Review

Non-invasive Multiregional Tumor Sampling Using Magnetic Nanoparticle-Loaded Macrophages

Michael Renteln *

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Keywords: Multiregional sequencing; multi-sample sequencing; targeted cancer therapy; OVERCOME; magnetotaxis; macrophages

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Non-Invasive Multiregional Tumor Sampling Using Magnetic Nanoparticle-Loaded Macrophages

Michael Renteln

University of Würzburg (Germany)
* Correspondence: mrenteln@gmail.com

Abstract: Some tumors occur in anatomical regions that are hard to biopsy with a needle. Such regions include the brain, spinal cord, liver, and lungs. For the latter two, magnetic nanoparticle-loaded macrophages could be intravenously infused and driven via an MRI machine into the tumor or tumors. Once there, they can be induced to phagocytose whole tumor cells. They would keep their target in a non-digested form by inhibiting phagosome maturation - and be directed via magnetotaxis or chemotaxis to an extraction point in the body where they can be more easily collected via needle.

Keywords: multiregional sequencing; multi-sample sequencing; targeted cancer therapy; overcome; magnetotaxis; macrophages

1. Introduction

Cancer has plagued multi-cellular organisms since their conception. Recently, I wrote a paper about how many cancer patients may have one or more mutations that are ubiquitous throughout their tumor(s) [1–3]. The rest may at least have a small set of subclonal mutations that together cover all sequenced regions of their tumors. These mutations could be targeted by an oncolytic vector with the broadest tropism possible that only replicates and becomes hyper-virulent after detecting said mutations. I called this strategy, "Oncolytic Vector Efficient Replication Contingent on Omnipresent Mutation Engagement" (OVERCOME).

To identify these mutations, multiregion, multi-sample sequencing should be employed for each patient. However, tumors in certain anatomical regions are not easy to biopsy - especially in a multiregional fashion. Such regions include the brain, the spinal cord, the liver, and the lungs.

Here, I explore another way of acquiring multiregion biopsies from tumors that are hard to reach via traditional means.

2. Mechanisms

Bioengineered macrophages could be used for this purpose. They could be loaded with magnetic nanoparticles (MNPs) and steered into the heart or lung tumors using an MRI machine [4]. Perhaps the macrophages should be induced via small molecule to chemorepel [5] each other once they have reached the target site or sites - in order to spread out more evenly throughout the tumor(s). A gene circuit possibly involving ARHGEF37 [6] or Cdc42 could be employed as well - to allow for vigorous, random movement within the tumour in addition to their chemorepulsion from each other.

In either case, once there, they could be induced via small molecule to express a chimeric antigen receptor for phagocytosis (CAR-P) [7]. Alternatively, they can be heated via an alternating magnetic field to induce gene expression of the CAR-P [8]. The CAR-P would target a ubiquitously expressed cell surface protein [9,10]. If necessary, SNIPRs [11] could be utilized to allow for targeting multiple ubiquitously expressed cell surface proteins to ensure phagocytosis of cancer cells throughout the tumor(s).

Inhibiting maturation of and lysosomal fusion with the specific phagosome carrying the target cell could be achieved in a variety of ways [12]. After giving the macrophages some time to collect a

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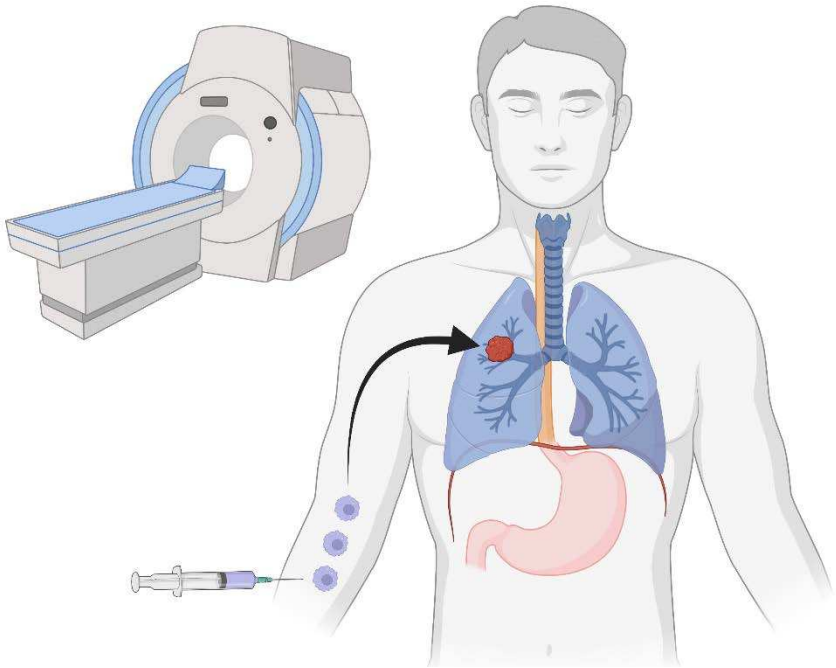
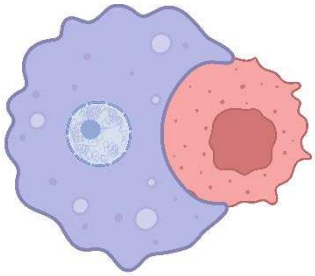
target - they would be drawn via magnetism or chemotaxis to an extraction point in the body. Perhaps they can be magnetically drawn to the peritoneal cavity [13] and withdrawn via needle.

Importantly, it was shown that whole cancer cells can be engulfed via this CAR-P method, as opposed to trogocytosis. However, trogocytosis was still more common. Thus, more work may be necessary before this strategy can be employed.

Figure 1 CAR-Ps.



(**A**)



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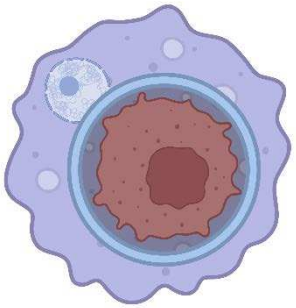

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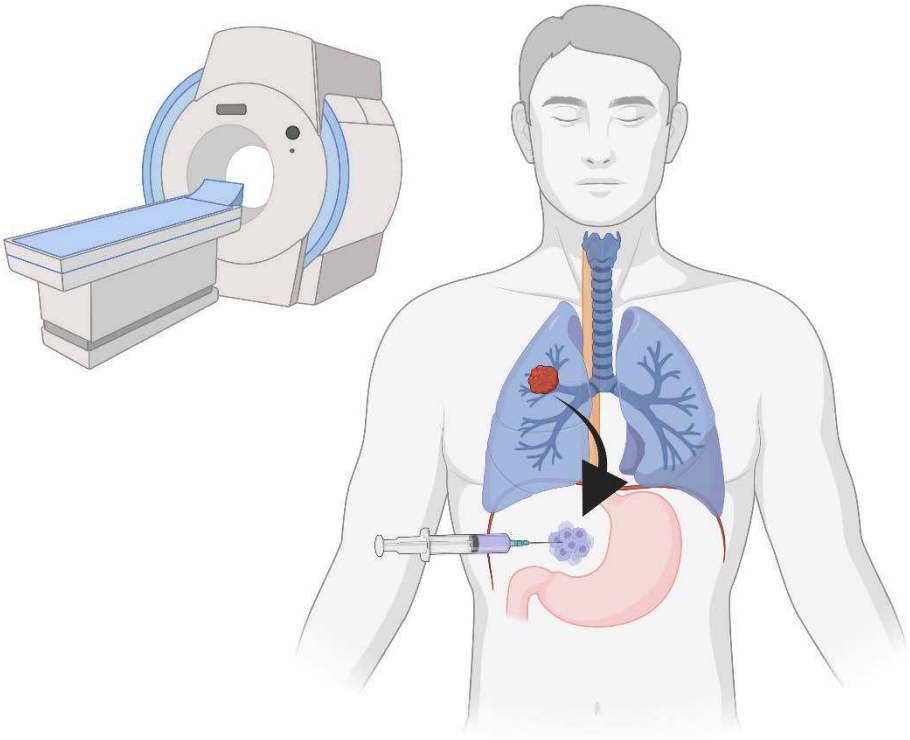

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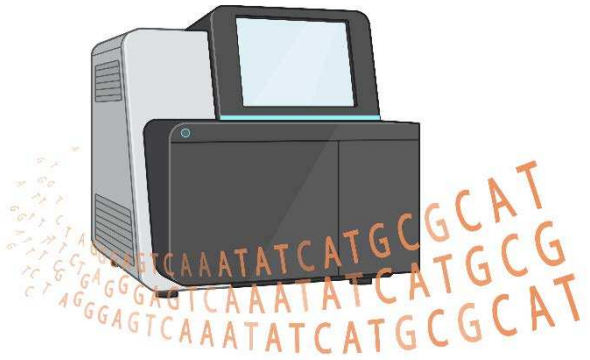

(**E**)

Figure 1. A) The bioengineered macrophages are magnetically steered into the tumor. B) The chimeric antigen receptor is induced, and macrophages phagocytose cancer cells in a patient's tumor. C) The cancer cell resides within a non-acidified phagosome that does not fuse with a lysosome. D) The macrophages are magnetically drawn to the peritoneal cavity; they are withdrawn via needle. E) The cancer cell genomes are sequenced to look for ubiquitous mutations.

An alternative solution would be to install the T-cell lytic granule system in the CAR-P macrophages [14,15]. They would lack granzyme B, but be able to rapidly and directionally secrete perforin to lyse the target cell while sparing the nucleus [16,17]. Then, they could phagocytose debris until they bind the nuclear envelope - and selectively phagocytose that and inhibit the maturation of the phagosome and phagosome-lysosome fusion. (The nucleus would be a smaller target than the cell as a whole.)

Yet another strategy would be for carrier, Irf8($^{-/-}$) [18] macrophages to ferry intracellular, phagocytic [19], magnetosome [20]-bearing bacteria to cancer cells. The bacteria would lyse the carrier macrophages once in the tumor region, invade tumor cells, and phagocytose their nuclei. Then, they would lyse the cancer cell - and could be directed chemotactically or magnetotactically to an extraction point.

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The macrophages could also simply attach to the cancer cells and magnetotactically pull them to an extraction point. It would be very important that they do not drop their cargo along the way. However, the cancer cell could theoretically replicate while being towed.

Finally, cell-cell fusion [21] could potentially be locally induced in the tumor - as long as the macrophage had many chromosomally-encoded, small molecule-controlled kill switches and could inhibit the nuclear activity of the cancer cell after fusion. Perhaps this could be achieved by rapidly surrounding it with a deacidified autophagosome with dominant negative Stx17 [22] - so it cannot fuse with lysosomes. Or, bacterial phagocytosis could be employed. While at least some of these strategies may carry an increased risk of metastasis - traditional needle biopsies may also increase the risk of causing metastasis.

3. **Conclusion**

With regard to the brain and spinal cord, perhaps magnetism is all that's required to cross the blood-brain and blood-spinal cord barrier - but perhaps not. Magnetic resonance-guided motorized transcranial ultrasound [23] could be used to open the blood-brain barrier at the tumor locale(s). The same technology is applicable to the spinal cord as well; MRI-guided ultrasound has already been tested in that context [24]. Instead of magnetism, perhaps a prodrug version of deschloroclozapine [25] can be employed that is blood-brain and blood-spinal cord barrier—permeable. It would be activated by an extracellular enzyme that is abundant in the brain and/or spinal cord [26] and serve as a chemoattractant for the macrophages [27]. This could be superior to magnetism because it might induce more crawling along the barrier endothelial cells until an infiltration point can be found. The question of egress from the central nervous system is also an issue. Chemotaxis and/or magnetotaxis may again be sufficient. Alternatively, maybe in the future, the CAR-Ps can somehow be directed to the lymphatic system in the brain and spinal cord and drain into a more easily accessible lymph node [28] for needle-based extraction.

This magnetism-based approach is also suitable for the macrophage-mediated delivery of oncolytic vectors to tumors that would be difficult to reach via needle, of course. This has already been accomplished in mice [4]. With regard to OVERCOME, vector replication in the carrier macrophages would be induced via small molecule, heat, or even magneto-mechanical actuation [29] once they have reached the tumor(s). Excess iron oxide nanoparticle deposition in the body from carrier macrophage lysis may not be ideal, but they should be degraded eventually [30].

Acknowledgments: The figure in this piece was created with BioRender.com.

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