Dynamics of Telomere Length Reflect the Clonal Suppression Seen with the Telomerase Inhibitor Imetelstat in Patients with Essential Thrombocythemia

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BACKGROUND

Telomerase Inhibition in Essential Thrombocythemia (ET)

In a phase-2 study, patients with ET treated with imetelstat demonstrated rapid and durable hematologic and molecular responses and suppression of clones with non-driver mutations (Baerlocher et al. NEJM 2015; Oppliger et al. ASH 2015).

The median *JAK2* V617F mutant allele burden was reduced by 71% at month 3, and *MPL* W515L/K and *CALR* mutant allele burdens were reduced by 15 to 66%. Most additional mutations in *ASXL1*, *CBL*, *DNMT3A*, *EZH2*, *IDH1*, *SF3B1*, *TET2*, *TP53* and *U2AF1* were also responsive to imetelstat except some *TP53* and *SF3B1* mutations.

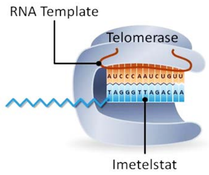
Telomerase and Telomere Length

Telomerase is transiently expressed in normal stem and progenitor cells and can maintain or elongate telomere length.

In most human somatic cells, however, telomerase is physiologically suppressed and telomere repeats are lost with each cell division. Telomere length (TL) can therefore be used to describe clonal dynamics of hematopoietic cells.

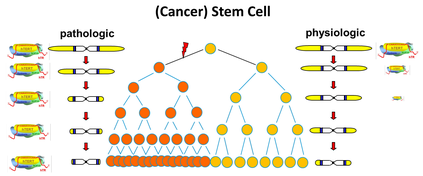
In contrast, telomerase is reactivated in 90% of human cancer cells, as in neoplastic cells of myeloproliferative neoplasms (MPN). Despite telomerase activity neoplastic cells with a high mitotic rate typically display low telomere length values (TLV).

Imetelstat



- First telomerase inhibitor in clinical studies
- 13-mer modified oligonucleotide with palmitoyl lipid tail
- Competitively binds to RNA template of telomerase
- Potent inhibitor of telomerase enzyme activity
- Long residence time in bone marrow, spleen and liver

Clonal Hematopoiesis



AIMS

- To measure TLV in subsets of leukocytes from ET patients treated with imetelstat in comparison to patients with MPN on standard of care (SOC) and healthy individuals
- To assess the TL dynamics in patients with ET treated with imetelstat and to correlate TLV with hematologic and molecular responses

METHODS

17 patients with ET treated with imetelstat (IM-ET) who were resistant or intolerant to prior therapies and 63 patients with MPN (16 ET, 34 PV, 13 MF) untreated or treated with standard of care (SOC-MPN) were included in the study. 44% were treated with hydroxyurea, 43% with aspirin, 41% with phlebotomy and 19% received another medication (as for example IFN-alpha, anagrelide).

All patients were diagnosed according to WHO 2008 criteria.

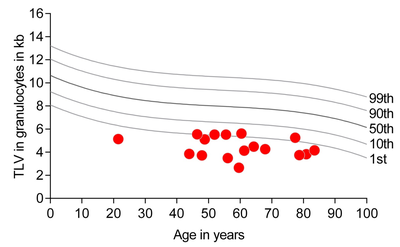
TLV were measured by automated multicolor flow-FISH in subsets of leukocytes (Baerlocher et al, Nat Protoc. 2006). TLV from >400 healthy individuals were used as reference population.

PATIENT CHARACTERISTICS

Characteristics	IM-ET N=17 Median or N (%)	SOC-MPN N=63 Median or N (%)		
		SOC-PV (N=34)	SOC-ET (N=16)	SOC-MF (N=13)
Age at Study Entry (years)	60	60	65	66
Gender (f)	9	15	13	6
Years Since Initial Diagnosis	7.6	4.7	2.1	1.5
Platelet Count at Study Entry (x $10^3/\mu L$)	809	397	881	267
Hemoglobin at Study Entry (x g/L)	124.0	166.5	137	119.5
WBC Count at Study Entry (x $10^3/\mu L$)	8.1	8.8	9.8	6.7
JAK2 V617F	8	28	11	6
Calreticulin mt	5	0	2	4
MPL W515^{mt}	2	0	1	0

RESULTS

Telomere Length Values (TLV) in IM-ET Patients at Baseline



All IM-ET patients showed low TLV at baseline, with 12 patients below the 1st percentile.

dTLV in granulocytes were significantly lower than in the corresponding lymphocytes (p<0.001).

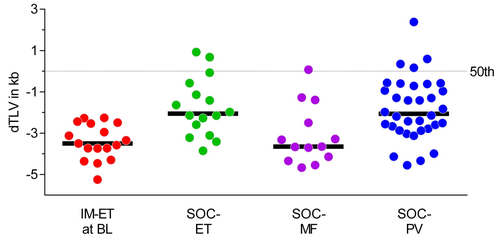
Features of IM-ET Patients by Baseline TLV

Features	Baseline TLV (Median = 4.2 kb)	
	Low < Median (n=8)	High ≥ Median (n=9)
Age, median (range)	60 (43-80)	60 (21-83)
Years Since Diagnosis, median (range) †	11 (2-25)	3 (0.26-21)
Prior Therapies No., median (range)	2.5 (2-4)	2 (1-3)
Hematologic Complete Remission†	6 (75%)	9 (100%)
Loss of Response (thromboembolic events or resistance to treatment)	3 (38%)	2 (22%)
Baseline Driver Mutation Burden, median*	40%	23%
Best Reduction in Driver Mutation Burden, median (n=15)*	-42%	-78%
Baseline Additional Mutation Burden, median (n=9) †	57%	38%

In patients with shorter baseline telomere length, driver mutation burden at baseline was significantly higher (p=0.03) and best reduction in driver mutation burden was significantly less (p=0.03).

* p < 0.05; † trend towards statistical significance

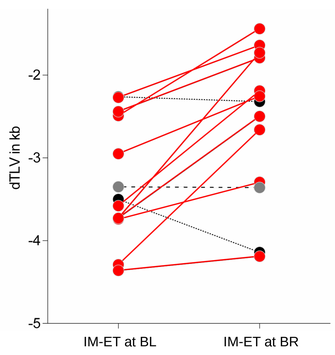
Age-adjusted TLV (dTLV) of IM-ET Compared to MPN Patients



The median dTLV in IM-ET patients (red) with a median of 2 prior therapies is significantly lower than in ET patients on SOC (green) and similar to dTLV of MF patients (purple).

dTLV: TL change in kb to the 50% percentile (corrected for age); BL: Baseline; 50th percentile

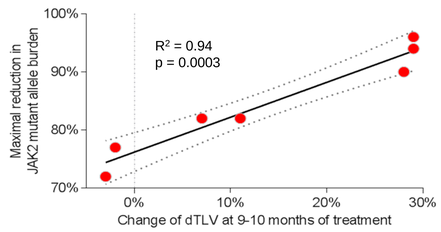
Higher dTLV after Treatment with Imetelstat in 10 Patients



The higher dTLV at BR reflects the reduction of neoplastic clones in relation to normal hematopoietic cells.

13 patients with at least two TL values (at baseline and best response)
BR: Best response of molecular driver mutation

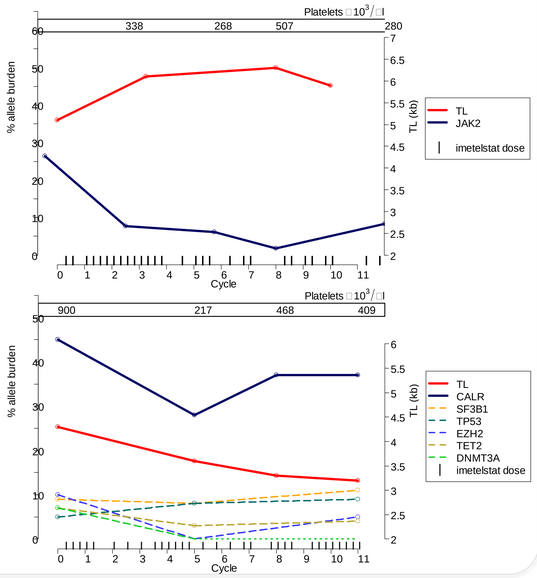
Changes of dTLV Correlate with Molecular Response to Imetelstat



Patient No	Change in TLV at 9 Months of Treatment (%)	Max. Reduction in JAK2 Mutant Allele Burden (%)	Additional Mutations (Variant Allele Frequency)	Total Additional Allelic Burden (%)
14	-2	-72	DNMT3A p.Ala644Thr (3%) DNMT3A p.Arg688His (4%) DNMT3A c.2597+1G>A (8%)	15
09	-2	-77	ASXL1 p.Gly646Trpfs (33%) U2AF1 p.Gln157Pro (28%) CBL c.1432-1G>A (5%)	66
03	4	-82	none	none
17	6	-82	ASXL1 p.Tyr591Ter (30%)	30
05	13	-94	none	none
16	23	-96	none	none
10	27	-90	DNMT3A p.Met880Val (45%) TET2 p.Met1772Cysfs (42%)	87

7 patients with JAK2V617F and at least two TL values (at baseline and best response)

Patient Courses and TL



CONCLUSIONS

- The lower TLV found in granulocytes of patients with MPN and especially with MF compared to healthy individuals reflect the higher mitotic history of malignant clones.
- In the ET imetelstat cohort, driver mutation burden at baseline and best reduction during treatment correlate significantly with baseline telomere length.
- We demonstrate higher TLV after 9-10 months of therapy and a significant correlation with the reduction in the driver mutational burden.
- TL dynamics analyzed in subsets of leukocytes by flow-FISH depict clonal behavior. Our data suggest that imetelstat treatment in ET patients can suppress neoplastic clones and favors recovery of normal hematopoiesis.