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Macrophages hold the key to cancer's inner sanctum

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A B S T R A C T

All malignancies contain tumor-associated macrophages (TAMs) that facilitate cancer growth by secreting chemicals to elicit angiogenesis and shield the cancer from the immune system. The abundance of TAMs is a reflection of invasiveness and metastatic potential. TAMs will actively ingest ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles following intravenous administration and will store them as large lysosomal aggregates which can be imaged with MRI and ultrasound and visualized or quantitated in tissue biopsies. Since the USPIO also enhances regional lymph nodes, it is possible to include this information for more accurate cancer staging. The USPIO aggregates surprisingly also serve as heat sinks and can enhance hyperthermic regimens with focal laser, focused microwaves, or high-intensity focused ultrasound (HIFU). The hyperthermic intervention can be chosen based upon accessibility for the selected energy source. By sustaining an intratumoral elevation of temperature for an effective period of time, ablation of a small or large fraction of the TAMs and cancer cells can be achieved. Thus, for aggressive cancer, USPIO is a *theragnostic agent*. Following USPIO-enhanced hyperthermia, the resulting debris will slowly reach the regional lymphatics and immune recognition may result. An effective vaccine or adjuvant could be injected peritumorally to improve immunorecognition of that patient's cancer. The field of immunotherapy is being intensely explored at present. Using the theragnostic properties of USPIOs that are accumulated in the TAMs may prove useful in further attempts to make immunotherapy successful. This intervention could be utilized at any stage of cancer therapy. Should immunological recognition occur, an abscopal response may be achieved for that patient and for his/her cancer. This would truly be personalized cancer therapy.

Introduction

There are more than 120 named cancers in the National Cancer Institute (NCI) database. Yet cancer originates in a patient's own cells and may differ, in important ways, from other cancers with the same pathological diagnosis. It is surprising that surgery is not effective at extending life [1]. The essence of personalized medicine is to treat the disease that the patient has, and, where cancer is concerned, and the treatment should give each patient a chance at cure. Since all aggressive cancers have TAMs, it might be possible to target those macrophages rather than the malignant cells. In order to position the proposed hypothesis in relation to what we know, a general review of the relevant cancer literature was conducted. Table 1 shows selected search terms in the PubMed database and Table 2 shows reported clinical protocols in the ClinicalTrials.gov database. Many cancer therapies, including immunotherapy, are heavily represented in these data bases. However, there is little representation of USPIO for cancer detection, staging, and inclusion in hyperthermic therapies or support of immunotherapy. As the reviews suggest, it is difficult to chemically target a cancer cell. Yet, if properly used, USPIOs easily accumulate in the TAMs within any cancer where they are present in adequate numbers.

Cancer therapy and immunogenic cell death

Adkins et al. [2] have recently published a comprehensive review of the chemistry, biology, and mechanisms of immunogenic cell death, including clinical studies. In brief, chemotherapy, radiation therapy, phototherapy, and hyperthermia have all had some success in both animal models as well as clinical studies (references cited therein). Radiation therapy seems to have the most clinical success, often in combination with chemotherapy, but even here success is uncommon.

Adkins notes several important aspects of hyperthermia. The efficacy is related to both the achieved temperature and how long it is sustained. In general, lower doses of heat (41–44 °C) cause apoptosis while higher temperatures (50–55 °C) cause direct necrosis. The interaction of temperature and time sustained is shown in Table 3. Cancer cells are not more sensitive to hyperthermia than normal cells. The shape of a tumor is rarely perfectly spherical, and it is difficulty is to heat specifically only the cancer region without damaging the surrounding normal tissues.

Hyperthermia goal

Sufficient heat, maintained for an adequate time will kill any cell, including cancer stem cells. The time-temperature interaction for outright cell destruction is strikingly nonlinear. And at any given period of heating, an elevation of temperature has powerful effects. Clinically, when hyperthermia is used to treat tumors, it is difficult to get effective heating throughout the tumor while sparing surrounding normal tissues.

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Table 1
The pub med trail.

Search Term	Citations
Cancer treatment	658,047
Cancer chemotherapy	255,420
TAMs	3912
TAM + metastasis	869
TAM + prognosis	698
TAM + MRI	40
TAM + MRI + USPIO	4

Table 2
Cancer studies in clintrials.gov.

	Registered Trials
Adjuvant	4656
Vaccine	1904
Macrophage	340
HIFU	103
Focal Laser	33
USPIO	16
TAM	10
Focused Microwave	6

Table 3
Time-temperatures for tumor ablation.

Temperature °C	Sustain for
48	15 min
52	60 s
55	10 s
60	5 s
65	1 s

But perhaps we don't need to ablate the entire cancer. We might only have to obtain differential heating of part of the tumor and the opportunity is presented by tumor-associated macrophages.

Tumor-associated macrophages

TAMs play a key role in malignancy. Recruited early, they generate the cytokines that enhance permeability of tumor vessels, and suppress immunologic attack [3]. They facilitate metastasis. The more TAMs there are in the cancer, the more malignant that cancer is. The publications of Fidler [4], Shih et al. [5], Zhang et al. [6] and Sica et al. [7] are recommended. The status of TAMs is not currently included in cancer staging, although it could be. It has proven difficult to chemically target the cancer cell, but it easy to target the TAMs.

Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles

Two different sizes of iron oxide nanoparticles have been used to identify macrophage phagocytosis in patients. Table 4 reviews the three that I have experience with. Macrophages ingest the nanoparticles via specific receptors in the cell membrane. Feridex® is much larger than the USPIOs and only has ready access to the macrophages in liver and spleen. The process is MR imageable, the wait time is short, and the dose required is low.

USPIOs are small enough to extravasate through the normal capillary beds in lymph node, bone marrow, and adrenal cortex—accumulating in normal macrophages in these tissues. It is their long blood life that allows them to accumulate to imageable amounts after one or more blood half-lives. Sequestration continues as long as the USPIOs are presented in the blood. Within the macrophage, the nanoparticles are stored in lysosomes where they accumulate to huge aggregates [see Fig. 2 & 4 in Levy et al. [8]]. A lysosome may accumulate more than 100,000 nanoparticles and there are often many loaded lysosomes in each macrophage. The enhanced permeability and retention of tumors facilitates the access of USPIOs to tumor-associated macrophages.

Table 4
Iron oxide nanoparticles.

		Not approved for imaging indications	
	Feridex IV* *No longer marketed	Ferumoxtran-10** **in clinical trials	Ferumoxytol injection Feraheme®
Target Tissue	Liver	Lymph Nodes	Treatment of anemia, imaging off-label
Administration	0.6 mg Fe/kg, 60 min infusion	3–5 mg Fe/kg Slow infusion	4–6 mg Fe/kg Slow infusion
Size nm	120–180	15–30	30
Relaxivity	R1 = 10 R2 = 120	R1 = 10 R2 = 65	R1 = 15 R2 = 90
Blood T ½	2 h	24–36 h	10–14 h

Metabolism of the ingested USPIOs is slow. USPIO aggregates can be imaged with MRI. There is an excellent review of the utility of USPIO in clinical MRI applications [9] as well as TAM imaging in animal models [10]. The report that USPIO aggregates are visualized with ultrasound [11] has not been seriously explored. The actual concentration of iron due to the aggregated USPIOs can be quantitatively estimated with special MRI sequences, but visual estimates would usually suffice. USPIO-loaded TAMs could be quantitatively and qualitatively assessed in tissue biopsy as part of cancer staging.

USPIO-enhanced hyperthermia

These large aggregates can actually serve as heat sinks for laser, microwave or high-Intensity focused ultrasound (HIFU). And this property does not require the magnetization of the nanoparticles that is necessary for MRI.

To demonstrate the importance of large USPIO aggregates, I performed an experiment in cooperation with TTY Biopharm (Taiwan). We made suspensions with MKN45 (gastric cancer cells) or RAW264.7 macrophages with a cellular concentration of 5%. The cells were incubated for 24 h with 0, 0.1, or 1 mg/ml USPIO concentration. Each preparation was then irradiated with a 1064 nM laser at 1.4 W/cm2 for 60 s. After 24 h of further incubation the preparations were then plated for microscopic determination of cell viability. The results are shown in Table 5. Note that, despite the same added USPIO, only the macrophages were killed in a dose-dependent manner.

The hypothesis

If we give USPIO intravenously to a patient with cancer containing tumor-associated macrophages,

Then

–Following at least one-blood half-life, these macrophages will

Table 5
USPIO uptake and laser ablation.

USPIO (mg/ml)	MKN45		RAW264.7	
	Fe (picogram per cell)	Viability (%)	Fe (picogram per cell)	Viability (%)
0	2.11	100%	0.52	98%
0.1	1.71	99%	9.72	73%
1	2.03	95%	28.24	20%

Footnote: Comparison of response to 60 s of laser exposure in MKN 45 cancer cells and RAW247.2 macrophages after 24 h USPIO loading.

accumulate very large numbers of iron oxide nanoparticles in their lysosomes

And

–MRI, weighted for T2 and/or T2* will identify all such cancers of sufficient size as well as the presence of regional nodal metastases;

–Rapid detection of USPIO in core biopsy samples with desktop NMR would confirm sampling of the cancer;

And

–Enhanced hyperthermia with the best choice of laser, HIFU, or focused microwaves for an accessible, USPIO-enhanced cancer will induce necrosis and apoptosis in the cancer

Resulting in

–the slow release of tumor debris which may, by itself, induce immunorecognition of that cancer

–the opportunity to increase immunodetection within the regional lymphagics by peritumoral administration of an effective immunoadjuvant or immunovaccine

It is bold to suggest that USPIOs can be theragnostic for all cancers where TAMS are present in sufficient numbers. I speculate that greater than 5% of the cancer volume as TAMS is sufficient to consider their potential utility. Prior imaging trials of ferumoxtran-10 and ferumoxutol showed safety and accuracy for detecting nodal metastasis. There is insufficient data to claim that they could predict invasiveness or metastasis, but TAM content is already known to do this. Utility in core biopsy would appear to be simply a matter of building the right desktop NMR where detection and semi-quantitation can be done in minutes. So far, the ability of large aggregates USPIO particles to serve as heat sinks for laser, microwave, or HIFU has not been confirmed. In animal models or clinical interventions. But other forms of iron oxide nanoparticles do serve this function for lasers [12] and HIFU [13]. Finally, the hypothesis is readily adaptable for animal and clinical cancer trials, especially with the addition of a likely effective immunoadjuvant.

The protocol for treatment of TAM-enriched cancers

1. At the earliest possible time in a cancer patient in need of therapy, administer USPIO and within 20–24 h perform whole body T2-and/or T2*-weighted MRI to locate all cancers with TAMs
2. Identify the best cancer to attempt enhanced hyperthermia. This includes location and choice of hyperthermic modality—interstitial laser, ultrasound-guided HIFU, or focused microwaves.
3. Use interstitial temperature measurement within the tumor (technology will evolve and may even be subject to simulation) to generate an intratumoral temperature that exceeds 50 °C and sustain it for the period necessary to ablate the tumor according to Table 5. Since MRI is not required for the heating, this can be done in an interventional suite using imaging guidance. There are special mobile platforms that will be able to collate prior medical images with interventional guidance such as ultrasound, CT, or endoscopy.
4. Locally administer an adjuvant that facilitates recognition of tumor debris in the draining lymph node as the tumor undergoes necrosis. This adjuvant is not yet fully characterized but there are numerous candidates.
5. Monitor the response of metastatic cancers using diagnostic USPIO-enhanced MRI or ultrasound. (N.B., if this is done within 2–4 weeks after step 1, it will not be necessary to reinject the USPIO).
6. Repeat the intervention with another adjuvant if tumor growth continues.
7. If an abscopal response is not achieved, refer the patient for other interventions as desirable.

Consequences and discussion

First, use the proposed treatment protocol regardless of whether the patient has already received other therapy. The USPIO would be administered in a clinical setting, but with an MRI already scheduled for a time that would be after 1–3 blood half-lives of the particular USPIO. Whole body T2 and/or T2* sequences would be performed, preferably using at least 1.5 T MRI. T2* can be used to quantitate iron content. This may be helpful in planning the hyperthermic treatment. Note that pre-USPIO and early T1 sequence MRI are not necessary and this reduces cost. It is expected that any tumor larger than about 1 cm that containing at least 5–10% TAMs would be detected. Nodal status would also be evaluated.

Clinical decisions would now be determined. We want to know if it is feasible to attempt enhanced hyperthermia to ablate at least part of one TAM-infiltrated cancer with the best choice of focal laser, focused microwave, or HIFU. I believe that the extensive efforts to develop cancer vaccines or adjuvants would guide the decision to inject it peritumorally upon completion of the enhanced hyperthermia. The intent here is to have the slow, sustained release of cancer debris and the immunologic-enhancing agent reach the lymph nodes in the draining lymphatics where regional dendritic cells, or APCs could recognize one or more of the cancer antigens being presented– thereby mounting an effective immunologic response. As the debris continues to be delivered over a time measured in weeks, it may be possible to use several injections of one or an alternative immunologic enhancer.

The dose response to temperature and time is shown in Table 3. The goal is not to ablate all the cancer, but merely to cause necrosis of TAMs and a portion of the nearby cancer cells. More ablation is probably desirable and a mixture of apoptosis and necrosis may provide a range of tumor antigens [2]. The applied energy will directly heat cells in the target, and the absorption of energy within the USPIO-enhanced TAMS will create much higher temperatures there. This heat will diffuse to the nearby cancer cells. We expect to have a spectrum of necrosis and apoptosis as well as viable cancer cells left in the target after enhanced hyperthermia. This outcome would be a problem if the goal were complete tumor destruction. The hypothesis is that we are only seeking to elicit an immunogenic response. If effective, any remaining viable cancer in the treated tumor will be immunologically targeted as well as the other tumors that have not been heated. A reasonable plan could be to generate an intratumoral temperature greater than 52 °C and sustain it for 60 s. It may be necessary to monitor the intratumoral temperature, but it is likely that simulations based upon the tumor USPIO content (this can be estimated from the tumor T2*) will provide approximate thermal response in relation to the treatment plan. As shown in Table 3, the conditions for cell destruction are quite flexible. The thermal response to laser, microwave, or HIFU will differ, but each has the capability of generating tumor necrosis that presents tumor debris to the regional lymphatics.

Brewer et al. [14] have recently shown that regional lymph node swelling is a potential biomarker of successful vaccination. In as much as the USPIO remains in both nodal and tumor-associated macrophages for at least 2–4 weeks, repeat MRI of the treated cancer and its regional lymph node as well as one or more of the untreated cancer lesions might provide evident of immunologic success. There may well be other methods to determine if the enhanced hyperthermic ablation of the target cancer has initiated an attack on all like cancers, i.e. an abscopal response.

Finding an effective immunoadjuvant

It is beyond the scope of this hypothesis to anticipate the proper focally-administered pharmaceutical to enhance immunologic recognition of one or more of the cancer fragments [15]. As noted in Tables 1 and 2, the effort to find such an enhancing agent is already vigorous. There may be many agents that are effective. For example, Chen et al. [16] found different efficacy of four agents in the same metastatic animal tumor mode. A single tumor was treated with photothermal therapy, survival was enhanced as follows—partial Freunds-7%; complete Freunds-18%; Cornybacterium parvum-9%; glycated chitosan-29%.

All of the technology necessary to test the hypothesis is available, although the use of USPIO for this purpose has not been approved. Both frumoxtran-10 and ferumoxytol have undergone extensive clinical trials and the later is available, as Feraheme® for the treatment of iron-deficiency anemia. It would be easy to include the theragnostic capability of USPIO in clinical cancer trials of immunotherapy.

The hyperthermic intervention, if successful, has immediate results as contrasted with the slow outcomes from chemotherapy or radiation therapy. And if it is accompanied by enhanced immunotherapy with a given adjuvant, USPIOs may capitalize on the TAMs that have been making the cancer more malignant.

Conflict of interest statement

Gerald Wolf had no financial and personal relationships with other people or organizations that could inappropriately influence (bias) his work. There were no sponsors. The single experiment reported was paid for by the author.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2018.10.028.

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