CAR-T and nanoCAR-T for multiple myeloma: Does smaller mean smarter ?

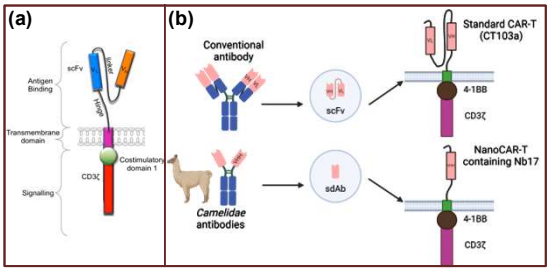
Mégane Jassin[1], Chloé Onkelinx[1], Bianca E Silva[1], Valentina Bocuzzi[1], Guillaume Marcion[1], Grégory Ehx[1], Frédéric Baron[1,2], Emmanuel Di Valentin[3], Jo Caers[1,2] and Nguyen Tham[1]

1 Laboratory of Hematology, GIGA-I3, University of Liège, Liège, Belgium
2 Department of Hematology, CHU de Liège, Liège, Belgium
3 Viral Vectors, GIGA, University of Liège, Liège, Belgium

INTRODUCTION & OBJECTIVE

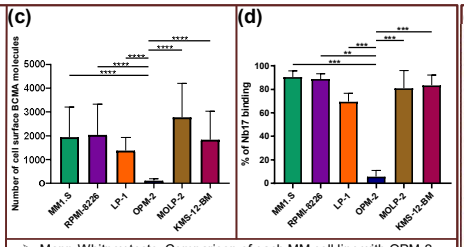
❖ **Multiple myeloma** (MM) is an incurable hematologic malignancy of plasma cells. **Chimeric antigen receptor T lymphocyte** (CAR-T) immunotherapy from second generation **(a)** has shown remarkable results in relapse patients. **Single-domain antibody** (sdAb) offers an excellent alternative to **scFv** due to the advantage of having a small and stable folding structure **(b)**. Despite the development of numerous nanoCAR-Ts, there is limited data demonstrating the direct differences and their mechanisms of action.

❖ This **project** aims to characterize the activity of a **nanoCAR-T** (containing sdAb, called Nb17) and a **standard CAR-T** (called CT103a) to elucidate the underlying mechanisms driving these differences. These findings may reveal new potential targets for novel CAR-T design strategies.

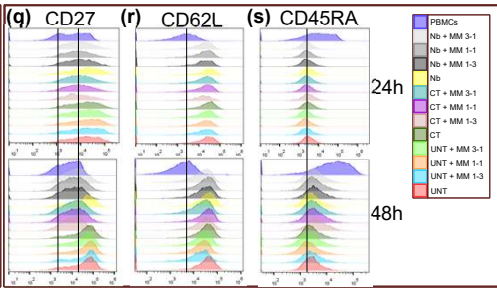


METHODS & RESULTS

1) Cell surface BCMA was **quantified** in six MM cell lines using the BD Quantibrite™Beads-PE kit, using OPM-2 as a negative control **(c)**. Then, the **binding capacity** of the sdAb (Nb17) to BCMA cell surface was analyzed on each MM cell lines **(d)**.

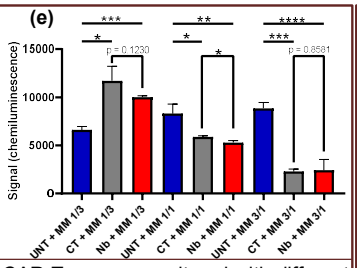


➢ Mann-Whitney tests: Comparison of each MM cell line with OPM-2

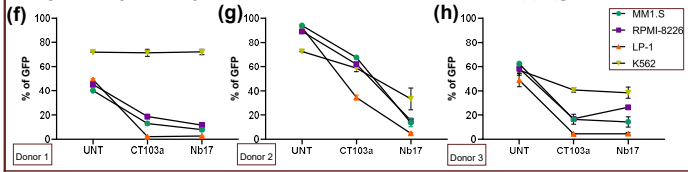


3) After 24h and 48h of co-culture with MM1.S using different ratios, **differentiation markers** were analyzed by flow cytometry. Compared to unactivated T cells from PBMCs, UNT and both CAR-Ts express CD27 **(q)** and CD62L **(r)** but do not express CD45RA **(s)**. Thus, cells are differentiated into **central memory T cells**.

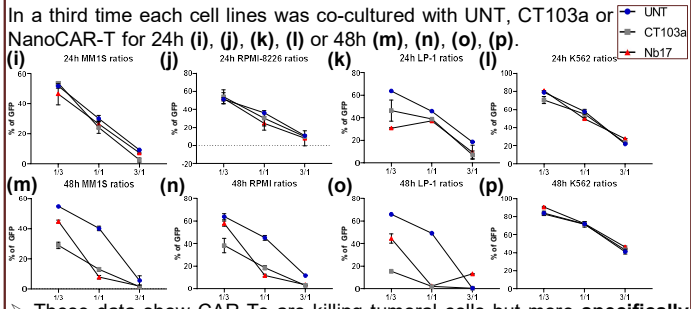
2) Killing assays were first performed by co-cultures of MM1.S with untransduced T cells (UNT) or CAR-Ts using different ratios (1/3, 1/1, 1/3) and analyses were done by **luciferase assay (e)**.



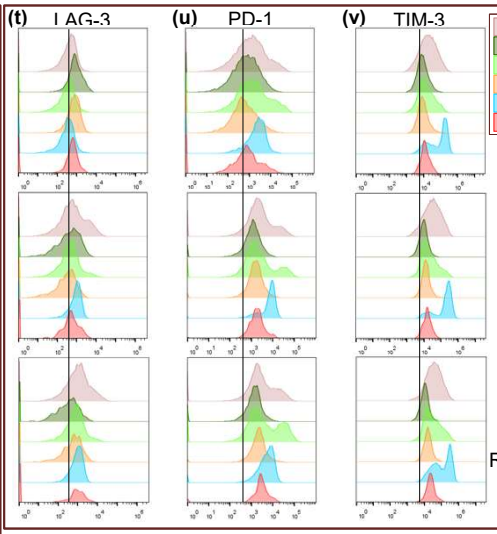
Secondly, using a ratio 1/1 UNT and CAR-Ts were co-cultured with different MM (MM1.S, RPMI-8226, LP-1) expressing BCMA or lymphoma (K562) cell lines (n=3 for each of the 3 donor). Co-cultured were performed for 48h using **flow cytometry** with GFP transduced tumoral cells **(f), (g), (h)**.



In a third time each cell lines was co-cultured with UNT, CT103a or NanoCAR-T for 24h **(i)**, **(j)**, **(k)**, **(l)** or 48h **(m)**, **(n)**, **(o)**, **(p)**.

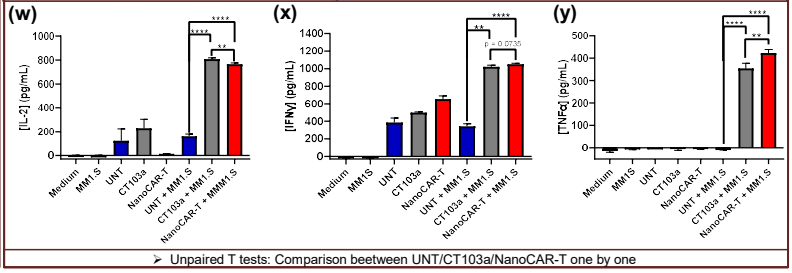


➢ These data show CAR-Ts are killing tumoral cells but more **specifically** BCMA cell surface positive cell lines.



4) Exhaustion markers of UNT and both CAR-Ts were analyzed using flow cytometry after being co-cultured with MM1.S for 24h, 48h, or after rechallenging of 48h after 48h of current co-culture. Following the timepoints the expression of LAG-3, PD-1 and TIM-3 is increasing **(t)**, **(u)**, **(v)**.

5) ELISAs were performed for different donors after 24h of co-cultures with MM1.S cells. Both CT103a and NanoCAR-T produce cytokines in comparison to untransduced T cells: IL-2 **(w)**, IFNγ **(x)** and TNFα **(y)**.



➢ Unpaired T tests: Comparison beetween UNT/CT103a/NanoCAR-T one by one

CONCLUSION

The nanoCAR-T generated in our lab demonstrates excellent anti-cancer efficacy equivalent to the standard CAR-T CT103a. Further investigation of activation and degranulation markers and transcriptomic levels may reveal mechanistic differences and potential improvements to CAR-T therapy.

Megane.Jassin@uliege.be