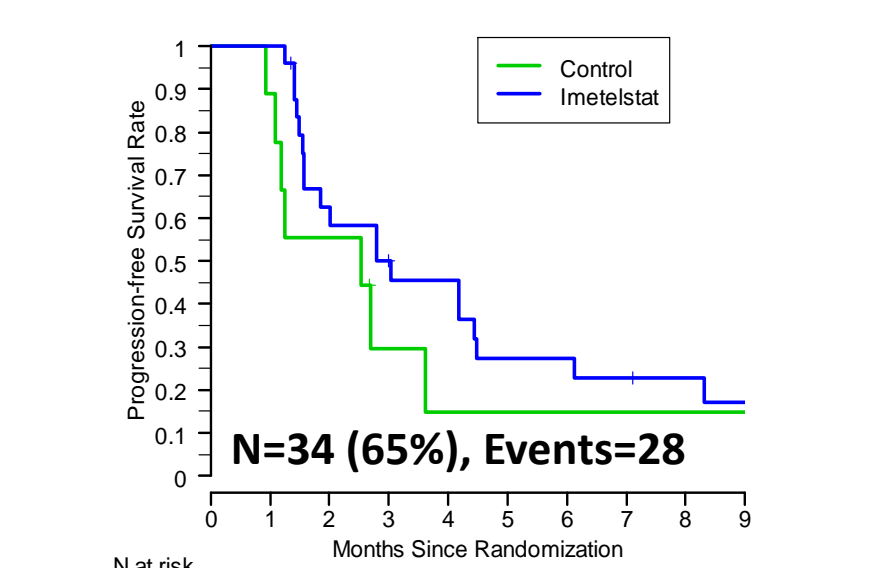
Median (95% CI)
 Control: 2.7 (1.09, NA)
 Imetelstat: 2.8 (1.55, 4.18)
 HR (95% CI): 0.86 (0.39, 1.88)
 P-value: 0.689

short telomeres[†]



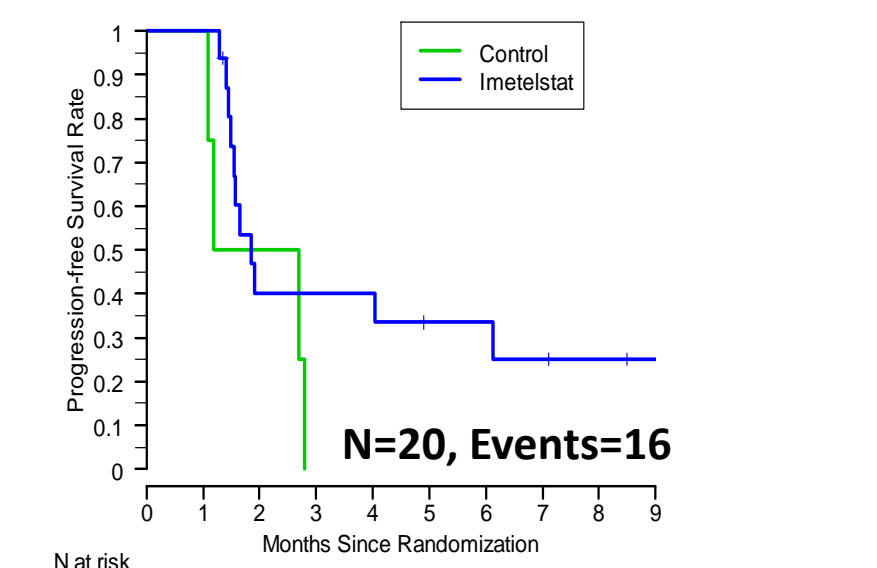
Median (95% CI)
 Control: 2.57 (1.18, 3.98)
 Imetelstat: 1.51 (0.72, NA)
 HR (95% CI): 0.97 (0.32, 2.93)
 P-value: 0.962

medium-long telomeres[†]



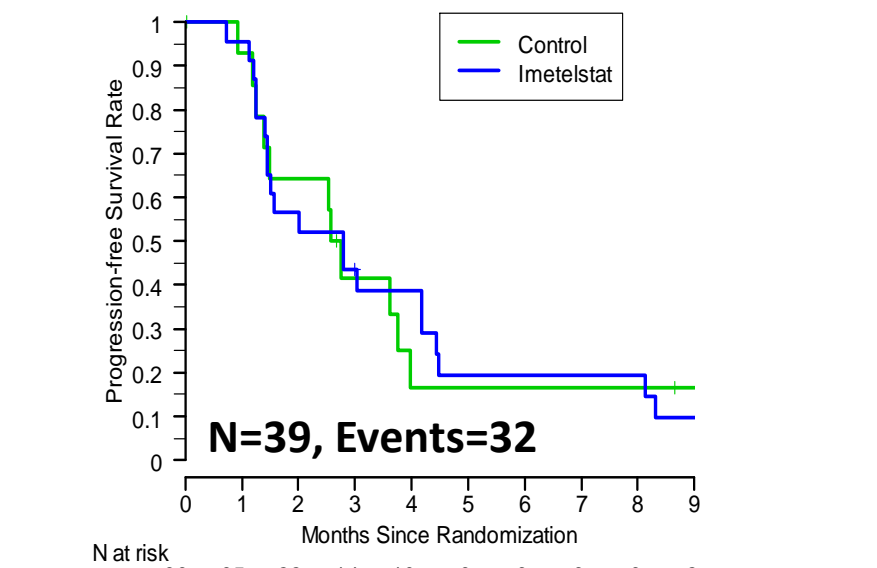
Median (95% CI)
 Control: 2.53 (0.92, 3.62)
 Imetelstat: 3.03 (1.58, 4.47)
 HR (95% CI): 0.55 (0.24, 1.27)
 P-value: 0.154

short telomeres[†]



Median (95% CI)
 Control: 1.94 (1.09, NA)
 Imetelstat: 1.84 (1.48, 6.12)
 HR (95% CI): 0.45 (0.14, 1.48)
 P-value: 0.177

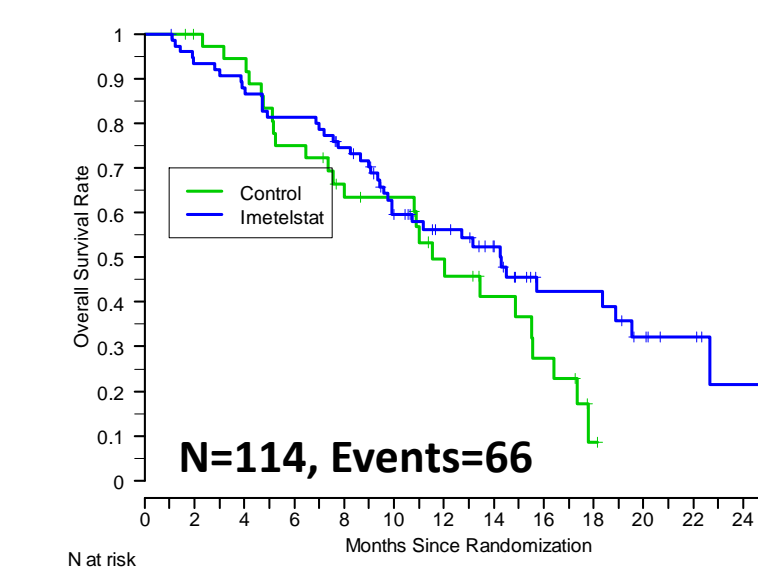
medium-long telomeres[†]



Median (95% CI)
 Control: 2.66 (1.25, 3.75)
 Imetelstat: 2.80 (1.45, 4.18)
 HR (95% CI): 0.92 (0.45, 1.89)
 P-value: 0.820

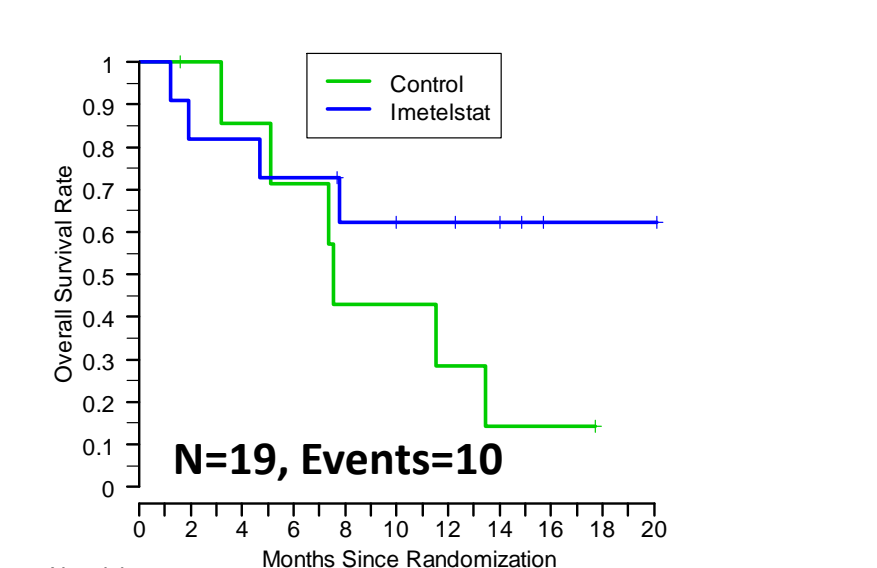
Mature OS

- February 11, 2013
- median follow-up = 10.5 months



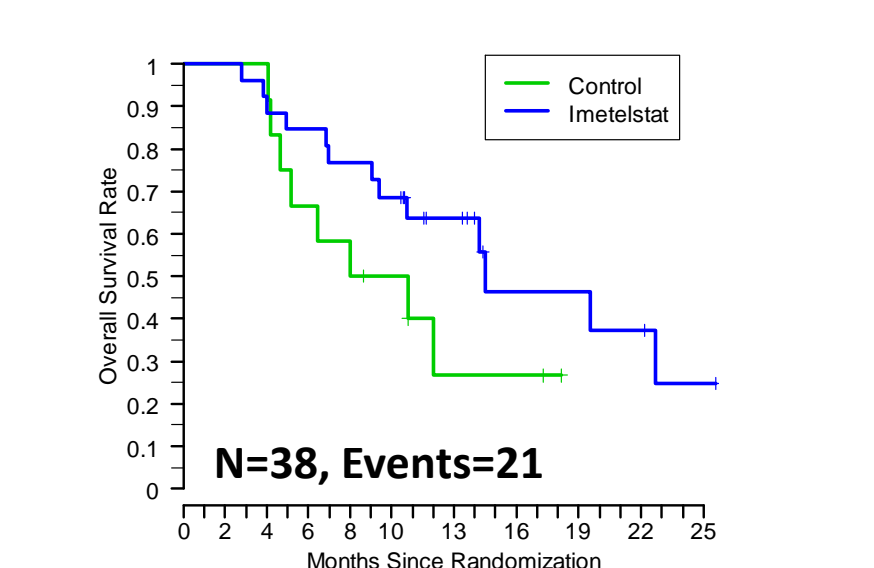
Median (95% CI)
 Control: 11.5 (7.6, 15.5)
 Imetelstat: 14.3 (9.9, 18.9)
 HR (95% CI): 0.68 (0.41, 1.12)
 P-value: 0.129

short telomeres[†]



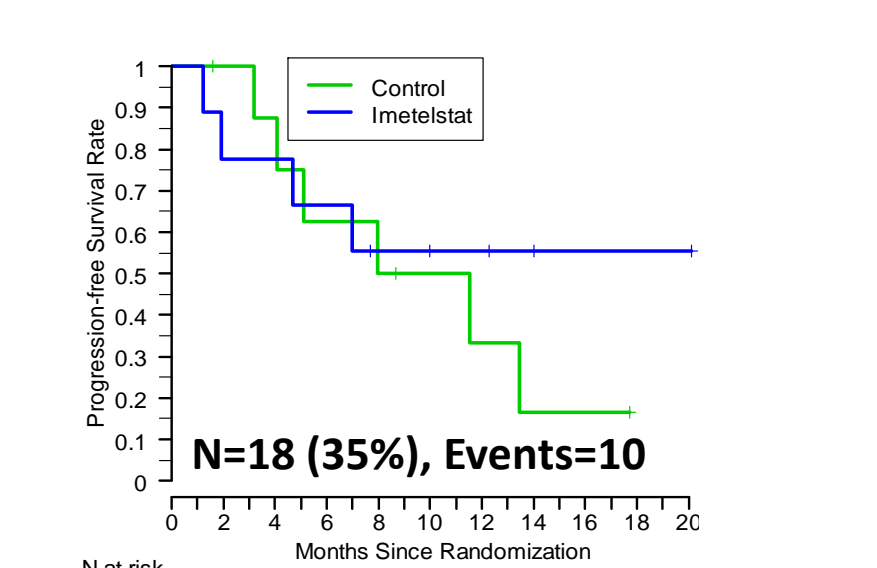
Median (95% CI)
 Control: 7.57 (3.19, 13.45)
 Imetelstat: NA (1.91, NA)
 HR (95% CI): 0.41 (0.11, 1.46)
 P-value: 0.155

medium-long telomeres[†]



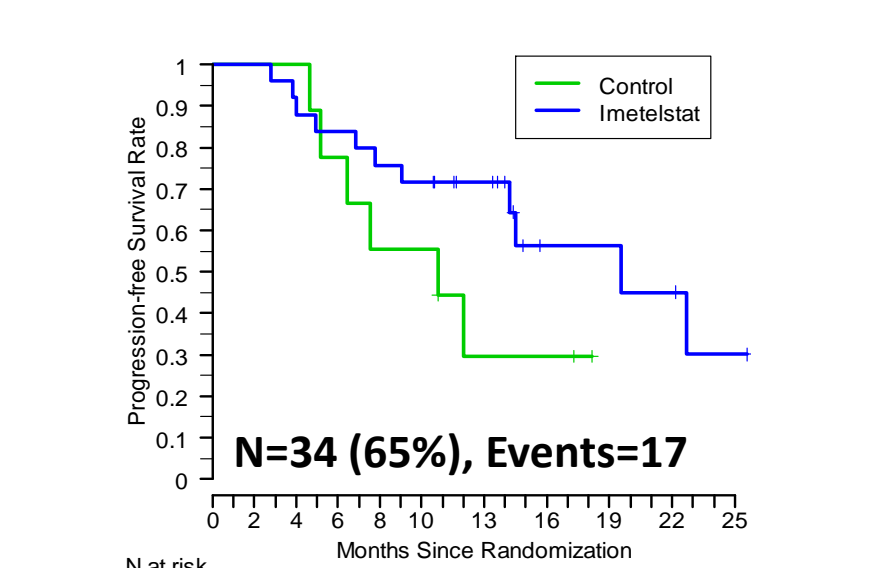
Median (95% CI)
 Control: 7.99 (4.18, NA)
 Imetelstat: 14.51 (9.41, NA)
 HR (95% CI): 0.51 (0.2, 1.28)
 P-value: 0.145

short telomeres[†]



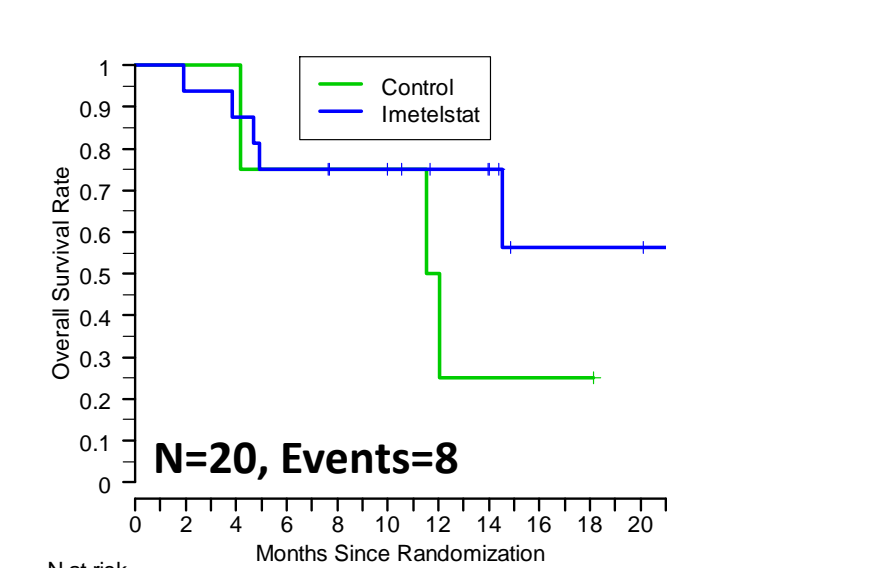
Median (95% CI)
 Control: 9.77 (3.19, NA)
 Imetelstat: NA (1.22, NA)
 HR (95% CI): 0.67 (0.19, 2.37)
 P-value: 0.529

medium-long telomeres[†]



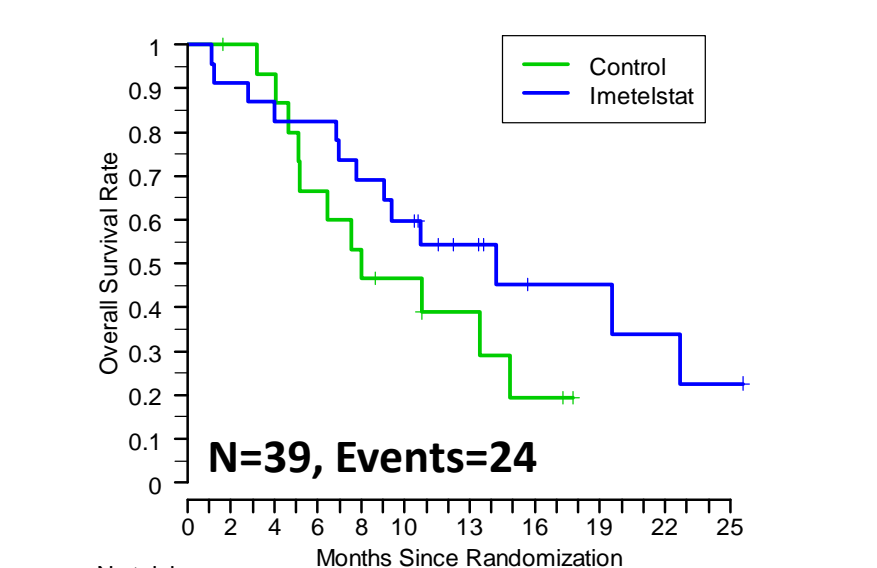
Median (95% CI)
 Control: 10.79 (4.67, NA)
 Imetelstat: 19.54 (9.08, NA)
 HR (95% CI): 0.47 (0.17, 1.33)
 P-value: 0.144

short telomeres[†]



Median (95% CI)
 Control: 11.79 (4.18, NA)
 Imetelstat: NA (4.93, NA)
 HR (95% CI): 0.44 (0.11, 1.87)
 P-value: 0.256

medium-long telomeres[†]



Median (95% CI)
 Control: 7.99 (4.67, 14.87)
 Imetelstat: 14.24 (7.76, 22.66)
 HR (95% CI): 0.58 (0.25, 1.36)
 P-value: 0.200