Effects of soluble and microvesicle-bound therapeutic anti-CD38 antibodies on immune effector cells in multiple myeloma

Angelo Corso Faini[1], Barbara Castella[2], Alberto Horenstein[1], Fabio Morandi[3] and Fabio Malavasi[1]

1 Laboratory of Immunogenetics, Department of Medical Sciences and Fondazione Ricerca Molinette, Turin, Italy; 2 Laboratorio di Immunologia dei Tumori del Sangue (LITS), Centro Interdipartimentale di Ricerca in Biologia Molecolare (CIRBM), University of Turin, Turin, Italy; 3 Stem Cell Laboratory and Cell Therapy Center, IRCCS Istituto Giannina Gaslini, Genova, Italy.

Background

- CD38 is a pleiotropic cell surface molecule with multiple functions[1].
- This molecule is highly expressed by multiple myeloma (MM) cells, and it was chosen as target of therapeutic monoclonal antibodies, such as daratumumab (dara).
- This work aims at investigating how dara interacts with target and immune cells, and what effects are induced on NK cells and MDSCs.

Materials and methods

- Experiments were conducted on MM-derived cell lines stabilized in our Laboratory and fresh human MM cells.
- Microvesicles (MVs) were collected through serial centrifugations.
- MVs surface phenotype and internalization were evaluated by flow cytometry.
- Functional experiments were performed on cells purified from PBMC or BMMC and analyzed by flow cytometry.

Results (I)

- As previously reported[2], CD38 engagement by dara on MM cells, is followed by the release of MVs with peculiar phenotype and cargo.
- MVs carry on their surface the therapeutic IgG, which becomes insolubilized. Their destiny is to be captured by FcR$^+$cells. As target we adopted NK cells and MDSCs, as prototype of positive and negative effectors.

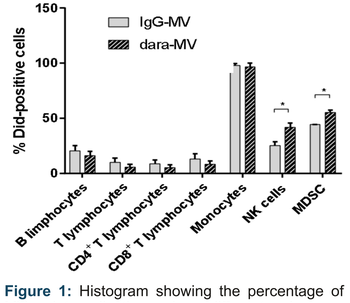


Figure 1: Histogram showing the percentage of MV internalization in different immune populations.

Results (II)

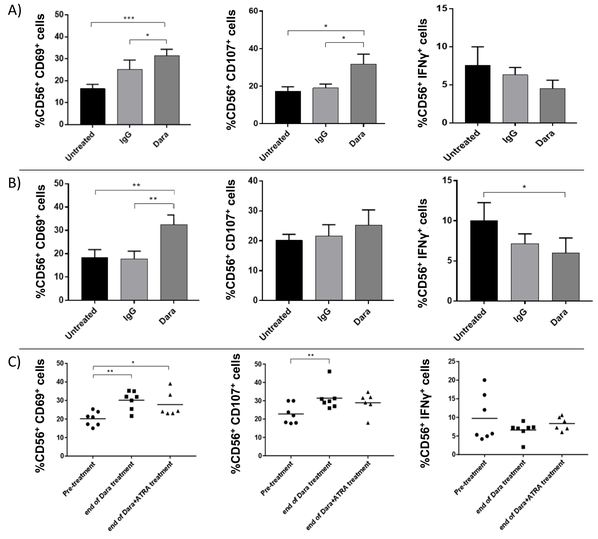


Figure 2: Histograms representing the modulation of CD107, IFNγ and CD69 on NK cells induced by soluble dara (A) and by dara insolubilized on cell line-derived (B) and patient-derived (C) MVs. Patients had been treated with dara- and dara+all trans retinoid acid (ATRA).

- Dara-covered MVs internalization is preferential in NK cells and MDSCs.
- To extend these observations, the activity of both soluble and insolubilized dara was assessed exposing NK cells and MDSC to dara.
- Results indicate that dara treatment of NK cells is followed by increased expression of CD69 (activation marker) and CD107 (degranulation marker) and decreased IFNγ production by the latter cells. NK proliferation was reduced by dara treatment, thus confirming *in vivo* observations.

Results (III)

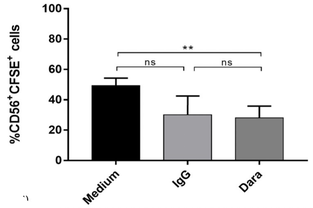


Figure 3: Histogram representing the modulation of proliferation induced by soluble dara. Values are expressed as percentage of CD56$^+$CFSE$^-$ cells.

- On human MDSCs, treatment with dara was followed by an increased expression of iNos.
- Dara also induced an increase in nitrite production by MDSCs.
- iNos and nitrites are markers of activation.

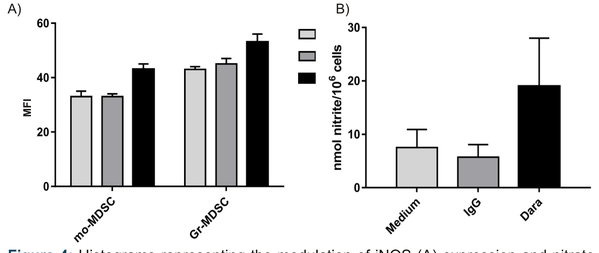


Figure 4: Histograms representing the modulation of iNOS (A) expression and nitrate production (B) on purified MDSCs induced by soluble dara. Values are expressed as mean fluorescence intensity of iNOS in CD14$^+$ (granulocytoid) or CD15$^+$ (monocytoid) MDSCs (A) or nmol of nitrate/10^6 cells produced (B).

- Dara-covered MVs and control IgG-covered MVs were also incubated with human dendritic cells and a high internalization rate was observed, particularly with dara-MVs.

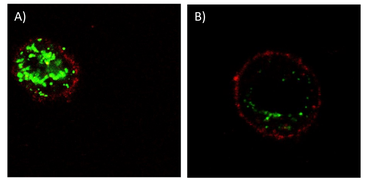


Figure 5: Human Dendritic Cells exposed to MV obtained from dara-treated human MM line BF01(A) and to MV obtained from the same cells treated with isotype-matched Ig (B).

Conclusions

- MVs obtained from dara-treated MM cells may be a particulate system to modulate the immune response, either locally in the bone marrow niche or at distance.
- Dara enhances NK degranulation and activation, while paradoxically decreasing their proliferation. These phenomena were observed either with the soluble or with the insolubilized form of the antibody.
- Our data may describe a mechanism underlying dara effectiveness and partially explain the onset of dara resistance.

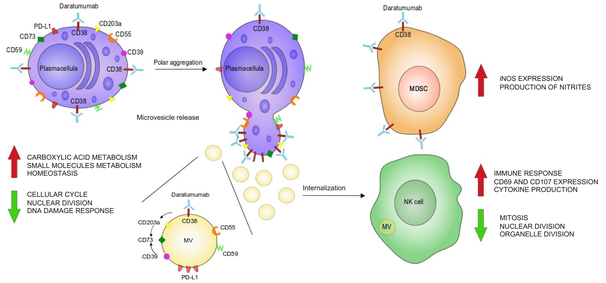


Figure 6: Schematic representation of the proposed model of interaction between dara, MVs and positive (NK cells) and negative (MDSCs) effectors.

References

1) Malavasi et al., *Physiological Review*, 2008
2) Malavasi et al., *British Journal of Hematology*, 2021

CONTACT INFORMATION
Angelo Corso Faini, MD
Email address: angelocorso.faini@unito.it
Phone: +39 3347266288

This work was supported by Fondazione Ricerca Molinette, Turin, Italy.