BONE MARROW FIBROSIS IS ASSOCIATED WITH NON-RESPONSE TO CD19 CAR-T THERAPY

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INTRODUCTION

- CD19-directed CAR-T cell therapy is used to treat relapsed/refractory (R/R) B-Acute Lymphoblastic Leukemia (B-ALL)
- However, 15% of patients do not respond (NR) to CAR-T treatment, and 30-50% of patients eventually relapse
- Many factors impact CAR-T response, including tumor antigen loss, failure of T-cell expansion, T-cell exhaustion, and CAR-T tumor trafficking/infiltration
- CAR-T cell Infiltration is impacted by physical barriers and and immunosuppressive microenvironments
- **There is no pre-infusion clinical test to predict patient response to CAR-T Therapy**

AIM

Bone Marrow (BM) is primary site of both disease and CAR-T action. Yet the pre-infusion BM microenvironment **has not** been studied in detail

We evaluated whether pre-infusion bone marrow biopsy (BMB) parameters can predict response to CAR-T cell therapy

CASES & CONTROLS

Samples (n = 104)	SR	NR	REL	CR (SR + REL)	Total
Pre	26	20	16	42	62
1M Post	20	11	11	31	42

Identified heavily pre-treated CD19 CAR-T treated R/R B-ALL patients with **> 20% tumor burden** at infusion
SR: Sustained Responders – Complete Response at 1 month (1M) post-infusion, no relapse at follow up timepoint
NR: Non-Responders – Persistent disease at 1M post infusion
REL: CD19-Relapsers – CR at 1M post infusion but subsequent CD19- relapse

METHODS

A subset (80%) of samples underwent transcriptome mRNA Expression Analysis – 19,398 gene probes
Differential Expression Analysis performed using DEseq2
Pre-Infusion BMBs were assessed for fibrosis using Reticulin Special Stain
Samples were scored for fibrosis utilizing the Bauermeister scoring scale
Artificial Intelligence (AI) Assisted Digital Image Analysis used to validate expert scoring
53 additional cases of primary B-ALL were stained with reticulin to determine baseline variation in fibrosis before initiation of front-line treatment

RESULTS

Extracellular Matrix (ECM) Pathways and Fibrosis-Associated Genes are significantly up-regulated in Pre-Infusion CAR-T Non-Responders

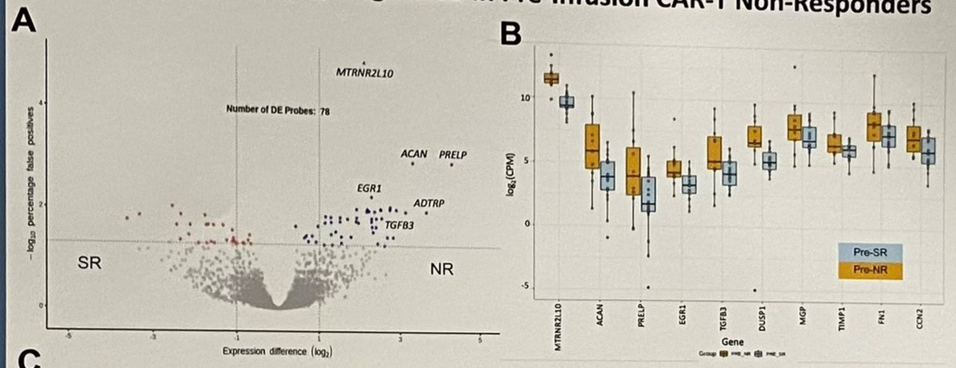


Figure 1:
- **Differential Expression (DE) and pathway enrichment analysis of NR and SR pre-CAR infusion samples showed significant NR up-regulation of genes involved in Extracellular Matrix (ECM), Stromal, and Fibrotic Development.**
- 50 genes were upregulated in NRs, while 28 genes were upregulated in SRs. **(A)**
- Fibrosis-Associated genes were prevalent within the top 20 most significantly upregulated genes in Pre-NR samples. Specific genes include *MTRNR2L10* (4.26x, p < 0.001), *ACAN* (9.81x, p = 0.001), *PRELP* (19.26x, p = 0.001), *EGR1* (4.85x, p = 0.005), *TGFB3* (5.67x, p = 0.009), *DUSP1* (4.51x, p = 0.009), *MGP* (5.11x, p = 0.0102), *TIMP1* (2.44x, p = 0.017), *FN1* (3.42x, p = 0.0337), *CCN2* (2.91x, p = 0.0452). **(B)**
- Several *TGFβ*-pathway related genes were present, including ***CCN2***, ***EGR1***, and ***TGFB3***. **(B)**
- Metascape pathway enrichment analysis showed upregulation of Collagen-containing ECM **(p < 0.0001)**, Connective Tissue Development **(p < 0.001)** and ECM Structural Constituent **(p < 0.001)** pathways. Upregulated genes found in these pathways include: *ACAN, CCN2, FN1, CCN1, MGP, ORM1, PRELP, TGFB3, TIMP1, TIMP3, GNAS, NPPL1, PLS3, DUSP1, EGR1, GNMT, RIN2, ADTRP, TRIB2*. **(C)**

Reticulin Fibrosis is increased in Non-Responders prior to infusion

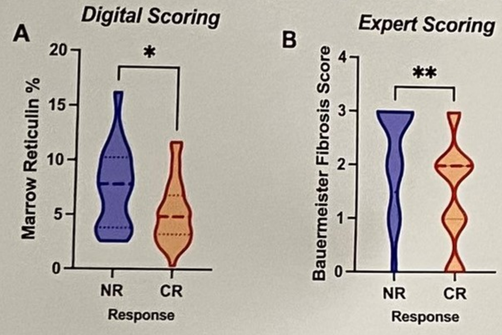


Figure 2:
- Marrow Reticulin % is significantly increased in Pre-Infusion NRs compared to CRs. Area assessed by AI-Assisted Analysis, p = 0.0277 (Mann-Whitney, Two-tailed), NR (n = 18, med = 7.828, sd = 3.715), CR (n = 39, med = 4.881, sd = 2,884) **(A)**
- Expert Scoring of using Bauermeister Reticulin Scoring Scale, demonstrated significantly increased reticulin network formation in Pre-NRs, (p = 0.0064 (Mann-Whitney, Two-tailed). **(B)**
- Score ≥2 indicates severe fibrosis. 76.4% of NRs vs 53.8% of CRs had severe fibrosis. **(B)**

Fibrosis is present at diagnosis in all untreated B-ALL subtypes

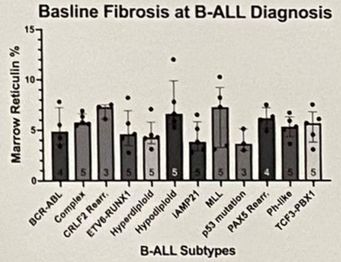


Figure 3:
- Fibrosis at diagnosis does not significantly differ among 13 major B-ALL subtypes (p = 0.1780, Kruskal-Wallis Test, $\bar{x}$ = 5.60%, med = 5.58%, sd = 1.92%).

NRs, but not CRs, have significantly increased fibrosis from baseline diagnosis levels

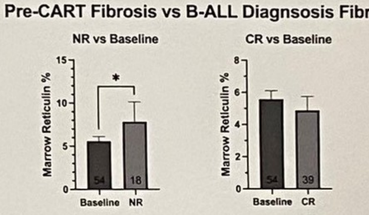
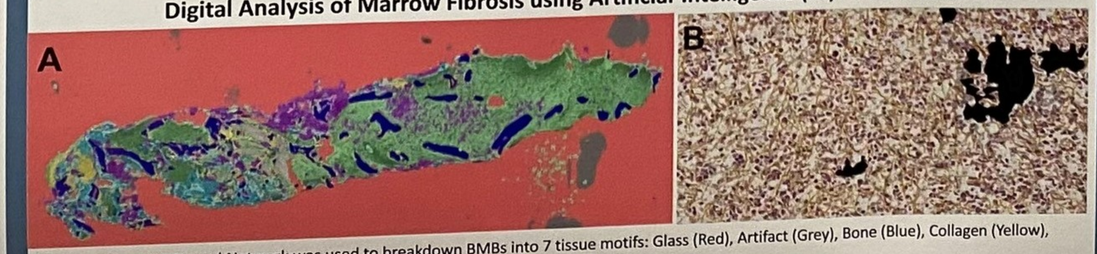


Figure 4:
- By the time of CAR-T infusion, fibrosis is significantly increased in NRs compared to baseline B-ALL fibrosis (p = 0.0424, Mann-Whitney, two-tailed). **(Left)**
- *Right*: Fibrosis in SRs is not significantly changed from baseline diagnosis levels at the time of CAR-T infusion (p = 0.1827, Mann-Whitney, two-tailed). **(Right)**

METHODS (CONT.)

Digital Analysis of Marrow Fibrosis using Artificial-Intelligence (AI) Neural Network



- Deep-Learning Neural Network was used to breakdown BMBs into 7 tissue motifs: Glass (Red), Artifact (Grey), Bone (Blue), Collagen (Yellow), Blood (Purple), Clots/Crushed Tissue (Blue), Marrow (Green). **(A)**
- Areas of reticulin staining (orange strands) were identified and quantified in relation to marrow area **(B)**

CONCLUSIONS

- **High levels of bone marrow fibrosis are associated with Non-Response to B-ALL CD19 CAR-T Therapy**
- Significant increase of fibrosis in NRs prior to CAR-T infusion compared to baseline B-ALL fibrosis levels may suggests that fibrosis is induced by multiple rounds of chemotherapy
- **Reticulin staining is a widely available assay that could be used as a heuristic tool to predict likelihood of CAR19 success**
- **Evaluation of pre-CAR bone marrow fibrosis levels in conjunction with additional markers may allow for more informed decision making, targeted therapeutic plan development, and patient outcome optimization in R/R B-ALL.**

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