Clinical decision making based on circulating (progenitor) cells:

Help or Struggle?

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Background (I)

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- More and more anti angiogenic treatments developed; some already used in daily practice
    - *RTKIs VEGFR*
    - *Monoclonal AB VEGF*
    - *VEGFR trap*
    - *VDA*
    - *mRNA antagonists/ ribozymes*
- Difficult to define optimal dose and select benefiting patients
- Preferable to prevent overtreatment and unneeded toxicity

Biomarker development

Background (II)

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Biomarkers in development

- Circulating cytokines
- Soluble VEGF receptors
- Circulating endothelial cells (CEC)
- Circulating endothelial progenitor cells (CEPC)
- Circulating progenitor cells (CPC)
- Plasma CD133 mRNA/DNA
- …

Background (II) Potential implications

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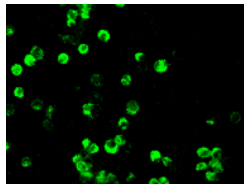
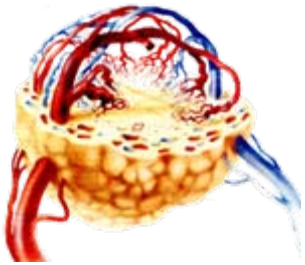
Circulating (Endothelial) Progenitor cells



Reflection of angiogenic activity of tumor



Correlation with disease status and response to therapy

Target of therapy

Measuring endothelial cells

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- Magnetic bead assay
- PCR based assays
- Flowcytometry analysis

Measuring endothelial cells

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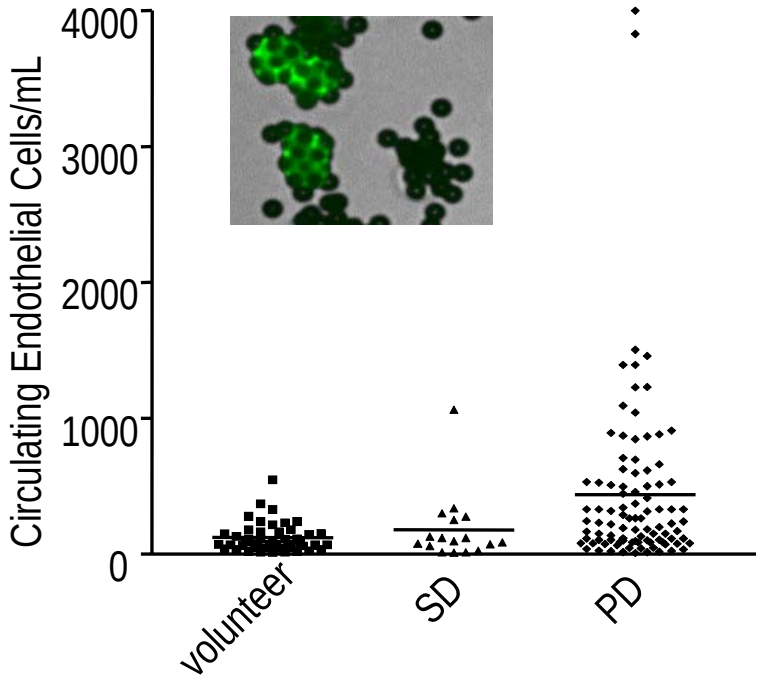
Magnetic bead assay

- Sensitive assay
- Possibility to sort cells and culture
- Time consuming
- Fresh material needed
- Selection based on 1 marker



L.V.Beerepoot, N.Mehra, E.E.Voest. *Ann Oncol* , 2004

Measuring endothelial cells

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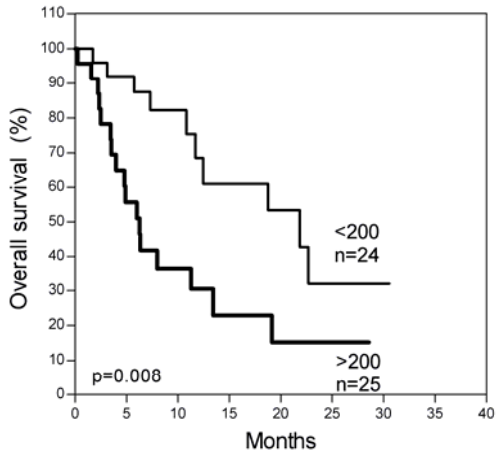
PCR based assays

- Very sensitive assay
- Selection based on 1 marker
- No identification on individual cells
- Relative numbers (copies)



Survival according to CD133 positivity in patients with bone metastases

N.Mehra, E.E.Voest. *Clin Cancer Res*, 2006.

Measuring endothelial cells

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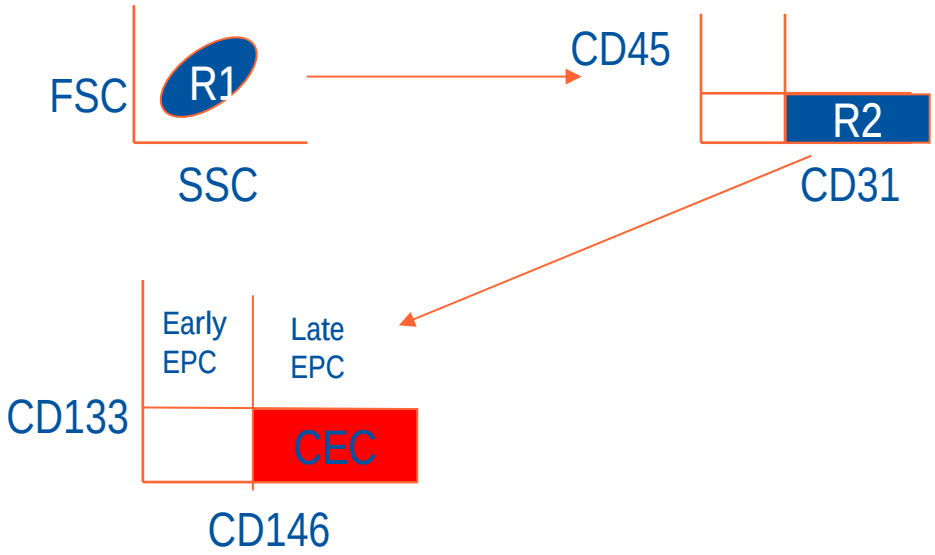
Flowcytometry analysis

- Selection based on multiple markers
- Frozen material
- Experience requiring method



D.G.Duda, R.K.Jain *Nat Protoc.* (2007).

Measuring endothelial cells

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Experience in more than 1000 samples, measured by one of the three modalities

Samples from:

- 450 patients in clinical trials
  - *Flowcytometry analysis in 214 patients*
    - Patients treated with chemotherapy
    - Patients treated with anti angiogenics
    - Patients treated with immunotherapy
    - Patients treated with surgery or RFA
- 90 volunteers

Important research questions

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Potential confounders		Research question
• Low number of cells of interest • Experience requiring procedure	→	What is the intraprocedure variability?
• Natural variation within patients • Storage procedure concerning fragile cells	→	What is the intrapatient variability?

• Variation between patients
  • *Natural variation*
  • *Variation caused by disease/ therapy*
  • *Variation caused by processing*
	→	What is the interpatient variability?
		What is the best representation of the data?

What is the intraprocedure variability?

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Comparison of duplo measurements

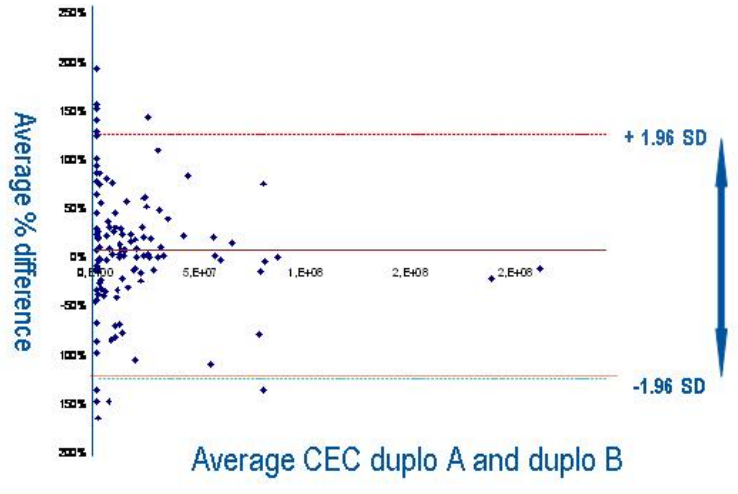
(N=214)

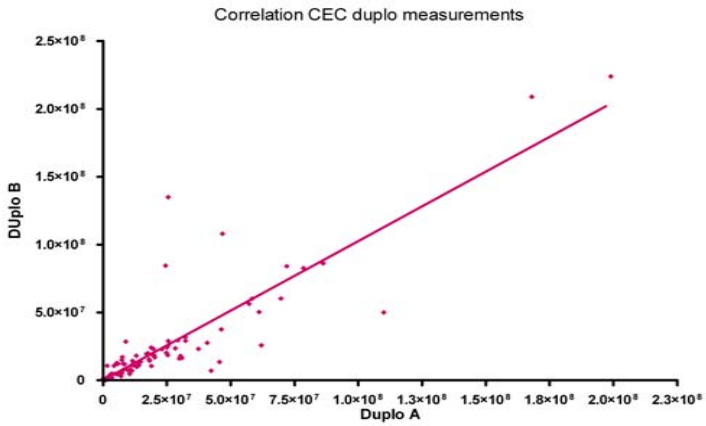
Bland Altman analysis

Good repeatability

Spearman's correlation

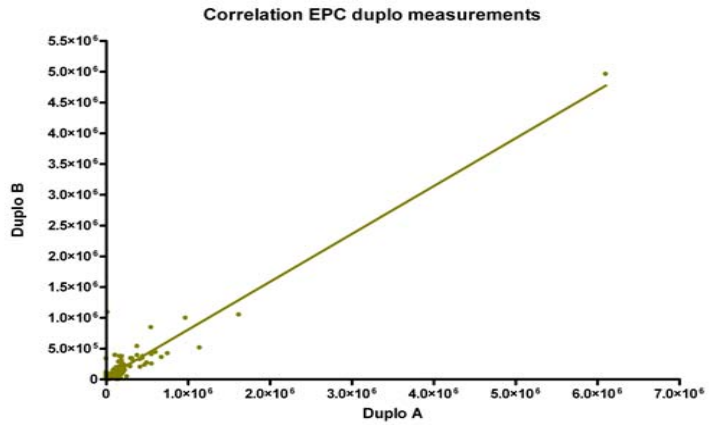
Good correlation





Spearman's rho 0.937

P=0.001



Spearman's rho 0.68

P=0.001

What is the intrapatient variability?

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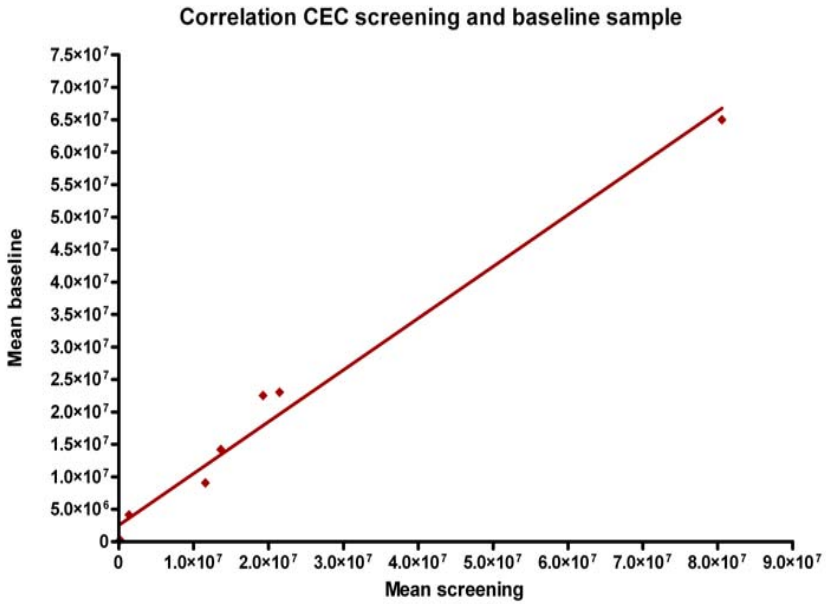
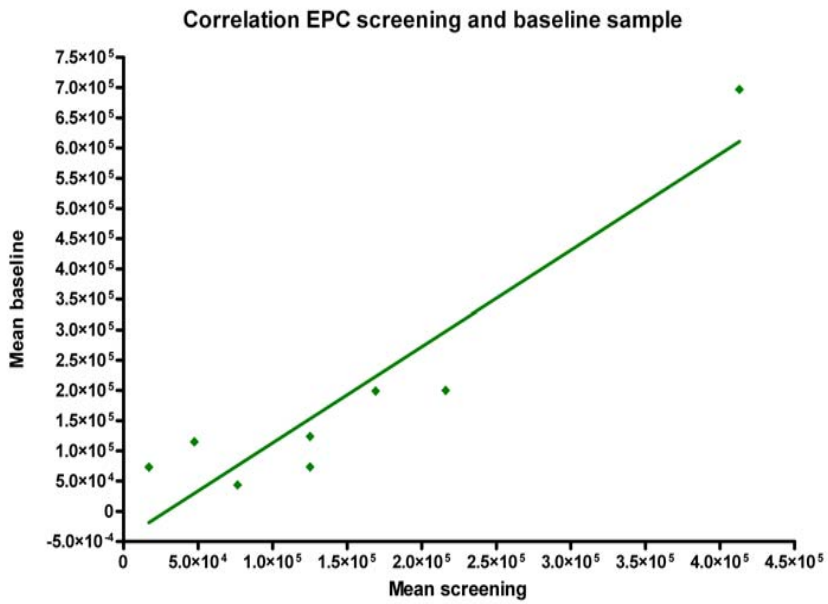
Comparison of baseline and screening sample in 1 patient

(samples taken no more than 7 days apart from each other; N=8)



Spearman's rho 0.99

P=0.001



Spearman's rho 0.86

P=0.001

What is the interpatient variability?

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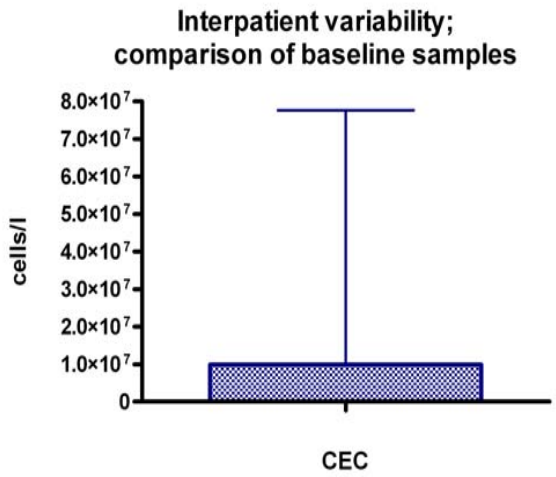

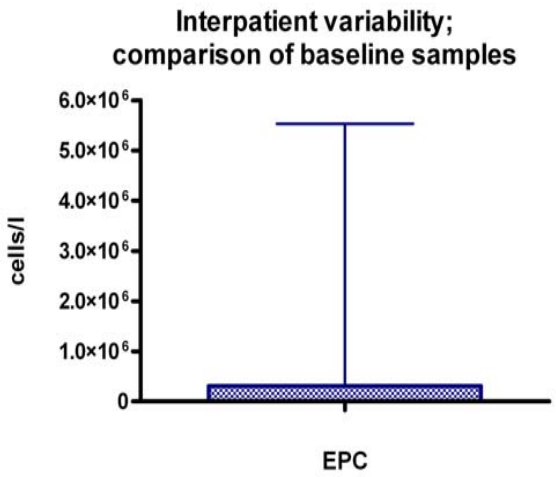

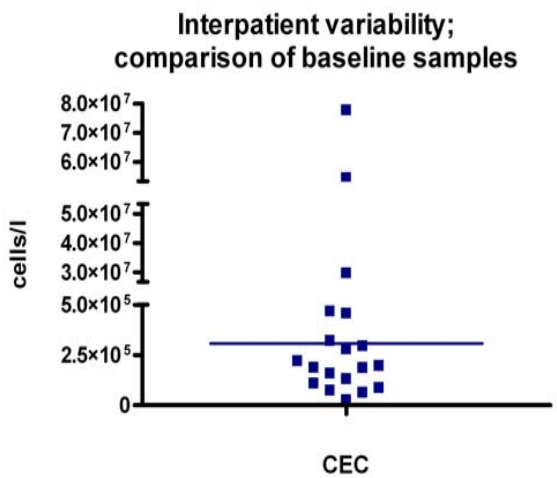

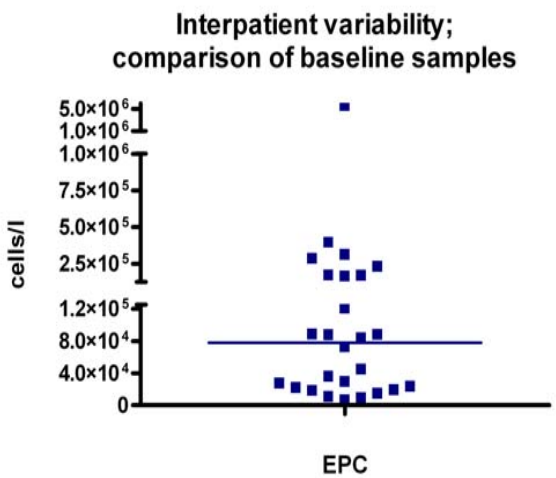

median $3{,}07.10^5$ cells/l

Interq. range: $1{,}45.10^5$-$1{,}52.10^7$

median $7{,}7.10^5$ cells/l

Interq. range: $2{,}01.10^5$ -$1{,}69.10^7$

Conclusions

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Measurement of circulating endothelial progenitor cells by flowcytometry

- *is a preferable method*

  enables specific phenotypic characterization of cells in stored samples
- *is a reliable method*

  the intraprocedure and intrapatient variability is acceptable

Interpatient variability is substantial

For all three implementations and especially reflection of biological behavior of these cells, absolute numbers are required

Normalization of data with respect to the mononuclear cell count at the same timepoint is essential

Conclusion

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Clinical decision making based on circulating (progenitor) cells:
Help or Struggle?

‘A Struggle with Perspective’

Acknowledgements

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