Immunosuppressive monocyte modulation of CART cell functions and impact on response to CART19

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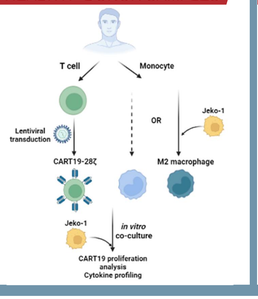
INTRODUCTION

- CD19 directed chimeric antigen receptor T (CART19) cell therapy has resulted in remarkable outcomes in B cell malignancies
- Four U.S. FDA approved CART19 cell therapy in multiple indications
- Durable remissions are **limited to 40%** of treated patients[1]
- Immunosuppressive tumor microenvironment, including **inhibitory myeloid cells**, contributes to failure of CART19 cell therapy
- Monocytes have been demonstrated to **suppress T cell expansion** during CART cell production[2,3], as well as to contribute to the development of CART cell toxicities and resistance[4].

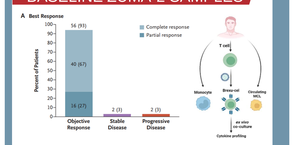
AIM

- Unravel the interactions between monocytes, CART19 cells, and tumor cells
- Determine how monocytes-CART19 cell interactions impact CART19 cell effector functions and outcomes

MODEL 1
IN VITRO CULTURES OF HEALTHY DONOR SAMPLES

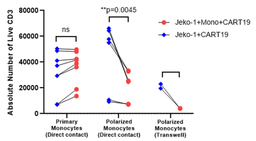


MODEL 2
EX VIVO CULTURES OF BASELINE ZUMA-2 SAMPLES



- ZUMA-2 clinical trial[5]
  - Brexu-cel (CART19-28Ζ)
  - Mantle cell lymphoma (MCL)
- 60 treated patients with at least 7 months of follow-up
  - 93% had objective response[5]
- Mantle cell lymphoma (MCL) is an optimal model :
  - autologous monocyte
  - Circulating tumor cells
- Study Sample
  - 6 responders (CR)
  - 3 non-responder
  - 2 relapsed disease post CART

EX VIVO M2 POLARIZED MACROPHAGES INHIBIT ANTIGEN-SPECIFIC PROLIFERATION OF HEALTHY DONOR CART19-28Z CELLS WITH NO DIRECT CONTACT REQUIRED

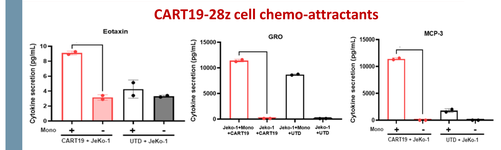


Primary monocytes (direct contact): 4 biological replicates, 2 technical replicates each
Polarized monocytes (direct contact): 3 biological replicates, 2 technical replicates each
Polarized monocytes (transwell): 1 biological replicate, 2 technical replicates
Paired t test
*ns: not significant (p>0.05), *p<0.05, **p<0.01*

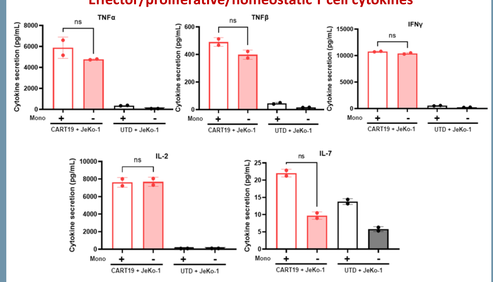
- Monocytes : CART19 : Jeko-1 = 1:2:2
  - Same well
  - Transwell
  - 3 days *in vitro*
- *Ex vivo* polarized M2 macrophages
  - Primary monocytes polarized with recombinant human GM-CSF for 7 days
  - Another 24-hour incubation with Jeko-1 cells at ratio of 1:2
  - Absolute T cell count was measured by flow cytometry

IMPACT OF HEALTHY DONOR ISOLATED MONOCYTES ON CART19-28Z CELL CHEMO-ATTRACTANTS AND CYTOKINES

CART19-28z cell chemo-attractants



Effector/proliferative/homeostatic T cell cytokines

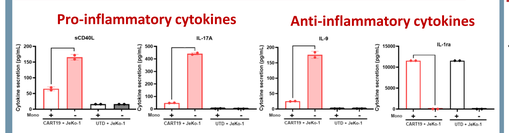


Supernatants were harvested after a 3-day co-culture in the presence or absence of monocytes and analyzed for cytokines using 38 plex multiplex; 2 biological replicates, 2 technical replicates each, 1 representative donor is shown, paired t test, ns: not significant (p>0.05); UTD: un-transduced T cells

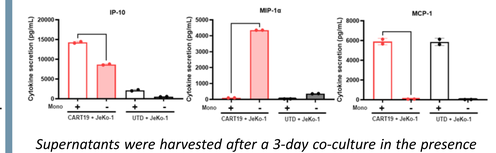
Trends of increased levels of **Eotaxin**, **GRO**, **IL-7**, and **MCP-3** were observed when CART19 cells were stimulated with CD19⁺ targets in the presence of freshly isolated monocytes

IMPACT OF M2 POLARIZED HEALTHY DONOR MACROPHAGES ON CART19-28Z CHEMO-ATTRACTANTS AND CYTOKINES

Pro-inflammatory cytokines



CART19-28z cell chemo-attractants

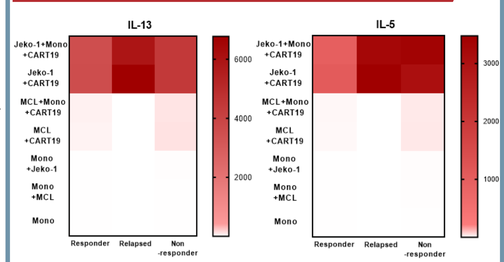


Supernatants were harvested after a 3-day co-culture in the presence or absence of monocytes and analyzed for cytokines using 38 plex multiplex; 2 biological replicates, 2 technical replicates each, 1 representative donor is shown, paired t test
ns: not significant (p>0.05); UTD: un-transduced T cells

Trends of increased levels of **IL-1ra**, **IP-10**, and **MCP-1** were observed when CART19 cells were stimulated with CD19⁺ targets in the presence of M2 polarized macrophages

Trends of decreased levels of **IL-17A**, **sCD40L**, **IL-9**, and **MIP-1α** were observed when CART19 cells were stimulated with CD19⁺ targets in the presence of M2 polarized macrophages

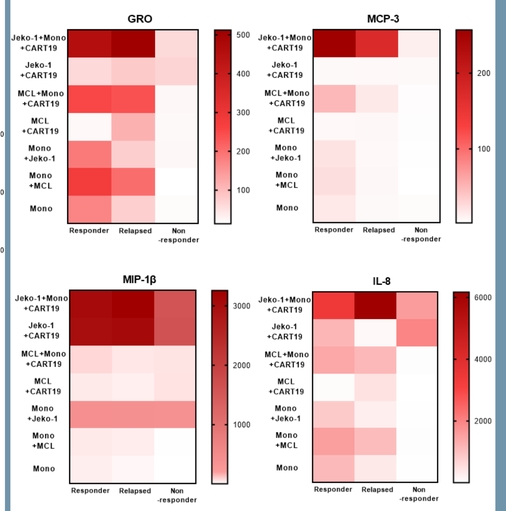
CART19-28Z CYTOKINES FOLLOWING AN *EX VIVO* CO-CULTURE OF BREXU-CEL, BASELINE AUTOLOGOUS MONOCYTES, AND PATIENT-MATCHED MCL IN RESPONDERS VS NON-RESPONDERS



*Supernatants were harvested after a 3-day co-culture of baseline **Brexu-cel from ZUMA-2 trial (products)**, baseline **autologous monocytes** (isolated from leukophoresis collections), and **baseline CD19⁺ MCL** (isolated from leukophoresis collections), or **CD19⁺ JeKo-1 cells**, and analyzed for cytokines using 38 plex multiplex;*
N=11 (6 responders, 3 non-responders, 2 relapsed disease)

Trends of increased levels of **IL-13** and **IL-5** in *ex vivo* cultures of baseline brexu-cel in the presence of autologous monocytes in non-responders

CART19-28Z CHEMO-ATTRACTANTS FOLLOWING AN *EX VIVO* CO-CULTURE OF BREXU-CEL, BASELINE AUTOLOGOUS MONOCYTES, AND PATIENT-MATCHED MCL IN RESPONDERS VS NON-RESPONDERS (RIGHT)



Trends of decreased levels of **GRO**, **MCP-3**, **MIP-1β**, **IL-8** in *ex vivo* co-cultures of baseline brexu-cel in the presence of autologous monocytes in non-responders

CONCLUSIONS

- *Ex vivo* polarized **immunosuppressive M2 macrophages** from healthy donor monocytes **inhibit** antigen specific **CART19 proliferation** in a **contact-independent** manner
- Monocytes from healthy donors possibly promote the *in vitro* tumor microenvironment to become more pro-inflammatory by secreting significantly higher level of T cell chemo-attractants and cytokines, including **eotaxin**, **GRO**, **MCP-3** and **IL-7**, in the presence of tumor cells and CART19
- *Ex vivo* polarized M2 macrophages promote the production of suppressive cytokines such as **IL-1ra**, while altering secretion level of immuno-modulating chemokine and cytokines, such as **IP-10**, **sCD40L**, **IL-17A**, **IL-9**, **MIP-1α** and **MCP-1**
- In an *ex vivo* co-culture of baseline CART19 products (brexu-cel), autologous monocytes, and tumor cells:
  - ✓ Trends of increased levels of **IL-13** and **IL-5** in the presence of monocytes in non-responders
  - ✓ Trends of decreased levels of **GRO**, **MCP-3**, **MIP1β** and **IL-8** in non-responders
- Baseline monocyte characteristics may play a role in modulation of CART19 function and clinical outcomes

Limitations: small sample size for relapse and non-response in the ZUMA-2 trial
Ongoing work: molecular understanding of baseline autologous monocytes

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ACKNOWLEDGEMENTS

The study was partly supported by Kite, a Gilead company, the National Institute of Health (R37CA266344, K12CA090628), the Department of Defense (CA201127), Regenerative Medicine Minnesota (091620 TR 012, 012819 EPC 003), Mayo Clinic Center for Individualized Medicine