Aldafermin quantitatively improves inflammation in a 24-week clinical trial in patients with nonalcoholic steatohepatitis

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INTRODUCTION

Pathologist-based scores are the standard for assessing efficacy in nonalcoholic steatohepatitis (NASH) clinical trials, but are limited by variability in interpretation and insensitivity to small changes, especially for lobular inflammation. Lobular inflammation score represents the number of inflammatory foci per microscopic field. The inflammatory foci consist of a mixture of immune cells including pro-inflammatory (M1) macrophages as the major cell type. In this study, samples from a Phase 2 clinical trial with Aldafermin (NGM282), an engineered fibroblast growth factor 19 analogue, were used to develop and test novel image analysis readouts for liver inflammation based on CD68, a macrophage lysosomal marker. One of the major challenges was to distinguish M1s from liver-resident Kupffer cells, both of which express CD68. To overcome this, a differentiation strategy was applied based on cell size, shape and CD163 co-expression.

AIM

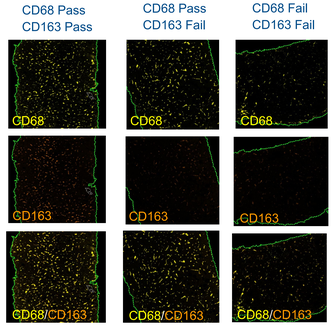
We developed and tested an image analysis method to quantify M1s in liver biopsies from a NGM282 Phase 2 clinical trial where liver inflammation was decreased based on the pathologist reads.

METHODS

- The design of clinical study (NCT02443116, Cohort 4) is illustrated above: Liver biopsies were collected at baseline and end of treatment from a 24-week trial (placebo (PBO), n = 22; 1-mg NGM282, n = 49). Serum ALT and AST levels at day 1 and week 24 were measured.
- A 6-plex immunohistochemistry (IHC) panel that included CD68 (Cell Signaling Technology, D4B9C) and CD163 (Cell Signaling Technology, D6U1J) was developed.
- Image analysis was designed using Visiopharm (Visiopharm A/S, Denmark). M1s and Kupffer cells were identified by CD68, and further differentiated based on size, CD163, and cell shape.
- The M1 foci class ("M1 foci") was developed based on the heatmap of M1s to account for focal accumulation of M1s.

RESULTS

Figure 1. Macrophage immunolabeling and quality control of CD68 and CD163.



B.

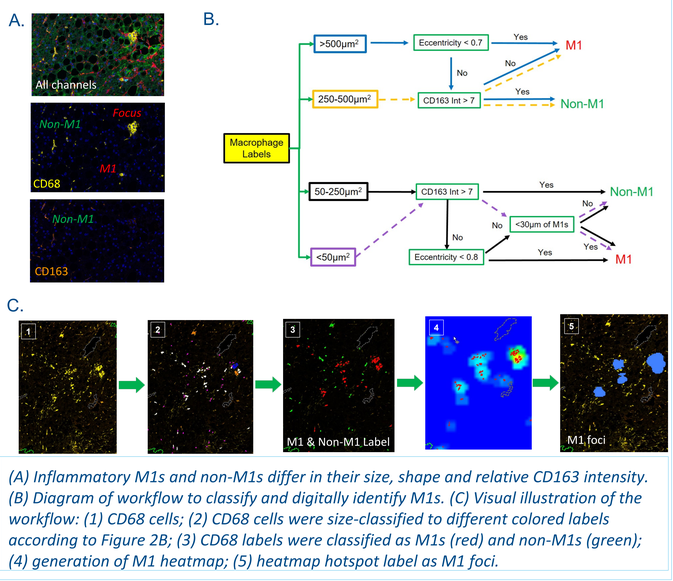
	CD68 Pass	CD68 Fail
CD163 Pass	94	0
CD163 Fail	39 (29%)	3 (2%)

133 qualified images

130 paired

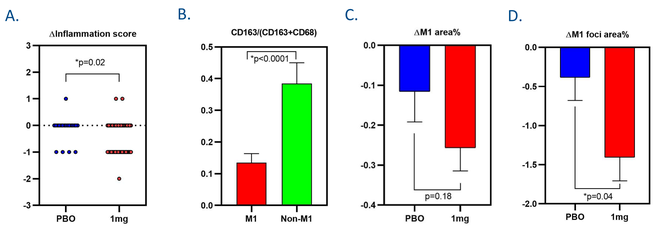
(A) Examples of images that failed or passed CD68 and CD163 quality control (QC). (B) 20 patients in PBO and 48 patients in 1mg-NGM282 group had histology sections available (136 in total). 133/136 passed CD68 QC. In the end, 130 paired and qualified images were used for analysis (18 in PBO and 47 in 1mg-NGM282).

Figure 2. Image analysis strategy to quantify hepatic M1 macrophages.



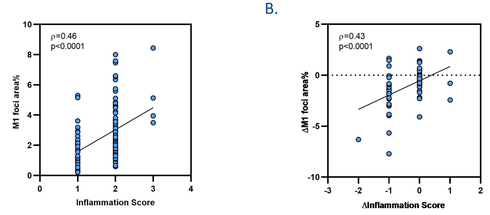
(A) Inflammatory M1s and non-M1s differ in their size, shape and relative CD163 intensity. (B) Diagram of workflow to classify and digitally identify M1s. (C) Visual illustration of the workflow: (1) CD68 cells; (2) CD68 cells were size-classified to different colored labels according to Figure 2B; (3) CD68 labels were classified as M1s (red) and non-M1s (green); (4) generation of M1 heatmap; (5) heatmap hotspot label as M1 foci.

Figure 3. NGM282 significantly reduced M1 foci area.



(A) NGM282 treatment significantly reduced inflammation score as measured using the criteria of the Clinical Research Network (CRN; Kleiner et al.). (B) M1s had a lower CD163/(CD163+CD68) ratio than non-M1s. (C and D) Image analysis revealed a trend of decrease in M1 cells label area% and a significant decrease of M1 foci area% by NGM282 treatment. Panel (A) uses Fisher's Exact Test for statistics analysis; Panels (B), (C) and (D) uses Mann-Whitney Test for statistics analysis with mean and standard deviation illustrated in the graphs.

Figure 4. M1 foci correlated with inflammation scores.



(A) M1 foci area% absolute value or (B) change at end of study vs. baseline were compared with inflammation score from pathologist reads. Spearman's ρ and p-value were illustrated.

CONCLUSIONS

- CRN reads and image analysis for M1 foci identified a decrease in liver inflammation after 24 weeks of treatment with NGM282. These changes were accompanied by an improvement in fibrosis and resolution of NASH in NGM282-treated patients at the end of the study.
- M1 foci image analysis provides support for the evaluation of inflammation reduction by NASH CRN.

DISCUSSION

- Pathologists' read of inflammation in the context of NASH is difficult to assess and has low intra-rater and inter-rater Kappa score (Davidson et al.).
- Multiplex immunofluorescence (Mx) affords observation of more details in histology slides compared to conventional staining.
- Challenges of Mx immunofluorescence includes epitope loss, time for assay and computational approach development, and regulatory acceptance.
- Both CRN score and image analysis showed significant decreases in inflammation with NGM282 treatment.
- Image analysis can aid pathologists to be more consistent in their assessment of inflammation.

REFERENCE

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