

Interleukin-6 (IL-6) Produced by Monocyte Activation Reduces Dendritic Cell (DC) Differentiation in Patients with Multiple Myeloma (MM) and Malignant Lymphoma (ML): Role of CNTO 328, an Anti-IL-6 Monoclonal Antibody (Mab) and Possible Clinical Applications.

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Abstract

We developed a serum-free process in a closed system using culture cassettes and bags for large-scale and clinical-grade DC vaccination, accepted by the "Afssaps-French drug Agency" (*Tarte K. et al. Leukemia 2000; 14:2152 & patent*).

Intermediate mature DCs are generated from mononucleated cells obtained by mobilized leukapheresis, followed by Mo selection using adherence in specific cassettes (CLINICell, Mabio).

Non-adherent cells are removed and Mo are cultured for 5 days (D) in X-VIVO15 medium (Cambrex) with 2% of human albumin, 100ng/ml of GM-CSF (Leukine, Berlex) and 25 ng/ml of IL-4 (CellGenix-Cellgen). At D5, immature DCs are harvested, pulsed with autologous tumor lysate (or peptides) for 4 h in X-VIVO15 medium + GM-CSF (100ng/ml) and maturation factors (TNF- α : 20ng/ml, CellGenix-CellGen, and PGE2: 100ng/ml; Prostin, Pharmacia). Maturation of DCs was allowed to proceed for 20 h with TNF- α and PGE2. Mo-conditioned media, or IL-6 as well as IL-1 are used for enhancing *ex vivo* DC maturation by different groups in spite of the fact that IL-6 has been described as a blocker of DC differentiation from CD34+ cells particularly in MM.

We demonstrated that in our process, IL-6 is produced by activated Mo during their selection (mean= 378pg/mL, range 37–1219).

The amount of the IL-6 released in the medium correlated with the % of CD14+ cells obtained at D5 (CD14<2.8%: mean IL-6=73.1 pg/mL; CD14>22.6%: mean IL-6=682.9 pg/mL), indicating that the intrinsic production of IL-6 is one major parameter of variability of the cellular product.

By adding IL-6 from D1 to D5, the percentage of CD14+ cells at D5 was enhanced by a mean of 23-fold in samples from patients with MM (n=7) and 17-fold in ML (n=7). The modifications of other DCs markers including CD1a, CD 84 and CCR7 were modest.

By using CNTO 328, an anti-IL-6 MAb (Centocor Inc) at 1–10 μ g/mL, we totally blocked the activity of added IL-6 and samples with high IL-6 intrinsic production, with a reduction of CD14+ cells at D5.

In contrast, neither IL-6 nor CNTO 328 had any activity on terminal DC maturation after D5. IL-6 and CNTO328 are tested on DC functions.

This means that in B-cell malignancies and other solid tumors with high levels of circulating IL-6: 1) anti-IL-6 treatment such as CNTO 328 may be associated with active immune therapy, including vaccinations; 2) mature and intermediate mature DCs are the only cells to be administered in vaccination programs because of a de-differentiation effect of immature DCs due to IL-6; 3) anti-IL-6 MAbs, particularly CNTO 328 could be added for *ex vivo* DC differentiation, instead of IL-6.

mean % (range) of CD14+ cells at Day5

samples	MM	ML
Control	2.9 (0.1–7.1)	12.2 (0–44.8)
IL-6 (100ng/mL)	20 (6–35)	34.2 (0–71.4)
IL-6+CNTO328 1µg/mL	2.8 (0.5–7.5)	15.7 (0–45.6)
IL-6+CNTO328 10µg/mL	0.4 (0–0.8)	6.8 (0–20.3)
CNTO328 1µg/mL	0.4 (0.1–0.7)	6.5 (0–19.5)
CNTO328 10µg/mL	0.2 (0–0.4)	5.3 (0–15.3)