PHARMACODYNAMIC MONITORING OF THERAPEUTIC CYTOTOXIC ANTI-CD33 IMMUNOCONJUGATE ON MYELOID CELLS SURFACE

Clinical Biomarkers in Phase I Dose Escalation Studies Of AVE9633 in Patients With Refractory/Relapsed CD33+ AML (Acute Myeloid Leukemia)

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BIOCYTEX

• INTRODUCTION

Flow cytometry (FC) is a method of choice to monitor **whole blood** (WB) or **bone marrow** (BM) cell subsets during clinical investigations of drugs which (1) induce changes in subsets proportions and/or (2) target and/or modulate the expression of biomarkers.

Using appropriate reagents and calibration, the absolute number of both bound- and free-receptor were measured at various time-points on cell subsets in WB or BM. This **quantitative technology** provides absolute measurement of mean numbers of molecules (or epitope sites) per cell, with a dynamic range from **100 to > 500 000 molecules/cell**.

The assay has been validated for AVE9633 and Mylotarg® monoclonal antibodies (MAb), and included in international multi-sites pharmacodynamic monitoring of a dose escalation study of AVE9633 administered as a single agent by intravenous infusion to patients with relapsed or refractory CD33+ AML. Pharmacodynamic properties of the immunoconjugate have been monitored, including:

(1) **CD33 density** before drug infusion on CD33+ myeloid subsets in WB and BM

(2) **CD33 occupancy** by AVE9633 (including assessment of Free vs Total target sites)

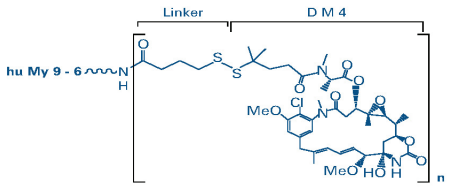
(3) **Modulation of CD33** on cell surface

(4) **DM4 molecules / IgG** ratio

(5) Functional expression of the *MDR1* gene product : **P-glycoprotein** (Pgp)

• MATERIALS AND METHODS

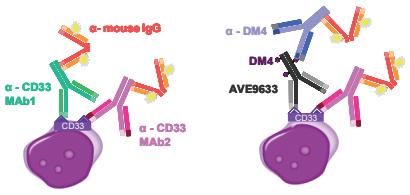
AVE9633/huMy9-6-DM4 is an immunoconjugate composed of a humanized CD33-antigen targeting MAb (IgG1), huMy9-6, conjugated to a tubulin inhibitor: the maytansine derivative "DM4". The CD33 antigen is expressed on the surface of myeloid cells. After binding to the CD33 antigen, the drug is internalized and its cytotoxicity is released within the target cell.



CD33 Density and Occupancy

Mouse Calibration

▶ MAb1 targets the same epitope as AVE9633
▶ MAb2 targets an independent epitope that is not masked by binding of AVE9633
▶ If AVE9633 is present, MAb1 can't bind to its epitope (occupied by AVE9633)
▶ Anti-DM4 MAb allows DM4 quantitation

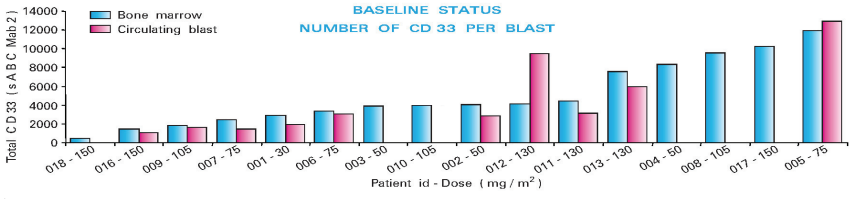


Human Calibration

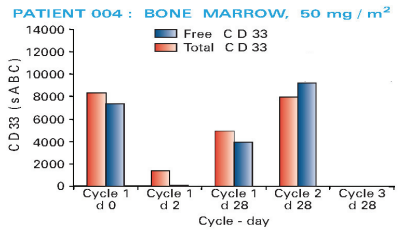
▶ AVE9633 is directly detected

• RESULTS

AVE9633 PHARMACODYNAMICS



▶ Great heterogeneity of CD33 density at baseline (sABC = specific Antibody Binding Capacity)

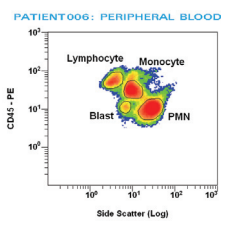


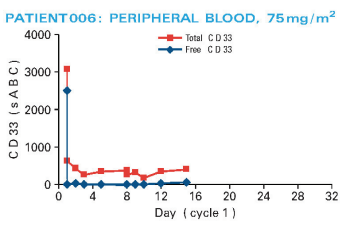
▶ Saturation of CD33 antigens on the surface of WB and BM blasts observed from 50 mg/m²

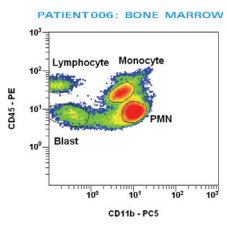
▶ Persistence of saturation depends on the dose of AVE9633 and the tumor burden

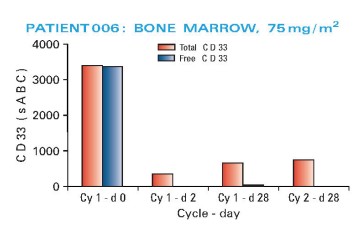
▶ Correlation between WB and BM for CD33 occupancy

▶ Down-regulation (probably by internalization)



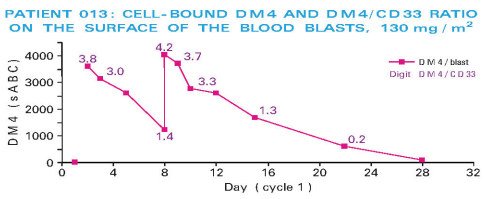






▶ Flow cytometry analysis allows isolation of blast sub-population(s) in WB and BM

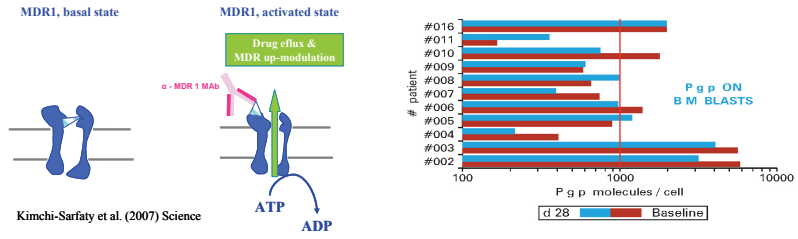
DM4/IgG RATIO



▶ Significant decrease of DM4/CD33 ratio on the surface of WB blasts between D1 and D8

▶ Increase after the second infusion at D8

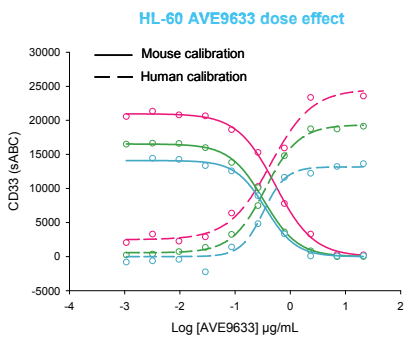
FUNCTIONAL EXPRESSION OF PgP

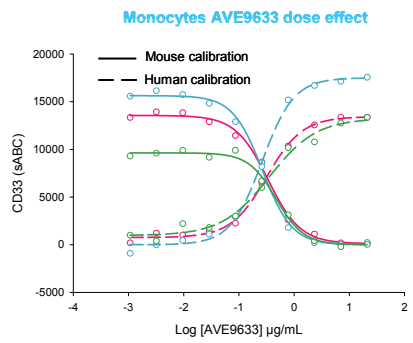


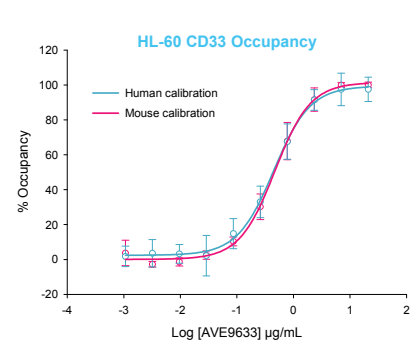
▶ Pgp results were comparable on blasts from BM and WB

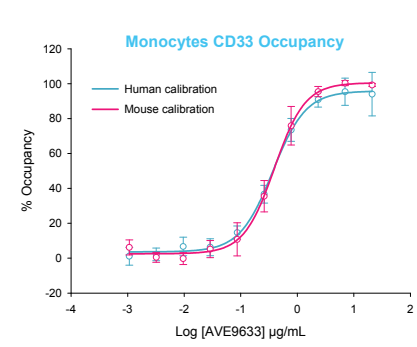
▶ No significant variation in Pgp expression after one cycle of AVE9633

MOUSE VS HUMAN CALIBRATION COMPARISON

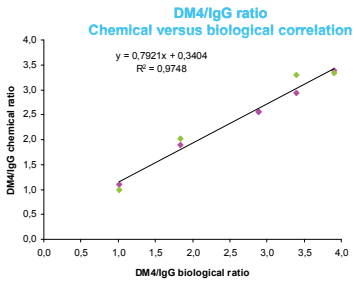






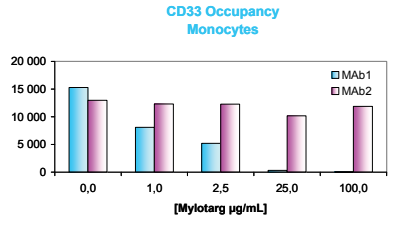


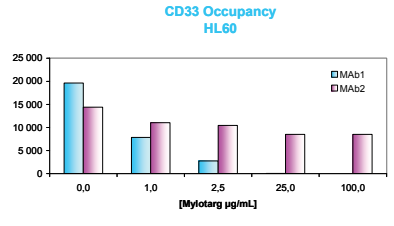
▶ Correlation between human and mouse calibration systems on monocytes and HL60-spiked WB



▶ Significant correlation between ratios determined by both chemical and biological methods

MYLOTARG™ QUANTIFICATION





▶ Mylotarg™ dose effect monitoring on monocytes and whole blood spiked HL60

• CONCLUSIONS

▶ BioCytex quantitative flow cytometry technique allows *in vivo* monitoring of receptor density, modulation and occupancy by drugs targeting specific cell types

▶ These results can be of great help to further design forthcoming clinical trials

▶ BioCytex offers dedicated tools including reporter and counter-staining MAbs, as well as calibrators for quantitation of cell-bound human or mouse IgG in both whole blood and bone marrow

(1) BioCytex, Marseille, France - (2) ImmunoGen, Inc, Cambridge, USA - (3) Sanofi-Aventis, Paris, France