This is the author's accepted manuscript of the article "**Marie Laviron, Martin Guilliams, In vivo macrophage engineering as novel therapeutic strategy against liver metastasis, Trends in Cancer, Volume 10, Issue 3, Pages 175-176, 2024**". The final authenticated version is available online at: https://doi.org/10.1016/j.trecan.2024.01.012.

In vivo macrophage engineering as novel therapeutic strategy against liver metastasis

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Abstract:

A recent study by Kerzel et al. reports a novel therapeutic strategy to engineer tumor-associated macrophages in vivo by inducing the expression of IFNα in these cells. This allows for better antigen-presentation and T cell activation, leading to controlled tumor growth in multiple murine models of liver metastasis.

Keywords: Liver metastasis, macrophage engineering, immunotherapy, tumor-associated macrophage (TAM)

Main text:

The breakthrough of immunotherapies has revolutionized cancer treatment, but its efficiency is still limited and the side effects are considerable. Most immunotherapies target T cells, one of the main cytotoxic effectors of the tumor microenvironment (TME). However, the enhancement of T cell response leads to strong inflammation, causing collateral tissue damage. Over the past decades, there has been a growing interest in targeting tumor-associated macrophages (TAMs) for anti-cancer therapies [2]. Macrophages are the most abundant immune population of the TME. They are highly heterogenous, and are associated with immunosuppressive functions and worse prognosis. Both tissue-resident macrophages and recruited monocyte-derived macrophages contribute to the pool of TAMs [1]. TAMs represent a promising target for immunotherapies, as they can modulate the adaptive immune response and increase local T cell responses within the tumor, without promoting excessive inflammation.

The liver is one of the most common organs for metastasis. Liver metastasis impacts on the local and the systemic immunity, making patients less responsive to immunotherapy [3]. These reduced immune functions hamper the elimination of liver metastases and of the primary tumor. Therefore, the reprogramming of liver macrophages represents an interesting therapeutic target.

In a recent study in *Cancer Cell*, Kerzel *et al.* developed a lentiviral vector (LV) platform that preferentially targets the liver to induce IFNα expression in macrophages *in vivo* (Fig.1) [4]. To ensure macrophage-specific expression, the authors used the Mrc1 promoter to drive the expression of their LV. Mrc1 is highly expressed by mouse and human Kupffer cells (KCs) [5]. Because of their location in the liver sinusoids, KCs can easily uptake the LV injected in the bloodstream. However, they are

surrounded by a network of cells, such as liver sinusoidal endothelial cells (LSEC), which also exhibit high phagocytic capacity [5]. MRC1 is highly expressed by LSECs in the mouse and the human liver [5]. The authors elegantly demonstrate that the incorporation of miRNA sequences downstream of IFNα prevents expression in LSECs and hepatocytes, while maintaining expression of IFNα in KCs. Administration of IFNα LV to mice challenged with experimental models of liver metastases was associated with delayed tumor growth and even complete remission in some mice.

One caveat of using the Mrc1 promoter is that it does not allow the discrimination between KCs and monocyte-derived TAMs. Single-cell studies in mouse and human, demonstrate that liver metastasis is associated with a strong recruitment of monocyte-derived TAMs, many of which express Mrc1 [3,6]. As discussed by the authors, the fate of KCs upon metastatic development is still unclear. The long-term expression of IFNα suggests that long-lived KCs are responsible for most of the IFNα production, but it would be interesting to validate this claim by using a KC-specific promoter to induce LV expression only in KCs. CD5L is highly expressed by human and mouse KCs [5] and not expressed by recruited TAMs in liver metastasis [3,6]. Therefore, CD5L appears to be a good KC-specific promoter. The authors observed that KCs are mainly located at the tumor border and in the healthy part of the liver. However, a recent study showed that KCs can lose their identity in diseased liver, which is associated with reduced expression of several KC-specific surface markers [7]. We hypothesize that some TAMs are KCs that lost their identity and have been rewired by the TME.

IFNα production in the TME is associated with enhanced antigen presentation by KCs and increased CD8+ T cell infiltration. Remarkably, the enhanced inflammatory TME after macrophage engineering does not lead to any collateral tissue damage. The distribution of TAMs and KCs is not altered by IFNα production. Instead, these cells change their activation state and display a distinct transcriptomic profile. The impact of IFNα is different in TAMs and KCs, as only KCs exhibit an increased score of antigen presentation. The authors also identified a large shift in the so-called monocyte-derived dendritic cell (MoDC) population. However, it is difficult to distinguish MoDCs from conventional DCs during inflammation [8]. Stimulation of cDC2s with IFNα induces a hybrid profile with the expression of many genes typically linked to monocyte-derived cells [9]. This population could also contain DC3s, a novel subset of inflammatory DCs [8]. Therefore, there might be additional heterogeneity within the MoDC population which might play a key role in the increased T cell responses.

Only a portion of the mice respond to the macrophage engineering therapy and the effect is partly mediated by CD8+ T cells (Fig.1). Spatial transcriptomics shows that although IFN signaling is activated in macrophages of all mice, resistant mice exhibit increased IL-10 signaling and increased T cell exhaustion and Treg and Tr1 infiltration at the front of the metastasis. One additional aspect that could be addressed is the co-localization between macrophages relative to CD8+ T cells. We hypothesize that the relative distribution of these populations at the time of LV injection could determine the outcome of the therapy as the combination of IFNα-LV and anti-CTLA4 administration leads to complete remission in all mice. As the authors suggested that CD8+ T cell activation might result from IFNα-dependent increased antigen-presentation, we could imagine that mice respond to the therapy only if CD8+ T cells are in close vicinity to KCs at the time of LV injection.

These findings by Kerzel *et al.* reveal the promise of targeting macrophages for cancer treatment. Another approach using chimeric-antigen receptor transduction of macrophages (CAR-M generation) to target HER2 in solid tumors showed a delayed tumor growth with prolonged survival [10]. CAR-M therapy was also associated with induction of antigen-processing and antigen-presentation markers and enhanced CD8 T cell recruitment and activation.

So far, macrophage engineering to treat cancer has only been tested on pre-clinical models. Although the results appear promising, the toxicity and the therapeutic efficacy still remain to be determined in patients. Additionally, the targeted pathway could be adapted to each tumor type. We are thus entering a new age of cancer immunotherapies based on macrophage engineering and we hope these innovative immunotherapies will soon be tested in patients.

Acknowledgements:
Funding: Ghent University BOF18-GOA-024 and BOF24/GOA/035 (M.G.), FWO project grants 3G000519 and G013823N (M.G.), MSCA IF fellowship MetaMacNiche 101109361 (M.L.) and FWO Postdoc Grant 12A3H24N (M.L.).

DECLARATION OF INTERESTS: The authors declare no competing interests.

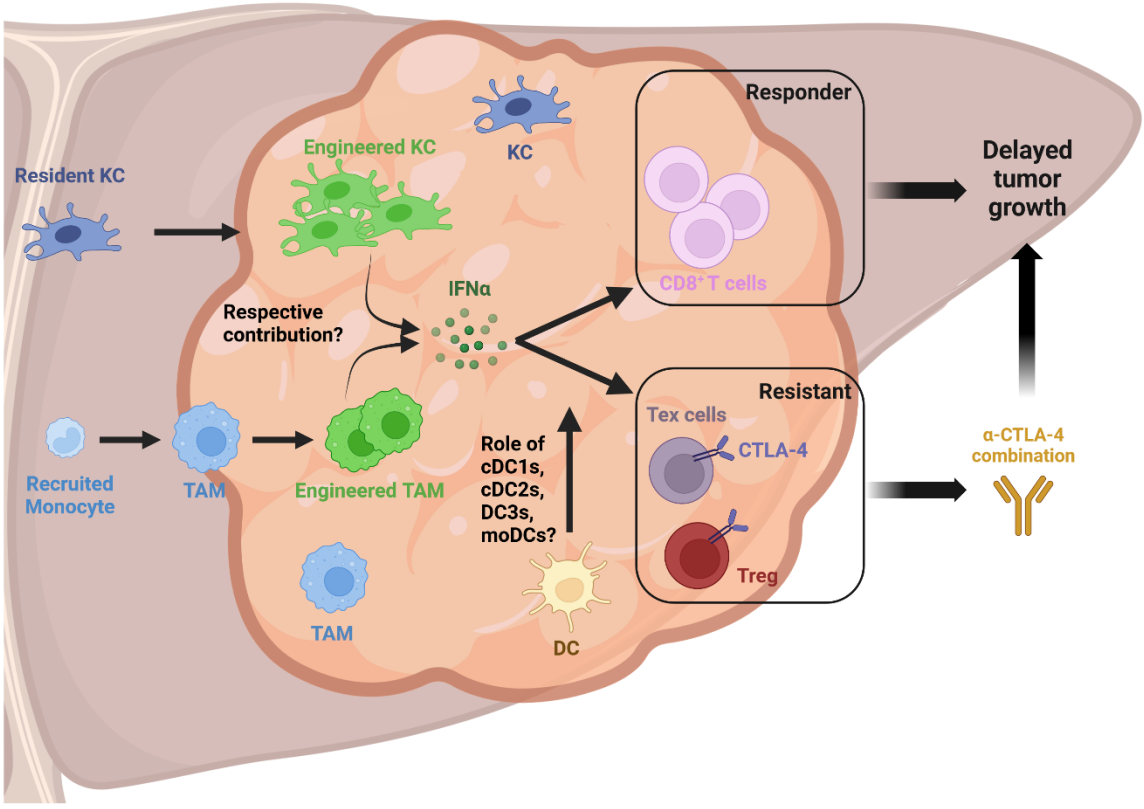

Figure legend:

Title: In vivo macrophage engineering enhances elimination of liver metastases

Macrophages engineered *in vivo* by lentiviral vectors produce IFNα that enhances tumor elimination in a portion of the mice. The combination with an anti-CTLA4 allows to overcome the resistance to IFNα production alone. The origin of the macrophage subset(s) responsible for the therapeutic effect, and the role of DCs in response to IFNα production remains to be determined.

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