

# Imetelstat, A Potent Telomerase Inhibitor, Inhibits the Spontaneous Growth of CFU-Meg

## In Vitro From Essential Thrombocythemia Patients but Not From Healthy Individuals

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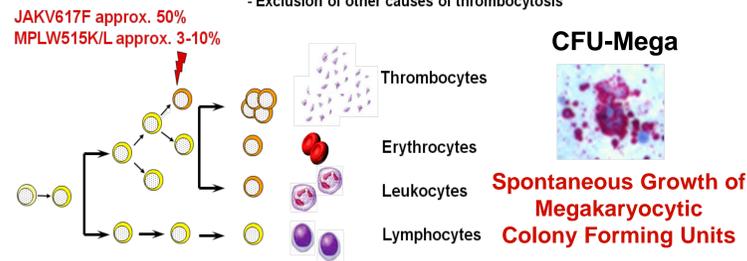
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### Background

### Results and Methods

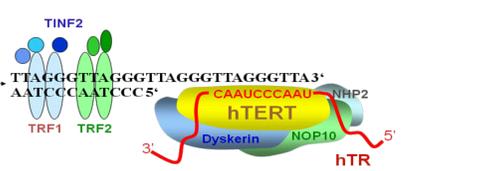
#### Essential Thrombocythemia

Diagnostic Criteria (WHO): -Thrombocytosis  $\geq 450$  G/L  
- Bone marrow: high number of large, mature megakaryocytes  
- Episodes of thrombosis and hemorrhage  
- Exclusion of other causes of thrombocytosis

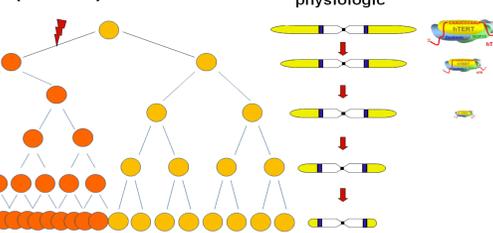


#### Telomerase

##### Telomere



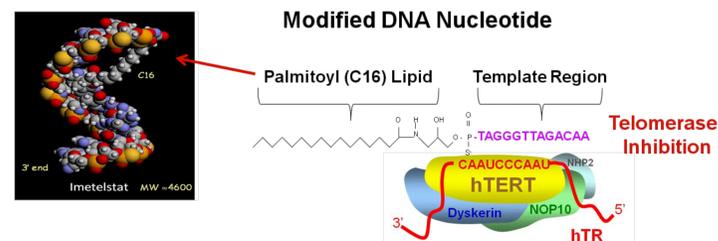
##### Tumor Cells (Cancer) Stem Cell



90% of human cancer cells express telomerase

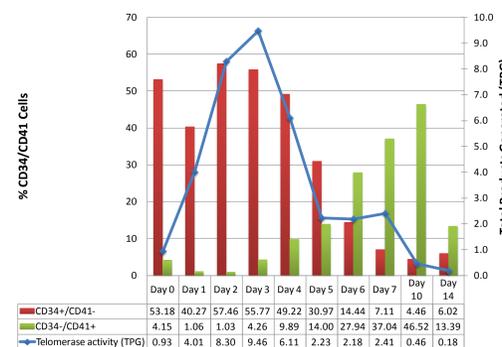
#### Telomerase Inhibitor (Imetelstat Sodium GRN163L)

##### Modified DNA Nucleotide

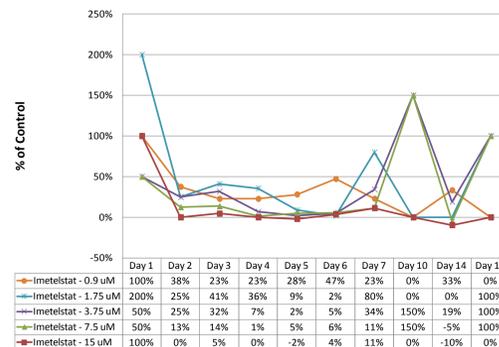


#### Cord Blood

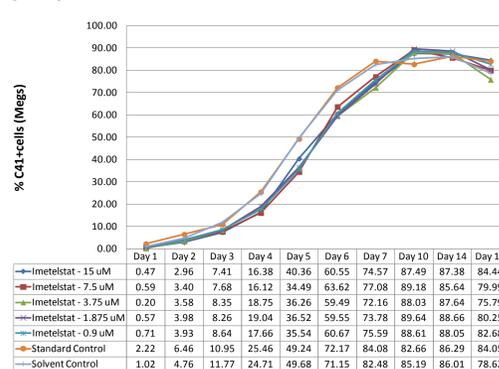
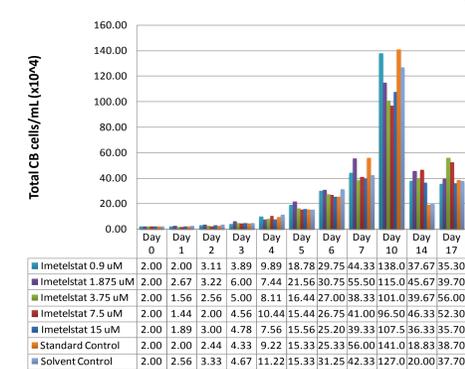
##### TA during Megakaryocytic Differentiation



##### Telomerase Inhibition by Imetelstat in CB Cells



#### Imetelstat Effect on Megakaryocyte Growth and Differentiation



#### Methods:

- CB cells were enriched for CD34+ expressing cells using a negative cell separation system
- Cells were incubated with imetelstat (1-15  $\mu$ M) in serum free liquid medium, StemSpan® SFEM containing a cytokine formulation designed for the development of meg progenitor cells
- CB cells were cultured for a total of 17 days; at various time points cells were enumerated, assessed by flow cytometry for differentiation markers (CD41) and for TA by TRAP assay.

#### ET Patients and Healthy Individuals

##### CFU-Mega (%) in Patients with ET

Patient ID	0 $\mu$ M [%]	0.1 $\mu$ M [%] $\pm$ SD [%]	1 $\mu$ M [%] $\pm$ SD [%]	10 $\mu$ M [%] $\pm$ SD [%]
1*	100	138 $\pm$ 5.7	119 $\pm$ 3.8	46 $\pm$ 1.9
2*	100	106 $\pm$ 4.3	48 $\pm$ 4.3	39 $\pm$ 4.3
3*	100	104 $\pm$ 5.7	96 $\pm$ 11.3	44 $\pm$ 5.7
4*	100	77 $\pm$ -	37 $\pm$ -	14 $\pm$ -
5	100	138 $\pm$ 33.7	81 $\pm$ 23.6	52 $\pm$ 6.7
6	100	117 $\pm$ 4.9	52 $\pm$ -	45 $\pm$ 45.6
7	100	33 $\pm$ 5.9	29 $\pm$ 0.0	13 $\pm$ 2.9
8*	100	141 $\pm$ 9.6	49 $\pm$ 13.4	14 $\pm$ -
9*	100	80 $\pm$ 14.1	40 $\pm$ 7.1	40 $\pm$ -
10	100	130 $\pm$ 1.6	66 $\pm$ 8.1	3 $\pm$ 0.4
11*	100	114 $\pm$ 0	95 $\pm$ 34.4	49 $\pm$ 7.6
n=11	100	107 $\pm$ 8.6	79 $\pm$ 11.8	33 $\pm$ 9.4

\* JAK2V617F-positive

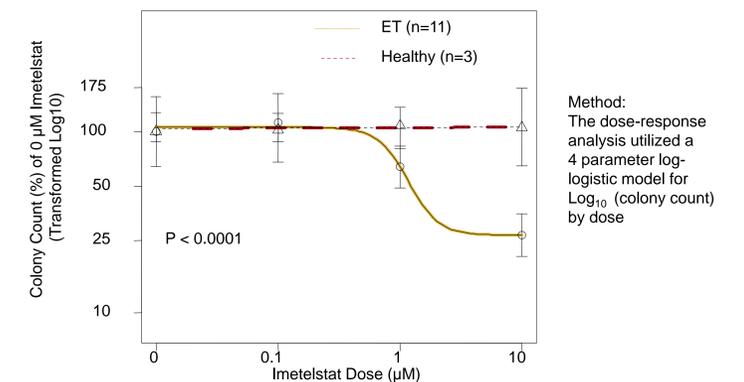
Spontaneous growth of CFU-Mega and inhibition by imetelstat

##### CFU-Mega (%) in Healthy Individuals

Donor ID	0 $\mu$ M [%] C+	0.1 $\mu$ M [%] $\pm$ SD [%] C+	1 $\mu$ M [%] $\pm$ SD [%] C+	10 $\mu$ M [%] $\pm$ SD [%] C+
1	100	93 $\pm$ 10	96 $\pm$ 5	86 $\pm$ 10
2	100	109 $\pm$ 58	109 $\pm$ 51	173 $\pm$ 13
3	100	111 $\pm$ 47	122 $\pm$ 20	78 $\pm$ 16
n=3	100	104 $\pm$ 38	109 $\pm$ 25	112 $\pm$ 13

Cytokine-stimulated growth of CFU-Mega and no inhibition by imetelstat

#### CFU-Mega Dose-Response Curves



Method: The dose-response analysis utilized a 4 parameter log-logistic model for Log<sub>10</sub> (colony count) by dose

#### Methods:

- Mononuclear cells (MNC) from 3 healthy individuals and from 11 ET patients (WHO 2009 criteria) were isolated from PB and suspended in IMDM or plated into collagen  $\pm$  cytokines (TPO, IL3, IL6, SCF, EPO) and treated with 0, 0.1, 1 and 10  $\mu$ M imetelstat or a mismatch control, and incubated for several hours (cell suspensions) or 10–12 days (collagen plus 5% CO<sub>2</sub>) at 37° C.
- Megs were stained and the number of CFU-Meg was scored
- TA was measured in MNC by TRAP assay

### Aims

- Investigate growth of megakaryocytes (Mega), of megakaryocytic colony-forming units (CFU-Mega) and of telomerase activity (TA) from cord blood (CB) and peripheral blood (PB) of healthy individuals and of patients with ET
- Inhibition of TA and suppression of formation of CFU-Mega by the telomerase inhibitor imetelstat

### Summary and Conclusions

- TA decreases with cellular differentiation despite further cellular proliferation
- Dose-dependent inhibition of TA by imetelstat in precursor cells does not inhibit cellular proliferation or CFU-Mega formation in healthy donors
- However, there is a dose-dependent suppression of CFU-Mega formation by imetelstat in patients with ET independent of the JAKV617F mutational status or cytoreductive therapy

- Our data suggest a specificity of imetelstat for malignant megakaryocytic cells
- The impact of imetelstat's clinical activity is being explored in an ongoing phase 2 study in ET patients who have failed at least one prior therapy or who refuse standard of care.

Conflicts of interest to disclose: Ning Go, Joi Ninomoto, Hooman Kashani, Monic J. Stuart: employees of Geron Corp.; Elisabeth Oppliger Leibundgut, Gabriela M. Baerlocher: service contract and research funding by Geron Corp.