Development of a mode of action-based testing suite for the screening of regenerative macrophage cell therapy candidates for end-stage liver disease and beyond

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Introduction

Patients with end-stage liver disease (ESLD) have extensive fibrosis and inflammation, which cause loss of organ function and portal hypertension, resulting in decompensation events and limited life expectancy (median ~2years after first decompensation; median ~9 months after second decompensation event)[1]
Currently, there are no licensed therapies to treat ESLD[1]. Macrophages with a pro-regenerative phenotype control inflammation and promote fibrosis remodeling, thereby coordinating liver regeneration and offering a potential therapeutic avenue in these patients[2,3,5].

Autologous, non-engineered, pro-regenerative macrophages have been tested in patients with advanced cirrhosis in the academic MATCH clinical studies, which show that the therapy is well tolerated and improves transplant-free survival[4,6,8]. Further enhancement of pro-regenerative macrophage potency via engineering is needed to ensure durable clinical responses in a more severe patient population with ESLD.

To ensure that the best candidate was progressed to clinical trial, Resolution Therapeutics proceeded with an extensive screening process to select the most effective payloads. The screening process consisted of establishing a mode of action (MoA) based screening platform and validating on a clinically relevant cell type, candidate screening and refinement, and finally, candidate selection.

Payloads were considered effective if they maintained or enhanced phenotypic attributes as well as enhanced anti-inflammatory and anti-fibrotic functions when compared to the clinically-tested non-engineered macrophages.

Method

RTX001 was envisioned as a monocyte-derived, **autologous macrophage** cell therapy that has undergone ***engineering*** to enhance macrophage function. Monocytes are derived from leukapheresis and cultured as per protocol[4,7] to derive macrophages which are then engineered ***ex vivo*** using mRNA before being cryopreserved.

Resolution established a modular *in vitro* platform based on RMT MoA (Fig 1) from which it could compare potential candidates against non-engineered macrophages (MATCH-like, clinically-tested regenerative macrophages). An extensive screening program with candidate genes selected from literature5 and previous preclinical experiments was then conducted (Fig 2).

Finally, we combined the selected candidate payloads, and we tested the combination of IL10 and MMP9 against IL10 alone and non-engineered (MATCH-like) macrophages (Fig 3), comparing their functional phenotype and secretion profile.

References

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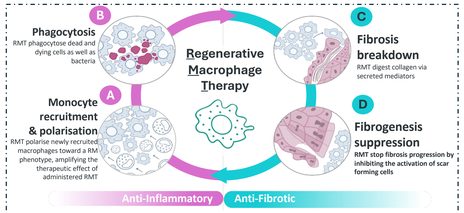
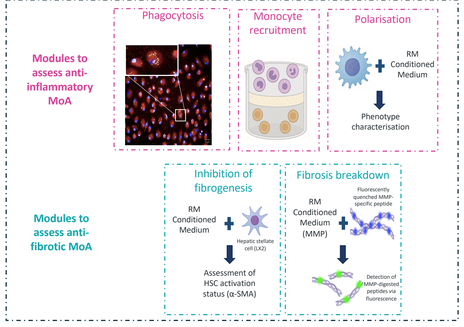
Results

Figure 1: Establishing and validating a MoA-driven testing suite

Establish comprehensive MoA understanding and its relationship with efficacy



Develop modular *in vitro* testing suite



Verify modular testing suite with clinically relevant cells

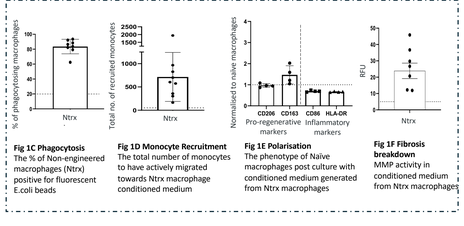


Fig 1C Phagocytosis The % of Non-engineered macrophages (Ntrx) positive for fluorescent E.coli beads

Fig 1D Monocyte Recruitment The total number of monocytes to have actively migrated towards Ntrx macrophage conditioned medium

Fig 1E Polarisation The phenotype of Naïve macrophages post culture with conditioned medium generated from Ntrx macrophages

Fig 1F Fibrosis breakdown MMP activity in conditioned medium from Ntrx macrophages

Figure 2: Candidate Screening identifies IL10 and MMP9 out of 8 potential candidates

Anti inflammatory candidate Selection

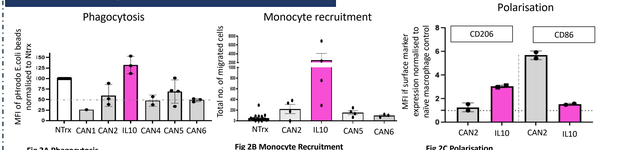


Fig 2A Phagocytosis MFI of candidate transfected macrophage ingested E.coli beads. IL10 is the leading candidate, with CAN1 and CAN4 deprioritised.

Fig 2B Monocyte Recruitment Total monocyte recruitment towards conditioned media of candidate transfected cells. IL10 is the leading candidate with CAN5 and CAN6 deprioritised.

Fig 2C Polarisation Phenotype of naive macrophages cultured in the conditioned medium derived from candidate Trx macrophages. IL10 trx macrophages have enhanced ability to polarise macrophages to a pro-regenerative phenotype vs NTrx. CAN2 deprioritised.

Anti fibrotic candidate selection

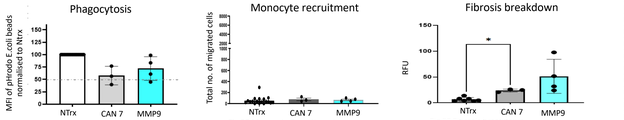


Fig 2D Phagocytosis MFI of candidate transfected macrophage ingested E.coli beads. MMP9 is the leading anti-fibrotic candidate.

Fig 2E Monocyte Recruitment Total monocyte recruitment towards conditioned medium of candidate transfected cells. Anti-fibrotic candidates show no uplift compared to non-transfected cells.

Fig 2F Fibrosis breakdown MMP activity in conditioned medium derived from non-transfected and anti-fibrotic candidate macrophages. MMP9 is the leading anti-fibrotic candidate.

Bicistronic rationale

Candidate	Phagocyt.	Mono. Recruitment	Polarisation	Fibrosis breakdown
IL10	++	++	++	Not tested (hypothesised low)
MMP9	+	-	Not tested (hypothesised low)	++

Results from anti-inflammatory and anti-fibrotic candidate screening, identified two lead candidates, IL10 and MMP9. We hypothesised that by combining IL10 and MMP9 into one candidate, we could improve potency in all aspects of the multi-modal MoA. To this end, we tested multiple linkers and optimised the positioning of the candidates in the construct (data not shown). We chose the optimal construct and progressed with candidate refinement and selection (Fig 3).

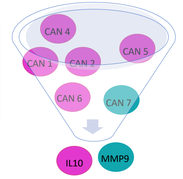


Figure 3: Candidate Refinement and Selection

Phenotypic Characterisation

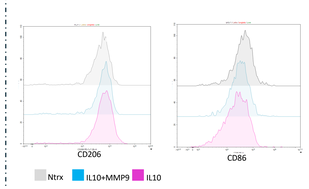


Fig 3A Phenotypic Characterisation (Flow overlay).CD206 (Left) and CD86 (right) expression in RTX001/IL10 engineered macrophages compared to a non-engineered control displayed as an overlay plot. NTRx; IL10 Trx; RTX001 (IL10+MMP9) Trx:

Fig 3B Phenotypic characterisation (Fold Change): CD86 (Left) and HLA-DR (Right) expression in RTX001/IL10 engineered macrophages compared to a non-engineered (NTrx) control. Data displayed as an MFI fold change from a Naive macrophage

Secretion profile

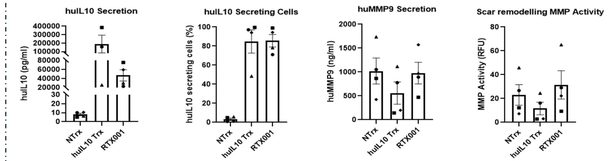


Fig 3C Human IL10 Secretion Measured secretion of human IL10 in the conditioned media of RTX001/IL10 engineered macrophages compared to non engineered controls (NTrx)

Fig 3D Engineering efficiency Percentage population of macrophages secreting human IL10 in RTX001/IL10 engineered macrophages compared to non engineered controls (NTrx)

Fig 3E Human MMP9 Secretion Measured secretion of human MMP9 in the conditioned media of RTX001/IL10 engineered macrophages compared to non engineered controls (NTrx)

Fig 3F MMP Activity Measured activity of human MMPs in the conditioned media of RTX001/IL10 engineered macrophages compared to non engineered controls (NTrx)

Functional *In Vitro* Characterisation

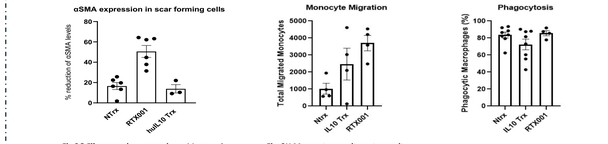


Fig 3G Fibrogenesis suppression: . Measured by αSMA protein levels in LX2 cells cultured with TGFβ and conditioned media from either RTX001/IL10 engineered macrophages or Non-engineered (NTrx) controls.

Fig 3H Monocyte recruitment capacity. PBMCs cultured in conditioned media from either RTX001/IL10 engineered macrophages or a Non-engineered (NTrx) control to induce monocyte migration

Fig 3I Phagocytic capacity. Percentage of RTX001/IL10 engineered and Non-engineered (NTrx) macrophages phagocytosing E.coli-coated beads.

Conclusions

- Engineering RMT to enhance their potency is required to treat patients with decompensated liver cirrhosis (ESLD). To ensure the best candidate progressed to clinical trial, a MoA-based *in vitro* testing suite was established and was contingent on three steps: 1) Understanding of RMT MoA in liver disease, 2) Build individual modules that reflect each aspect of RMT MoA and 3) Verify the platform with a clinically relevant cell type.
- Candidate screening on the MoA testing suite identified that IL10 and MMP9 outperformed the other anti-inflammatory and anti-fibrotic candidates, respectively.
- Macrophages engineered with a combination of IL10 and MMP9, outperformed macrophages engineered with IL10 alone in their ability to recruit monocytes, phagocytose and reduce the activation of scar forming cells. Importantly, in all cases, IL10 and MMP9 engineered macrophages outperformed the non-engineered, clinically validated macrophages.
- IL10 and MMP9 combination was nominated as the candidate to progress to clinical development (RTX001).
- RTX001 (IL10 and MMP9 engineered RM) is now being tested in Resolution's first in human clinical trial – EMERALD – in the UK and Spain.

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