Inecalcitol Induces CD38 Expression in Multiple Myeloma and Myeloid Cell Lines

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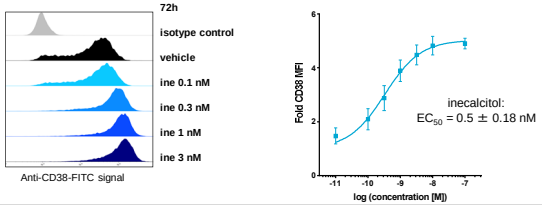
ABSTRACT

Inecalcitol (14 epi-, 19 nor-, 23 yne-, 1,25 dihydroxy-cholecalciferol) is a vitamin D receptor agonist characterized by a high anti-proliferative effect and a low calcemic potential (Okamoto et al., 2012; Ma et al., 2013) allowing its administration at high oral doses to human cancer patients (Medioni et al., 2014). The present findings show that inecalcitol induces the expression of CD38 at the surface of five multiple myeloma (MM) and four acute myeloid leukemia (AML) cell lines. It suggests that inecalcitol may enhance the clinical response of MM patients to anti-CD38 therapeutics such as daratumumab, and may even render AML patients sensitive to daratumumab and the like.

METHODS: FLOW CYTOMETRY

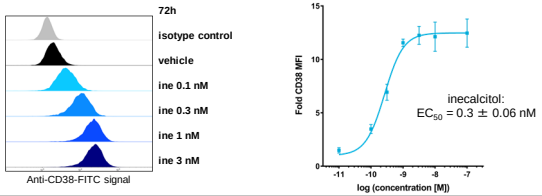
Cells were treated for the indicated times with inecalcitol. CD38 was detected on the surface of native cells by fluorescently labeled anti-CD38 antibodies (eBioscience clone HIT2-FITC unless otherwise specified). After cell wash, fluorescence was measured on an ACEA NovoCyte Flow Cytometer. Results are expressed as fold CD38 MFI (Mean Fluorescence Intensity) with the vehicle (0,1% EtOH) as a reference. Error bars are SEM of at least 3 independent experiments.

Concentration-dependent increase in CD38 on the surface of MM.1S cells



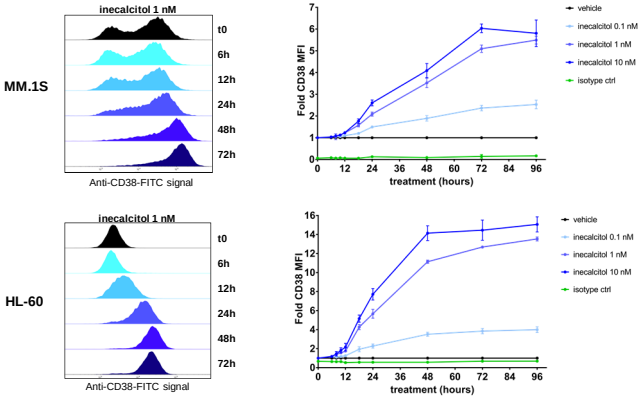
After treatment with inecalcitol for 72h, all MM.1S cells become CD38+ and CD38 labeling on their surface is multiplied by 5, with an EC_{50} of 0.5 nM.

Concentration-dependent increase in CD38 on the surface of HL-60 cells



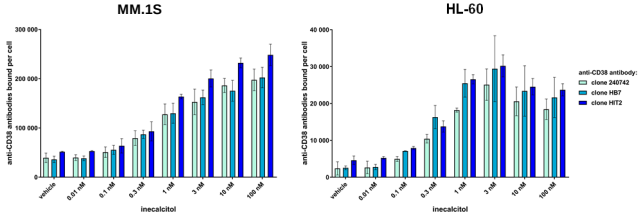
CD38 labeling on untreated HL-60 cell surface is very low. After treatment with inecalcitol for 72h, it increases dramatically (12-fold), with an EC_{50} of 0.3 nM.

Time course of the stimulation of CD38 by inecalcitol on MM.1S and HL-60 cells



The increase in CD38 surface labeling is time and concentration dependent in both cell lines. It is detectable by flow cytometry from 8h on. In MM.1S cells, the 5-fold increase plateau is reached after 72h of inecalcitol treatment. In HL-60 cells, the 14-fold increase plateau is reached after 48h of inecalcitol treatment.

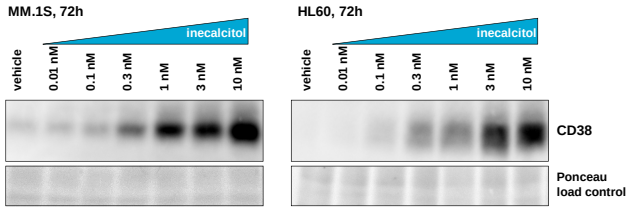
Number of anti-CD38 antibodies bound on cell surface



Quantitative cytometry using CellQuant calibrator beads (Biocytex) and 3 anti-CD38 monoclonal antibodies that bind 3 different extra-cellular epitopes.

An average of 40,000 / 3,000 anti-CD38 antibodies bind on untreated MM.1S / HL-60 cells respectively, these numbers increase up to 140,000 / 23,000 after 72h 1 nM inecalcitol treatment of MM.1S / HL-60 cells (3.5 / 8-fold respectively).

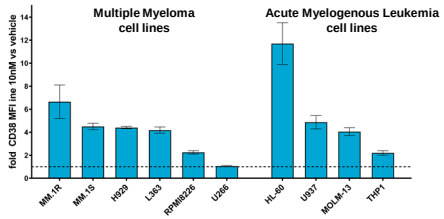
Total CD38 protein up-regulation by inecalcitol



Western blot of SDS total cell lysates

Total cellular protein levels of CD38 increase in a concentration dependent manner following inecalcitol treatment for 72h, demonstrating that inecalcitol regulates CD38 amount and not just membrane localization of the protein.

MM and AML cell lines Screen for CD38 stimulation by inecalcitol



	Cell line	CD38 status of untreated cells*	CD38 MFI: fold ine 10 nM compared to vehicle control	CD38 increase after 72h inecalcitol: EC_{50}
Multiple Myeloma (MM)	MM.1R	+	6.6	*in progress*
	MM.1S	++	4.5	0.5 nM
	H929	++	4.4	*in progress*
	L-363	+	4.2	3.4 nM
	RPMI-8226	+++	2.2	0.9 nM
	U266	+	1.1	-
Acute Myelogenous Leukemia (AML)	HL-60	-	11.7	0.3 nM
	U-937	-	4.9	0.5 nM
	MOLM-13	+	4	0.8 nM
	THP1	++	2.2	*in progress*

* fold compared to isotype control antibody: 0-2,5 = -; 2,5-10 = +; 10-25 = ++; 25-50= +++

All tested MM and AML cell lines but U266 showed up-regulation of CD38 on their surface following 72h inecalcitol treatment.

CONCLUSION:

- **Inecalcitol is a vitamin D receptor agonist with high anti-proliferative and pro-differentiating properties, and with a low calcemic potential, currently in Phase II clinical trial in France and in the United States in AML patients unfit for chemotherapy.**
- **It has been shown that inecalcitol increases the expression of the CD38 antigen at the surface of CD38 positive multiple myeloma (MM) cell lines *in vitro*; therefore, inecalcitol could potentiate the clinical response of MM patients to a therapeutic anti-CD38 antibody.**
- **It has also been discovered that inecalcitol induces the expression of the CD38 antigen at the surface of CD38 negative acute myeloid leukemia (AML) cell lines *in vitro*; therefore, inecalcitol could render AML patients sensitive to a therapeutic anti-CD38 antibody.**

Disclosures: S. Benjamin, C. Planquette: *Hybrigenics:* Employment, Equity Ownership. **R. Delansorne:** *Hybrigenics:* Employment, Equity Ownership, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties: Inventor, but no royalties.